RICE UNIVERSITY

Anoplolepis gracilipes invasion of the Samoan Archipelago: Can mutualisms with native species amplify ecological consequences?

By

Amy Marie Savage

A THESIS SUBMITTED
IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE

Doctor of Philosophy

APPROVED, THESIS COMMITTEE

Jennifer A. Rudgers, Co-Chair
Assistant Professor, Ecology and Evolutionary Biology

Kenneth D. Whitney, Co-Chair
Assistant Professor, Ecology and Evolutionary Biology

Tom E. X. Miller, Faculty Fellow, Ecology and Evolutionary Biology

Dennis D. Cox, Professor, Statistics

HOUSTON, TEXAS
May 2011

Abstract

Anoplolepis gracilipes invasion of the Samoan Archipelago: Can mutualisms with native species amplify ecological consequences?

by

Amy Marie Savage

Integrating mutualism into the study of ecological communities is likely to be critical to understanding community dynamics and to predict the consequences of anthropogenic changes to ecosystems. Invasive species are among the greatest of these threats to global biodiversity. Throughout the Pacific, the invasive ant *Anoplolepis gracilipes* associates mutualistically with *Morinda citrifolia*, a native plant with extrafloral nectaries (EFN). I tested the hypothesis that these interactions can mediate invader impacts *Anoplolepis gracilipes* abundances were positively correlated with the dominance of EFN-bearing plants per site and negatively correlated with the species richness of native ants. Additionally, *A. gracilipes* displayed a higher magnitude of responses to nectar than other dominant ants. Mutualisms also had significant impacts on the structure of arthropod communities. These effects were strongest when *A. gracilipes* dominated local ant assemblages. These results suggest that novel mutualisms between invasive and native species can facilitate the impacts of invasions on communities.

Acknowledgments

I would like to thank my advisors, Ken Whitney and Jennifer Rudgers for support, feedback, patience and assistance of many kinds over the course of this project. Thanks also to land owners and managers in Independent and American Samoa. Sally Johnson, Olivia Barnes, Jeremy Caves, Kevin Dougherty, Rebecca Sandidge, Mark Schmaedick, and Samani Tupufia provided invaluable field assistance. I am also grateful to the undergraduate students, graduate students and post-doctoral associates in the Rudgers-Whitney lab, Special thanks to Kerri Crawford, Jeff Ahern, Scott Chamberlain, A. J. Davitt, Juli Carillo, Christopher Gabler, and Katherine Horn for providing a wonderful support network. Thanks also to the members of the ANTS discussion group. Finally, the National Geographic Society, Sigma Xi Scientific research society, and Rice University provided funding that was critical to the project's success.

Contents

List of Tables

List of Figures

Chapter 1

Elevated dominance of extrafloral nectary-bearing plants is associated with increased abundances of an invasive ant and reduced native ant richness

ABSTRACT:

Invasive ants can have substantial and detrimental effects on co-occurring community members, especially other ants. However, the ecological factors that promote both their population growth and their negative influences remain elusive. Opportunistic associations between invasive ants and extrafloral nectary (EFN)-bearing plants are common and may fuel population expansion and subsequent impacts of invasive ants on native communities. We examined three predictions of this hypothesis, compared ant assemblages between invaded and uninvaded sites and assessed the extent of this species in the Samoan Archipelago (six islands and 35 sites. We surveyed abundances of the invasive ant *Anoplolepis gracilipes*, other ant species, and EFN-bearing plants. *Anoplolepis gracilipes* was significantly more widely distributed in 2006 than in 1962, suggesting that the invasion of *A. gracilipes* in Samoa has progressed. Furthermore, (non-*A. gracilipes*) ant assemblages differed significantly between sites invaded and uninvaded sites. *Anoplolepis gracilipes* workers were found more frequently at nectaries than other plant parts, suggesting that nectar resources were important to this species. There was a strong, positive relationship between the dominance of EFN-bearing plants in the community and *A. gracilipes* abundance on plants, a relationship that co-occurring ants did not display. High abundances of *A. gracilipes* at sites dominated by EFN-bearing plants were associated with low species richness of native plant-visiting ant species. *Anoplolepis gracilipes* did not display any significant relationships with the diversity of other non-native ants. Together, these data suggest that EFN-bearing plants may promote negative impacts of *A. gracilipes* on co-occurring ants across broad spatial scales. This study underscores the potential importance of positive interactions in the dynamics of species invasions. Furthermore, they suggest that conservation managers may benefit from explicit considerations of potential positive interactions in predicting the identities of problematic invaders or the outcomes of species invasions.

Keywords: Ant Invasions, Mutualism, Extrafloral Nectar, Ant Communities, Island Populations, *Anoplolepis gracilipes*

1.1. Introduction

The introduction of invasive species to novel habitats poses a major threat to global biodiversity and the maintenance of ecosystem processes (Walker and Steffen 1997, Mack et al. 2000, Reaser et al. 2007). Invasions have fundamentally altered community structure across marine, freshwater and terrestrial habitats and have led to significant declines in populations of native species (Sakai et al. 2001, Callaway and Moran 2006, Snyder and Allendorf 2006). Tropical oceanic islands, which are characterized by high levels of endemism, are particularly vulnerable (Fritts and Rodda 1998, Myers et al.

2000, Reaser et al. 2007). Among insect groups, invasive ants have had especially strong effects on native island communities, causing broad-scale changes in island ecosystems, altering community dynamics across multiple trophic levels, and ultimately leading to the dissolution of important ecosystem processes (Holway et al. 2002, O'Dowd et al. 2003). One factor that may contribute to these negative effects is the propensity of invasive ants to associate facultatively with carbohydrate-excreting plants and arthropods, thus displacing native ants in mutualistic associations (Lach 2003, Ness and Bronstein 2004).

Native ant species appear particularly vulnerable to the impacts of invasive ants (Holway et al. 2002). For example, Hoffmann et al. (1999) found that the invasive ant *Pheidole megacephala* was associated with reductions of 50% to >95% in the abundance of co-occurring ants across invaded sites. Such negative effects may occur through direct interactions with native ants, such as increased levels of aggression (e.g., Rowles and O'Dowd 2007) and usurpation of nesting sites (Fluker and Beardsley 1970). Invasive ant species may also affect native ant populations indirectly. For example, invaders may depress prey populations or restrict access to carbohydrate resources such as extrafloral nectar and hemipteran honeydew (Ness and Bronstein 2004). Because of the strong ecological consequences of ant invasions, it is important to identify factors that contribute to their progression.

Empirically, invasions have often been observed to include a lag phase in which the invader is not abundant enough to negatively influence co-occurring species (Mack et al. 2000, Simberloff and Gibbons 2004). The transition from this lag phase to an exponential growth phase marks a critical step in species invasions, one that often leads to the most extreme negative consequences for native communities. For example, when yellow crazy ants (*Anoplolepis gracilipes*: Formicinae) were introduced to Christmas Island, they remained at low population densities during a lag phase that lasted >70 years (O'Dowd et al. 2003). In the 1990's, this species began displaying invasive characteristics. Within ~10 years, *A. gracilipes* had killed up to one-third of the island's endemic red land crabs (*Gecarcoidea natalis*). Furthermore, *A. gracilipes* actively tended scale insects, and correlative evidence suggests that the combination of increased carbohydrate resources for the ants and population expansion of scale insects led to the death of native canopy trees (O'Dowd et al. 2003). The transition out of a benign lag phase is an important component of the process of invasion; however, the ecological factors that promote this transition remain unclear.

For invasive ants, mutualisms with carbohydrate-excreting plants or arthropods can potentially provide a constant resource to 'fuel' the growth of ant populations. Several authors have suggested that these mutualistic associations structure ant communities, especially in the tropics (Davidson et al. 1993, Heil and McKey 2003, Lach 2003, Blüthgen and Stork 2007). The carbohydrates provided by honeydew-excreting insects and extrafloral nectary (EFN)-bearing plants are predicted to be more important for invasive ants than native ants in many systems (Holway et al. 2002, Ness and Bronstein 2004, Styrsky and Eubanks 2007). For example, Lach (2007) showed that invasive Argentine ants (*Linepithema humile*) were more responsive to native honeydew-excreting scale insects than were co-occurring ant species. Additionally, we have found that experimental increases in nectar levels led to higher forager recruitment and numerical dominance of the invasive ant, *Anoplolepis gracilipes,* whereas co-occurring non-invasive ants did not respond to changing nectar availability (Savage et al. *In Press*).

However, few experiments or broad-scale geographic surveys have explored whether carbohydrate subsidies drive invasive ant abundances or impacts (but see Eubanks 2001, Kaplan and Eubanks 2005).

In this study, we investigated the potential for EFN-bearing plants to promote the invasion of the yellow crazy ant, *Anoplolepis gracilipes,* in the Samoan Archipelago. Although *A. gracilipes* was first recorded in Samoa in 1925, the species was present only near ports and plantations at very low abundances (Emery 1925 *cited in* Wilson and Taylor 1967). Recent evidence suggests that their populations may be expanding in Samoa. Lester and Tavite (2004) collected *A. gracilipes* in pitfall traps around the port of Apia (Upolu). They found that *A. gracilipes* reached abundances of 191-1,060 per trap within 24 h. However, to assess the progression of the *A. gracilipes* invasion, it is critical to understand the abundance and dominance *A. gracilipes* across a broad geographic range and quantify the current composition of ant assemblages across both *A. gracilipes*-invaded and uninvaded sites. Importantly, ants have access to a variety of carbohydrate-excreting plant species in Samoa, which could provide resources for *A. gracilipes* population growth. These species include four native EFN-bearing plants: *Hibiscus tiliaceous* (Malvaceae), *Ipomea pres-caprae* (Convolvulaceae), *Erythrina fusca* (Fabaceae), and *Morinda citrifolia* (Rubiaceae) and one non-native EFN-bearing plant, *Clerodendrum fragrans* (Lamiaceae).

In this study, we addressed the following specific questions: (1) Has the *A. gracilipes* invasion progressed in Samoa? Wilson and Taylor (1967) assessed ant assemblages in Samoa in 1962. We predicted that *A. gracilipes* would be more widespread in our (2006) survey, indicating that the *A. gracilipes* invasion has progressed over the past ~40 years; (2) Do ant assemblages differ in invaded vs. uninvaded sites? We expected *A. gracilipes* presence to lead to an overall simplification of co-occurring ant assemblages; (3) Are *A. gracilipes* foragers more abundant at extrafloral nectaries than other plant parts? Ants may visit EFN-bearing plants to forage for invertebrates, nectar, or other resources. Additionally, plants can alter local biotic and abiotic conditions, by providing shade and shelter from wind and natural enemies. Predominance of ants at nectaries (relative to other plant parts) would be consistent with a primary role for extrafloral nectar in attracting ants to plants. (4) Does the dominance of extrafloral nectary-bearing plants across communities correlate with the abundance and/or composition of local plant-visiting ant assemblages? If resources provided by EFN-bearing plants are important factors in ant population growth generally, then the abundances of all ant species should be higher at sites with greater dominance of EFN-bearing plants. However, if extrafloral nectar is more important to some ants (e.g. *A. gracilipes*) than it is to others, then high proportions of EFN-bearing plants per site should lead to numerical dominance of one or a few ant species. We expect these differences to be apparent both on EFN-bearing plants and, more generally, at non-EFN-plant locations, e.g. on other plants and on the ground. Finally, we asked: (5) Is the diversity or abundance of plant-visiting ants lower at sites with high abundances of *A. gracilipes*? If populations of *A. gracilipes* reach high abundances at high nectar levels, and this numerical dominance is associated with a reduced diversity or abundance of other ant species, then patterns would support the hypothesis that opportunistic associations between *A. gracilipes* and native EFN-bearing plants: (i) promote the *A.*

gracilipes invasion of island communities and (ii) reduce the biodiversity and abundance of other ants, suggesting a need for future experimental tests.

1.2 Methods

Survey Sites

In July 2006, we surveyed thirty-five sites spanning the political entities of Independent and American Samoa and including the islands of Upolu, Savaii, Nuutele, Tutuila, Ofu and Olosega (Figure 1, Appendix 1). On the large islands of Upolu (1115 km^2) and Savaii (1700 km^2), sites were located every 15 km around the perimeter of the island. On the small islet of Nuutele, with no villages, we surveyed three sites. In Tutuila (140 km^2), we conducted surveys at five sites. We also conducted two surveys on the small island of Ofu and one on its neighboring island, Olosega (together, Ofu and Olosega are 9 km^2) (Figure 1, Appendix 1).

Survey Methodology

All surveys were conducted between 10hr and 16hr, and thus were focused on diurnally active ants. At each site, we first used a meter tape to delineate a 100m-long belt transect that was 2m wide. Every 5m along each transect, we examined all herbaceous and woody plants within an area of 0.5m X 2m and recorded the total number of plants. For each site, we calculated the percentage of plants that were EFN-bearing. We recorded four native EFN-bearing plants: *Hibiscus tiliaceous* (Malvaceae), *Ipomea pres-caprae* (Convolvulaceae), *Erythrina fusca* (Fabaceae), and *Morinda citrifolia* (Rubiaceae) and one non-native, EFN-bearing plant, *Clerodendrum fragrans*. This plant was only found at one site (Taputimu, in Tutuila). We recorded the number and identity of ants on each plant, and on plants bearing extrafloral nectaries, we recorded ant location (numbers on nectary bodies vs. numbers on other parts of the plant). Finally, we assessed the activity of ground foragers using 10 X 10cm cards placed on the ground (Abbott 2006) <5cm from the center of the belt transect, and on non-EFN-bearing plants. We collected type specimens at each site for every ant species recorded. All ants were later identified in the laboratory using Wilson and Taylor (1967), Shattuck (1999), and Andersen (2000). We then determined the geographic origin of each ant species using Wilson and Taylor (1967), Andersen (2000), and Wetterer and Vargo (2005). Species were categorized as native if they were reported as native to Samoa and/or the islands of the South Pacific Ocean.

Data Analysis

(1) Has the *A. gracilipes* invasion progressed in Samoa?

Wilson and Taylor assessed the distribution of ant species, including *A. gracilipes* (under previous name, *Anoplolepis longipes*), in Samoa in 1962 (Wilson and Taylor 1967). They surveyed 55 sites on the two major islands of Savaii and Upolu and observed *A. gracilipes* at eight sites (Figure 1). We compared results from our survey of *A. gracilipes* incidence on these two islands with their account using a Fisher's exact test to test for differences in the proportion of *A. gracilipes*-invaded sites between the two surveys (Proc Freq; SAS, version 9.1 (SAS Institute 2003)). While the two surveys did not overlap completely, many sites were shared (Figure 1). To the extent that both the earlier survey and our survey represent unbiased sampling across the two islands, one would expect no differences between the surveys if indeed the invasion had not progressed. Wilson and Taylor (1967) also reported other accounts of *A. gracilipes* incidence from the literature. However, we only used data from their direct observations, since that 'snapshot' of ant abundances was most comparable to our survey.

(2) Do Ant assemblages differ in invaded vs. uninvaded sites?

To test for the differences in ant species composition between *A. gracilipes*-invaded and uninvaded sites, we conducted a 2-way Analysis of Similarities (ANOSIM), with Bray-Curtis distance measures and 9999 iterations, using Primer, version 6.1.10 (Clarke and Gorley 2007). This procedure detects differences in species assemblages between two or more groups (Clarke et al. 2006). We included the factors of invasion status (*A. gracilipes*-invaded or uninvaded) and island. Because this analysis requires replicates within each combination of invasion status and island, we only included sites from Savaii and Upolu (12 invaded and 12 uninvaded sites). *Anoplolepis gracilipes* workers were excluded from the data. Significantly greater variation between invaded vs. uninvaded sites relative to within invaded/uninvaded sites would indicate that the community composition of non-*A. gracilipes* ants differs with the presence/absence of *A. gracilipes*. We used scree plots to determine the number of axes that maximized the amount of information presented in the ordination and minimized its stress (McCune and Grace 2002).

Next we used Primer v. 6.1.10 (Clarke and Gorley 2007) to construct non-metric multi-dimensional scaling (NMDS) plots, using Bray-Curtis distances and 9999 iterations. To determine how individual ant species contributed to differences between invaded and uninvaded sites, we used SIMPER (Primer v. 6.1.10, Clarke and Gorley 2007).

Finally, we used ANOVA with the independent factor of *A. gracilipes* invasion status to test for differences between invaded and uninvaded sites in the abundances of all ants and the proportion of native ants per plant. Density of all plants per site was used as a covariate in these analyses. As with many response variables in this study, residuals were non-normally distributed, and normality could not be obtained through transformations. Therefore, randomization tests were used to evaluate differences among treatments (Manly 1991). Randomization tests determine p-values by comparing an observed test statistic (e.g. F-ratio from ANOVA) to a distribution of the test statistic that is expected under the null hypothesis. To create the expected distribution, the response variable values from treatments being compared are pooled, permuted, and randomly assigned to the treatments for 9999 iterations. We used randomization test equivalents of ANOVA by embedding Proc GLM code within a SAS randomization test macro program (Cassell 2002).

(3) Are *A. gracilipes* foragers more abundant at extrafloral nectaries than other plant parts?

To test the prediction that there would be more *A. gracilipes* workers on nectaries than any other part of the plant, we used ANOVA (Proc GLM; SAS version 9.1 (SAS Institute 2003)). The independent factors were location (two levels: nectaries vs. stems, branches, leaves, or fruit), and site (15 levels), and the response variable was the number of *A. gracilipes* workers per plant. Plants without extrafloral nectaries were excluded, resulting in a total of 240 plants in the analysis. As above, we used the Cassell (2002) randomization procedure because residuals violated the assumption of a normal distribution.

(4) Do the levels of extrafloral nectar resources across communities correlate with the abundance and/or composition of local ant assemblages?

We tested the prediction that the abundance of plant-visiting ants increases as the proportion of EFN-bearing plants per site increases using ANCOVA (Proc GLM; SAS version 9.1 (SAS Institute 2003)). This indirect estimate of extrafloral nectar provides a conservative test: since EFN-bearing plant species may differ from each other in nectar production, any observed correlation between the proportion of EFN-bearing plants and ants likely indicates a strong EFN-ant signal. The independent factors were the percentage of EFN-bearing plants per site, ant type (*A. gracilipes*, other non-native ants, or native ants), and their interaction. The response variable was ant abundance per site and the analysis was conducted separately for *A. gracilipes*-invaded and uninvaded sites (n=15 and n=20, respectively). Total plant density per site was used as a co-variate in these analyses. A significant interaction for ant type × percentage EFN-bearing plants would indicate that different categories of ants exhibit different associations with EFN-bearing plants; we predicted that *A. gracilipes* would demonstrate the strongest positive association. We then used multiple regression analysis (Proc REG; SAS version 9.1) to examine the slope of the relationship between the percentage of EFN- bearing plants and total plant density per site and the individual abundances of (i) *A. gracilipes*, (ii) other non-native ants, or (iii) native ants. Species that occurred in five or fewer sites, or were represented by five or fewer individuals were excluded from this analysis, resulting in a total of 10 individual regressions. Therefore, we used a Bonferroni-corrected α of 0.0005 to determine significance. For (ii) and (iii) we pooled all native and non-native ants, respectively, and examined these relationships separately for the invaded vs. uninvaded sites. We embedded ANCOVA and regressions in a randomization procedure (as described above).

Plants bearing EFN can be considered a resource, and dominant ants sometimes exclude co-occurring species from resources (Andersen 1992). We therefore tested whether patterns of ant abundance on EFN-bearing plants reflect abundances at a site generally. Preliminary regressions examined whether dominance of EFN-bearing plants had similar relationships to the proportion of the ant community comprised of *A. gracilipes* (i) at "resource locations", that is, on EFN-bearing plants and (ii) at "non-resource locations", that is, on the ground and on non-EFN-bearing plants. At both location types, the proportion of *A. gracilipes* was significantly higher when EFN-bearing plants were numerically dominant (resource locations: β=1.23, *P*=0.0017; non-resource locations: β=0.58, *P*=0.0471). Consequently, while we restrict our conclusions to plant-visiting ants, our measures of ant abundances on all plants (EFN-bearing + non-EFN-bearing) are likely indicative of general, site-level patterns.

(5) Is the diversity or abundance of co-occurring ants lower at sites with high abundances of *A. gracilipes*?

We predicted that the diversity of co-occurring, plant-visiting ants would be negatively related to *A. gracilipes* abundance across the subset of invaded sites. To test this prediction, we first calculated species richness, the Shannon-Weiner diversity index (H) and evenness (J) (Begon et al. 2006) for: (i) all ants, excluding *A. gracilipes*, (ii) other non-native ants, and (iii) native ants. We then used multiple regression analysis (following methods for question 2) to examine the relationships between the abundance of *A. gracilipes* and richness, H, or J for each of the two categories of co-occurring ant species (n = 35 sites). The abundances of *A. gracilipes*, richness, H, and J values were all log-transformed. Again, we used the randomization procedure described above to conduct statistical tests, due to residuals with significantly non-normal distributions. Finally, we used a Bonferroni-corrected α of 0.00625 to determine statistical significance, because a total of eight tests were conducted.

1.3 Results

(1) Has the *A. gracilipes* invasion progressed in Samoa?

We surveyed 24 sites and observed *A. gracilipes* at 12 sites on the islands of Savaii and Upolu. In contrast, Wilson and Taylor (1967) found *A. gracilipes* in 8 of 55 sites in their 1962 survey of the same two islands. The incidence of *A. gracilipes* was significantly higher in our 2006 survey (Fisher's exact test, P=0.0016, Figure 1). This pattern was strongest on the island of Upolu (Figure 1).

(2) Do Ant assemblages differ in invaded vs. uninvaded sites?

We recorded a total of 21 species of plant-visiting ants during our survey of 35 sites across six islands (Appendix 2). Ten species were native to Samoa and/or the islands of the South Pacific Ocean, while the remaining eleven (including *A. gracilipes*) were non-native. Two species were only present in sites that were uninvaded by *A. gracilipes:* the native species *Vollenhovia samoensis* was only observed in one uninvaded site, while the non-native *Plagiolepis alluaudi* was only present in four uninvaded sites. Additionally, the native species *Tetraponera guineense* and the non-native species *Monomorium destructor* were both only observed at one invaded site. The absolute abundance of individuals observed in invaded sites (14.17 ± 1.73) was significantly higher than that of uninvaded sites (8.6 ± 0.1.5, randomization ANCOVA, *A. gracilipes* invasion status: P=0.0137, plant density: P=0.6736). Native ants represented 22% (± 2.5 %) of the individuals at *A. gracilipes*-uninvaded sites, compared with 14% (± 3.0 %) at *A. gracilipes*- invaded sites (*A. gracilipes* invasion status: P=0.0730, plant density: P=0.8505). At invaded sites, *A. gracilipes* workers comprised 60% (± 1.20 %) of all individuals.

The composition of non-*A. gracilipes* ant assemblages differed between *A. gracilipes*-invaded and uninvaded sites on the large islands of Upolu and Savaii (2-way crossed ANOSIM, Global r=0.171 , P=0.037; Figure 2). However, islands did not significantly differ in species composition (r=0.039, P=0.139). Eight ant species contributed to more than 90% of the dissimilarity between *A. gracilipes*-invaded and uninvaded sites, and six of these had decreased abundances in *A. gracilipes*-invaded sites, relative to uninvaded sites (SIMPER, Table 1).

(3) Are *A. gracilipes* foragers more abundant at extrafloral nectaries than other plant parts?

There were more *A. gracilipes* workers on nectaries than on the stems and leaves of the five species of EFN-bearing plants (ANOVA, location: $P<0.00001$, site: $P<0.00001$, location × site: $P=0.3333$). This pattern was present even though nectaries typically made up <1% of the total plant surface area. For example, active nectary bodies comprised on average 0.34% (± 0.04% SE) of the surface area of five *M. citrifolia* plants measured on Savaii. Thus, ant abundance would be expected to be higher on stems and leaves if ants were randomly distributed on the plant.

(4) Do the levels of extrafloral nectar resources across communities correlate with the abundance and/or composition of local ant assemblages?

At *A. gracilipes*-invaded sites, the total abundance of all ants on plants was four times greater at sites with high proportions of EFN-bearing plants than at sites with no EFN-bearing plants (linear regression, $r= 0.65$, $P=0.0094$). *Anoplolepis gracilipes* and other ants displayed different relationships with extrafloral nectar availability, as evidenced by a significant interaction between ant type and the proportion of EFN-bearing plants (ANCOVA, Ant type, $P=0.3869$, percentage EFN-bearing plants per site, $P=0.0214$, and Ant type × percentage EFN-bearing plants per site, $P=0.0036$) at *A. gracilipes*-invaded sites. *Anoplolepis gracilipes* displayed a strong, positive relationship with the proportion of EFN-bearing plants per site (Table 2; Figure 3A). However, after Bonferroni correction, there were no significant relationships between the percentage of EFN-bearing plants and the abundances of either native or other (non-*A. gracilipes*) non-native ants, across both *A. gracilipes*-invaded and uninvaded sites (Table 2, Figure 3B-C). Plant density was not significantly correlated with abundances of any ant species (Table 2).

(5) Is the diversity or abundance of co-occurring ants lower at sites with high abundances of *A. gracilipes*?

Within invaded sites, higher *A. gracilipes* abundances were associated with a lower diversity of plant-visiting native ants (Figure 4A, Table 3), although this effect was not significant after Bonferroni corrections were applied. Decomposing the native ant diversity indices into species richness and evenness, we found that while both were negatively correlated with *A. gracilipes* abundances, this effect was only significant for species richness (Figure 4B-C, Table 3). In contrast, *A. gracilipes* abundances were not significantly related to the diversity, richness and evenness of other non-native ants, nor were they related to abundances of either native or other non-native ants (Table 3).

One common non-native ant species (*Paratrechina longicornis*) reached high abundances at some sites; abundances were occasionally even higher than those of *A. gracilipes* (Appendix 2). Therefore, the negative association between *A. gracilipes* and other ants could conceivably be driven by *P. longicornis,* if it positively co-varied with *A. gracilipes*. However, there was no significant relationship between *P. longicornis* and the diversity of native ants (Multiple regression, r^2=0.15, *P. longicornis:* β=0.1401, *P*=0.3239, plant density: β=0.0050, *P*=0.1722), or between the abundances of *A. gracilipes* and *P. longicornis* (Multiple regression, r^2=0.62, *A. gracilipes:* β=-0.0288, *P*=0.8548, plant density: β=-0.0189, *P*=0.0042). These patterns suggest that native ant diversity is related to the abundance of *A. gracilipes* but not of *P. longicornis*.

1.4 Discussion

Invasive ants readily associate with extrafloral nectary-bearing plants in their introduced ranges (Lach 2003, Ness and Bronstein 2004). These interactions may provide fuel for the growth of invasive ant populations, and thereby mediate the negative influences of invasive ants on other species, especially co-occurring ant species. We examined this hypothesis using surveys of the invasive ant, *Anoplolepis gracilipes*, co-occurring ants, and extrafloral nectary (EFN)-bearing plants in the Samoan Archipelago. We found that *A. gracilipes* workers were more abundant on extrafloral nectaries than other plant parts. In addition, overall ant abundance was higher at sites with higher proportions of EFN-bearing plants, with stronger positive associations for *A. gracilipes* than for other ant species. Furthermore, the species richness of plant-visiting native ants was lower at sites with high *A. gracilipes* abundance. These correlative data indicate that negative influences of *A. gracilipes* on co-occurring ants may be mediated by the amount of available extrafloral nectar resources.

If extrafloral nectar resources are valuable to invasive ants (including *A. gracilipes)*, then we predicted that workers should spend more time at nectaries than on the stems and leaves of EFN-bearing plants. If, however, *A. gracilipes* workers are randomly distributed across all parts of the plants, then it is less likely that nectar is responsible for the presence of *A. gracilipes* on EFN-bearing plants. Our finding that *A. gracilipes* was more abundant at nectaries than other plant parts supported the prediction that extrafloral nectar is important to this species. Moreover, in prior work, we manipulated nectar availability and showed that increasing nectar levels altered ant recruitment, tending and foraging behaviors, most strongly for the invasive species, *A.*

gracilipes (Johnson et al. *in review*). Similarly, Lach (2007) demonstrated that *Linepithema humile* (the Argentine ant) was more likely to forage on floral nectar of *Protea nitida* if native honeydew-excreting arthropods were found on the plants, a response that native ants did not display. In another study, she showed that *A. gracilipes* was not only more likely to be found on floral nectaries, but also collected much more floral nectar than other non-native ants (including *L. humile*; Lach 2005). Taken together, these data suggest that carbohydrate-rich resources may be particularly important for invasive ants. Furthermore, the importance of nectar resources to *A. gracilipes* provides a possible explanation for the patchy distribution of this species across the Samoan Archipelago. In a similar broad-scale investigation, Eubanks (2001) found patchy distributions of the red imported fire ant (*Solenopsis invicta*) in agricultural systems of the Southeastern United States. He suggested that the presence of ant-tended aphids explained much of this variation - a supposition that was later supported by manipulative experiments (Eubanks 2001, Kaplan and Eubanks 2005).

We predicted that EFN-bearing plants provide important resources to ant assemblages, leading to higher abundances of ants as extrafloral nectar levels in the community increase. The finding that *A. gracilipes* was strongly, positively correlated with the dominance of EFN-bearing plants supports this prediction and corresponds with other studies of invasive ants, particularly in the subfamilies Formicinae (e.g., *A. gracilipes*, *Paratrechina longicornis*) and Dolichoderinae (e.g., *Linepithema humile*) (Holway et al. 2002, Ness and Bronstein 2004). For example, *L. humile* only became dominant in the ant community of a South African vineyard after the introduction of honeydew-excreting insects (Addison and Samways 2000). Additionally, *A. gracilipes* have been observed tending honeydew-excreting scale insects in high abundances in both agricultural (Greenslade 1972) and forested (Abbott and Green 2007) habitats. Recently, authors have suggested that EFN-bearing plants may influence invasive ants in a similar manner to these honeydew-excreting arthropods (Lach 2003, Ness and Bronstein 2004). However, to our knowledge, this study is the first to provide evidence that invasive ants positively co-vary with the proportion of EFN-bearing plants in communities, indicating the potential importance of extrafloral nectar to invasion dynamics.

Unlike the abundance of *A. gracilipes,* which displayed a positive linear relationship to EFN-bearing plants, the abundances of other plant-visiting ant species did not vary positively with EFN frequency across sites. These data suggest that, by supporting the population growth of the invader, EFN-bearing plants may negatively affect other ants. Consistent with this idea, we found that the species richness of native ants was lower at sites with high abundances of *A. gracilipes*, and that the composition of co-occurring ant species significantly differed between *A. gracilipes*-invaded and uninvaded sites. Most of the ant species that contributed to these differences (including three native species) experienced declines in abundance when *A. gracilipes* workers were present. If these dynamics are indeed occurring, then the presence of EFN-bearing plants may serve to reduce the abundance of non-invasive ants and simplify ant assemblages, despite the increased resources provided by the plants.

Although most prior studies have focused on the ground-foraging (rather than plant-foraging) ant community, a negative association between invasive and native ants has been commonly observed following ant invasions (Holway et al. 2002). For example, Abbott (2006) found that *A. gracilipes* was associated with reduced abundances of co-occurring ants. Similarly, Sarty et al. (2007) documented lower species richness of ant communities on islands of Tokelau that were invaded by *A. gracilipes*. Additionally, Lester and Tavite (2004) presented anecdotal evidence that ants foraging for extrafloral nectar in Tokelau were negatively influenced by *A. gracilipes*. Specifically, when *A. gracilipes* were observed visiting EFN, no other ant species occurred on the plant. In contrast, multiple ant species foraged for extrafloral nectar in sites where *A. gracilipes* was absent. These studies, in combination with the data presented here, indicate that *A. gracilipes* may have strong, negative effects on co-occurring ant assemblages---spanning both plant- and ground-foragers. Similar consequences have been found for ant assemblages in locales invaded by the Argentine ant (*Linepithema humile*; Holway et al 1998, Sanders et al. 2001), the red imported fire ant (*Solenopsis invicta*; Porter and Savignano 1990), and the big headed ant (*Pheidole megacephala*; Hoffmann et al. 1999). Because ants are important to the population dynamics of a variety of plants and animals (Wilson and Hölldobler 1990), a simplification of ant assemblages caused by ant invasions could lead to cascading declines of species across multiple trophic and taxonomic levels (Sanders and Platner 2007, Moya-Laraño and Wise 2007).

We have examined some alternate explanations for the patterns observed in this study, and are able to reject some of them, while others need further testing. First, plant density could potentially affect ant abundance, independent of the dominance of EFN-bearing plants. Plants can modify local abiotic conditions, provide refuge and housing, and often host insect prey. However, these general effects of plant density were apparently relatively unimportant in our system, compared to the dominance of EFN-bearing plants. We found no significant relationships between plant density and metrics of *A. gracilipes* abundance. Moreover, our experimental data (Johnson et al *in revision*) demonstrated that ants, and especially *A. gracilipes*, responded strongly to experimentally-manipulated nectar levels. Second, honeydew-excreting insects could also influence ant assemblages (O'Dowd et al. 2003, Styrsky and Eubanks 2007). However, we found no significant differences in the numbers of ant-tended Hemiptera across sites (Savage, unpublished data). We have also experimentally demonstrated that ants are significantly less likely to tend honeydew-excreting insects when nectar is available (Johnson et al. *In review*). Finally, other aspects of the environment, such as soil characteristics, resource availability or the availability of nest sites for ants, could also co-vary with both *A. gracilipes* abundance and the frequency of EFN-bearing plants. Manipulative experiments that tease apart the effects of EFN-bearing plants, invasive ants, honeydew-excreting insects, and their interaction on co-occurring ant communities are currently underway to determine the mechanisms leading to the patterns reported here. Additionally, it will be important to ascertain the influence of *A. gracilipes* on other (i.e. non-ant) arthropods and plants.

Conclusions

Understanding geographic variation in the abundance and community-level impacts of invasive species will be critical to predicting the population expansion of invaders and preventing local extirpation of native species. Importantly, novel positive interactions that include non-native species may alter the effects of invaders on co-occurring community members (Richardson et al. 2000, Stachowicz 2001, Bruno et al. 2005). In this study, we documented variation in the abundance of the invasive ant, *A. gracilipes*; the abundance of co-occurring ants, and the abundance of EFN-bearing plants across 35 sites and six islands of the Samoan Archipelago. These broad-scale surveys are consistent with the hypothesis that plant-derived, carbohydrate-rich resources can increase both the local abundance of *A. gracilipes* and the negative impacts of this invader on native ant species. These findings suggest that conservation managers may benefit from explicit considerations of potential positive interactions in predicting the identities of problematic invaders or the outcomes of species invasions.

1.5 Acknowledgements:

Many thanks to Samani Tupufia, Tepa Suaesi, Mark Schmaedick, and Joshua Seamon for logistical support and field assistance. Land owners in both Independent and American Samoa graciously allowed us to conduct surveys on their property. Thanks to Anika Vinze for assistance in the laboratory. Comments from Scott Chamberlain and Phil Lester greatly improved the quality of this manuscript. This work was funded in part by a National Geographic Research and Exploration Grant (8237-07), the Rice University Wray-Todd Fellowship to A.M.S. and Rice University startup funds to J.A.R. and K.D.W.

1.6 References:

Abbott, K. L. (2006). Spatial dynamics of supercolonies of the invasive yellow crazy ant, *Anoplolepis gracilipes*, on Christmas Island, Indian Ocean. Diversity and Distributions **12**: 101-110.

Abbott, K. L., and P.T. Green. (2007). Collapse of an ant-scale mutualism in a rainforest on Christmas Island. Oikos **116**: 1238-1246.

Andersen, A. N. (1992). Regulation of momentary diversity by dominant species in exceptionally rich ant communities of the Australian seasonal tropics. American Naturalist **140:** 401-420.

Andersen, A. N. (2000). The ants of Northern Australia: A guide to the monsoonal fauna. CSIRO Publishing, Collingwood, VIC Australia.

Begon, M., C. R. Townsend, and J. L. Harper. (2006). Ecology: From individuals to ecosystems, 4th ed. Blackwell Publishing Inc., Oxford, UK.

Bluthgen, N., and N. E. Stork. (2007). Ant mosaics in a tropical forest in Australia and elsewhere: A critical review. Austral Ecology **32**: 93-104.

Bruno, J. F., J. D. Fridley, K. D. Bromberg, and M. D. Bertness. (2005). Insights into biotic interactions from studies of species invasions. *In:* Sax, D. F., J. J. Stachowicz, and S. D. Gaines (eds). Species Invasions: Insights into Ecology, Evolution and Biogeography Sinauer Associates Inc. Publishers, Sunderland, MA USA.

Callaway, R. M., and J. L. Moran.(2006). What have exotic plant invasions taught us over the past 20 years? Trends in Ecology and Evolution **21**: 369-375.

Cassell, D. L. (2002). A randomization-test wrapper for SAS PROCS. In SAS Institute Inc. (ed). Proceedings of the 27th Annual SAS User's Group International Conference. SAS Institute.

Clarke, K. R., P. J. Somerfield, L. Airoldi, and R. M. Warwick. (2006). Exploring interactions by second-stage community analyses. Journal of Experimental Marine Biology and Ecology **338**: 179-192.

Clarke, K.R., and R. N. Gorley. (2007). Primer, version 6.1.10: User manual and tutorial. Primer-E, Plymouth.

Davidson, D. W., S. C. Cook, R. R. Snelling, T. H. Chua. (2003). Explaining the abundance of ants in lowland tropical rainforest canopies. Science **300:**969-972.

Eubanks, M. D. (2001). Estimates of the direct and indirect effects of red imported fire ants on biological control. Biological Control **21**:35–43.

Emery, C. (1925). Hymenoptera: Formicidae: Formicinae. Genera Insect **183**: 1-302.

Fluker, S. S. and J. W. Beardsley. (1970). Sympatric associations of three ants: *Iridomyrmex humile, Pheidole megacephala* and *Anoplolepis longipes* (Hymenoptera: Formicidae) in Hawaii. Annals of the Entomological Society of America **63:** 1290-1296.

Fritts, T.H. & Rodda, G.H. (1998) The role of introduced species in the degradation of island ecosystems: A case history of Guam. Annual Review of Ecology and Systematics **29**: 113-40.

Greenslade P. J. M. (1971). Interspecific competition and frequency changes among ants in Solomon Islands coconut plantations. Journal of Applied Ecology **8**: 323–52.

Heil, M., and D. McKey. (2003). Protective ant-plant interactions as model systems in ecological and evolutionary research. Annual Review of Ecology, Evolution and Systematics **34**: 425-453.

Hoffmann, B. D., A. N. Andersen, and G. J. E. Hill. (1999). Impact of an introduced ant on native rain forest invertebrates: *Pheidole megacephala* in monsoonal Australia. Oecologia **120**: 595-604.

Holway, D. A., A. V. Suarez, and T. J. Case. (1998) Loss of intraspecific aggression in the success of a widespread invasive social insect. Science **282**: 949–52.

Holway, D.A, L. Lach, A.V. Suarez, N.D. Tsutsui and T.J. Case. (2002). The ecological causes and consequences of ant invasions. Annual Review of Ecology and Systematics **33**:181-233.

Johnson, S. D., A. M. Savage, K. D. Whitney, and J. A. Rudgers. *In review*. Extrafloral nectar availability alters the recruitment, dominance and behavior of the invasive ant, *Anoplolepis gracilipes* Ecological Entomology.

Kaplan, I., and M. D. Eubanks. (2005). Aphids alter the community-wide impacts of fire ants. Ecology **86**: 1640-1649.

Lach, L. (2003). Invasive ants: unwanted partners in ant-plant interactions? Annals of the Missouri Botanical Gardens **90**: 91-108.

Lach, L. (2005). Exploitation and interference competition in the nectar-thieving behavior of three invasive ant species. Insectes Sociaux **52**: 257-262.

Lach, L. (2007). Mutualism with a native membracid facilitates pollinator displacement by Argentine ants. Ecology **88:** 1994-2004.

Lester, P. J., and A. Tavite. (2004). Long-legged ants, *Anoplolepis gracilipes* (Hymenoptera: Formicidae), have invaded Tokelau, changing composition and dynamics of ant and invertebrate communities. Pacific Science **58**: 391-401.

Mack, R. N., D. Simberloff, W. M. Lonsdale, H. Evans, M. Clout, and F. A. Bazzaz. (2000). Biotic invasions: Causes, epidemiology, global consequences, and control. Ecological Applications **10**: 689-710.

Manly, B. F. J. (1991). Randomization and Monte Carlo Methods in Biology. Chapman and Hall, London.

McCune, B. and J. B. Grace. (2002). Analysis of Ecological Communities, 2nd edition. MJM Software Design Oregon USA.

Moya-Laraño, J., and D. H. Wise. (2007). Direct and indirect effects of ants on a forest-floor food web. Ecology **88**: 1454-1465.

Myers, N., R. A. Mittermeier, C. G. Mittermeier, G. A. B. da Fonseca, and J. Kent. (2000). Biodiversity hotspots for conservation priorities. Nature **403**: 853-856.

Ness, J. H., and J. L. Bronstein. (2004). The effects of invasive ants on prospective ant mutualists. Biological Invasions **6**: 445-461.

O'Dowd, D. J, P. T. Green, and P. S. Lake. (2003). Invasional 'meltdown' on an oceanic island. Ecology Letters **6**: 812-817.

Porter SD, Savignano DA. (1990). Invasion of polygyne fire ants decimates native ants and disrupts arthropod community. Ecology **71**: 2095–106.

Reaser, J. K, L. A. Meyerson, Q. Cronk, M. De Poorter, L. G. Eldrege, E. Green, M. Kairo, P. Latasi, R. N. Mack, J. Mauremootoo, D. O'Dowd, W. Orapa, S. Sastroutomo, A. Saunders, C. Shine, S. Thrainsson, and L. Vaiutu. (2007). Ecological and socioeconomic impacts of invasive alien species in island ecosystems. Environmental Conservation **34**: 98-111.

Richardson, D.M.,N. Allsopp, C. M. D'Antonio, S. J. Milton, and M. Rejmanek. (2000). Plant invasions: The role of mutualisms. Biological Reviews **75**: 65-93.

Rowles, A. D., and D. J. O'Dowd . (2007). Interference competition by Argentine ants displaces native ants: implications for biotic resistance to invasion. Biological Invasions **9**: 73-85.

Sakai, A. K., F. W. Allendorf, J. S. Holt, D. M. Lodge, J. Molofsky, K. A. With, S. Baughman, R. J. Cabin, J. E. Cohen, N. C. Ellstrand, D. E. McCauley, P. O'Neil, I. M. Parker, J. N. Thompson, and S. G. Weller. (2001). The population biology of invasive species. Annual Review of Ecology and Systematics **32**: 305-332.

Sanders, D., and C. Platner. (2007). Intraguild interactions between spiders and ants and top-down control in a grassland food web. Oecologia **150**: 611-624.

Sarty, M., K. L. Abbott, and P. J. Lester. (2007). Community level impacts of an ant invader and food mediated coexistence. Insectes Sociaux **54**: 166-173.

SAS Institute. (2003). SAS version 9.1.3. SAS Institute, Cary, North Carolina, USA.

Sax, D. F., J. J. Stachowicz, and S. D. Gaines (eds). Species Invasions: Insights into Ecology, Evolution and Biogeography. Sinauer Associates Inc. Publishers, Sunderland, MA USA.

Shattuck, S. O. (1999). Australian ants: Their biology and identification. CSIRO Publishing, Collingwood, VIC Australia.

Snyder, W. E., and F. W. Allendorf. (2006). Ecological effects of invasive arthropod generalist predators. Annual Review of Ecology Evolution and Systematics **37**: 95-122.

Stachowicz, J. J. (2001). Mutualism, facilitation, and the structure of ecological communities. Bioscience **51**:235-246.

Styrsky, J. D., and M. D. Eubanks. (2007). Ecological consequences of interactions between ants and honeydew-producing insects. Proceedings of the Royal Society B-Biological Sciences **274**:151-164.

Walker, B., and W. Steffen. (1997). An overview of the implications of global change for natural and managed terrestrial ecosystems. Conservation Ecology **2**: 2-10.

Wetterer, J. K., and D. L. Vargo. (2005). Ants (Hymenoptera: Formicidae) of Samoa. Pacific Science **57**: 409-419.

Wilson, E. O. and B. Hölldobler (1990). The Ants. Harvard University Press, Cambridge, MA USA.

Wilson, E. O., and R.W. Taylor. (1967). The ants of Polynesia (Hymenoptera: Formicidae). Pacific Insects Monograph **14**: 1-10.

1.7 Tables

Table 1.1: Summary of differences in the abundances of the non-*Anoplolepis gracilipes* ant species that contributed to ~90% of the dissimilarity between *A. gracilipes*-invaded vs. uninvaded sites. Only sites from Savaii and Upolu were used in this analysis.

Species	**Mean (± SE) Abundance (Uninvaded)**	**Mean (± SE) Abundance (Invaded)**	**Difference (%)**	**Contribution to Dissimilarity (%)**
<u>Non-Native</u>				
Monomorium floricola	3.40 ± 0.70	1.61 ± 0.41	- 53	32.7
Paratrechina longicornis	2.08 ± 0.59	2.61 ± 1.01	+ 27	25.01
Monomorium pharoensis	0.80 ± 0.27	0.65 ± 0.25	- 19	10.78
Tetramorium simillimum	0.77 ± 0.32	0.36 ± 0.08	- 53	8.69
<u>Native</u>				
Tetramorium tonganum	0.41 ± 0.33	0	- 100	4.47
Solenopsis papuana	0.24 ± 0.24	0.18 ± 0.13	- 25	4.03
Pheidole sexspinosa	0.27 ± 0.24	0.11 ± 0.11	- 59	3.37
Camponotus chloroticus	0.13 ± 0.08	0.24 ± 0.18	+ 85	2.32

Table 1.2: Relationships between the abundances of the most common ant species and (i) the proportion of EFN-bearing plants per site and (ii) the density of all plants per site. β is the partial regression coefficient. The number of *A. gracilipes*-invaded / uninvaded sites in which each species was recorded is also presented. Ant species that occurred in five or fewer sites or were represented by five or fewer individuals were excluded from these regression analyses. Because ten separate tests were conducted, we used a Bonferroni-corrected α of 0.005 to determine significance and bolded significant relationships.

Species	**Subfamily**	**Presence at Invaded/ Uninvaded sites**	**Proportion of EFN-bearing plants per site**		**Plant Density**		**r^2**
Non-Native			β	*P*	β	*P*	
Anoplolepis gracilipes	Formicinae	15 / --	**27.33**	**0.0027**	0.1831	0.2251	0.48
Paratrechina longicornis	Formicinae	13 / 18	1.72	0.0151	-0.05	0.5569	0.14
Paratrechina bourbonica	Formicinae	2 / 4	-0.34	0.0396	0.02	0.9063	0.33
Monomorium floricola	Myrmicinae	15 / 18	0.98	0.0071	-0.03	0.9745	0.09
Monomorium phoaroensis	Myrmicinae	11 / 14	1.13	0.6227	0.003	0.4849	0.06
Tetramorium simillimum	Myrmicinae	10 / 15	-0.61	0.4892	0.003	0.0999	0.03
Native:							
Camponotus chloroticus	Formicinae	4 / 7	0.14	0.0090	-0.005	0.1019	0.06
Tapinoma minutum	Dolochoderinae	3 / 6	0.06	0.0169	0.03	0.5427	0.31
Solenopsis papuana	Myrmicinae	3 / 5	-0.62	0.1724	0.007	0.0595	0.07
Tetramorium tonganum	Myrmicinae	3 / 5	0.30	0.0871	0.004	0.1445	0.01

Table 1.3: Relationships between non-*A. gracilipes* ant abundance and diversity measures and two variables that differed among sites: *A. gracilipes* abundance and plant density. "Non-native ants" refer to all non-native ants excluding *A. gracilipes*. Ant abundances were log transformed prior to multiple regression analysis. Because eight separate tests were conducted, we used a Bonferroni-corrected α of 0.00625 to determine significance and bolded significant relationships. N = 15 *A. gracilipes*-invaded sites.

Dependent Variable	*A. gracilipes* Abundance		Plant Density		r^2
	β	*P*	B	*P*	
Native ant					
Abundance	-0.3959	0.0932	0.0096	0.2199	0.22
Diversity (H)	-0.1603	0.0209	0.0052	0.0311	0.42
Richness	**-0.3982**	**0.0016**	0.0066	0.0781	0.58
Evenness (J)	-0.1240	0.0809	0.0042	0.0886	0.28
Non-native ant					
Abundance	-0.0596	0.7189	-0.0136	0.0325	0.45
Diversity (H)	0.0422	0.3554	-0.0016	0.2966	0.11
Richness	0.0474	0.3150	-0.0021	0.2067	0.14
Evenness (J)	-0.0069	0.7909	0.0005	0.6139	0.02

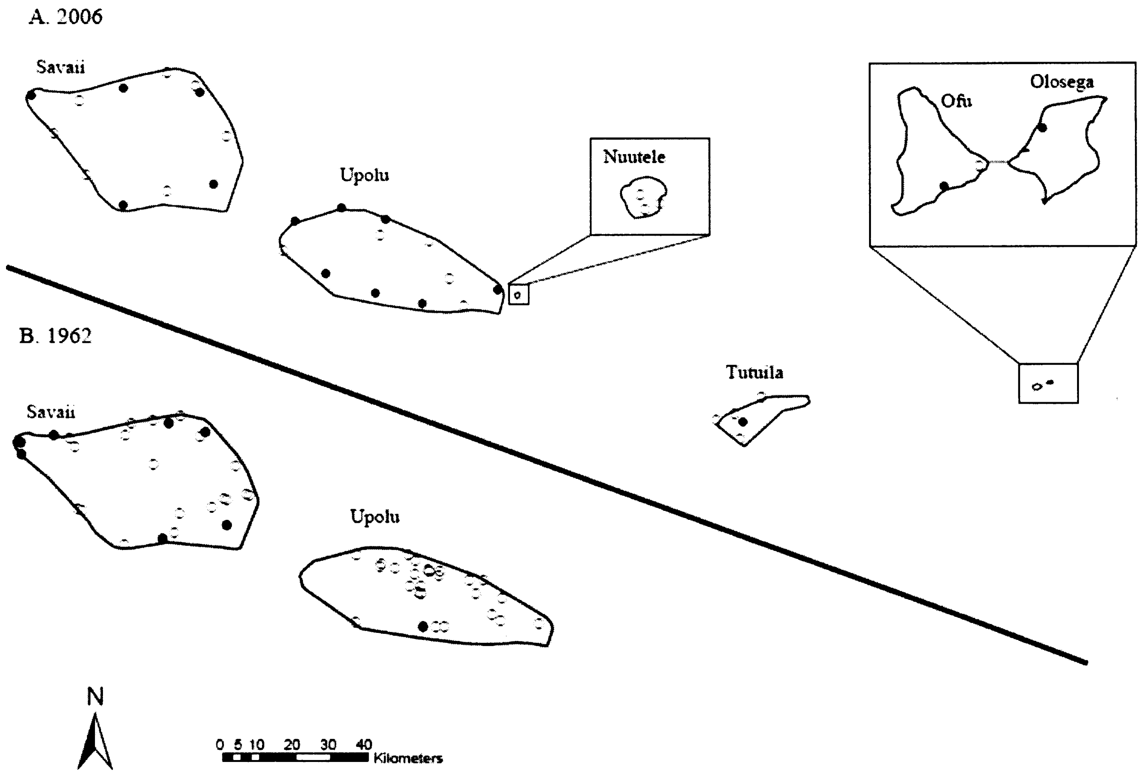


Figure 1.1: Map of sites surveyed in (A) 2006 (the current study) and (B) 1962 (Wilson and Taylor 1967). Sites invaded by *A. gracilipes* are represented by filled symbols and *A. gracilipes*-uninvaded sites are depicted with empty symbols. Note that Wilson and Taylor did not visit Nuutele, Tutuila, Ofu, or Olosega.

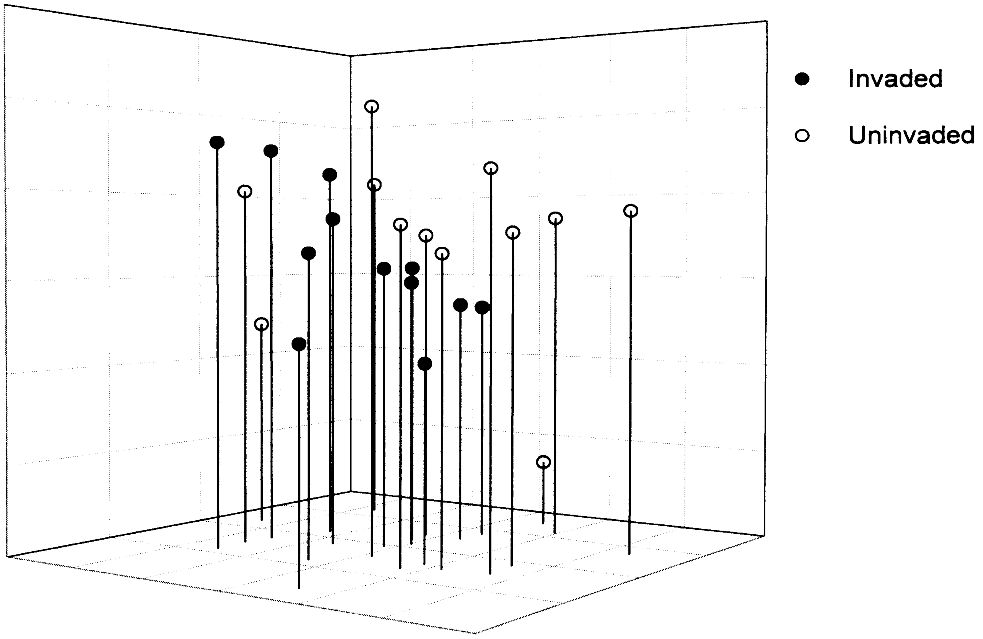


Figure 1.2: Ordination plot comparing non-*Anoplolepis gracilipes* ant species composition between *A. gracilipes*-invaded sites (filled circles, n = 12 sites) and uninvaded sites (open circles, n = 12 sites) in Savaii and Upolu. This plot was created using non-metric multidimensional scaling (NMDS) with Bray-Curtis distance measures and 9999 iterations. *Anoplolepis gracilipes* invasion status significantly contributed to differences between sites (2-way Crossed ANOSIM, global r=0.171, P=0.037), however, the island on which sites were located did not (global r=0.039, P=0.139). The NMDS had a 3-D stress value of 0.08 (Primer v.6).

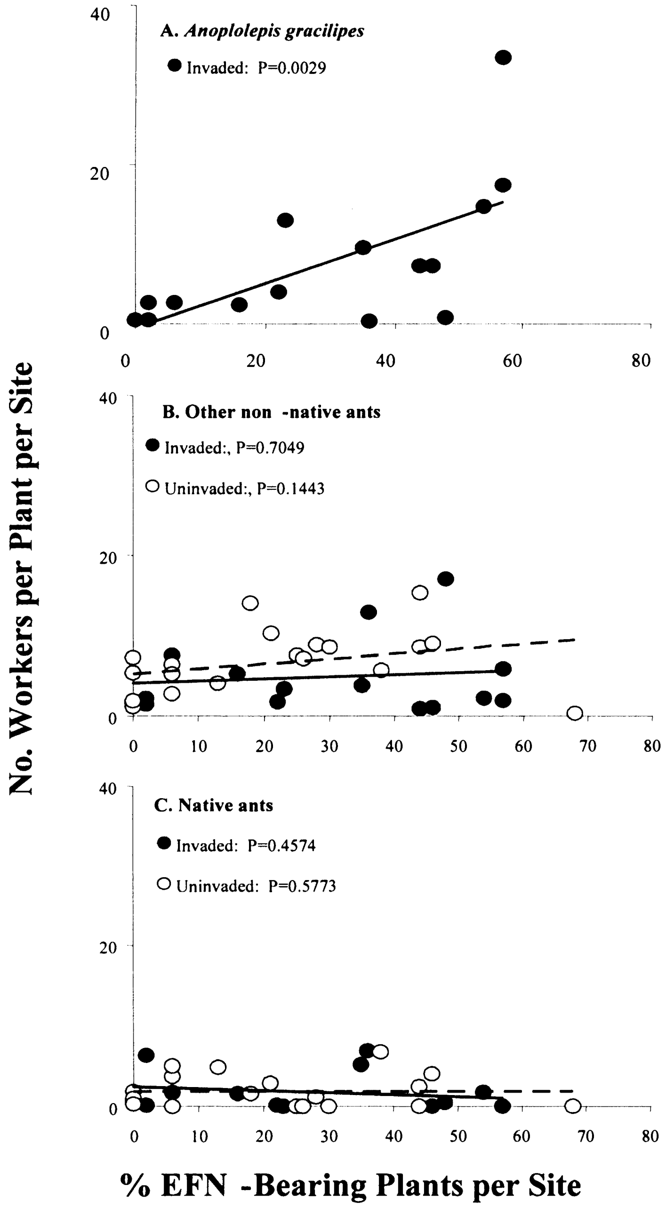


Figure 1.3: Relationship between the percentage of EFN-bearing plants per site and the mean abundance of (A) *Anoplolepis gracilipes*, (B) other non-native ants, and (C) native ants (n = 15 invaded sites and n=20 uninvaded sites).

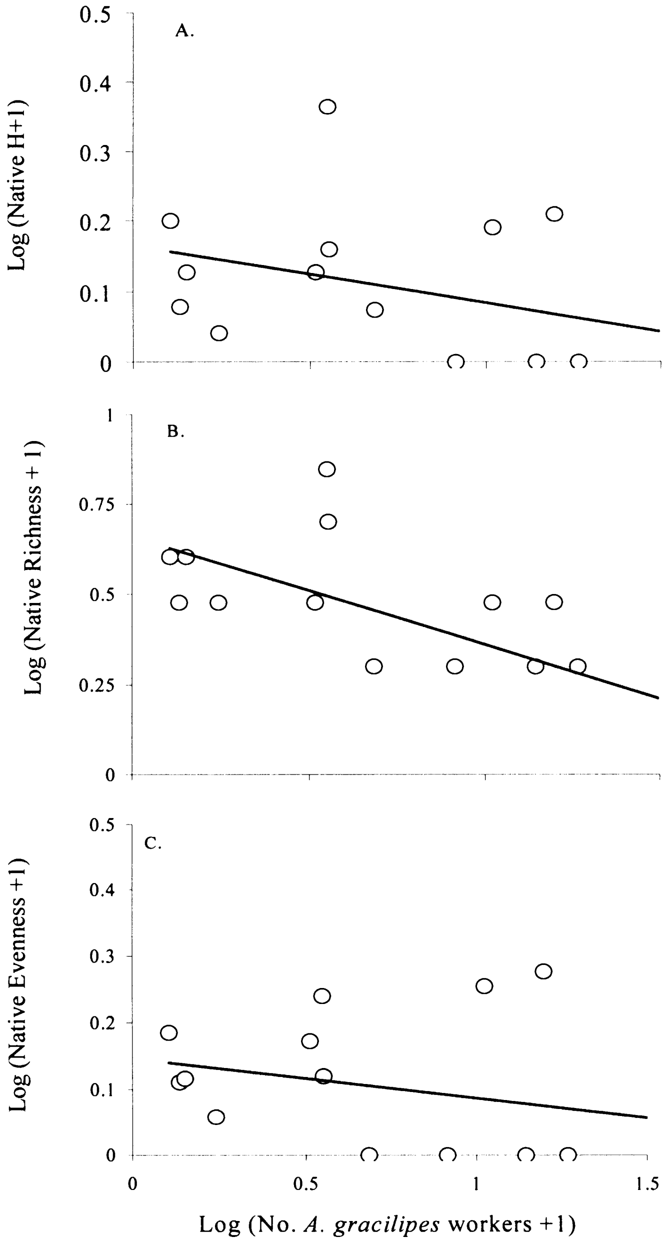


Figure 1. 4: Relationship between *Anoplolepis gracilipes* abundance and (A) native ant species diversity (Shannon-Weiner H), (B) native ant species richness, and (C) native ant species evenness (Shannon-Weiner J). Note log transformations of all variables. (n = 15 invaded sites). After Bonferroni correction, only the relationship in Panel B remains significant.

Chapter 2

Do invasive ants respond more strongly to carbohydrate availability than co-occurring non-invasive ants? A test along an active *Anoplolepis gracilipes* invasion front

ABSTRACT

Invasions by non-native insects can have important ecological impacts, particularly on island ecosystems. However, the factors that promote the success of invaders relative to co-occurring non-invasive species remain unresolved. For invasive ants, access to carbohydrate resources via interactions with both extrafloral nectary (EFN)-bearing plants and honeydew-excreting insects may accelerate the invasion process. A first step toward testing this hypothesis is to determine whether invasive ants respond to variation in the availability of carbohydrate resources, and whether this response differs from that of co-occurring, non-invasive ants. We investigated the effect of carbohydrate subsidies on the short-term foraging and hemipteran tending behaviors of the invasive ant *Anoplolepis gracilipes* (Formicidae) and co-occurring ant species on an EFN-bearing plant by experimentally manipulating carbohydrate levels and tracking ant recruitment. We conducted experiments in two years at two sites: one site was invaded by *A. gracilipes* prior to 2007 and the other became invaded during the course of our study, allowing pre- (2007) and post-invasion (2009) comparisons. Short-term increases in carbohydrate availability increased the density of *A. gracilipes* workers on plants by as much as 400% and reduced tending of honeydew-excreting insects by this species by up to 89%, with similar responses across years. In contrast, ants at the uninvaded site in 2007 showed a weak and non-significant forager recruitment response. Across all sites, *A. gracilipes* workers were the only ants that responded to carbohydrate manipulations in 2009. Furthermore, ant-carbohydrate dynamics at a site newly invaded by *A. gracilipes* quickly diverged from dynamics at uninvaded sites and converged on those of the site with an established invasion. These findings suggest that carbohydrate resources may be particularly important for *A. gracilipes* invasions, and underscore the importance of species interactions, particularly putative mutualisms, in facilitating exotic species invasions.

Key Words: Ant-plant, mutualism, honeydew, extrafloral nectar, species invasions

2.1 Introduction

Invasive species pose a major threat to global biodiversity and may significantly alter the composition of native communities as well as important ecosystem processes and services (Fritts and Rodda 1998, Pimentel et al. 2001, Holway et al. 2002, Abbott 2006, Dunham and Mikheyev 2010). Among insect groups, invasive ants have had especially strong, negative effects on native communities, altering community dynamics across multiple trophic levels and leading to the dissolution of important ecosystem processes (reviewed by Holway et al. 2002). Currently, nine of the ~150 ant species that have been introduced around the world are considered invasive (McGlynn 1999). Five of these species were included in the IUCN's list of the world's one-hundred worst invasive species: *Solenopsis invicta* (the red imported fire ant), *Wasmania auropunctata* (the little fire ant), *Linepithema humile* (the Argentine ant), *Pheidole megacephala* (the big-headed ant) and *Anoplolepis gracilipes* (the yellow crazy ant) (Lowe et al. 2000). Although few ant species have become invasive, when they have, there have been widespread and strong negative impacts on native communities. For example, some ant invasions have been associated with reductions of >90% in native ant abundances (Porter and Savignano 1990, Hoffmann and Parr 2008, but see King and Tschinkel 2008). Invasive ants may also negatively affect other native invertebrates, birds, and reptiles (Holway et al. 2002).

Identifying factors that promote ant invasions is of critical importance. At least two common traits are shared among invasive ant species: a wide diet breadth and the ability to form high-density supercolonies in which intraspecific competition is low (reviewed by Holway et al. 2002). A third factor that may promote the success of invasive ants is their ability to exploit carbohydrate resources (Lach 2003). Invasive ants commonly exhibit tending behavior towards extrafloral nectary (EFN)-bearing plants (Koptur 1979, Lach 2003, Ness and Bronstein 2004) and honeydew-excreting insects (Helms and Vinson 2002, Styrsky and Eubanks 2007). It has been hypothesized that these carbohydrate-rich resources promote ant invasions by providing high-energy fuel for greater activity, growth, and the establishment of dominant supercolonies (Holway et al. 2002, Lach 2003). Furthermore, carbohydrates may drive the aggressive behaviors of invading ants. For example, laboratory colonies of the invasive Argentine ant (*Linepithema humile*) became more aggressive and had higher exploration rates when provided with sucrose (Grover et al. 2007). The relative importance of diet breadth, supercolony formation, and carbohydrate exploitation in ant invasions remains unclear; however, comparisons between the ecology of invasive ants and that of co-occurring, non-invasive ants may help to disentangle these factors.

Here we focus on the mechanism of carbohydrate exploitation and compare responses of invasive and non-invasive ants in an island ecosystem. Like many island groups, the Samoan Archipelago (South Pacific Ocean) is dominated by non-native ants. Our recent survey of six islands and 35 sites across the archipelago revealed that the yellow crazy ant, *Anoplolepis gracilipes,* was the most dominant non-native species (Savage et al. 2009). Furthermore, *A. gracilipes* abundance was positively correlated with natural levels of EFN availability and negatively associated with the species richness of native ants across sites. Neither of these associations were displayed by any other non-native ant species (Savage et al. 2009).

Here, we used manipulations of nectar to evaluate the hypothesis that invasive ants respond differently to plant-based carbohydrate resources than co-occurring, non-invasive ants. We conducted experiments along an active invasion front, yielding the ability to examine non-invasive ant behavior both before and after the arrival of the invasive species. Specifically, we replicated nectar manipulations (i) at a site with an established *A. gracilipes* population in 2007 and 2009, (ii) at a nearby site both before (2007) and after (2009) *A. gracilipes* had invaded, and (iii) at the same nearby site, but in an area outside of the invasion front that remained free of *A. gracilipes* in 2009. We conducted our experiments on *Morinda citrifolia*, an EFN-bearing shrub that reaches ecological dominance in the lava fields in Savaii where this study was conducted. Previous work demonstrated that both *A. gracilipes* and co-occurring ants commonly forage at the nectaries of *M. citrifolia* (Savage et al. 2009).

Our experiments addressed the following specific questions: (1) Do invasive *A. gracilipes* workers differ from co-occurring non-invasive ants in their response to increasing carbohydrate levels? While we expected most ants to recruit to experimental manipulations of carbohydrate resources, we predicted that *A. gracilipes* workers would display the strongest positive response. Next, in addition to nectar provided by *M. citrifolia*, the ants in this study had access to honeydew-excreting insects that used *M. citrifolia* as a host plant. Therefore, we also asked (2) Does increasing plant-based carbohydrate levels change the honeydew excreting insect-tending behaviors of *A. gracilipes* and/or non-invasive ants? We expected that the proportion of ants tending honeydew-excreting insects would decline with increasing plant-based carbohydrates, irrespective of ant species identity, if our artificial treatments designed to mimic plant nectar were more attractive than honeydew-excreting insects rewards (as predicted by Becerra and Venable 1989). We expected that ant tending behaviors would remain unchanged if plant-based and honeydew-excreting insects-based resources were equivalently attractive to ants. Because our experiments were conducted both before and after a new *A. gracilipes* invasion as well as at a previously invaded site, our study also provided insight into how ant responses may vary during different stages of *A. gracilipes* invasions.

2.2 Methods

Study Organisms

A pan-tropical 'tramp ant,' *Anoplolepis gracilipes* has a broad diet breadth, can form supercolonies, and is considered invasive in many island groups, including Samoa, Hawaii, Christmas Island, and Tokelau (Holway et al. 2002, Lester and Tavite 2004, Abbott 2006). Although its native range is thought to include Africa or Asia, this species' origin is currently undetermined (Wetterer 2005). In Samoa, *Anoplolepis gracilipes* workers were first recorded in 1925 at very low abundances near ports and plantations (Emery 1925 *cited in* Wilson and Taylor 1967). However, recent studies have demonstrated that this species is currently widely distributed in Samoa, occasionally occurring at very high densities (Lester and Tavite 2004, Savage et al. 2009). For example, Lester and Tavite (2004) found that *A. gracilipes* reached abundances of 191-1,060 per trap within 24 h.

Morinda citrifolia (Nonu, Rubiaceae) is an abundant EFN-bearing plant that commonly co-occurs with *A. gracilipes* in Samoa (Savage et al. 2009). Native to Southeast Asia, *M. citrifolia* may have spread to many Pacific islands via historical migrations of indigenous peoples ~3000 years ago (Whistler 1993). This plant possesses annular disk nectaries clustered on an inflorescence (Plate 1, Waki et al. 2007). Some of these nectaries are surrounded by a small white floret, and are thus functionally floral. However, other nectaries begin secreting nectar soon after bud burst and never become surrounded by a floret, making them functionally extrafloral. Finally, floral nectaries continue to produce nectar after florets drop; these nectaries are therefore post-floral (A. Savage, personal observation 2007-2009). Here, we adopt the inclusive term 'nectary body' to refer to the pre-fruit structure that bears all three nectary types (Plate 1). At our sites in Samoa, nectary bodies contained 2 to >50 nectaries and reached a maximum size of 80 cm^3. *Morinda citrifolia* plants produce nectary bodies year-round, the three nectary types are commonly active simultaneously, and we observed ants visiting all nectary types.

Study Sites

Experiments were conducted on the island of Savaii, Samoa during July-August 2007 and September 2009. Study sites were located on lava flows dating from 1907-1911, and plant communities were dominated by *M. citrifolia.* In 2007, we studied one site invaded by *A. gracilipes* in the village of Saleaula (13°27'31" S, 172°19'19" W) and a similar reference site lacking *A. gracilipes* in the village of Mauga (13°29'11" S, 172°19'07" W), where the dominant ant species was *Pheidole megacephala* (Table 1). The two sites were separated by ~5 km and had similarly low levels of anthropogenic disturbance, almost entirely limited to the collection of *M. citrifolia* fruit by local people. We returned to both sites monthly from July 2007 to June 2008. In November 2007, we first recorded the arrival of *A. gracilipes* in the southern portion of the previously uninvaded Mauga site. By August 2009, *A. gracilipes* workers were abundant and dominant over ~1.5 km^2 in Mauga ("Mauga South"). However, at the northernmost end of the site ("Mauga North"), there were no *A. gracilipes* workers and *Pheidole megacephala* remained the dominant ant species (Table 1), corresponding to the front of the *A. gracilipes* invasion.

Despite the co-occurrence of other non-native ants at our sites, we only classified *A. gracilipes* as highly invasive because, when present, *A. gracilipes* reached very high abundances and comprised >98% of all ground foragers (see below, Table 1). Across both invaded and uninvaded sites, no other ant species approached these high abundances (Table 1). Additionally, in a recent survey of 35 sites spanning six islands of the Samoan archipelago, we documented a total of eleven non-native ant species. However, *A.*

gracilipes was the only non-native ant species significantly associated with lower native ant species richness (Savage et al. 2009).

Experimental Design: 2007

At each site, we established five 4m × 4m plots. Plots were located a minimum of 8 m apart (range 8-11 m) to reduce the probability that ants in different plots would belong to the same colony. Within each plot, five *M. citrifolia* plants of similar size were selected and randomly assigned to one of five carbohydrate availability treatments (see next section). We also measured total branch length and evaluated differences in the abundances of ant-tended hemipterans between sites. For each plant, we haphazardly selected 5 leaves, 2 stems and 2 nectary bodies and counted the number of aphids (Aphididae), scale insects (Coccididae), and mealybugs (Pseudococcididae). Finally, ant assemblages were assessed outside treatment plots (<0.5m from edge of plots) using 10 x10cm cards placed on the lava surface and observed for 30s, following methods in Abbott (2005). We used this approach because the lava was impenetrable; thus, it was not possible to set out pitfall traps. Ant assemblage assessments were conducted on 12 October 2007 at Mauga and at 15 October 2007 at Saleaula (Table 1).

To control variation in ambient nectar availability, we bagged all nectary bodies in each plot using dark poplin fabric secured at the base with a plastic cable tie. Bags were ~1.5-2X bigger than nectary bodies to minimize contact between nectaries and bagging material. These bags excluded ants from floral, extrafloral and postfloral nectaries.

To inform the design of our carbohydrate treatments, we assessed natural nectar production and concentration for *M. citrifolia*. We excluded insect visitors from nectary bodies for 24 h and collected nectar in microcapillary tubes. Average nectar production per plant per day was 2249 μL ± 642 SE (range = 645-5226, *n* = 6 plants). The concentration of *M. citrifolia* nectar using a field refractometer (EZ-Red B1, EZ Red Co., Deposit, New York) was 28.06 % ± 1.04 % SE (*n* = 8 plants).

We manipulated carbohydrate availability by adding artificial nectary tubes that were either empty or contained 2000 μL of a 30% sucrose solution. In a previous study, Freeman et al. (1991) demonstrated that *M. citrifolia* nectar is dominated by sucrose, with sucrose contributing an average of 72.6-88.9% to total nectar carbohydrates. Therefore, we considered the 30% sucrose solution in our artificial nectaries to be a reasonable approximation of naturally-occurring *M. citrifolia* nectar. Treatments manipulated the number of filled artificial nectar tubes with five levels: 0, 5, 10, 15, or 20 filled tubes. To control for the presence of the nectary tube structures, all plants received 20 artificial nectary tubes (2000 μL, MCT-200-C Microtubes, Axygen Scientific, Union City, California) with a small hole in the lid, through which a cotton wick was threaded and allowed to reach the bottom of the tube; wicks were trimmed to ~3 mm. The 20 tubes were strung on a strip of poplin fabric (~2cm wide) in randomized order, then wrapped around the main stem and branches of *M. citrifolia* to mimic the distribution of nectary bodies. To maximize the potential to detect ant responses, our nectar treatments substantially increased nectar availability, e.g. the 5 tube treatment produced 450% higher nectar levels (10,000 μL/day) than would occur naturally on an average plant.

Artificial nectaries were established between 1530-1700 (five replicates) on 26 July 2007 at the Saleaula, and 1500-1700 on 8 August 2007 (two replicates) and 1700-1830 on 9 August 2007 (three replicates) at Mauga. During this time period, there was no measurable rainfall and average daily temperatures at Avao weather station ranged from 28°C to 30°C). At both sites, artificial nectaries were refreshed once after the first census at 16h, due to depletion of nectar in the tubes.

Counts of ant visitors to plants were obtained at three times: 16h, 24h and 40h after the initial establishment of the nectar treatment. All counts occurred during daylight hours due to cultural restrictions. While this sampling scheme provided a good estimate of the relative foraging rates for diurnal and crepuscular foragers, it did not account for the nocturnal activities of local ant assemblages. During counts, each individual ant was classified by behavior (tending hemipterans versus any other behavior), by species, and by location (at artificial nectaries, nectary bodies, stems and leaves, or hemipteran aggregations). Counts took ~5 minutes per plant. We collected specimens of each ant species from nearby non-treatment plants and identified them using Wilson and Taylor (1967) and Shattuck (1999).

Experimental Design: 2009

We returned to the same villages during 16-17 September 2009 (Saleaula) and 24-25 September 2009 (Mauga) to conduct similar experiments. We selected three sites for these trials: (i) Saleaula; (ii) Mauga South (inside *A. gracilipes* invasion front); and (iii) Mauga North (outside *A. gracilipes* invasion front). The sites selected at Mauga were separated by ~150 m. Average daily temperatures during these dates ranged between

26°C and 29°C, and there was no measurable precipitation at the Avao weather station. As in 2007, treatments were applied at random to individual plants chosen haphazardly from among plants of similar size bearing at least two nectary bodies. Treatment plants were again located in blocks that were separated from each other by ~10m (*n*=5 blocks for Saleaula and Mauga South and *n*=3 blocks for Mauga South due to the small local extent of the new invasion). We measured plant height, counted the total number of nectary bodies, and counted the number of honeydew-excreting insects per plant. Prior to nectar manipulations, we also assessed ant assemblages using card counts (as described for 2007).

The experimental design for 2009 was the same as for 2007, with the following exceptions. The carbohydrate manipulations were modified to more closely mimic natural variation in *M. citrifolia* extrafloral nectar availability at the per plant scale. Thus, the levels were 0, 50, 100, 150, and 200% of ambient levels per plant. To reduce nectar, we again secured bags to the base of nectary bodies (as described above), but bags were constructed from lightweight organza instead of poplin. We bagged all nectary bodies, regardless of treatment assignment to control for any effects of the bags. However, we cut holes in control bags to allow ants to access actual *M. citrifolia* nectar. Thus, for plants in the 50% treatment, we cut holes in half of the bags, and all bags had holes in the 100%, 150% and 200% treatments.

As in 2007, we used artificial nectaries to supplement nectar levels (50 μL per tube - Seal-Rite microcentrifuge tubes, USA Scientific 1605-0000, Ocala, FL USA, filled with a 30% sucrose solution). We inserted a 5 μL microcapillary tube into the centre of each microcentrifuge tube instead of using string in order to reduce evaporation of the sucrose solution. Tubes were affixed to trees using twist ties, all plants received 10 tubes, and as in 2007, control tubes were empty. Thus, the 150% treatment had 5 empty tubes and 5 filled tubes for a total supplement of 2500 μL over the course of the experiment (48 h). Tubes were not replenished at any time during the experiment. As a result, some of the tubes were empty by the end of the experiment. However, this was rare, since microcapillary tubes reduced evaporation rates. Air bubbles occasionally formed inside the microcapillary tubes. Therefore, all microcapillary tubes were cleared (removed and then re-inserted) ~15 minutes before each census.

We conducted six censuses for each plant: morning (~600 – 800), mid-afternoon (~1200-1400), and evening (~1600-1800) over two consecutive days. Response variables were the same as in 2007.

Data analyses

Because we used different methods in 2007 and 2009, we analyzed responses for each year separately. To test (1) Do invasive *A. gracilipes* workers differ from co-occurring non-invasive ants in their response to increasing carbohydrate levels?, we examined ant density (number of workers per plant) for each of two ant dominance status categories: the most abundant ant species per site (dominant) or all other co-occurring ants (subordinate). Using dominance status allowed us to compare the responses of both dominant and non-dominant ants between both invaded (*A. gracilipes*-dominated) and reference (*P. megacephala*-dominated) sites. Data were analyzed by site and year using repeated measures general linear models following recommendations in von Ende (2001). Models included the following independent variables: time elapsed since the addition of nectar tubes (16, 24, 40 h for 2007 or 12, 18, 24, 32, 36, 48 h for 2009), the random effect of block, the fixed effects of ant dominance status and the carbohydrate treatment (0, 5, 10, 15, 20 filled tubes for 2007 or 0, 50, 100, 150, 200% ambient levels for 2009), and all interactions with time (SAS Inc., 2003, version 9.1, Cary, NC). Ant dominance status and block were treated as categorical factors, and the carbohydrate treatment was treated as a continuous factor. Plant size (total branch length for 2007 or height for 2009), the number of nectary bodies per plant, and the abundance of honeydew-excreting insects per plant (2009 only) were tested as possible covariates, but none had significant effects on ant abundances, perhaps because plants were of similar sizes. Thus, we did not include these covariates in the final analyses. When the factor of elapsed time had no significant influence on ant responses to our treatments, we averaged across time to simplify data presentation. Statistical analyses met assumptions of multivariate normality of residuals and homogeneity of variances following square-root transformation of ant density.

Using dominance status as a factor in the previous analyses allowed us to compare the within-site differences of *A. gracilipes* and co-occurring non-invasive ants. However, we needed a separate test to compare *A. gracilipes* with other non-invasive ants across sites with different invasion statuses. Using the analysis described above, we first confirmed that dominant and subordinate ants at sites where *A. gracilipes* were absent did not differ in their responses to our carbohydrate manipulations (see below). We then pooled their responses and compared them to *A. gracilipes* responses at invaded sites, again using repeated measures analysis of covariance. Subordinate ants at *A. gracilipes*-invaded sites were excluded from these cross-site comparisons. Site was treated as a categorical factor, our carbohydrate treatments were again treated as continuous, and block was incorporated in the model as a nested factor within site. When ant responses to carbohydrate manipulations were significantly different between sites, as indicated by a statistically significant site by carbohydrate treatment interaction, then for each site we conducted regressions of ant density per plant as a function of carbohydrate treatment.

To test (2) Does increasing plant-based carbohydrate levels change the honeydew-excreting insects-tending behaviors of *A. gracilipes* and/or non-invasive ants?, we examined the proportion of workers tending honeydew-excreting insects using the same repeated measures analyses as described for question (1). Proportion data were arcsine square-root transformed to meet assumptions of normality and heterogeneity.

2.3 Results

Assessments of ground and plant foragers outside of treatment blocks confirmed that the relative abundances of *A. gracilipes* and co-occurring non-invasive ants were similar both on *M. citrifolia* plants and on the ground, although there were more ants observed on plants than on the ground (Table 1). These data confirmed that the patterns below are likely indicative of site-level ant assemblage characteristics. However, we focused on the plant-foraging ant community in order to present our results conservatively.

(1) Do invasive *A. gracilipes* workers differ from co-occurring non-invasive ants in their response to increasing carbohydrate levels?

Experimental increases in carbohydrate availability strongly increased the short-term densities of *A. gracilipes* on plants but had no significant effects on other ant species. In 2007, *A. gracilipes* recruited to increasing carbohydrate availability at

Saleaula, with an increase in worker density per plant of ~400% for the 20 tubes treatment relative to 0 tubes. However, few workers of other ant species were observed on treatment plants and their numbers did not respond to carbohydrate availability (Supplementary Table 1a, Figure 1a). Meanwhile, at Mauga, the reference site, there was no significant difference between the response of the dominant species (*P. megacephala*) and co-occurring, subordinate ant species. Although both dominant and subordinate ants were somewhat more abundant on plants with high carbohydrate levels at Mauga, the carbohydrate treatment effect was not statistically significant for either (Supplementary Table 1a, Figure 1b).

In 2009, *A. gracilipes* workers again responded strongly to our carbohydrate manipulations, while workers of co-occurring ant species were infrequent visitors to *M. citrifolia* plants and non-responsive to carbohydrate treatments. This effect was consistent at both the site with the established *A. gracilipes* invasion (Saleaula) and in the newly-invaded region of Mauga South (Supplementary Table 1a, Figures 1c-d). However, outside of the *A. gracilipes* invasion front in Mauga (Mauga North), neither the dominant or subordinate ants significantly recruited to increasing carbohydrates, although both dominant *P. megacephala* and subordinate ants were observed on *M. citrifolia* plants (Supplementary Table 1a, Figure 1e). Over the course of the experiments in both 2007 and 2009, there were some cases in which the magnitude of the carbohydrate treatment effect strengthened through time. This resulted in significant 2-and-3-way interactions with time, but did not change the relative rankings of treatments during any given census (Supplementary Table 1a).

Across-site differences in the response of *A. gracilipes* versus non-invasive ants to carbohydrate treatments were significant in both 2007 and 2009 (Supplementary Table 1b). Across both years and sites, *A. gracilipes* displayed a strong, positive foraging recruitment response to increasing levels of carbohydrates (Saleaula 2007: r=0.74, P<0.0001; Saleaula 2009: r=0.80, P<0.0001; Mauga South 2009: r=0.93, P<0.0001). However, there was only a slight, positive response of ant assemblages at the site that lacked *A. gracilipes* in 2007 (Mauga 2007: r=0.45, P=0.0232), and no foraging response of non-*A. gracilipes* workers in 2009 (Mauga North 2009: r=0.37, P=0.1800).

(2) Does increasing plant-based carbohydrate levels change the honeydew-excreting insects-tending behaviors of A. gracilipes *and/or non-invasive ants?*

Carbohydrate manipulations altered ant behaviors toward honeydew excreting insects (honeydew-excreting insects). At Saleaula in 2007, the percentage of *A. gracilipes* workers that tended honeydew-excreting insects decreased from 42% (± 7%) in the zero filled tubes treatment to 9% (± 3%) in the treatment with 5 filled tubes (450% ambient levels) and remained below 10% for all other carbohydrate levels (Figure 2a). Only one non-*A. gracilipes* worker (*Monomorium pharoensis*) was observed tending honeydew-excreting insects during the entire course of the experiment, resulting in a significant ant dominance status x carbohydrate treatment interaction (Supplementary Table 2, Figure 2a). Ants at the reference site displayed similar patterns, with the only discernable honeydew-excreting insects-tending occurring at the zero tubes treatment by the species *Pheidole megacephala* and *Paratrechina longicornis*. However, both dominant *P. megacephala* workers and workers from co-occurring ant species responded similarly by abandoning tending when carbohydrates increased (Supplementary Table 2, Figure 2b).

Anoplolepis gracilipes workers did not respond significantly differently to carbohydrate treatments in terms of honeydew-excreting insects tending behaviors than ants at the reference (uninvaded) site in 2007 (Supplementary Table 2).

Carbohydrate treatments for the 2009 trials were closer to ambient levels (0-200%). However, even with these more subtle carbohydrate subsidies, there was a significant linear decrease in honeydew-excreting insects tending as nectar levels increased at both Saleaula (~81% reduction) and within the *A. gracilipes* invasion front at Mauga South (~89% reduction; Supplementary Table 2, Figure 2c-d). Outside of the invasion front (Mauga North), there was no effect of the carbohydrate treatment on honeydew-excreting insects tending rates, which were generally low (Supplementary Table 2, Figure 3d). Outside of the invasion front, more *P. megacephala* workers were observed tending honeydew-excreting insects than any other ant species, there was no consistent effect of our carbohydrate treatment through time, and this high variability resulted in a significant interaction between time and the carbohydrate treatment (Supplementary Table 2). In contrast to the 2007 trials, there was a significant difference between the honeydew-excreting insects tending responses of *A. gracilipes* and ants at the uninvaded site. Across both Saleaula and Mauga South, *A. gracilipes* workers displayed a strong, negative response to carbohydrate treatments (Saleaula 2009: $R^2=0.6117$, $P<0.0001$; Mauga South 2009: $R^2=0.9230$, $P<0.0001$). Conversely, ants outside of the *A. gracilipes* invasion front in Mauga did not change their honeydew-excreting insects tending behaviors as carbohydrate availability increased (Mauga North 2009: $R^2=0.0742$, $P=0.3259$).

2.4 Discussion

Many invasive ants readily form facultative associations with carbohydrate-excreting plants and insects. These putatively mutualistic interactions may accelerate the progression of non-native ant invasions, particularly if invasive ant species respond more strongly to carbohydrate resources than co-occurring non-invasive ant species (Holway et al. 2002). As an initial test of the predictions of this nectar subsidy hypothesis, our experiments created variability in plant-based carbohydrate resources and demonstrated that short-term increases in carbohydrate availability can increase the density of foraging ants on plants. Importantly, the effects of carbohydrate manipulations were significantly stronger for invasive *A. gracilipes* ants than for non-invasive ants, a pattern that was consistent across and within five site-year combinations along an active *A. gracilipes* invasion front.

Importance of carbohydrate resources to invasive ants

For all cases in which *A. gracilipes* dominated the local ant assemblage, this species responded strongly to experimentally increased carbohydrate levels while non-invasive ants that co-occurred at the same or at nearby sites did not respond strongly. Several mechanisms could underlie this difference in response between the invader species and other ant species. First, a strong *A. gracilipes* response could be a simple consequence of dominance, since dominant ant species can exclude subordinate ants from resources (Andersen 1992). However, when *A. gracilipes* was absent from the site, there was no significant influence of carbohydrate manipulations on the total density of other ant species observed on *M. citrifolia* plants, including the dominant species, *Pheidole* *megacephala*. Recently, Lach (2005) evaluated the relative foraging behaviors of co-occurring *A. gracilipes, P. megacephala* and *Linepithema humile* (Argentine ant, also considered a dominant invasive in many regions) at floral nectaries across multiple sites in Hawaii. Consistent with our results, *A. gracilipes* visited flowers at the highest densities and were the least likely of the three species to abandon nectaries. Moreover, *P. megacephala* visited flowers at the lowest densities and were the most likely to abandon nectaries of the three species Lach compared. Together, these results suggest that nectar may be particularly important for invasive *A. gracilipes* workers, and particularly unimportant for *P. megacephala* workers. Additional studies that manipulate carbohydrate availability for ant species that vary in their degree of invasiveness will help to elucidate the mechanism(s) of these patterns.

The importance of carbohydrate resources to ants has been hypothesized to vary during the temporal progression of ant invasions. Specifically, some research has suggested that increased carbohydrate availability may allow exotic ant invasions to progress past benign lag phases (Holway et al. 2002, O'Dowd et al. 2003). For example, the diets of invasive *L. humile* workers shifted toward plant-based resources and honeydew-producing hemipterans only after invading populations became established (Tillberg et al. 2007). However, we rarely know exactly when a species is introduced into a new region or the rate of progression for any particular invasion, making it difficult to evaluate the validity of this hypothesis. In our study, the approximate date of arrival of *A. gracilipes* to Mauga was known. In less than two years, *A. gracilipes*-dominated ant assemblages at this site displayed a response to carbohydrate manipulations that closely resembled the response of ants at a site with an older, established *A. gracilipes* invasion

(Saleaula). The responses of ants in *A. gracilipes*-dominated regions differed markedly from those in areas that were not dominated by *A. gracilipes*. This finding suggests that strong recruitment to carbohydrates may be a characteristic of invasive *A. gracilipes* generally, rather than specific to a particular stage of its invasion. However, replication of the age of the invasion would be needed to directly address this question.

Extrafloral nectar availability and Hemiptera tending by ants

During the 2007 trials, all ants - regardless of site - reduced honeydew-excreting insects tending behaviors when they were provided with additional carbohydrates. For these trials, carbohydrate supplements were, at minimum, 450% of ambient *M. citrifolia* nectar levels and the only treatment level in which an appreciable proportion of ants tended honeydew-excreting insects was zero. This abandonment of tending was displayed by ants at both the invaded and the reference (uninvaded) site. Interestingly, the zero filled tubes treatment represented a carbohydrate level that was lower than ambient levels. This suggests that the attractiveness of honeydew-excreting insects to local ant assemblages increases as other sources of carbohydrates on *M. citrifolia* plants decline. Because artificial nectaries closely mimicked the composition of naturally-occurring *M. citrifolia* nectar (Freeman et al. 1991), but lacked the additional amino acids that are more commonly found in hemipteran honeydew (e.g. Blüthgen et al. 2004), we interpret this result as a preference for plant-based carbohydrate resources over those derived from honeydew-excreting insects. Because changes in ant behavior occurred at both the *A. gracilipes*-dominated and *P. megacephala*-dominated sites, *A. gracilipes* and other ant species appeared to respond similarly to the choice between plant nectar and hemipterans on *M. citrifolia*. As with overall forager recruitment to *M. citrifolia* plants, *A. gracilipes* workers displayed a stronger response to carbohydrate manipulations in terms of reduced honeydew-excreting insects tending than did co-occurring non-invasive ants within the same site. However, the amount of artificial nectar that we provided ants in the 2007 trials was higher than ambient levels produced by *M. citrifolia* plants, making it difficult to extrapolate to ant behaviors under more natural conditions.

In response to more realistic nectar manipulations, *A. gracilipes* workers again displayed a strong negative honeydew-excreting insects tending response, while ants outside of the *A. gracilipes* invasion front in 2009 no longer changed their honeydew-excreting insects tending as carbohydrate resources increased. These 2009 trials not only included more realistic levels of artificial nectar, but also allowed ants to access actual *M. citrifolia* nectar. Tending of honeydew-excreting insects at both *A. gracilipes*-dominated sites decreased linearly as carbohydrate availability increased. At sites that were not dominated by *A. gracilipes,* this trend was less clear, but a weak trend for reduced honeydew-excreting insects tending with increased carbohydrate availability remained. These results suggest that an increase in the plant's nectar resources can distract ants from the honeydew resources of hemipterans and redirect foraging efforts to the potentially more abundant or higher quality plant-derived nectar, as proposed by Becerra and Venable (1989). Furthermore, the fact that honeydew-excreting insects were abandoned at high levels of carbohydrate availability suggests that there may be a limit to the amount of carbohydrate resources that *A. gracilipes* and co-occurring non-invasive ants will collect. Perhaps at high levels of carbohydrate availability, workers become more protein-starved (e.g. Ness et al.2009) or costs associated with collecting carbohydrates (in terms of time or effort expended) outweigh the benefits provided by collecting both honeydew and nectar. Alternatively, these patterns may be due to the ease of obtaining artificial nectar from tubes, relative to honeydew and EFN. However, the linear decline in tending behaviors during the 2009 trials makes this unlikely because tending behaviors were reduced when ants were allowed access to different levels of natural plant nectar. More generally, if decreased tending of honeydew-excreting insects leads to declines in the abundance of hemipterans, this change in ant behavior could cascade to the plant as well as to other community members (Styrsky and Eubanks 2007).

Conclusions

Our experiments showed that increases in carbohydrate resources can produce strong, short-term changes in the relative foraging densities and honeydew-excreting insects tending behaviors of ants on plants. Furthermore, invasive *A. gracilipes* workers were more responsive to carbohydrate subsidies than were other ant species at our sites. These findings provide a first step towards confirming that interactions with carbohydrate-excreting species promote ant invasions. If further studies spanning more sites and different invasion histories confirm the strong patterns reported here, then ant-plant protective mutualisms may have community-wide consequences by promoting *A. gracilipes* invasions. Previous studies have found impacts of this species to cascade to canopy tree survival and seedling establishment (O'Dowd et al. 2003), and to affect the abundance, behaviors and reproduction of native birds (Davis et al. 2008) and crabs (O'Dowd et al. 2003, McNatty et al. 2009).

Caution should be exercised in the interpretation of these results for two reasons. We tested the responses of *A. gracilipes* workers and workers from co-occurring non-invasive ant species to carbohydrate subsidies along an invasion history gradient, spanning two different years. However, within each year, each invasion category was only represented by one (or two) replicate. Future studies with greater replication are therefore needed to determine the generality of our findings. Additionally, we focused on plant-foraging ants, not on population-level dynamics (which would have included ground foragers and nests).

Nonetheless, our results demonstrate that ant species can differ markedly in their responses to carbohydrate-rich resources. Evidence for stronger responses of invasive *A. gracilipes* compared to other ant species marks a first step toward understanding the importance of carbohydrate resources in fueling this and other ant invasions.

2.4 Acknowledgements

We thank Levao Fa'a Malo and Paogo Malo in the villages of Saleaula and Mauga for graciously allowing us to use their land for our experiments. Thanks also to Meki Tauai, Maiava Veni, Pati Liu, Afele Faiilagi and Susau Siolo in the Samoan Ministry of Natural Resources and Environment for logistical and field assistance. We also thank Phil Lester and three anonymous reviewers for thoughtful comments on previous versions of this manuscript. This work was supported by the National Geographic Society grant 8237-07, by the Godwin Assistant Professorship to J.A.R., and by a Rice University Wray-Todd Fellowship to A.M.S.

2.5 References

Abbott, K.L. (2005) Supercolonies of the invasive ant, *Anoplolepis gracilipes,* on an oceanic island: forager activity patterns, activity and biomass. *Insectes Sociaux,* **52**, 266-273.

Abbott, K.L. (2006) Spatial dynamics of supercolonies of the invasive yellow crazy ant, *Anoplolepis gracilipes*, on Christmas Island, Indian Ocean. *Diversity and Distributions,* **12**, 101-110.

Andersen, A.N. (1992) Momentary diversity by dominant species in exceptionally rich ant communities of the Australian seasonal tropics. The American Naturalist **140,** 401-420.

Becerra, J.X.I. & Venable, D.L. (1989) Extrafloral nectaries-a defense against ant-Homoptera mutualisms? *Oikos*, **55**, 276-280.

Blüthgen, N., Gottsberger, G., & Fielder, K. (2004) Sugar and amino acid composition of ant-attended nectar and honeydew sources from an Australian Rainforest. *Austral Ecology*, **29**, 418-429.

Dunham, A. E. and A. S. Mikheyev. (2010) Influence of an invasive ant on grazing and detrital communities and nutrient fluxes in a tropical forest. Diversity and Distributions **16**, 33-42.

Freeman, C. E., R. D. Worthington, and M. S. Jackson. (1991) Floral nectar sugar compositions of some south and southeast Asian species. Biotropica **23**, 568-574.

Fritts, T.H. & Rodda, G.H. (1998) The role of introduced species in the degradation of island ecosystems: A case history of Guam. *Annual Review of Ecology and Systematics,* **29**, 113-40.

Grover, C.D, Kay, A.D., Monson, J.A., Marsh, T.C., & Holway, D.A. (2007) Linking nutrition and behavioral dominance: carbohydrate scarcity limits aggression and activity in Argentine ants. *Proceedings of the Royal Society B-Biological Sciences,* **274**, 2951-2957.

Helms, K.R. & Vinson, S.B. (2002) Widespread association of the invasive ant *Solenopsis invicta* with an invasive mealybug. *Ecology,* **83**, 2425-2438.

Hoffmann, B. D., and Parr, C.L. (2008) An invasion revisited: the African big-headed ant (*Pheidole megacephala*) in northern Australia. *Biological Invasions*, **10**, 1171-1181.

Holway, D. A., Lach, L., Suarez, A.V., Tsutsui, N.D. & Case, T.J. (2002) The causes and consequences of ant invasions. *Annual Review of Ecology and Systematics,* **33**, 181-233.

King, J. R. and W. R. Tschinkel. (2008) Experimental evidence that human impacts drive fire ant invasions and ecological change. Proceedings of the National Academy of Sciences of the United States of America **51**, 20339-20343.

Koptur, S. (1979) Facultative association between weedy vetches bearing extrafloral nectaries and weedy ants in California. American Journal of Botany **66**, 1016-1020.

Lach, L. (2003) Invasive ants: Unwanted partners in ant-plant interactions? *Annals of the Missouri Botanical Garden,* **90**, 91-108.

Lach, L. (2005) Interference and exploitation competition of three nectar-thieving invasive ant species. Insectes Sociaux **52**, 257-262.

Lester, P.J. & Tavite, A. (2004) Long-legged ants, *Anoplolepis gracilipes* (Hymenoptera: Formicidae), have invaded Tokelau, changing composition and dynamics of ant and invertebrate communities. *Pacific Science,* **58**, 391-401.

Lowe, S., Browne, M.& Boudjelas, S. (2000) 100 of the world's worst invasive alien species. *Aliens,* **12**, 1–12.

McGlynn, T. P. (1999) The worldwide transfer of ants: geographical distribution and ecological invasions. *Journal of Biogeography,* **26**, 535-548.

McNatty, A., K.L. Abbott, and P.J. Lester (2009) Invasive ants compete with and modify the trophic ecology of hermit crabs on tropical islands. Oecologia **160**, 187-194.

Ness, J.H. & Bronstein, J.L. (2004) The effects of invasive ants on prospective ant mutualists. *Biological Invasions,* **6**, 445-461.

Ness, J. H., W. F. Morris and J. L. Bronstein. (2009) For ant-protected plants, the best defense is a hungry offense. Ecology **90**, 2823-2831.

O'Dowd, D.J., Green, P.T. & Lake, P.S. (2003) Invasional 'meltdown' on an oceanic island. *Ecology Letters,* **6,** 812-817.

Pimental, D., McNair, S., Janecka, J., Wightman, J., Simmonds, C., O'Connell, C., Wong, E., Russel, L., Zern, J., Aquino, T. & Tsomondo, T. (2001) Economic and environmental threats of alien plant, animal, and microbe invasions. *Agriculture, Ecosystems, and Environment,* **84**, 1-20.

Porter, S. D. & Savignano, D. A. (1990) Invasion of polygyne fire ants decimates native ants and disrupts arthropod community. *Ecology*, **71**, 2095-2106.

Sarty, M., Abbott, K.L., & Lester, P.J. (2007) Community level impacts of an ant invader and food mediated coexistence. *Insectes Sociaux,* **54**, 166-173.

SAS Institute. (2003) *SAS version 9.1.3.* SAS Institute, Cary, North Carolina, USA.

Savage, A. M., Rudgers, J. A., & Whitney, K.D. (2009) High abundances of extrafloral nectary-bearing plants are associated with increased abundances of an invasive ant and reduced native ant richness. *Diversity and Distributions* **15**, 751-761.

Shattuck, S. O. (1999) *Australian Ants: Their biology and identification. Monographs on invertebrate taxonomy,vol. 3.* CSIRO Publishing, Collingswood, Victoria, Australia.

Styrsky, J.D. & Eubanks, M.D. (2007) Ecological consequences of interactions between ants and honeydew-producing insects. *Oecologia,* **149**, 465-473.

Tillberg, C.V., Holway, D.A., LeBrun, E.G., & Suarez, A.V. (2007) Trophic ecology of invasive Argentine ants in their native and introduced ranges. *Proceedings of the National Academy of Sciences of the United States of America,* **104**, 20856-20861.

von Ende, C. N. (2001) Repeated measures analysis: growth and other time dependent measures. In *Design and Analysis of Ecological Experiments* (ed. by S. M. Scheiner & J. Gurevitch), pp. 134-157. Oxford University Press, New York, New York.

Waki, J., Olkpul, T., Komolong, M.K. (2007) A descriptor list for morphological characterization of Noni (*Morinda citrifolia* L.). *The South Pacific Journal of Natural Science,* **10,** 61-66.

Wetterer, J. K. (2005) Worldwide distribution and potential spread of the long-legged ant, *Anoplolepis gracilipes* (Hymenoptera: Formicidae). *Sociobiology,* **45**, 77-96.

Whistler, W. A. (1993) *Flowers of the Pacific Island Seashore: A Guide to the Littoral Plants of Hawaii, Tahiti, Samoa, Tonga, Cook Islands, Fiji and Micronesia.* University of Hawaii Press, Honolulu, HI USA.

Wilson, E. O., & Taylor, R.W. (1967) The ants of Polynesia (Hymenoptera: Formicidae). *Pacific Insects Monograph,* **14**,1-10.

2.7 Tables

Table 2.1: Densities of *Anoplolepis gracilipes* and other co-occurring ants at study sites in both 2007 and 2009. During the 2007 experiment, Saleaula and Mauga were invaded and uninvaded by *A. gracilipes*, respectively. The southern region of Mauga became invaded by 2009 (see text for more information). Densities (mean ± SE) on the ground are reported per 10 cm^2 and per sub-sampled plant for *Morinda citrifolia* plants (see text for more detail).

	2007				**2009**			
	Density of *A. gracilipes*		Density of other ants		Density of *A.gracilipes*		Density of other ants	
Site	Ground	*Morinda citrifolia*	Ground	*Morinda citrifolia*	Ground	*Morinda citrifolia*	Ground	*Morinda citrifolia*
Saleaula	15.38 ± 3.99	29.44 ± 4.37	0.22 ± 0.22	2.33 ± 6.43	30.5 ± 6.43	53.6 ± 7.69	12.4 ± 2.49	3.67± 2.01
Mauga	0	0	0	6.40 ± 0.80	---	---	---	---
Mauga North	---	---	---	---	0	0	9.4 ± 1.60	11.32 ± 0.98
Mauga South	--	---	---	---	38.75 ± 1.32	53.21 ± 4.65	0	0

2.8 Figures

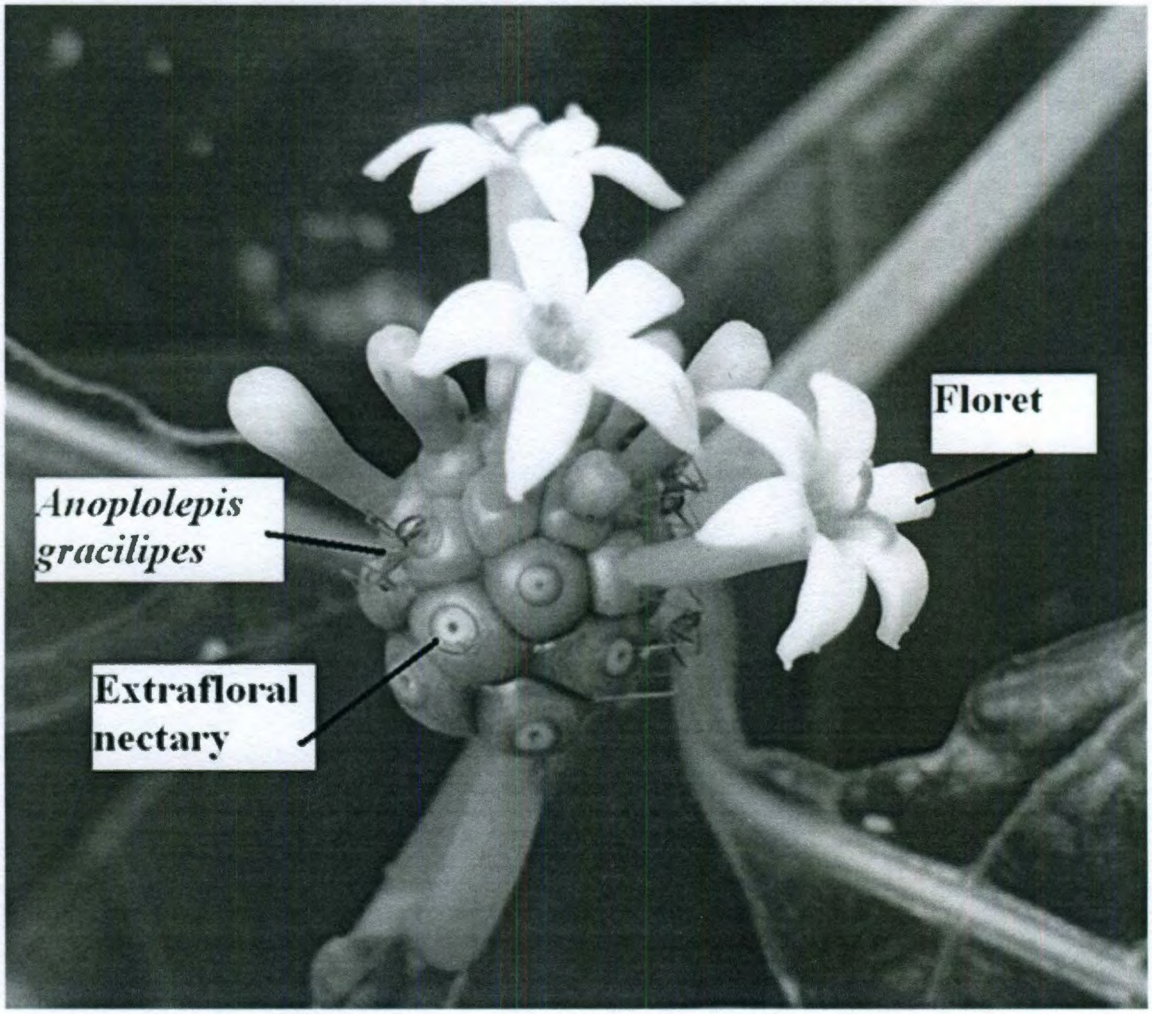


Figure 2.1: *Morinda citrifolia* plant showing florets and extrafloral nectaries, and *Anoplolepis gracilipes* workers. We refer to the entire structure (inflorescence and nectaries) as a 'nectary body' (see text for more details).

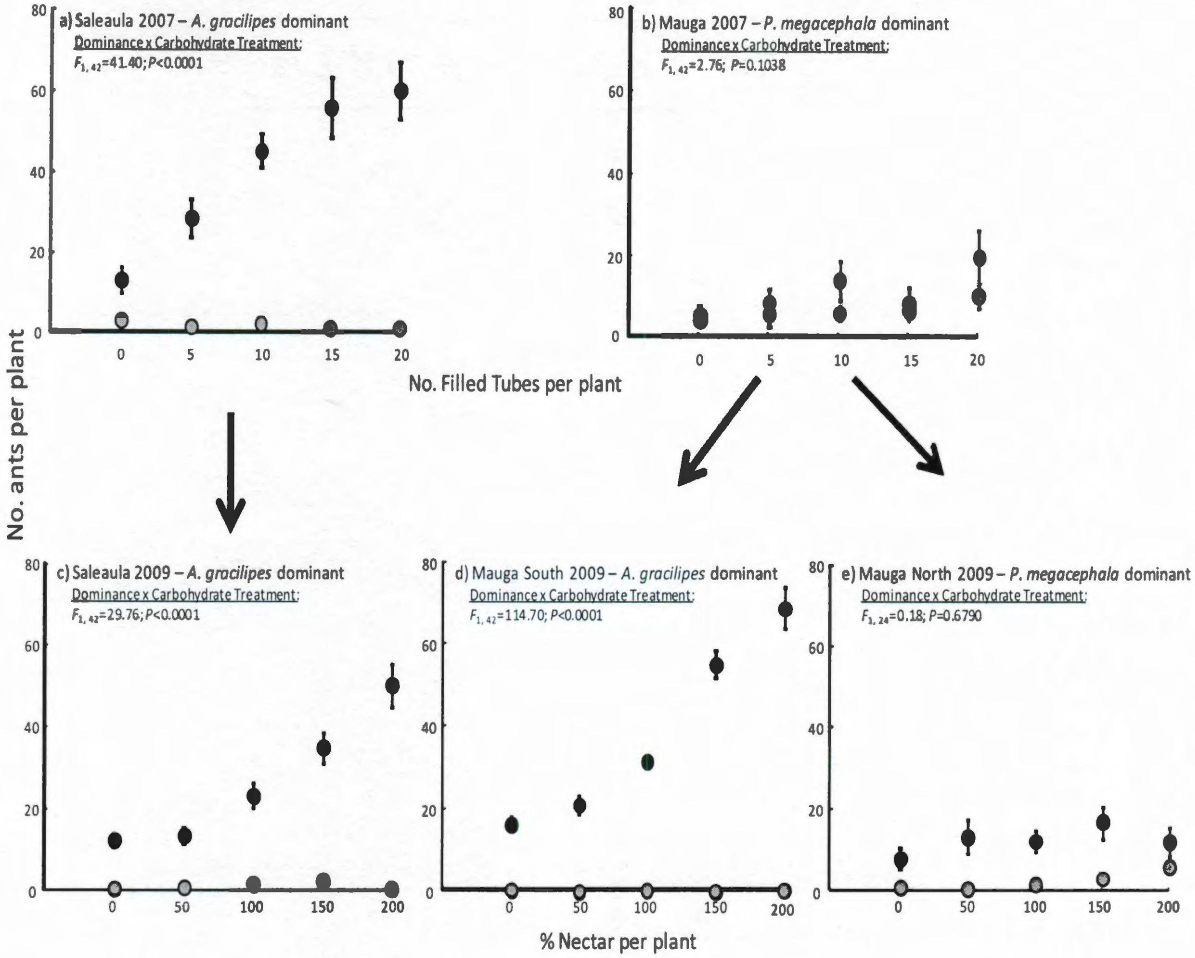


Figure 2.2: Ant Density as a function of the carbohydrate treatment. Black circles (●) represent the abundance of the dominant species in a site per plant and grey circles (◉) represent the combined abundances of all subordinate ant species per plant. Error bars represent ± 1 SE of the mean.

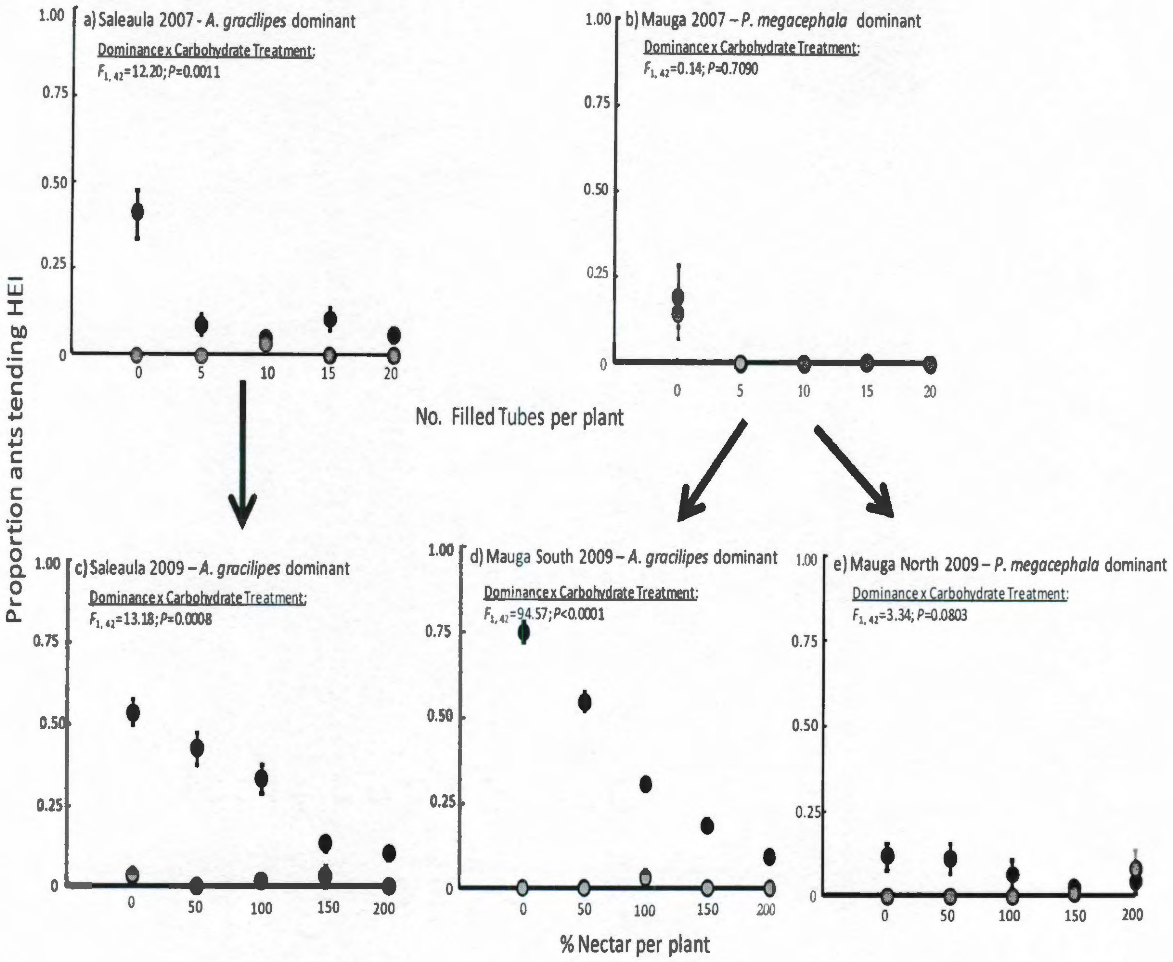


Figure 2.3: Proportion of ants tending honeydew-excreting insects (honeydew-excreting insects) as a function of the carbohydrate treatment. The proportion of the dominant species at each site is denoted with black circles (●) and the proportion of all subordinate ants that were observed tending honeydew-excreting insects are represented by grey circles (●). Error bars represent ± 1 SE of the mean.

Chapter 3

Mutualistic, trait-mediated indirect interactions in invasions: a highly invasive ant has unique behavioral responses to plant nectar

ABSTRACT

Exotic species often form beneficial, facultative associations with indigenous species. However, we still have a limited understanding of the influences that these positive associations may have on the dynamics and impacts of species invasions. Highly invasive species may respond differently than less-invasive species to resources that are exchanged in mutualisms, leading to trait-mediated indirect interactions between native species via invaders that may reshape native communities. In this study, we tested the hypothesis that the highly invasive ant species, *Anoplolepis gracilipes*, exhibits stronger trait changes in response to increasing levels of nectar than co-occurring, less-invasive ant species. Across two islands in the Samoan Archipelago, we located multiple sites dominated by *A. gracilipes* and multiple sites dominated by other, less invasive species. At each site, we manipulated nectar levels on a common extrafloral nectary-bearing shrub and assessed short-term changes in ant worker recruitment and aggression. We found that the recruitment response of the highly invasive ant species *A. gracilipes* was not unique: other dominant ant species also increased recruitment in response to increasing nectar levels. However, *A. gracilipes* did show unique changes in aggressive behaviors: as nectar levels increased, the proportion of prey discovered, attacked and removed by *A. gracilipes* workers and the speed at which they performed these aggressive behaviors all increased strongly. Other ant species showed no such responses. In addition, fewer subordinate ants persisted on plants at sites invaded by *A. gracilipes.* These results suggest that mutualisms between invasive ants and native plants can modify interactions between invaders and co-occurring arthropods, possibly leading to more negative consequences for native communities. They also underscore the importance of incorporating both positive species interactions and indirect pathways into our studies of both community ecology and invasion biology.

Key Words: Aggression, ant-plant mutualisms, *Anoplolepis gracilipes,* forager recruitment, invasive species, *Morinda citrifolia,* nectar, trait-mediated indirect interactions

3.1 Introduction

Although traditionally studied as pairwise associations, interspecific mutualisms involve interactions among diverse assemblages in complex, multispecies communities. The outcome of mutualisms can depend strongly upon the identity of the interacting partners (Bronstein 1994). For example, *Acacia drepanolobium* trees associate with several species of ant bodyguards; these ant species not only differ in the costs and benefits provided to host trees, but also influence plant host fitness most strongly at different phenological stages. Consequently, the fitness of *A. drepanolobium* plants is highest when the plants associate with multiple ant species throughout their lifetime (Palmer et al. 2010). Significant variation in the costs and benefits associated with different mutualist partners has been reported for other arthropod-plant protection mutualisms (Rudgers and Strauss 2004, Whitney 2004, Miller 2007), mycorrhizal mutualisms (Maherali and Klironomos 2007, Mangan et al. 2010), pollination mutualisms (Gomez et al. 2010), and seed dispersal mutualisms (Whitney 2005, Manzaneda and Rey 2008).

Because mutualisms are embedded in complex communities, variation in partner identity may have significant consequences for community structure and function. The community-wide consequences of mutualisms are still poorly understood. However, recent studies have demonstrated that the abundance, diversity, and composition of local communities can be strongly influenced by the presence of mutualistic interactions (Stachowicz 2001, Bruno et al. 2003, Hay et al. 2004, Rudgers et al. 2007, Lach 2008, Matthews et al. 2009). Furthermore, variation in the degree and type of benefits exchanged in mutualisms can have cascading effects on the structure and dynamics of communities. For example, Rudgers et al. (2010) demonstrated that geographic variation in the benefits provided to plants by ant guards was associated with significant differences in the composition and abundance of plant-associated arthropods that were not directly involved in the mutualism. We have a limited understanding of the mechanisms that underlie these community-level responses to variation in mutualist partner identity. However, interaction modifications via trait-mediated indirect pathways are likely to be important. These effects occur when the presence of one species modifies one or more traits of another species, with consequent effects on other community members through interaction modification (Werner and Peacor 2003).

Species invasions provide ideal systems for investigating the importance of species identity in ecological interactions. Invasive species often display exaggerated traits compared to native species (e.g. Pysek and Richardson 2007, van Kleunen et al. 2010), and these traits may influence co-occurring community members. Additionally, novel interactions between invaders and native species provide a contrast to the same associations involving native species that share a long history of interaction. When exotic species invade novel habitats, they may respond differently than native species to the resources or services exchanged in mutualisms. Moreover, it is likely that different exotic species differ in their responses to mutualist-derived resources, although such a pattern has never been explicitly described, to our knowledge. If a highly invasive species displays different traits when it displaces native or less-invasive species in facultative mutualisms and this trait change results in community-level effects, then trait-mediated pathways may be important mechanisms underlying the detrimental impacts of species invasions. However, surprisingly few studies have investigated the role of trait-mediated indirect interactions in invasions. White et al. (2006) recently surveyed the ecological literature and found only two studies that experimentally demonstrated that (i) novel positive interactions between an invader and a native species influenced co-occurring community members via interaction modification and (ii) these effects were trait-mediated. In both cases, the presence of an invasive plant altered pollinator visits to native plant species (Grabas and Laverty 1999, Chittka and Schurkens 2001). Since non-native species frequently form positive associations with native species (Bruno et al. 2003), we could be underestimating the importance of indirect, trait-mediated mechanisms in driving the dynamics and impacts of species invasions.

In this study, we investigated positive interactions between ants and extrafloral nectary (EFN)-bearing plants in Samoa. Like many other island ecosystems, Samoa is dominated by non-native ant species (Wetterer and Vargo 2003). However, in a recent survey (Savage et al. 2009), we found that the only non-native ant species that positively co-varied with the abundance of EFN-bearing plants was the highly invasive species *Anoplolepis gracilipes.* A pan-tropical 'tramp ant,' *A. gracilipes* has strong negative impacts on native island flora and fauna in the Pacific and Indian Oceans (Holway et al. 2002, Hill et al. 2003, Lester and Tavite 2004, Abbott 2006, Savage et al. 2009). *Anoplolepis gracilipes* invasions are hypothesized to be driven by subsidies from carbohydrate-excreting plants and insects (Holway et al. 2002, Davidson et al. 2003, Lach 2003, Savage et al. 2009). However, the precise mechanisms underlying the invasive impacts of *A. gracilipes* are poorly understood. Recently, we experimentally demonstrated that *A. gracilipes* displayed a strong recruitment response to nectar (Savage et al. *in press*) and that the influence of ant-plant mutualisms on arthropod communities is altered when *A. gracilipes* displaces other ants as mutualist partners (Savage et al. *in preparation*). Together, these results suggested the hypothesis that unique responses of *A. gracilipes* to nectar might underlie the particularly strong negative impact of *A. gracilipes* on arthropod communities.

Carbohydrate subsidies may facilitate ant invasions through both density and trait-mediated indirect pathways. Here, we focus on the prediction that carbohydrate-rich, mutualist-derived resources (such as plant nectar) influence the behaviors of highly invasive ants more strongly than the behaviors of co-occurring less invasive ants. We hypothesized two separate, but non-mutually exclusive, trait-mediated indirect pathways whereby these interactions could influence arthropod community structure. First, we hypothesized that (1) as nectar resources were experimentally increased, the highly invasive ant species *A. gracilipes* would recruit more workers to EFN-bearing plants than other non-native ant species. We expected that increased *A. gracilipes* recruitment would be associated with increased activity outside the nest and increased negative impacts on co-occurring arthropods (via predation and/or the threat of predation) relative to less-invasive ant species (dotted lines, Fig. 1a). Second, we hypothesized that (2) as nectar resources were increased, highly invasive *A. gracilipes* workers would become more aggressive toward co-occurring arthropods than less invasive ants. Increased levels of aggression could also lead to stronger negative community-wide impacts of *A. gracilipes* relative to less-invasive ants (dashed lines, Fig. 1a). Importantly, both of these pathways are not dependent upon changes in the density of invasive ants through increased colony growth (Oliver et al. 2008), because they require only a change in the allocation of workers to tasks (nectar foraging vs. other behaviors) or a change in worker aggression, respectively. Thus, we consider both of these hypothesized indirect interactions between nectar-producing plants and the arthropod community to be trait-mediated.

3.2 Methods

Study sites

We conducted nectar manipulation experiments from 16 June 2009 - 26 September 2009 on the islands of Tutuila, American Samoa and Savaii, Independent Samoa (Table 1). In a previous survey spanning six islands of the Samoan archipelago, we found that *A. gracilipes* were least abundant and widespread in Tutuila and most abundant and widespread in Savaii (Savage et al. 2009). We selected eight sites dominated by *A. gracilipes* and six sites dominated by other ant species (with *A.*

gracilipes absent). Data on foraging recruitment from three of the 14 sites were used in a previous study of an *A. gracilipes* invasion front in northeastern Savaii (Savage et al. *In press*). On Savaii, sites were separated by 150 m-66.7 km (mean 27.12 ± 9.46 km); sites on Tutuila were separated by 150m – 35.1 km (mean 16.11 ± 3.90 km).

Because it would be logistically difficult (and perhaps unethical) to experimentally manipulate the identity of the dominant ant species at a site, there is the risk that observed differences in ant species responses to nectar manipulations are confounded by different environmental conditions at sites dominated by different species. To partially address this concern, we examined spatial autocorrelation of ant species responses (forager recruitment and per-worker aggressive responses to experimentally manipulated nectar levels, see below). We constructed Euclidean distance matrices of (i) geographic distances between sites and (ii) dissimilarities between relevant test statistics per site per response variable. We used betas from regressions for the forager recruitment responses and chi square values for aggressive responses. We then conducted Mantel tests using the RELATE function in Primer v. 6.1.10 (Clark and Gorley 2007) with Spearman rank correlations and 9,999 iterations. We conducted these analyses both across all sites and for sites within each island (for a total of three tests per response variable, see Online Appendix 1). We interpret a lack of spatial autocorrelation in these responses as an indication that we are measuring differences in ant species biology as opposed to differences in environmental conditions.

During the time of the experiments, average monthly rainfall was 0.1325 mm (± 0.19 mm) and average daily temperatures ranged between 27.08°C and 29.86°C in

Tutuila (Pago Pago Weather station); temperatures ranged between 25.83°C and 29.17°C with no measurable rainfall in Savaii (Avao Weather Station).

Study organisms

A pan-tropical 'tramp ant,' *Anoplolepis gracilipes* has a broad diet breadth, can form supercolonies, and is considered invasive in many island groups, including Samoa, Hawaii, Christmas Island, and Tokelau (Holway et al. 2002, Lester and Tavite 2004, Abbott 2006). Although its native range is thought to include Africa or Asia, this species' origin is currently undetermined (Wetterer 2005). In Samoa, *A. gracilipes* workers were first recorded in 1925 at very low abundances near ports and plantations (Emery 1925 *cited in* Wilson and Taylor 1967). However, recent studies have demonstrated that this species is currently widely distributed in Samoa, occasionally occurring at very high densities (Lester and Tavite 2004, Savage et al. 2009). For example, Lester and Tavite (2004) found that *A. gracilipes* reached abundances of 191-1,060 per trap within 24 h. At sites in which *A. gracilipes* is absent from local ant assemblages in Samoa, other non-native ants are ecologically dominant (both on EFN-bearing plants and the ground; Table 1). These species all are less invasive than *A. gracilipes* and represent a range of invasiveness. Using a modification of the methods of Ward et al. (2008), we calculated invasiveness scores based upon literature accounts of the biological traits associated with invasiveness and the ecological impacts of the invaders (Table 1). We report each species' score as a percentage (species score/maximum possible score *100). Scores ranged from 25% (*Tetramorium bicarinatum)* to 100% (*A. gracilipes*) (Table 1).

Across the Samoan Archipelago, ants have access to multiple resources for carbohydrates. However, the most common and dominant extrafloral nectary-bearing plant is *Morinda citrifolia* ("Nonu", Rubiaceae), which is particularly common in disturbed habitats in Samoa (Savage, unpublished data). *Morinda citrifolia* may have spread to many Pacific islands from Southeast Asia via historical migrations of indigenous peoples ~3000 years ago (Whistler 1993). However, since the seeds can float in ocean water for months while remaining viable (Nelson 2006), this plant may have arrived in the Samoan Archipelago without human assistance. Consequently, we consider this plant to be native to the islands of Samoa. *Morinda citrifolia* possesses annular disk nectaries clustered on an inflorescence, hereafter 'nectary body' (Waki et al. 2007, Savage et al. *in press*). At our sites in Samoa, nectary bodies contained 2 to >50 nectaries and reached a maximum size of 80 cm^3. *Morinda citrifolia* plants produce nectary bodies year-round. Ants frequently visit nectaries of this plant, which are often dominated by *A. gracilipes* (Savage et al 2009).

Characterization of the ant assemblage at each site

At each site, we first assessed local ant assemblages by haphazardly selecting three *M. citrifolia* plants and counting the number and identity of ants on each plant. We used a comprehensive count of all ants per plant completed in ~5 minutes/plant. We also counted ants on the ground ~1m from each plant, using a 10 x 10 cm white paper card and counting the number of ants that crossed the card in 30sec (following methods in Abbott 2005). We used the card approach because ~60% of all sites were located on impenetrable lava fields, making it impossible to set out pitfall traps.

Experimental design

We located 5m x 5m blocks of plants at each site, focusing on areas dominated by *M. citrifolia.* Depending upon the size of the site and the naturally-occurring densities of *M. citrifolia* plants, we established 3-5 blocks per site. Blocks were separated from each other by ~8m to increase the independence of observations. Within each block, we haphazardly selected five *M. citrifolia* plants that were similar in size and within 1m of each other to be used for nectar manipulations. We first counted the number of nectary bodies, aphids (Aphididae), scale insects (Coccididae), and mealybugs (Pseudococcididae) on each plant and measured the height of the main stem and the diameter at the base of each plant to the nearest cm. Each plant was then randomly assigned to a nectar availability treatment (see below).

Hypothesis 1: As nectar levels increase, highly invasive ants will recruit more workers to EFN-bearing plants than less invasive ants

We manipulated nectar availability at the plant scale: 0, 50, 100, 150, and 200% of ambient levels per plant. To reduce access to nectar, we secured bags constructed of lightweight organza material to the base of nectary bodies with a plastic cable tie. Bags were ~1.5-2X larger than nectary bodies to minimize contact between nectaries and bagging material. We bagged all nectary bodies, regardless of treatment assignment, to control for any effects of the bags. However, in all treatments except 0%, we cut holes (~3-6cm) in bags to allow ants to access actual *M. citrifolia* nectar. Thus, for plants in the 50% treatment, we cut holes in half of the bags, and all bags had holes in the 100%, 150% and 200% treatments.

We used artificial nectaries (Seal-Rite microcentrifuge tubes, USA Scientific 1605-0000, Ocala, FL USA, filled with 500 µL of a 30% sucrose solution) to supplement nectar levels. In a previous study, Freeman et al. (1991) demonstrated that *M. citrifolia* nectar is dominated by sucrose, with sucrose contributing an average of 72.6-88.9% to total nectar carbohydrates. To inform the design of our nectar availability treatments, we assessed natural nectar production and concentration for *M. citrifolia*. We excluded insect visitors from nectary bodies for 24 h and collected nectar in microcapillary tubes. Average nectar production per plant per day was 2249 µL ± 642 SE (range = 645-5226, *n* = 6 plants). The concentration of *M. citrifolia* nectar, using a field refractometer (EZ-Red B1, EZ Red Co., Deposit, New York), was 28.06 % ± 1.04 % SE (*n* = 8 plants). Therefore, we considered the 30% sucrose solution in our artificial nectaries to be a reasonable approximation of naturally-occurring *M. citrifolia* nectar. We inserted a 5 µL microcapillary tube into the center of each microcentrifuge tube to allow ants to access sucrose in the artificial nectaries. Air bubbles occasionally formed inside the microcapillary tubes. Therefore, all microcapillary tubes were cleared (removed and then re-inserted) ~15 minutes before each census. Tubes were affixed to plants using twist ties, and all plants received 10 tubes. The 0, 50, 100, 150 and 200% treatment levels contained 0, 0, 0, 5 and 10 filled tubes, respectively. Thus in each experiment, we provided local ant assemblages with 0, 0, 0, 2500 or 5000 µl of artificial nectar per plant over the course of 48hrs. Short-term pulses of nectar availability allowed us to disentangle trait- from density-mediated effects of nectar on local arthropod assemblages, since ant population growth responses to nectar would have required much more time.

Response variables. We conducted six ant censuses per plant: morning (~6:00-8:00), mid-afternoon (~12:00-14:00), and evening (~1600-18:00) over two consecutive days. All censuses occurred during daylight hours due to cultural restrictions. While this sampling scheme provided a good estimate of the relative foraging rates for diurnal and crepuscular workers, it did not account for the nocturnal activities of local ant assemblages. Counts took ~5 minutes per plant. We collected specimens of each ant species from nearby non-treatment plants and identified them using Wilson and Taylor (1967) and Shattuck (1999).

Data analyses. We compared ant responses at sites dominated by *A. gracilipes* versus sites dominated by other non-native ants. To do this, we assigned ants at each site to one of two dominance categories: dominant (the most abundant ant species per site) or subordinate (all other co-occurring ants; see Table 1). Subordinate ants at sites dominated by *A. gracilipes* included species that were dominant at *A. gracilipes*-uninvaded sites and other species that were subordinate both in the presence and absence of *A. gracilipes.* We then conducted repeated measures general linear models on ant density (number of workers per plant) following recommendations in von Ende (2001), with separate tests for sites invaded by *A. gracilipes* vs. not invaded. Models included the following independent factors: ant dominance status (dominant or subordinate), nectar treatment (0, 50, 100, 150 or 200% ambient levels), site, block (nested in site), and all interactions with time (Proc GLM, SAS Inc., 2003, version 9.1, Cary, NC). Ant dominance status, site and block were treated as categorical factors, and the nectar availability level was treated as a continuous factor. Plant size (height x diameter at base), the number of nectary bodies per plant, and the abundance of honeydew excreting insects per plant were used as covariates. Because the repeated factor (elapsed time) had no significant influence on ant responses to our treatments, we pooled data across time to simplify data presentation. Statistical analyses met assumptions of normality of residuals and homogeneity of variances at each time following square-root transformation of ant density.

To examine the responses of the different species of dominant ants to our nectar treatments, we then conducted a second ANCOVA using the average abundances of dominant ant species on plants. Because the interaction term between species identity and the nectar treatment was significant, we conducted post-hoc linear regressions within each site. This allowed us to determine which ant species responded significantly to manipulations of nectar availability. Because we conducted 6 separate tests (one for each dominant ant species including *A. gracilipes*), we applied sequential Bonferroni corrections (Holm 1979). Finally, previous findings suggested that *A. gracilipes* invasions were associated with significant declines in co-occurring, plant-foraging ant species (Savage et al. 2009). Therefore, we also used t-tests to determine if the abundance of subordinate ants on *M. citrifolia* plants differed between sites invaded or not invaded by *A. gracilipes.*

Hypothesis 2: As nectar resources increase, highly invasive ants will become more aggressive toward co-occurring arthropods than less invasive ants

To examine the relative effects of nectar levels on ant aggression, we followed the recruitment trials (above) with aggression trials ~12 h after the last recruitment survey. We replenished all filled tubes, so that each plant's nectar availability was at the same level at the start of the aggression trials as it was at the start of the recruitment trials (0, 50, 100, 150 or 200%). Approximately 6 h later, we placed an *M. citrifolia*-feeding larva within 3cm of a nectary body (1 larva per plant, 3-5 replicates per nectar treatment, resulting in 15-25 aggression trials per site). In Tutuila, we collected nitidulid (Coleoptera) larvae from fallen *M. citrifolia* fruits ~24 hours before aggression trials were conducted. These larvae were weighed to the nearest 0.1mg and randomly assigned to nectar availability treatments. Although nitidulid larvae were common in Tutuila, they were rare in Savaii, so we instead used nectary-body feeding pyralid (Lepidoptera) larvae there. We used digital calipers to measure the length of pyralid larvae (collected from non-treatment plants) to the nearest 0.01 mm prior to aggression trials. For both test prey, there were no significant differences among the nectar treatments in the size of the larvae that were presented to ants (Nitidulidae: F = 0.01, *P* = 0.9210; Pyralidae F = 0.08, *P* = 0.7773). To assess potential differences in ant responses to prey types, we conducted an additional test using both nitiduids and pyralids which co-occurred at one site in Savaii (Saleaula_North). We found no significant differences in the response of ants to the two different larvae at that site (F = 0.71, *P* = 0.4027). Therefore, we pooled ant responses across both target prey types in the analyses (below).

Response variables. Based upon prior observations of ant behaviors (Savage, unpublished data), we determined that the interaction between ants and herbivorous larvae occurs quickly; therefore trial length was 150 seconds (unless the larva was removed from the plant by ants before the elapsed time). For each trial, we recorded the time it took ants to discover, attack, and/or remove each larva from treatment plants. Discovery occurred when ants approached the larva and tapped it with their antennae. Attack occurred when ants bit, stung, or sprayed formic acid at the larva. Removal occurred when ants forcibly ejected the larva from the plant, either by throwing it off the plant or by carrying it away from the plant.

Data analyses.

We examined aggressive behaviors both in terms of the proportion of prey larvae that were subject to ant aggression and in terms of the time required for the aggressive behaviors to be initiated. We used logistic regression (Proc LOGISTIC, SAS Inc. 2003, version 9.1 Cary, NC) with a binomial distribution and a chi square test to evaluate proportional data. To examine the time it took ants to perform aggressive behaviors, we conducted survival analyses (Proc LIFEREG, SAS Inc. 2003, Version 9.1, Cary, NC) with a Weibull distribution and Wald Chi square test statistics. This type of analysis allowed us to account for right-censored data.

We first examined the relative aggression displayed by different ant species in terms of the overall proportions of prey discovered, attacked, and removed and the time to perform these behaviors. To do this, we conducted logistic regressions and survival analyses (as described above) with *A. gracilipes* invasion status and site (nested in *A. gracilipes* invasion status) as categorical factors. For these tests we used the full dataset and pooled data across all nectar treatment levels.

Next, we assessed the influence of the nectar treatment on the relative aggression displayed by *A. gracilipes* and other dominant ant species towards prey larvae. Because we were interested in the independent effects of nectar on different stages of ant aggression, we examined prey discovery, attack and removal in terms of the proportion of all larvae that were discovered, the proportion of discovered larvae that were attacked, and the proportion of attacked larvae that were removed from *M. citrifolia* plants. Similarly, we examined the time to discovery of all prey, the time to attack of discovered prey, and the time to removal of attacked prey. In all models (both logistic regressions and survival analyses), the factors included *A. gracilipes* invasion status, the nectar treatment, and their interaction. We also included site (nested in *A. gracilipes* invasion status) and the forager recruitment response of the dominant ant species for each plant (as estimated in the recruitment trials, above) as covariates. Inclusion of the latter covariate allowed us to isolate the effects of per-worker aggressiveness from the effects of worker number on the proportions of prey discovered, attacked, and removed and on the time to perform these behaviors.

Finally, when the interaction between *A. gracilipes* invasion status and the nectar treatment was significant, we conducted post-hoc linear and quadratic regressions, with the nectar treatment as the independent variable and the average proportion of prey discovered, attacked, and removed and the time to perform these behaviors as dependent variables. When both linear and quadratic models were significant, we used F-tests to evaluate model fit and present only the best-fit model.

3.3. Results

Lack of spatial autocorrelation in ant responses to nectar

Analyses of spatial autocorrelation demonstrated that the ant responses to nectar described below are unlikely to be driven by spatial variation in environmental conditions, but are rather the result of species-specific differences in ant behaviors. Specifically, there was no significant spatial autocorrelation in forager recruitment responses (all tests: $P>0.21$), the proportion of prey discovered (all tests: $P>0.48$), the proportion of prey attacked (all tests: $P>0.44$), the proportion of prey removed (all tests: $P>0.20$), the time to prey discovery (all tests: $P>0.18$), the time to prey attack (all tests: $P>0.11$) or the time to prey removal (all tests: $P>0.13$; Online Appendix 1).

Hypothesis 1: As nectar levels increase, highly invasive ants will recruit more workers to EFN-bearing plants than less-invasive ants

We predicted that the highly invasive species *A. gracilipes* would demonstrate significantly stronger recruitment of workers to increasing nectar availability than other ant species (Fig. 1a). Within sites invaded by *A. gracilipes,* this prediction was supported. *Anoplolepis gracilipes* workers recruited strongly to *M. citrifolia* plants as experimentally manipulated nectar levels increased, with 281% more *A. gracilipes* workers observed on plants with the highest nectar levels (200%) compared to those with no nectar (Fig. 2a). In contrast, co-occurring subordinate ants were rarely observed on *M. citrifolia* plants, regardless of nectar availability level (Fig. 2a, Table 2a: Dominance × Nectar Treatment, $P < 0.0001$). The number of nectary bodies per plant also significantly influenced the abundance of ants per plant (Table 2a). However, the response of ants to the nectar treatment was not significantly influenced by this covariate, as evidenced by a non-significant interaction between the number of nectary bodies and the nectar treatment. Similarly, ant responses to treatments did not vary over time (Table 2a) at sites dominated by *A. gracilipes*.

Contrary to our predictions, other less-invasive ant species also recruited strongly to nectar resources (Fig. 2b, Table 2b). Although individual species varied substantially (see below), the composite response of the five less-invasive (but dominant) ant species (Fig. 2b) was positively related to nectar availability. Subordinate ants at sites lacking *A. gracilipes* did not display a significant response to experimentally elevated nectar levels, as evidenced by a significant Dominance × Nectar Treatment interaction (Table 2b, $P = 0.0024$). However, significantly more subordinate ants were observed foraging on *M. citrifolia* plants at sites without *A. gracilipes* than at sites with *A. gracilipes* (mean number of subordinate ants ± s.e.; *A. gracilipes* invaded sites = 0.90 ± 2.16; *A. gracilipes* uninvaded sites = 10.36 ± 2.50; t-test t=-7.2, P<0.0001). At sites uninvaded by *A. gracilipes*, ant responses were not significantly influenced by any of the covariates, nor did they vary over time (Table 2b).

Although the average densities of all dominant ant species increased as experimentally-manipulated nectar levels increased (Nectar treatment, $F_{1,69}$=67.91, P<0.0001; Fig. 2), this response varied substantially among species (Figs 2a, 3; Species identity x Nectar treatment: $F_{5,69}$=5.49, P=0.0003). The two species that responded most strongly to nectar manipulations were *A. gracilipes* (Fig. 2a; linear regression, r=0.97, P=0.0052, Sequential Bonferroni P_{crit}=0.01) and *Tapinoma melanocephalum* (Fig. 3d; linear regression, r=0.98, P=0.0034, P_{crit}=0.0083). These species have very different invasiveness scores (100 and 30.5%, respectively). *Pheidole megacephala* and *Paratrechina longicornis* displayed more variation in response to increasing nectar levels, although trends remained positive (*Ph. megacephala:* Fig. 3a, linear regression, r=0.90, P=0.0383, P_{crit} = 0.0125; *Pa. longicornis*, Fig. 3b, r=0.87, P=0.0543, P_{crit}

=0.0167). Finally, *Solenopsis geminata* (Fig. 3c; linear regression, r=0.73, P=0.1573, P_{crit}=0.025) and *Tetramorium bicarninatum* (Fig. 3e; linear regression, r=0.65, P=0.2395, P_{crit}=0.05) did not significantly respond to nectar manipulations.

Hypothesis 2: As nectar resources increase, highly invasive ants will become more aggressive toward co-occurring arthropods than less invasive ants

We predicted that highly invasive *A. gracilipes* workers would not only display higher overall levels of aggression, but also increase their aggressiveness more strongly in response to nectar than co-occurring less invasive ants (Fig. 1a). Overall aggression levels (pooling across nectar levels) were, in fact, higher for *A. gracilipes* than for other dominant ant species. Compared to sites where other ant species were dominant, the average proportion of prey larvae that were discovered (χ^2=6.53, P=0.0106), attacked (χ^2=7.20, P=0.0073), and removed (χ^2=6.42, P=0.0113) was 27%, 203% and 460% higher, respectively, at sites where *A. gracilipes* was dominant. On average, *A. gracilipes* workers also discovered prey 37% faster (χ^2=12.0, P=0.0005), attacked prey 49% faster (χ^2=24.45, P<0.0001), and removed prey 44% faster (χ^2=25.8, P<0.0001*)* than the average time taken by other dominant ants (Fig. 5).

In support of our hypothesis, the amount of nectar strongly influenced aggressive behaviors of *A. gracilipes*, while aggression of other dominant ant species was unresponsive to increasing nectar (Table 3; Fig. 6). The number of foragers recruiting to nectar had a significant effect on the proportion of and time to prey discovery and the time it took ants to attack discover prey (Table 3). However, the influence of nectar on ant aggression – particularly for *A. gracilipes* – was significant even when accounting for these numerical effects, indicating that nectar availability influenced per-capita worker aggression. There was a significant effect of the interaction between the nectar treatment and *A. gracilipes* invasion status for all responses with the exception of the proportion of prey removed (Table 3). Specifically, at the highest nectar level (200%), *A. gracilipes* workers discovered 205% more prey in 89% less time (Fig. 6a, b), attacked 32% more discovered prey in 93% less time (Figs 6c, d), and removed attacked prey in 76% less time (Figs 6e, f) than on plants with the lowest nectar level (0% treatment). Overall, prey removal by *A. gracilipes* increased from 19% to 81% from the lowest to the highest nectar level. In contrast, the aggressive behaviors of other dominant ants did not show significant responses to nectar manipulations (Fig. 6).

The strongest responses of *A. gracilipes* to increasing nectar levels occurred in terms of the proportion of larvae that were discovered (*P*=0.0068, r=0.9987; Fig. 6a, Table 3a) and the time it took workers to discover prey items (*P*=0.0037, r=-0.9995; Fig. 6b, Table 3b). Once larvae were discovered, there was a marginally significant positive effect of the nectar treatment on the proportion of larvae attacked by *A. gracilipes* (*P*=0.0594, r=0.8634; Fig. 6c) and a marginally significant negative effect of the nectar treatment on time to attack (*P*=0.0606; r=-0.8615; Fig. 6d). Once attacked by *A. gracilipes* workers, 94% (± 2.4%) of larvae were removed from *M. citrifolia* plants regardless of nectar level (Fig. 6e). The time it took *A. gracilipes* workers to remove larvae dropped significantly as nectar levels increased (*P*=0.0481, r=-0.8814; Fig. 6f).

3.4 Discussion

When exotic species are introduced to novel habitats, they often form beneficial associations with indigenous species. However, we know little about the ways that these positive interactions affect invaders themselves or their interactions with other community members. Trait-mediated indirect interactions via mutualistic associations may contribute strongly to the negative impacts associated with species invasions. In this study, we predicted that a highly invasive ant species would exhibit a strong response to increasing nectar levels in terms of recruitment and aggressive behaviors. We also predicted that these responses would be much weaker in co-occurring less-invasive ant species. We expected that differential responses of highly invasive and less-invasive ants would then lead to negative consequences for plant-foraging arthropods (Fig. 1a). In fact, we found that multiple non-native dominant ant species responded positively to increasing nectar levels in terms of forager recruitment. However, *A. gracilipes* was the only dominant ant species that increased the degree of aggressiveness as nectar levels increased (Fig. 1b), resulting in more rapid attacks on and greater removal of herbivorous arthropods. Thus, trait-mediated indirect effects between native nectar-producing plants and herbivorous arthropods were present and differed depending on the identity of the ant partner.

Effects of nectar subsidies on forager recruitment

Carbohydrate-rich resources, such as plant nectar or hemipteran honeydew, may promote ant invasions by providing a high-energy food that fuels greater activity and growth and furthers the establishment of dominant supercolonies (the 'carbohydrate subsidy hypothesis', Lach 2003, Savage et al. 2009). If these carbohydrate-rich resources are, in fact, important factors in the progression of ant invasions, then highly invasive species should respond more strongly to increasing nectar resources than co-occurring less-invasive species. A previous study in northeastern Savaii (Savage et al. in press) found that recruitment responses of *A. gracilipes* were much stronger than a less-invasive ant, *Pheidole megacephala*. In this study, we manipulated plant nectar and tested the recruitment responses of a much larger number of ant species (including four previously untested dominant species) across a much wider array of sites in both Savaii and Tutuila. We found that some species conformed to this prediction of the carbohydrate subsidy hypothesis, while other species did not. The former included the highly invasive ant species, *A. gracilipes,* which recruited very strongly to increasing levels of nectar. In addition, two species with relatively low invasiveness scores (*Solenopsis geminata* and *Tetramorium bicarinatum*) supported the prediction as they did not significantly increase recruitment of foragers to *M. citrifolia* plants in response to our nectar treatments. Finally, *Paratrechina longicornis* appeared to support the prediction as both had an intermediate degree of invasiveness and displayed an intermediate response to increasing nectar availability. However, two species clearly did not conform. *Tapinoma melanocephalum* was the species that recruited most strongly to increasing nectar (Fig. 3), but it was among the least invasive dominant species that we examined. *Pheidole megacephala,* which had the highest invasiveness score after *A. gracilipes,* displayed a weak response to increasing levels of nectar.

Importantly, we did not have sufficient replication within-species (with the exception of *A. gracilipes*) to determine if this was a general trait for these species. Future work that tests this prediction across a range of invasiveness (including native species), with multiple sites per dominant ant species will help to determine the generality of these findings.

Importantly, long-term responses to nectar could differ from short-term responses. It is likely that sustained increases in nectar availability (in contrast to the pulses used in this experiment) would lead to increased colony growth (also see Oliver et al. 2008). Larger colony size could have important effects on co-occurring community members (i.e., density-mediated indirect effects), especially in the context of invasions. Therefore, studies that manipulate nectar availability over longer time periods will be critical to gaining a thorough understanding of the effects of carbohydrate-rich resources on ant invasions.

Effects of nectar subsidies on ant aggression

In addition to increased forager recruitment, another trait-mediated pathway whereby carbohydrate-rich resources may influence ants and their impacts on other species is through increased levels of aggression (Fig. 1). Based upon the carbohydrate subsidy hypothesis, we predicted that highly invasive ants would respond to increasing carbohydrate levels by increasing the likelihood or speed of attacks on co-occurring arthropods. Again, this prediction is contingent upon the assumption that less-invasive ants do not respond in the same way to carbohydrate availability. In this study, we explicitly tested this prediction for the first time, by manipulating levels of plant nectar and observing the aggression displayed by highly invasive and co-occurring less-invasive ants.

We found that the highly invasive ant *A. gracilipes* displayed unique increases in aggression in response to increasing nectar availability, a response not found for other dominant ant species in Samoa. As nectar levels increased, there was a general increase in the likelihood of prey discovery and attack and a general reduction in the amount of time it took *A. gracilipes* workers to discover, attack and remove prey. The strongest responses of *A. gracilipes* were in terms of prey discovery, which may be due to higher overall activity levels when nectar availability is high. However, the effects of nectar on *A. gracilipes* attack and removal suggests that increased carbohydrate availability can influence other aspects of aggressive behaviors-at least for this species. Interestingly, other ant species-including *Tapinoma melanocephalum,* which responded strongly to nectar in terms of forager recruitment-did not display these aggressive responses, even in the absence of *A. gracilipes.* Thus, our findings support the prediction that highly invasive ant species respond more strongly to carbohydrate availability in terms of increased aggression than less invasive ants. Furthermore, this trait difference can have consequences for co-occurring arthropods (Figs. 1b, 6) and thus could potentially scale up to community-level effects.

Any changes in aggressive responses of *A. gracilipes* to increasing nectar resources could be simply explained by the forager recruitment responses that we demonstrated in the first experiment. With more ants recruiting to nectar, the likelihood of prey discovery should be higher, due to increased encounter rates. In fact, we found that increased forager recruitment did explain some of the variation in ant aggression across nectar levels. However, we also found that significant effects of nectar on aggression remained even after accounting for these numerical effects, indicating that nectar influences per-capita worker aggression in *A. gracilipes*.

Together with our findings, results from other recent studies suggest that carbohydrates can strongly influence ant behaviors with consequences for co-occurring arthropods, and that these effects can be conditional on ant identity. For example, Grover et al. (2007) found that both activity levels and intraspecific aggression of *Linepithema humile* (Argentine ants) were higher for lab colonies that were fed a diet rich in carbohydrates than under a protein-rich diet. Similarly, Pringle et al. (*In press*) showed that native plant-inhabiting ants in a neotropical lowland rainforest were more aggressive towards plant-feeding herbivores when fed a diet rich in carbohydrates. However, Kay et al. (2010) examined the aggression exerted by *L. humile* towards heterospecific ants and found no influence of diet, although carbohydrate-rich diets resulted in greater colony growth (a density-mediated effect). In the deserts of the Southwest United States, Ness et al. (2009) demonstrated that four species of native ants were more aggressive towards novel prey when fed supplemental carbohydrates than when fed protein or given no supplements; however, no invasive ant species were tested in this study. Finally, Lach and Hoffmann (*In press*) demonstrated that invasive *A. gracilipes* workers were more likely to attack prey than workers of one native, dominant ant species (*Oecophylla smaragdina*), but only on plants bearing EFN; responses did not differ on plants that did not secrete extrafloral nectar.

In most of the cases in which *A. gracilipes* has been reported to have detrimental impacts on co-occurring community members, this species has also associated with plants or insects that secrete carbohydrate-rich food in the form of nectar or honeydew (Addison and Samways 2000, Holway et al. 2002, Hill et al. 2003, O'Dowd et al. 2003, Lester and Tavite 2004, Savage et al. 2009). The amplification of aggressive behaviors that we detected in this study may provide a mechanism that underlies this pattern. Similar tests of aggression in response to carbohydrate availability across the invaded range of *A. gracilipes* will help to elucidate the generality of these findings. Furthermore, it will be important to conduct studies that manipulate nectar over longer time spans in order to ascertain the relative importance of density and trait-mediated effects of nectar on *A. gracilipes* invasions.

Conclusions

Mutualisms are common interspecific interactions that can influence the structure and dynamics of communities and the functioning of ecosystems (Bronstein 1994, Stachowicz 2001, Rudgers and Clay 2008). However, we know surprisingly little about the mechanisms that underlie many of these community-wide effects. Importantly, indirect, trait-mediated pathways are likely to be important mechanistic components of the effects of mutualisms on communities, just as they are for antagonistic interactions. In this study, we demonstrated that (i) resources exchanged in mutualisms between ants and EFN-bearing plants can change behaviors that ants exert towards other community members and (ii) these changes are more extreme for invasive *A. gracilipes* than co-occurring less invasive ants. These altered behaviors could scale up to affect community structure. In studies of antagonistic interactions, variation in aggression has been shown to influence both species co-existence (Frye 1983, Logan 1984, Morrison 1996) and the displacement of native species by exotics (Carpintero and Reyes-Lopez 208). Thus, if they represent a widespread pattern, our findings suggest that trait-mediated indirect interactions associated with novel mutualisms between invaders and native species could contribute to the success and detrimental impacts of species invasions.

3.5 Acknowledgements

Many thanks to J.A. Rudgers for help with experimental design and data analysis and for thoughtful comments on the manuscript. We also thank local land owners in Savaii and Tutuila for graciously allowing us to use their land for our experiments. Thanks to Mark Schmaedick for logistical and field assistance. This work was supported by National Geographic Society grant 8237-07 and by a Rice University Wray-Todd Fellowship to A.M.S.

3.6 References

Abbott, K.L. (2005) Supercolonies of the invasive ant, *Anoplolepis gracilipes,* on an oceanic island: forager activity patterns, activity and biomass. *Insectes Sociaux,* **52**, 266-273.

Abbott, K.L. (2006) Spatial dynamics of supercolonies of the invasive yellow crazy ant, *Anoplolepis gracilipes*, on Christmas Island, Indian Ocean. Diversity and Distributions, **12**, 101-110.

Addison, P. & Samways, M.J. (2000) A survey of ants (Hymenoptera: Formicidae) foraging in the western cape vineyards of South Africa. African Entomology, 8, 251–260.

Bronstein, J.L. (1994) Conditional outcomes in mutualistic interactions. Trends in Ecology and Evolution, **9**, 214-217.

Bruno, J. F., Stachowicz, J. J., & Bertness, M. D. (2003) Inclusion of facilitation into ecological theory. Trends in Ecology and Evolution, **18**, 119-126.

Carpintero, S. & Reyes-Lopez, J. (2008) The role of competitive dominance in the invasive abilities of the Argentine ant (*Linepithema humile*). Biological Invasions 10: 25-35.

Chittka, L. & Schurkens, S. (2001) Successful invasion of a floral market. Nature, 411: 653.

Clarke KR, Gorley RN (2007) Primer, version 6.1.10: user manual and tutorial. Primer-E, Plymouth.

Davidson, D. W., Cook, S. C., Snelling, R. R., & Chua, T. H. (2003) Explaining the abundance of ants in lowland tropical rainforest canopies. Science, **300**, 969-972.

Freeman C. E., Worthington R. D. & Jackson M. S. (1991). Floral nectar sugar compositions of some south and southeast Asian species. Biotropica **23,** 568–74.

Frye, R. J. (1983) Experimental evidence of interspecific aggression between two species of kangaroo rat (*Dipodomys*). Oecologia 59: 74-78.

Gomez, J. M., Abdelaziz, M. Lorite, J., Munoz-Pajares, A. J., & Perfectti, F. (2010) Changes in pollinator fauna cause spatial variation in pollen limitation. Journal of Ecology, **98**, 1243-1252.

Grabas, G. P., & Laverty, T. M. (1999) The effect of purple loosestrife (*Lythrum salicaria* L.; Lythraceae) on the pollination and reproductive success of sympatric co-flowering wetland plants. Ecoscience **6**, 230-242.

Grover, C.D, Kay, A.D., Monson, J.A., Marsh, T.C., & Holway, D.A. (2007) Linking nutrition and behavioral dominance: carbohydrate scarcity limits aggression and activity in Argentine ants. Proceedings of the Royal Society B-Biological Sciences, **274**, 2951-2957.

Hay, M. E., Parker, J. D., Burkepile, D. E., Caudill, C. C., Wilson, A. E., Hallinan, Z.P., & Chequer, A. D. (2004) Mutualisms and aquatic community structure: the enemy of my enemy is my friend. Annual Review of Ecology, Evolution and Systematics, **35**, 175-197.

Hill, M., Holm, K., Vel, T. Shah, N. J., & Matyot, P. 2003. Impact of the introduced yellow crazy ant *Anoplolepis gracilipes* on Bird Island, Seychelles. Biodiversity and Conservation, **12**, 1969-1984.

Holm, S. (1979) A simple sequentially rejective multiple test procedure. Scandinavian Journal of Statistics 6, 65-70.

Holway, D. A., Lach, L., Suarez, A.V., Tsutsui, N.D. & Case, T.J. (2002) The causes and consequences of ant invasions. *Annual Review of Ecology and Systematics,* **33**, 181-233.

Kay, A. D., Zumbusch, T., Heinen, J.L., Marsh, T.C., & Holway, D.A. (2010) Nutrition and interference competition have interactive effects on the behavior and performance of Argentine ants. Ecology. **91**,57-64.

Lach, L. 2003. Invasive ants: unwanted partners in ant-plant interactions? Annals of the Missouri Botanical Garden **90,** 91-108.

Lach, L. (2008) Argentine ants displace floral arthropods in a biodiversity hotspot. Diversity and Distributions, **14**, 281–29.

Lach, L. and Hoffmann, B. D. *In Press.* Are invasive ants better plant-defense mutualists? A comparison of foliage patrolling and herbivory in sites with invasive yellow crazy ants and native weaver ants. Oikos

Lester, P.J. & Tavite, A. (2004) Long-legged ants, *Anoplolepis gracilipes* (Hymenoptera: Formicidae), have invaded Tokelau, changing composition and dynamics of ant and invertebrate communities. Pacific Science, **58**, 391-401.

Logan, A. (1984) Interspecific aggression in heratypic corals from Bermuda. Coral Reefs 3: 131-138.

Mangan, S. A., Herre, E. A., & Bever, J. D. (2010) Specificity between Neotropical tree seedlings and their fungal mutualists leads to plant-soil feedbacks. Ecology, **91**, 2594-2603.

Maherali, H. & Klironomos, J. N. 2007. Influence of phylogeny on community assembly and ecosystem functioning. Science **316:** 1746-1748.

Manzaneda, A. J., & Rey, P. J. (2008) Geographic variation in seed removal of a myrmecochorous herb: influence of variation in functional guild and species composition of the disperser assemblage through spatial and temporal scales. Ecography, **31**, 583-591.

Matthews, C. R., Bottrell, D. G., & Brown, M. W. (2009) Extrafloral nectaries alter arthropod community structure and mediate peach (*Prunus persica*) plant defense. Ecological Applications, **19**, 722-730.

Miller, T. E. X. (2007), Does having multiple partners weaken the benefits of facultative mutualism? A test with cacti and cactus-tending ants. Oikos, **116**: 500–512.

Morrison, L. W. (1996) Community organization in a recently assembled fauna: the case of Polynesian ants. Oecologia 107: 243-256.

Nelson, S. C. (2006), *Morinda citrifolia* (Noni), v.4. *In* Elevitch, C. R. (ed.), Species profiles for Pacific Island forestry. Permanent Agricultural Resources (PAR), Holualoa, HI

Ness, J. H., W. F. Morris, and Judith L. Bronstein. (2009) For ant-protected plants, the best defense is a hungry offense. Ecology. **90**, 2823 - 2831.

O'Dowd, D.J., Green, P.T. & Lake, P.S. (2003) Invasional 'meltdown' on an oceanic island. *Ecology Letters,* **6,** 812-817.

Oliver, T. H., Pettitt, T., Leather, S. R. and Cook, J. M. (2008) Numerical abundance of invasive ants and monopolisation of exudate-producing resources – a chicken and egg situation. Insect Conservation and Diversity, 1: 208–214.

Palmer, T. M., Doak, D. F., Stanton, M. L., Bronstein, J. L., Kiers, E. T., Young, T. P., Goheen, J. R., & Pringle, R. M. (2010) Synergy of multiple partners, including freeloaders, increases host fitness in a multispecies mutualism. Proceedings of the National Academy of Sciences

Pringle, E. G., Dirzo, R. & Gordon, D. M. (*In press*) Indirect benefits of symbiotic coccoids for an ant-defended myrmecophytic tree. Ecological Monographs.

Pysek, P. & Richardson, D. M. (2007) Traits associated with invasiveness in alien plants. Biological Invasions, 193: 97-125.

Rudgers, J. A. & Strauss, S. Y. (2004) Selection mosaic in the facultative mutualism between ants and wild cotton. Proceedings of the Royal Society of London, Series B, **271**, 2481-2488.

Rudgers, J. A., Holah, J., Orr, S. P., & Clay, K. (2007) Forest succession suppressed by an introduced plant-fungal symbiosis. Ecology, **88**, 18-25.

Rudgers, J. A., Savage, A. M., & Rua, M. A. (2010) Geographic variation in a facultative mutualism: consequences for local arthropod composition and diversity. Oecologia,**163**, 985-996.

SAS Institute. (2003) *SAS version 9.1.3*. SAS Institute, Cary, North Carolina, USA

Savage, A. M., Rudgers, J. A., and Whitney, K. D. (2009) Elevated dominance of extrafloral nectary-bearing plants is associated with increased abundances of an invasive ant and reduced native ant richness. Diversity and Distributions, **15**, 751-761.

Savage, A. M., Johnson, S. D., Whitney, K. D., & Rudgers, J. A. *In Press.* Do invasive ants respond more strongly to nectar availability than co-occurring non-invasive ants? A test along an active *Anoplolepis gracilipes* invasion front. Austral Ecology.

Shattuck, S. O. (1999) Australian Ants: Their biology and identification. Monographs on invertebrate taxonomy,vol. 3. CSIRO Publishing, Collingswood, Victoria, Australia.

Stachowicz, J.J. (2001) Mutualism, facilitation, and the structure of ecological communities. BioScience, **51**, 235–246.

van Kleunen, M., Weber, E., & Fischer, M. (2010) A meta-analysis of trait differences between invasive and non-invasive plant species. Ecology Letters, 13: 235-245.

von Ende, C. N. (2001) Repeated measures analysis: growth and other time dependent measures. In Design and Analysis of Ecological Experiments (ed. by S. M. Scheiner & J. Gurevitch), pp. 134-157. Oxford University Press, New York, New York.

Waki, J., Olkpul, T., Komolong, M.K. (2007) A descriptor list for morphological characterization of Noni (*Morinda citrifolia* L.). The South Pacific Journal of Natural Science, **10,** 61-66.

Ward, D.F., Stanley, M.C., Toft, R.J., Forgie A.S. & Harris, R.J. (2008) Assessing the risk of invasive ants: a simple and flexible scorecard approach. Insectes Sociaux **55**, 360-363.

Werner, E. E. & Peacor, S. D. (2003) A review of trait-mediated indirect interactions in ecological communities. Ecology, **84**, 1083-1100.

Wetterer, J. K. (2005) Worldwide distribution and potential spread of the long-legged ant, *Anoplolepis gracilipes* (Hymenoptera: Formicidae). *Sociobiology,* **45**, 77-96.

Wetterer, J. K. & Vargo, D. L. (2003) Ants (Hymenoptera: Formicidae) of Samoa. Pacific Science, 57: 409-419.

White, E. M., Wilson, J. C. and Clarke, A. R. (2006), Biotic indirect effects: a neglected concept in invasion biology. Diversity and Distributions, **12**, 443–455.

Whistler, W. A. (1993) Flowers of the Pacific Island Seashore: A Guide to the Littoral Plants of Hawaii, Tahiti, Samoa, Tonga, Cook Islands, Fiji and Micronesia. University of Hawaii Press, Honolulu, HI USA.

Whitney, K. D. (2004) Experimental evidence that both parties benefit in a facultative plant-spider mutualism. Ecology, **85**, 1642-1650.

Whitney, K. D. (2005) Linking frugivores to the dynamics of a fruit color polymorphism. American Journal of Botany **92**, 859-867.

Wilson, E. O., & Taylor, R.W. (1967) The ants of Polynesia (Hymenoptera: Formicidae). *Pacific Insects Monograph,* **14**,1-10.

Tables:

Table 3.1: Descriptions of ant assemblages at sites on the islands of Savaii (S) in Independent Samoa and Tutuila (T) in American Samoa. Invasiveness scores (Ward 2008) are reported as percentages of the total possible score. Densities are reported from surveys on the ground and on *M. citrifolia* plants.

Site	Dates of Experiments (2009)	Dominant Species	Invasiveness Score (%)	Density (mean ± SE)			
				Dominant Species		Subordinate Species	
				Ground	*M. citrifolia*	Ground	*M. citrifolia*
Falealupo (S)	September	*Anoplolepis gracilipes*	100	23.5 ± 6.36	46.5 ± 8.66	1.0 ± 0.58	2.0 ± 2.16
Mauga_West (S)	June	*Anoplolepis gracilipes*	100	21.25 ± 2.72	46.50 ± 4.80	2.0 ± 0.91	4.75 ± 1.65
Mauga_South (S)	September	*Anoplolepis gracilipes*	100	38.78 ± 1.32	53.21 ±4.65	0	0
Saleaula_East (S)	June	*Anoplolepis gracilipes*	100	28.0 ± 1.47	39.25 ± 2.14	2.5 ± 1.04	1.75 ± 0.63
Saleaula_North (S)	September	*Anoplolepis gracilipes*	100	17.5 ± 2.96	33.0 ± 4.14	0	1.0 ± 0.41
Saleaula_South (S)	September	*Anoplolepis gracilipes*	100	15.38 ± 3.99	29.44 ± 4.37	0.22 ± 0.22	2.33 ± 1.0
Futiga (T)	August	*Anoplolepis gracilipes*	100	12.50 ± 3.23	15.13 ± 3.59	3.23 ± 0.48	1.38 ± 0.63
Masausi_East (T)	August	*Anoplolepis gracilipes*	100	8.13 ± 1.03	36.50 ± 5.17	0	2.0 ± 0.71
Mauga_North (S)	September	*Pheidole megacephala*	94	9.4 ± 1.60	11.32 ± 0.98	2.31 ± 0.86	5.61 ± 2.15
Fagatogo (T)	July	*Pheidole megacephala*	94	6.75 ± 3.90	8.25 ± 2.78	0.25 ± 0.25	2.78 ± 4.42
Tafuna (T)	July	*Solenopsis geminata*	69	12.25 ± 1.75	10.50 ± 3.07	1.50 ± 0.65	2.50 ± 1.19
Gataivai (S)	September	*Paratrechina longicornis*	40	15.75 ± 5.11	21.75 ± 4.53	8.75 ± 5.91	10.0 ± 4.08
Masausi_West (T)	August	*Tapinoma melanocephalum*	30.5	11.25 ± 3.01	56.25 ± 4.0	3.25 ± 2.36	3.40 ± 1.68
Ililli (T)	July	*Tetramorium bicarinatum*	25	2.25 ± 0.63	0.75 ± 0.48	7.0 ± 1.58	4.50 ± 2.53

Table 3.2a: Results from a repeated measures analysis of covariance of ant density per plant at sites invaded by *Anoplolepis gracilipes.*

Source of Variation	df	F	P
Dominance (Dominant/Subordinate)	1, 296	194.27	**<0.0001**
Nectar Treatment	1, 296	35.74	**<0.0001**
Dominance x Nectar Treatment	1, 296	139.80	**<0.0001**
Site	7, 296	4.55	**<0.0001**
Block (Site)	28, 296	1.46	0.0730
Plant size	1, 296	1.74	0.1878
Abundance of honeydew-excreting insects (HEI)	1, 296	1.57	0.2113
Number of nectary bodies	1, 296	11.25	**0.0009**
Nectar Treatment x Abundance of HEI	1, 296	0.00	0.9498
Nectar Treatment x Number of nectary bodies	1, 296	4.65	0.0519
Time	5, 292	0.67	0.6459
Time x Dominance	5, 292	3.28	**0.0068**
Time x Nectar Treatment	5, 292	1.23	0.2954
Time x Dominance x Nectar Treatment	5, 292	0.76	0.5761
Time x Site	25, 168	6.77	**<0.0001**
Time x Block (Site)	70, 168	1.34	**0.0080**
Time x Plant size	5, 292	0.29	0.9181
Time x Abundance of HEI	5, 292	0.50	0.7786
Time x Number of nectary bodies	5, 292	0.77	0.5744
Time x Nectar Treatment x Abundance of HEI	5, 292	0.95	0.4465
Time x Nectar Treatment x Number of nectary bodies	5, 292	0.63	0.6785

Table 3.2b: Results from a repeated measures analysis of covariance of ant density per plant at sites uninvaded by *Anoplolepis gracilipes.*

Source of Variation	df	F	P
Dominance (Dominant/Subordinate)	1, 172	125	0.2647
Nectar Treatment	1, 172	3.39	0.0671
Dominance x Nectar Treatment	1, 172	9.47	**0.0024**
Site	5, 172	2.88	**0.0159**
Block (Site)	14, 172	0.46	0.9521
Plant size	1, 172	0.00	0.9892
Abundance of honeydew-excreting insects (HEI)	1, 172	1.34	0.2485
Number of nectary bodies	1, 172	0.06	0.8015
Nectar Treatment x Abundance of HEI	1, 172	2.95	0.0878
Nectar Treatment x Number of nectary bodies	1, 172	1.31	0.2538
Time	5, 168	1.14	0.3387
Time x Dominance	5, 168	2.19	0.0573
Time x Nectar Treatment	5, 168	0.67	0.6455
Time x Dominance x Nectar Treatment	5, 168	1.05	0.3887
Time x Site	25, 76	6.16	**<0.0001**
Time x Block (Site)	17, 76	0.98	0.5289
Time x Plant size	5, 168	0.16	0.9753
Time x Abundance of HEI	5, 168	0.49	0.7855
Time x Number of nectary bodies	5, 168	0.61	0.6885
Time x Nectar Treatment x Abundance of HEI	5, 168	1.00	0.4214
Time x Nectar Treatment x Number of nectary bodies	5, 168	0.53	0.7503

Table 3.3a: Analyses of proportions of prey that were discovered, attacked or removed across sites invaded and uninvaded by *A. gracilipes.* Logistic regression with a binomial distribution and a chi square test were used to compare the proportions of prey discovered, the proportion of discovered prey that were attacked and the proportion of attacked prey that were removed.

	Proportion prey removed		Proportion of discovered prey attacked		Proportion of attacked prey removed	
Factor	X^2	*P*	X^2	*P*	X^2	*P*
***A. gracilipes* invasion status**	0.216	0.6418	0.8705	0.3508	0.0023	0.9616
Nectar treatment	6.140	**0.0132**	0.2762	0.5992	0.3366	0.5618
Invasion status x Nectar treatment	14.86	**0.0001**	4.8001	**0.0285**	0.1961	0.6579
Forager recruitment	9.000	**0.0027**	1.2926	0.2556	0.0033	0.9245
Site (Invasion status)	12.81	0.2347	8.534	0.5768	3.03	0.9807

Table 3.3b: Analyses of times to discovery, attack and removal of prey items across sites invaded and uninvaded by *A. gracilipes.* To examine the time it took ants to perform aggressive behaviors, we conducted survival analyses with a Weibull distribution and Wald Chi square test statistics.

	Time to Disvovery		Time to attack of discovered prey		Time to removal of attacked prey	
Factor	X^2	*P*	X^2	*P*	X^2	*P*
A. gracilipes invasion status	2.997	0.0834	0.2994	0.5843	0.7080	0.4001
Nectar treatment	7.387	**0.0066**	7.421	**0.0064**	0.1309	0.7175
Invasion status x Nectar treatment	39.90	**<0.0001**	24.279	**<0.0001**	7.147	**0.0075**
Forager recruitment	16.84	**<0.0001**	5.265	**0.0218**	2.104	0.1469
Site (Invasion status)	30.47	**0.0007**	27.443	**0.0022**	16.919	0.0762

3.7 Figures:

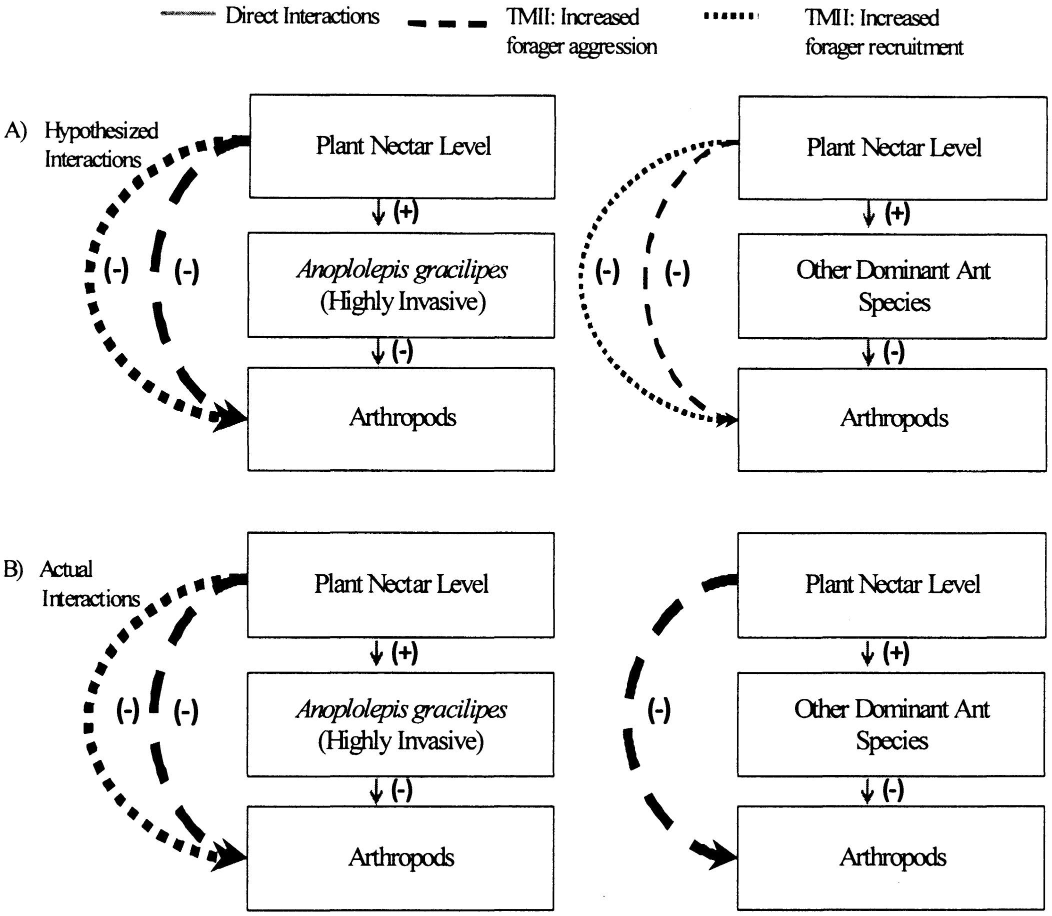


Figure 3.1: Interaction web diagrams depicting (A) hypothesized and (B) actual trait-mediated indirect interactions (TMII) between plant nectar and herbivores. Solid lines represent direct interactions and dotted lines represent TMII. Thicker lines represent stronger effects. (A) We predicted that nectar would affect traits of both highly invasive and less-invasive dominant ant species similarly, but with stronger effects for highly invasive ant species. (B) Actual patterns detected

in our experiments. Other species of dominant ants in addition to *A. gracilipes* responded to plant nectar by increasing forager recruitment, but *A. gracilipes* was the only species to increase aggression in response to increasing nectar levels.

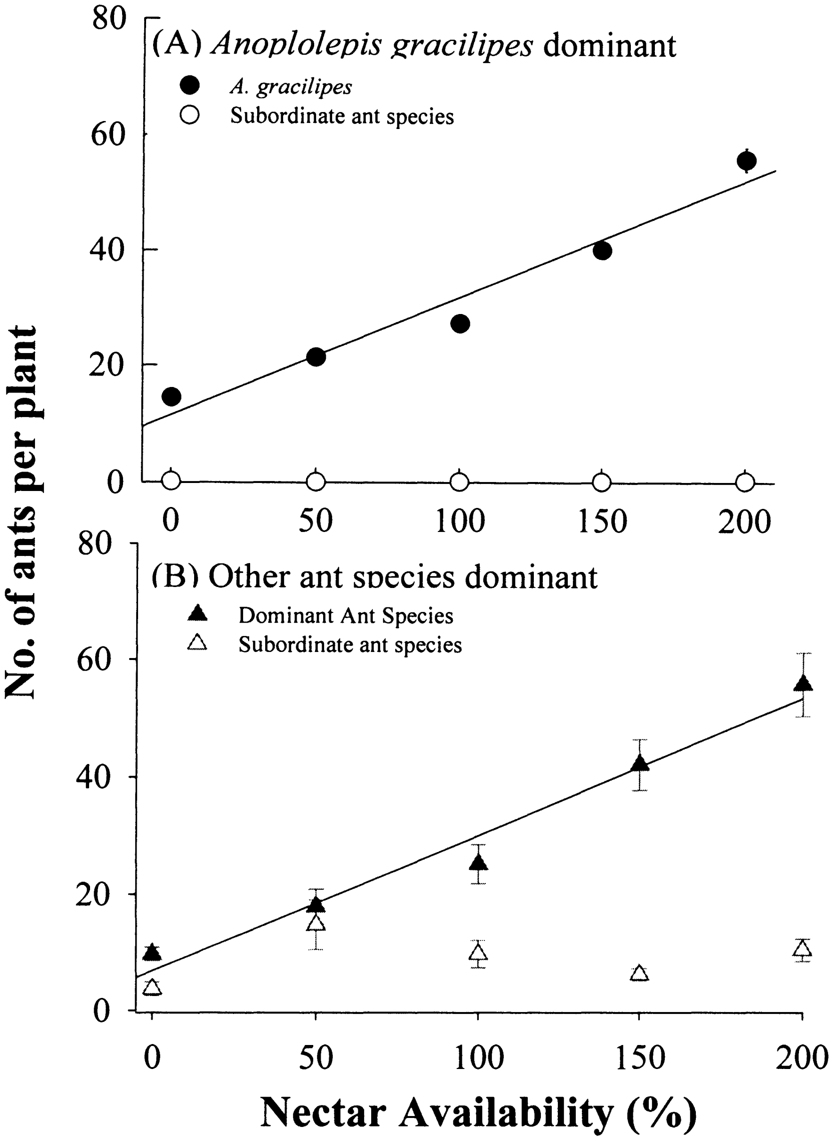


Figure 3.2: Forager recruitment of dominant and subordinate ants to increasing nectar levels at sites dominated by invasive *A. gracilipes* (a) and those dominated by other ant species (b). Error bars represent ± 1 SE of the mean.

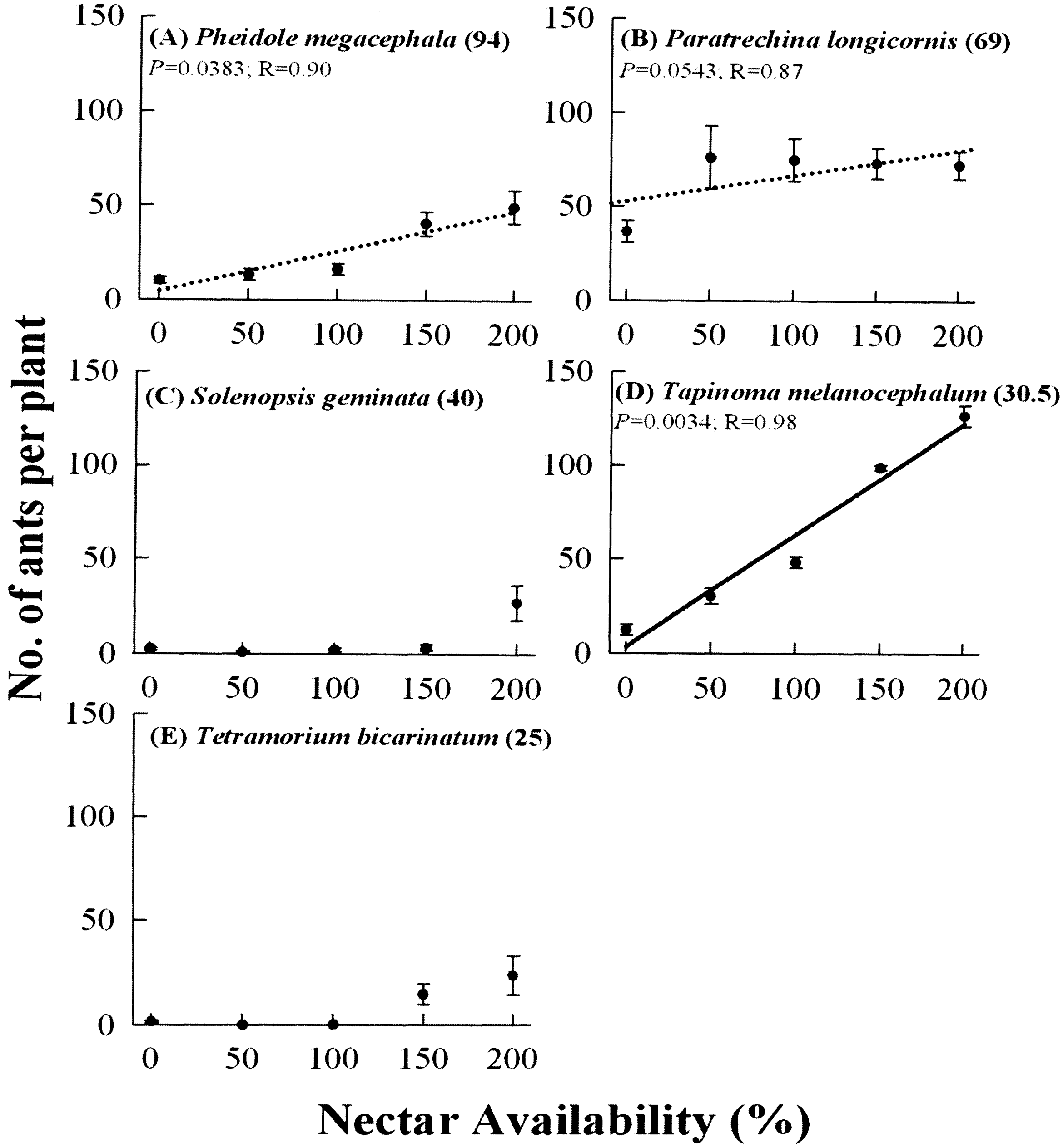


Figure 3.3: Forager recruitment of dominant ant species to *Morinda citrifolia* plants as a function of nectar availability and ant species identity. Species are organized from most invasive to least invasive (invasiveness scores noted parenthetically; see also Table 1). Errors represent ± 1 SE of the mean. Relationships that remained significant following

sequential Bonferroni corrections ($P<0.05$) are depicted with a solid line and marginally significant trends after Bonferroni corrections ($0.05<P<0.10$) are depicted with a dotted line.

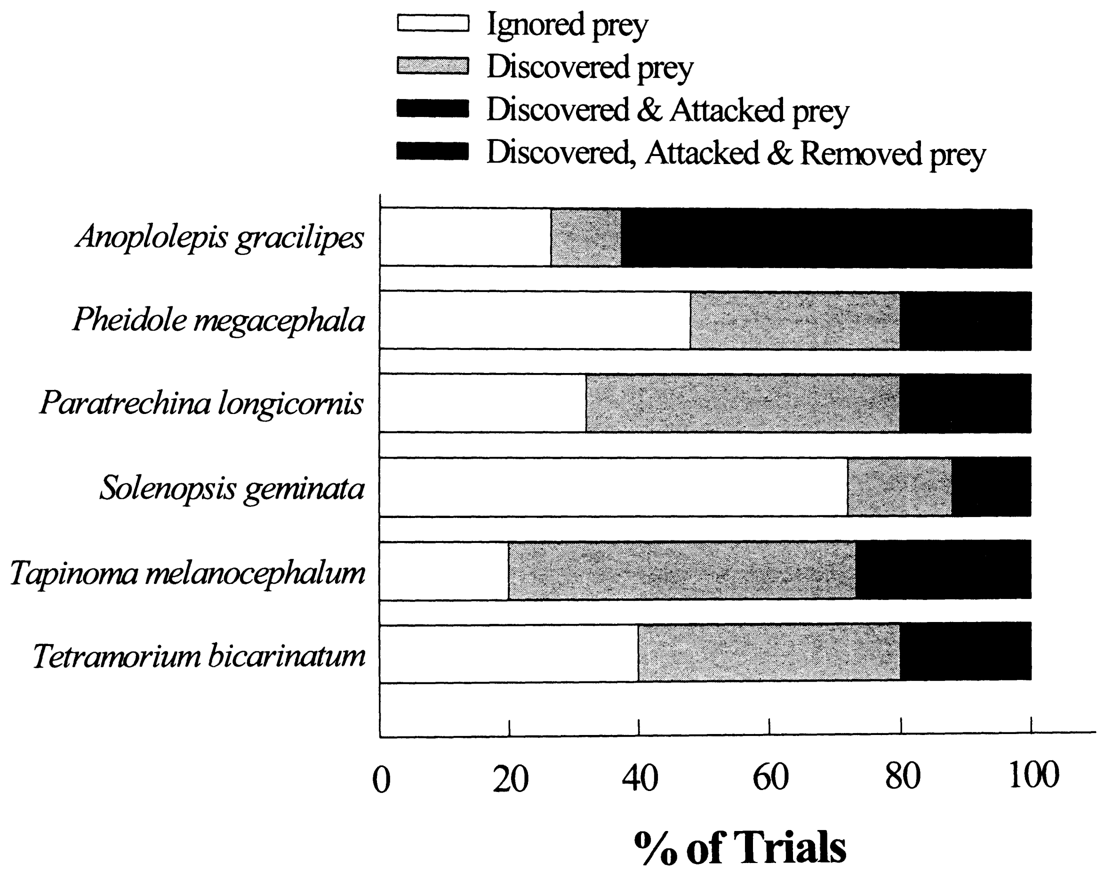


Figure 3.4: **Behavioral responses of dominant ant species to herbivorous larvae introduced near *Morinda citrifolia* nectary bodies.** Discovery was defined as antennation with no further aggressive behaviors. Attacks occurred when workers bit, stung, or sprayed formic acid at larvae, but did not remove them from plants. Removals occurred when workers either carried prey off plants or physically ejected the larva from the plant. Trials lasted 150 seconds, and prey were scored as ignored if no ants approached the larva within this time period.

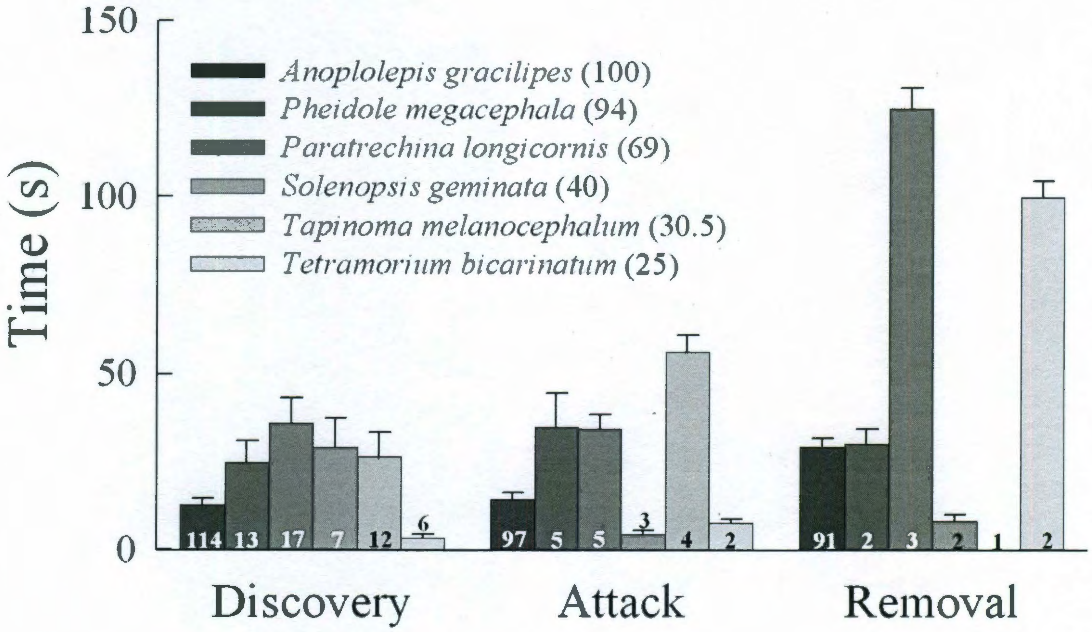


Figure 3.5: Time to discovery, attack and removal of prey by *Anoplolepis gracilipes* and other dominant ant species. Shorter bars represent more rapid responses. Each species' invasiveness score is noted parenthetically in legend (see also Table 1). Error bars represent ± 1SE of the mean, and numbers inside bars represent the total number of observations of each behavior per species.

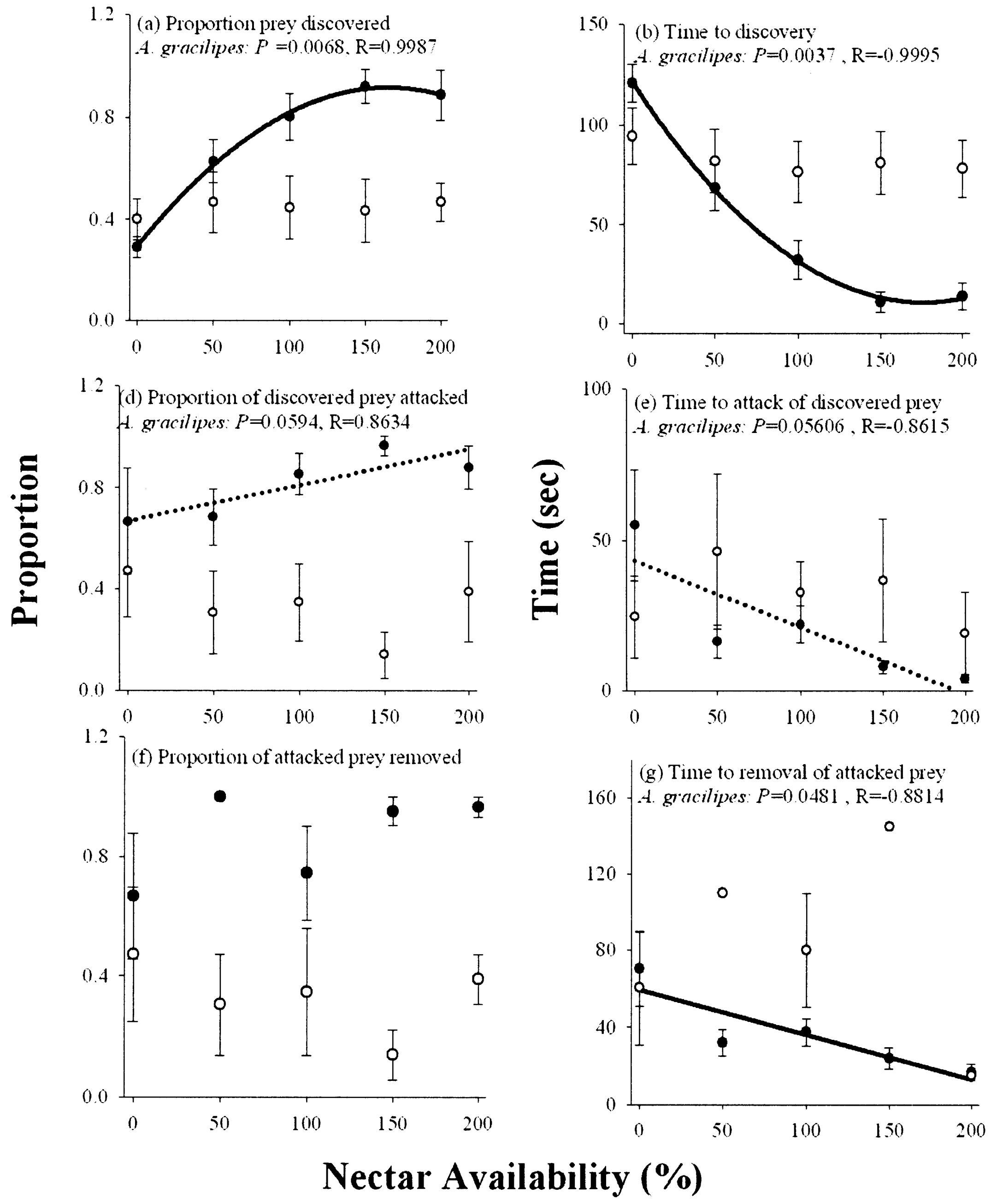


Figure 3.6: Behavioral responses of dominant ant species to experimentally manipulated nectar levels. Filled circles represent responses of *Anoplolepis gracilipes* and empty

circles represent other dominant ant species. Error bars represent ± 1SE of the mean. All relationships between nectar level and behavioral responses of non-*A. gracilipes* ants were *ns*. Significant relationships ($P<0.05$) are depicted with a solid line and marginally significant trends ($0.05<P<0.10$) are depicted with a dashed line.

Chapter 4:

Community-wide consequences of a novel mutualism between an invasive ant and a native plant

Abstract:

Non-native species invasions can have substantial, negative impacts on co-occurring species. However, we still have a limited understanding of the ecological factors that promote the progression and facilitate the negative consequences of invasions. For invasive ants, protective associations with plants that bear extrafloral nectaries (EFN) and honeydew-excreting insects may play a key role in fueling both the population expansion and the subsequent negative impacts of highly invasive species. However, this 'nectar subsidy' hypothesis has not yet been examined experimentally. Here, I test this hypothesis, using the yellow crazy ant (*Anoplolepis gracilipes*) invasion of the Samoan archipelago as a study system. The factors in this plot-level factorial experiment were ant access to a common EFN-bearing shrub (*Morinda citrifolia*) and nectar levels of the pant. Treatment plots were replicated across sites that had *A. gracilipes*-dominated ant assemblages and those that were not dominated by *A. gracilipes* . Three and six months after treatment application, I surveyed arthropods on *M. citrifolia* plants, and sampled plot-wide arthropod communities using sweep nets. I found that arthropod communities were significantly altered by mutualisms between ants and *M. citrifolia* plants. These effects of the mutualism on co-occurring arthropods were strongest when *A. gracilipes* dominated local ant assemblages. Additionally, arthropods from different feeding guilds displayed variable

responses to experimental manipulations. Specifically, herbivores responded most strongly to manipulations of ant access to plants and detritivores responded most strongly to increasing nectar levels, with the strongest responses for both groups occurring at the plant level. Conversely, carnivore responses were only evident at the plot level, but were strong for manipulations of both ant access and nectar levels. These results demonstrate that positive interactions (including mutualisms) can strongly influence community-wide ecological dynamics and that there can be substantial community-level consequences when these positive associations are disrupted by highly invasive species.

4.1 Introduction

Although the ecological importance of positive species interactions has been historically underappreciated relative to that of antagonistic interactions (Bronstein 1994), mutualisms appear to be increasingly likely to influence co-occurring species and communities (Bruno et al. 2003, Savage and Peterson 2007, Rudgers and Clay 2008, Matthews et al. 2009, Rudgers et al. 2010). Mutualistic interactions are embedded in complex multispecies communities which are mostly composed of species that are not directly involved in the mutualism. Thus, mutualist partners can simultaneously form associations with and exert ecological influences on other community members (Hofsetter et al. 2007, Morris et al. 2007). Consequently, dynamics that occur within guilds of partners that form mutualisms may influence community-wide patterns of species co-occurrence and relative abundances across ecological landscapes. The community-wide consequences of mutualisms are still poorly understood. However, recent studies have demonstrated that the abundance, diversity, and composition of local communities can be strongly influenced by the presence of mutualistic interactions (Stachowicz 2001, Bruno et al. 2003, Hay et al. 2004, Rudgers et al. 2007, Lach 2008, Matthews et al. 2009). Furthermore, variation in the degree and type of benefits exchanged in mutualisms can have cascading effects on the structure and dynamics of communities. For example, Rudgers et al. (2010) demonstrated that geographic variation in the benefits provided to plants by ant guards was associated with significant differences in the composition and abundance of plant-associated arthropods that were not directly involved in the mutualism.

Novel mutualisms that arise when exotic species are introduced to naïve habitats provide ideal systems for examining questions about the influences of mutualisms on communities. The global anthropogenic transport of species outside their native ranges is essentially a natural experiment at a community-wide scale, replicated world-wide. Studies of invasive species have played a significant role in advancing our understanding of the traits that allow populations to establish and expand (Veit and Lewis 1996, Sakai et al. 2001, Pangle et al. 2007, Colautti et al. 2010) as well as ecological factors that serve as barriers to this growth (Parker 1997, DeWalt et al. 2004, Jonjangs et al. 2006, Menke and Holway 2006, Shinen et al. 2009). Species invasions can also inform our understanding of the interactions that occur among species and their consequences for local communities. For example, Lau and Strauss (2005) investigated competition between native *Lotus* and invasive *Medicago* plants through both direct and indirect pathways. They demonstrated that indirect effects via increased abundances of shared herbivores can have stronger influences on plant fitness than direct competitive interactions. Studies of novel mutualisms may provide similar insights into the mechanisms and consequences of mutualisms for co-occurring species. Novel interactions between invaders and native species additionally provide a contrast to the same associations involving native species that share a long history of interaction. This dynamic allows us to examine the ways that new mutualisms may shape their communities in ecological time. Finally, experimental manipulations of access to or variation in resources exchanged in mutualisms across invaded and uninvaded communities can provide a powerful tool that decouples the effects of novel mutualistic interactions from the effects of the presence of non-native species.

Novel mutualisms may also modify the negative impacts of invasive species on native communities. Exotic species invasions have fundamentally altered community structure across marine, freshwater and terrestrial habitats and have led to significant declines in populations of native species (Sakai et al. 2001, Callaway and Moran 2006, Snyder and Allendorf 2006). Among insect groups, invasive ants have had especially strong effects on native communities, causing broad-scale changes in ecosystems, altering community dynamics across multiple trophic levels, and ultimately leading to the dissolution of important ecosystem processes (Holway et al. 2002, O'Dowd et al. 2003). One factor that may contribute to these negative effects is the propensity of invasive ants to associate facultatively with carbohydrate-excreting plants and arthropods, thus displacing native ants in mutualistic associations (Lach 2003, Ness and Bronstein 2004). In fact, recent studies have suggested that these mutualisms can provide carbohydrate subsidies that may play a key role in ant invasions, by both facilitating population expansion and mediating negative impacts of invasive ants (Lach et al. 2003, O'Dowd et al. 2003, Savage et al. 2009, *In press*).

In this study, I investigated novel mutualisms between a highly invasive ant species (*Anoplolepis gracilipes*), and a dominant extrafloral nectary (EFN)-bearing shrub (*Morinda citrifolia*) on the island of Savaii (Independent Samoa). Recent evidence suggests that mutualist-derived, carbohydrate-rich resources may be particularly important factors in *A. gracilipes* invasions (Lach 2005, Savage et al. 2009, *In Press, In review*). For example, Savage et al. (2009) found that across the Samoan Archipelago, *A. gracilipes* displayed both significant negative associations with the species richness of native ants and strong positive associations with the dominance of EFN-bearing plants (mostly *M. citrifolia*-Savage et al. 2009). Furthermore, Savage and Whitney (*in* review) experimentally demonstrated that *A. gracilipes* workers not only responded strongly to increasing nectar resources by recruiting more workers to EFN-bearing plants (a trait shared by other dominant ants in Samoa), but also intensified their aggressive behaviors towards plant-visiting arthropods (a trait that was unique to *A. gracilipes*) as nectar levels increased (Savage et al. *In review*).

Here, I test the hypothesis that these differences in dynamics within ant-*M. citrifolia* mutualisms can have consequences for co-occurring arthropod communities. Specifically, I asked (1) How do mutualisms between *M. citrifolia* and ants influence co-occurring arthropod communities?; and (2) Do these effects differ when *A. gracilipes* dominates local ant assemblages and *M. citrifolia* plants? These mutualisms may influence arthropods that visit *M. citrifolia* plants differently than co-occurring arthropods that are not directly associated with the plant or ants. Therefore, I examined arthropod communities at two spatial scales-on *M. citrifolia* plants and in plots dominated by *M. citrifolia* plants (but including plants that do not bear EFN and bare ground).

I characterized arthropod communities in Samoa and their responses to experimental manipulations of ant- *M. citrifolia* mutualisms across sites dominated by *A. gracilipes* and sites dominated by other ant species. Based upon previous studies (including 2009 surveys of ant assemblages in Samoa), I predicted that communities dominated by *A. gracilipes* would be more simplified than those dominated by other ant species. I expected these differences to be evident at both the plant and plot levels. To evaluate the carbohydrate subsidy hypothesis, I experimentally manipulated both ant access to and nectar levels of *M. citrifolia* plants. I predicted that (i) when ants were excluded from plants, the diversity of other arthropods (except honeydew-excreting insects) would increase as nectar levels increased, because there would be increased carbohydrate resources available to these other community members. However, when ants were allowed on plants, I expected (ii) general declines in the abundance and diversity of these other arthropods, since they would be subjected to more ant aggression as nectar levels increased. Previous work (Savage et al. *In press, In review*) demonstrated that *A. gracilipes* workers respond more strongly to nectar than co-occurring ants, especially in terms of aggressive behaviors. Consequently, I predicted (iii) that there would be a greater magnitude in the responses of arthropod communities at sites dominated by *A. gracilipes* than at sites dominated by other ant species. Finally, I expected (iv) that there would be stronger patterns of arthropod responses at the plant level than at the plot level because there would be an increased likelihood of contact between ants and other arthropods on *M. citrifolia* plants relative to entire plots.

4.2 Methods

Study organisms

A pan-tropical 'tramp ant,' *Anoplolepis gracilipes* has a broad diet breadth, can form supercolonies, and is considered invasive in many island groups, including Samoa (Holway et al. 2002, Lester and Tavite 2004, Abbott 2006, Savage et al. 2009). Although its native range is thought to include Africa or Asia, this species' origin is currently undetermined (Wetterer 2005). In Samoa, *A. gracilipes* workers were first recorded in 1925 at very low abundances near ports and plantations (Emery 1925 *cited in* Wilson and Taylor 1967). However, recent studies have demonstrated that this species is currently widely distributed in Samoa, occasionally occurring at very high densities (Lester and Tavite 2004, Savage et al. 2009). At sites in which *A. gracilipes* is absent from local ant assemblages in Samoa, other non-native ants are ecologically dominant (both on EFN-bearing plants and the ground) (Savage and Whitney *in review*). These species include *Pheidole megacephala, Paratrechina longicornis, Solenopsis geminata, Tapinoma melanocephalum,* and, *Tetramorium bicarinatum* (Savage and Whitney *in review*).

Across the Samoan Archipelago, ants have access to multiple resources for carbohydrates. However, the most common and dominant EFN-bearing plant is *Morinda citrifolia* (Nonu, Rubiaceae), which is particularly common in disturbed habitats (Savage, unpublished data). *Morinda citrifolia* may have spread to many Pacific islands from Southeast Asia via historical migrations of indigenous peoples ~3000 years ago (Whistler 1993). However, since the seeds can float in ocean water for months while remaining viable (Nelson 2006), this plant may have arrived in the Samoan Archipelago without human assistance. Consequently, I consider Samoa to be within the native range of this plant. *Morinda citrifolia* possesses annular disk nectaries clustered on an inflorescence that produce floral, extrafloral and post-floral nectar (hereafter 'nectary body' ; Waki et al. 2007, Savage et al. *in press*). At our sites in Samoa, nectary bodies contained 2 to >50 nectaries and reached a maximum size of 80 cm^3. *Morinda citrifolia* plants produce nectary bodies year-round. Ants frequently visit nectaries of this plant, which are often dominated by *A. gracilipes* (Savage et al 2009).

Study sites

I conducted manipulative experiments on the island of Savaii, Samoa from 15 October 2007 – 18 May 2008. Study sites were located on lava flows dating from 1907-1911, and plant communities were dominated by *M. citrifolia.* During the time of the experiments, average daily rainfall on the island of Savaii was 88.2 ± 0.0.01 mm and the average daily temperature was 30.4 ± 0.09 °C (Avao Weather Station; http://www.wunderground.com/global/stations/91757.html). Experiments were conducted across sites numerically dominated by *A. gracilipes* (6 sites) and sites dominated by other ant species (five sites). *Anoplolepis gracilipes* comprised >85% of all individuals in local ant assemblages at sites where it was dominant and <5% of local ant assemblages at sites dominated by other ant species (*Paratrechina longicornis* and *Pheidole megacephala*). Sites were separated by 20 m-73 km (mean 33.76 ± 10.12 km).

Experimental Design:

Treatment application: Within each site, I established seven 4x4m plots which I used for my factorial experiments. Plots were located a minimum of 8 m apart (range 8-11 m) to reduce the probability that ants in different plots would belong to the same colony. All plots had a minimum of three *M. citrifolia* plants (mean 10.1 ± 0.65 plants per plot). I randomly assigned plots to a treatment level and applied treatments to all *M. citrifolia* plants in the plot. The factors in this experiment were ant access to *M. citrifolia* plants (permitted or excluded) and nectar level (reduced, ambient or supplemented nectar). Each site also included one plot that received no experimental manipulations, to provide a true control for comparison with the procedural control (ants permitted, nectar ambient).

To exclude ants, I first attached paper banding material (Tanglefoot Co., Grand Rapids, MI USA) around the base of *M. citrifolia* plants, secured with self-clinging, non-adhesive tape (Hugo's Amazing Tape, Commerce, CA USA). Next, I applied Tree Tanglefoot Pest Barrier (Tanglefoot Co) to the banding material around the entire base of the plants. To control for any effects of the banding material and/or Tanglefoot Pest Barrier, I used the same banding methods in control plots, but only painted a single strip of Tanglefoot on one side of plant stems, to allow ants to access plants. Because neighboring plants could provide vegetations bridges that would allow ants to bypass the Tanglefoot Pest Barrier, I also trimmed vegetation directly touching *M. citrifolia* plants in all plots.

To reduce ants' access to nectar, I secured bags constructed of lightweight organza material to the base of nectary bodies with a plastic cable tie. Bags were ~1.5-2X larger than nectary bodies to minimize contact between nectaries and bagging material. I bagged all nectary bodies, regardless of treatment assignment, to control for any effects of the bags. However, I cut holes (~3-6cm) in the bags of plants in treatment plots with ambient or supplemented nectar to allow ants to access plant nectar. To supplement *M. citrifolia* nectar, I constructed artificial nectaries. Specifically, I inserted a 5μL microcapillary tube into the center of a microcentrifuge vial (MCT-200-C Microtubes, Axygen Scientific, Union City, California). Vials were affixed to plants using twist ties, and all plots received 30 vials. For the supplemented nectar treatment, vials were filled with 2000 μL of a 30% sucrose solution; in all other plots tubes were not filled. In a previous study, Freeman et al. (1991) demonstrated that *M. citrifolia* nectar is dominated by sucrose, with sucrose contributing an average of 72.6-88.9% to total nectar carbohydrates. The concentration of *M. citrifolia* nectar using a field refractometer (EZRed B1, EZ Red Co., Deposit, New York) was 28.06 % ± 1.04 % SE (*n* = 8 plants). Therefore, I considered the 30% sucrose solution in the artificial nectaries to be a reasonable approximation of naturally-occurring *M. citrifolia* nectar. I used measures of ambient nectar production at one site (Saleaula) as a baseline level of *M. citrifolia* nectar production per plant. To do this, I excluded insect visitors from nectary bodies for 24 h (using organza bags), then collected nectar in microcapillary tubes. Average nectar production per plant per day was 2249 μL ± 642 SE (range = 645-5226, *n* = 6 plants).

Treatment maintenance: I maintained experimental treatments every two weeks for six months. I maintained both ant exclusion plots and control plots by trimming vegetation that had formed bridges across the Tanglefoot barrier and replacing old Tanglefoot with a fresh coat. In all plots, I also added bags to newly formed nectary bodies and replaced old bags as needed (i.e. when nectary bodies grew too large for the old bag or the bag was otherwise damaged). Finally, I replenished fake nectaries with fresh sucrose and replaced any tubes that were damaged (this was generally rare).

Ant and plant assessments:

Local ant assemblages: At each site, I assessed local ant assemblages by haphazardly selecting three *M. citrifolia* plants within the buffer zones between treatment plots and counting the number and identity of ants on each plant. I used a comprehensive count of all ants per plant completed in ~5 minutes/plant. I also counted ants on the ground ~1m from each plant, using a 10 x 10 cm white paper card and counting the number of ants that crossed the card in 30sec (following methods in Abbott 2005). I used the card approach because sites were located on impenetrable lava fields, making it impossible to use pitfall traps. These assessments were conducted three times: prior to treatment application, three months after treatments were applied and six months after treatments were applied. I used initial assessments of ground foragers to assign *A. gracilipes* dominance status to each site. I considered sites in which *A. gracilipes* workers comprised ≥85% (range: 85.1-100%; mean: 95.6% ± 2.3%) of all individuals to be numerically dominated by this species and those in which *A. gracilipes* workers represented ≤5% (range: 0-4.7%; mean: 0.94% ± 0.94%) of all individuals to be numerically dominated by other ant species.

Morinda citrifolia *plants:* Within each plot, I measured traits of *M. citrifolia* plants in order to account for natural differences in resource abundance. To determine the best metric for evaluating size of *M. citrifolia* plants, I haphazardly selected 10 *M. citrifolia* plants outside of treatment plots at one site (Gataivai_East). I then measured the height of the main stem, the diameter at the base of the main stem, the number of branches, the length and diameter at the base of branches, the number of leaves, the length and width of five leaves, the number of nectary bodies and the height and diameter of five nectary bodies for each plant. I used these values to calculate total plant volume (volume = (height of main stem x diameter at base of main stem) +((average branch length x average branch diameter) x.total number of branches) + ((average leaf length x average leaf width) x total number of leaves) +((average nectary body height x average nectary body diameter) x total number of nectary bodies)). Using Spearman correlations to determine the plant traits that were highly correlated with total plant volume, I found that the product of the height of the main stem and the diameter at the base of the main stem was highly correlated with total plant volume ($r^2=0.91$, $P<0.0001$). Therefore, I measured these plant traits prior to treatment application, three months after treatments were applied and six months after treatments were applied. I also counted the number of nectary bodies per plant during each interval to account for variation in nectar production per plant.

Assessments of arthropod responses:

I examined the responses of the arthropod community three and six months after treatment application in two ways: (i) surveys of arthropods on *M. citrifolia* plants; and (ii) sweepnet samples of all vegetation in the plots. Surveys were conducted on all *M. citrifolia* plants in each plot; however, all plants were sub-sampled. Specifically, I recorded the abundance and morphospecies identity of all arthropods on five leaves, 2 stems, and 3 nectary bodies per plant (~2-5 minutes per plant). For the surveys, I standardized the data by calculating the average abundance of each morphospecies per plant part per plant per plot, because some plants did not have all 5 leaves, 2 stems and 3 nectary bodies for the surveys and because plots varied in the number of plants. I then calculated the cumulative abundances for each morphospecies using the sum of these averages over the three and six month surveys. I collected type specimens for each unique morphospecies, which were stored in 70-95% ethanol. I used a canvas sweepnet (diameter=38 cm; 91.5 cm long handle) to collect specimens from all vegetation in the plots. To do this, I systematically swept the plots from ground level to ~2m, using 12 sweeps per plot. For each census, I determined the cumulative abundances of each morphospecies per plot from the sweepnet samples.

All type specimens and sweepnet samples were later transported to a laboratory, where I used a stereomicroscope to sort and identify them. I used Shattuck (1999) and Wilson and Taylor (1967) to identify ant species. Specimens from Hemiptera, Hymenoptera. Coleoptera, Diptera, Lepidoptera, Orthoptera, Araneae, and Thysanoptera were identified to the family level. I also examined arthropod responses by arthropod trophic group. Herbivores, carnivores and detritivores not only likely respond differently to ant-plant protective mutualisms, but also influence distinct community and ecosystem processes. Therefore, when possible, I assigned each unique morphospecies into one of the following trophic groups: (i) ants (ii) carnivores (no ants); (ii) detritivores; (iii) honeydew-excreting herbivores, (iv) other herbivores and (v) nectivores, which included pollinators and obligate nectar feeders. However, there were too few nectivores to permit analyses of their relative abundance and diversity. Additionally, I expected honeydew-excreting insects to respond differently to the experimental treatments than other arthropods since they can form their own mutualistic associations with ants. Therefore, I excluded these herbivores from analyses of local arthropod community responses described below.

Data Analyses

For all analyses, I conducted separate tests for survey data and sweepnet data. Similarly, I also separated out ants and so that I could assess their responses separately since I manipulated ant access to plants.

Characterization of sites dominated by A. gracilipes *vs. sites dominated by other ants:* I first examined differences in local arthropod assemblages between sites dominated by *A. gracilipes* and those dominated by other ant species. Although I cannot infer a causal role of *A. gracilipes* invasion status for any differences between these sites, such analyses allowed me to gain a better understanding about the arthropod communities that were subject to the experimental manipulations. To evaluate these data, I split sites into dominance status (*A. gracilipes* vs. other ant species) and then conducted separate ANOVAs for plant-level surveys and plot-level sweepnet sampling. The factors in these models were *A. gracilipes* invasion status, site (nested in *A.gracilipes* invasion status), and plot (nested in site-only used for plant-level data). Response variables for these initial analyses were total abundance, morphospecies richness, Shannon-Weiner morphospecies evenness (J), and Shannon-Weiner morphospecies diversity of all arthropods. When these responses were significant, I conducted additional ANOVAs with the abundances of each trophic group as response variables. I used SAS v. 9.2 (SAS Institute 2002) for all analyses. Plant-level data were analyzed with PROC MIXED procedures, with plot included as a random factor and *A. gracilipes* invasion status and site treated as categorical factors. Plot level data were analyzed using PROC GLM, with both *A. gracilipes* invasion status and site treated as categorical factors. All data met assumptions of normality and homogeneity of variances following log transformation of raw data.

Assessment of β and α-diversity: I examined arthropod community responses to our treatments both among (β-diversity) and within (α-diversity) plots. To assess β-diversity, I conducted a permuted dispersion test (PERMDISP), which examines the average distance to centroids among groups. For this analysis, the groups were defined by *A. gracilipes* invasion status x ant access to *M. citrifolia* plants x the nectar availability treatment (a total of twelve groups). Prior to conducting PERMDISP, data were transformed to presence/absence for each morphospecies. To examine the effects of our experiments on overall α-diversity, I conducted a permuted ANOVA (PERMANOVA). The factors in this model were *A. gracilipes* invasion status, ant access *to M.* citrifolia plants, the nectar availability treatment and all interactions. To visualize differences between arthropod communities, I then performed an NMDS ordination using a Bray-Curtis distance matrix and 100 restarts. Morphospecies that represented fewer than 5 individuals (from sweepnet samples) or less than 1% of the total arthropod abundances (surveys) were excluded from these analyses. I used Primer-E v.6.1.13 with the PERMANOVA+ v. 1.0.3 extension (Clarke and Gorley 2009) for these analyses. I was unable to include site and plot in PERMDISP models. Therefore, I did not use these factors in PERMANOVA models, in order to more directly compare these tests. However, I also conducted additional analyses on relative abundances and diversity statistics within local communities (below) and the models for these tests included site and plot (for survey data).

Assessment of arthropod abundances and diversity statistics: To disentangle effects of my experiments on different components of α-diversity, I first calculated the total abundance, morphospecies richness, morphospecies evenness (Shannon-Weiner J'), and morphospecies diversity (Shannon Weiner H') of all trophic groups for each plot. For all responses, I next tested for differences between the true control plots (no manipulation) and the procedural control plots (ants permitted, with a single strip of Tanglefoot, all nectary bodies bagged with holes in the bags). There were no significant differences between these control plots (all $P > 0.20$), consequently, I pooled arthropod responses for all control plots. I then split data by invasion status and conducted ANCOVA for each response variable (including the diversity statistics described above, proportional representations of each trophic group, the abundance of *A. gracilipes,* and the abundance of other ants) using SAS v. 9.1.2 (SAS Institute 2003). I conducted separate tests for plant-level survey data and plot-level sweepnet samples. For survey data, I used PROC MIXED with the independent factors of ant access to *M. citrifolia* plants (permitted or excluded), the nectar treatment (reduced, ambient and supplemented), their interaction, and site. All factors were treated as categorical. I additionally included plot (nested in site) as a random factor in these models. Covariates for these analyses were the number of nectary bodies per plant and plant size (height of the main stem x diameter at the base of the main stem). All data met assumptions of normality of residuals and homogeneity of variances following log transformations of raw data.

I used PROC GLM to examine arthropod responses at the plot level. This model included the same factors as the plant-level analyses described above; however, plots were the units of replication so were not used as factors in the analyses. I additionally included the density of *M.citrifolia* plants per plot the average size of *M. citrifolia* plants per plot, and the average number of nectary bodies per plant per plot as covariates. For many response variables in the plot-level data set, residuals were non-normally distributed, and normality could not be obtained through transformations. Therefore, randomization tests were used to evaluate differences among treatments plot-level assessments of arthropod community responses (Manly, 1991). Randomization tests determine *P*-values by comparing an observed test statistic (e.g. F-ratio from ANOVA) to a distribution of the test statistic that is expected under the null hypothesis. To create the expected distribution, the response variable values from treatments being compared are pooled, permuted and randomly assigned to the treatments for 9999 iterations. We used randomization test equivalents of ANOVA by embedding Proc GLM code within a SAS randomization test macroprogram (Cassell, 2002).

4.3 Results:

Characterization of sites dominated by A. gracilipes *vs. sites dominated by other ants:*

Plant-level: I predicted that the abundance and diversity of arthropods at sites dominated by *A. gracilipes* would be simplified relative to arthropod communities at sites dominated by other ant species. This prediction was supported for *M. citrifolia*-associated arthropod communities. Plant surveys revealed that the abundance (F=22.44, P<0.0001), morphospecies richness (F=14.49, P=0.0002), morphospecies evenness (F=8.00, P=0.0048), and morphospecies diversity (F=16.22, P<0.0001) of plant-associated arthropod communities were lower at sites dominated *A. gracilipes* (Figure 4.1a). Site also significantly affected all of these aspects of local arthropod community structure (P<0.0001 for all responses). Decomposing local arthropod communities into trophic groups, I found that herbivores (F=5.37, P=0.0208), carnivores (F=5.56, P=0.0186), and detritivores (F=26.42, P<0.0001) all had significantly lower abundances at sites dominated by *A. gracilipes* (Figure 4.1b). Site was also an important factor influencing the abundances of herbivores, carnivores and detritivores (all $P<0.0001$).

Plot-level: In contrast to my prediction that similar effects would occur at the plot-level, we did not detect any significant differences in the abundance, morphospecies richness, morphospecies evenness or morphospecies diversity of arthropod communities at the plot-level (Figure 4.1c). Similarly, there were no significant differences between the abundances of any trophic groups at sites dominated by *A. gracilipes,* relative to sites dominated by other ant species (Figure 4.1d).

Response of A. gracilipes *and other dominant ants to experimental manipulations:*

Plant-level: Based upon previous research (Savage and Whitney *In review*), I expected both *A. gracilipes* and other dominant ants to respond strongly to increasing levels of *M. citrifolia* nectar. At sites dominated by *A. gracilipes*, this prediction was supported at the plant level. There were significant effects of both the ant access ($P<0.0001$) and nectar level ($P=0.0003$) treatments, but not their interaction. Specifically, *A. gracilipes* workers had much lower abundances in exclusion plots and increased linearly in control plots as nectar levels increased (Figure 4.2a). While other dominant ants responded similarly to the ant access treatment, they were unresponsive to nectar manipulations at the plant-level (Figure 4.2b).

Plot-level: My predictions for plot-level ant responses were the same, except that I expected to see a lower magnitude of ant responses to the experimental manipulations. In fact, I found similar responses of both *A. gracilipes* and other dominant ant species to the ant access treatment (Figure 4.2c-d). Additionally, non-*A. gracilipes* workers did not respond to manipulations of *M. citrifolia* nectar at the plot-level, which is consistent with their plant-level response (Figure 4.2d). The nectar level treatment did significantly influence plot-level abundances of *A. gracilipes* workers, however this response was, indeed, weaker at the plot level than it was at the plant level (P=0.0399; Figure 4.2c).

Assessment of β-diversity responses of local arthropod communities:

Plant-level: I predicted that α and β-diversity of local arthropod communities would differ among treatments, and that these effects would be stronger when *A. gracilipes* dominated local ant assemblages. This prediction was supported in terms of β-diversity for plant-visiting arthropod communities. On *M. citrifolia* plants, the greatest differences among plots occurred when nectar levels were ambient at sites dominated by *A. gracilipes* (Figure 4.3a). However, the opposite effect occurred at sites dominated by other ant species, where plots with ambient nectar levels were most similar across all sites (Figure 4.3b). Presence of ants on *M. citrifolia* plants served to dampen these effects (Figure 4.3a-b; Status x Ant x Nectar: *P*=0.0001).

Plot-level: Different responses were evident at the plot level. At sites dominated by *A. gracilipes*, there was an increase in dissimilarity among plots at the highest nectar level when ants were permitted on plants, but a sharp reduction in β-diversity when ants were excluded from *M. citrifolia* plants (Figure 4.3c). In contrast, a completely different pattern emerged at sites dominated by other ant species. At these sites, dissimilarity among sites peaked at ambient nectar levels when ants were permitted on *M. citrifolia* plants but was lowest when ants were excluded from the plants at these ambient levels (Figure 4.3d; Status x Ant x Nectar: *P*=0.0086).

Assessment of overall α-diversity responses of local arthropod communities:

Plant-level: In congruence with the findings for β-diversity, patterns of overall α-diversity also varied significantly across different treatment levels and were modified by *A. gracilipes* invasion status. At the plant-level *A. gracilipes* invasion status (*P*=0.0001), ant access to *M. citrifolia* plants (*P*=0.0002), the nectar availability treatment (*P*=0.0001), the interaction between

A. gracilipes invasion status and ant access (*P*=0.0006), the interaction between *A. gracilipes* invasion status and the nectar availability treatment (*P*=0.0001), the interaction between ant access to *M. citrifolia* plants and the nectar availability treatment (*P*=0.0078) and the three-way interaction between *A. gracilipes* invasion status, ant access to *M. citrifolia* plants and the nectar availability treatment (*P*=0.0306) all significantly influenced overall arthropod composition on *M. citrifolia* plants (Figure 4.4a-b).

Plot-level: Similar patterns emerged at the plot-level, although they were not as strong. The only factors that significantly influenced the composition of local arthropod communities across entire plots were the interaction between *A. gracilipes* invasion status and the nectar availability treatment (*P*=0.0464) and the three way interaction between *A. gracilipes* invasion status, ant access to *M. citrifolia* plants, and the nectar availability treatment (*P*=0.0025). However there were non-significant trends that suggest that *A. gracilipes* invasion status (*P*=0.0580), the interaction between ant access to *M. citrifolia* plants and the nectar availability treatment (*P*=0.0577) may also be important to these arthropod communities (Figure 4.4c-d).

Assessment of arthropod abundances and diversity statistics:

Plant-level: The previous analyses provided a good overall assessment of the responses of local arthropod communities to my experiments across sites dominated by *A. gracilipes* and those dominated by other ant species. However, I needed to examine these data in greater detail in order to determine the relative contribution of different components of these complex communities to such broad-scale responses. I expected the the arthropod community to respond to ant-*M citrifolia* mutualisms by increasing in abundance and diversity as nectar levels increased when ants were excluded from the plants and decreasing in abundance and diversity at increasing nectar levels in the presence of ants. Furthermore, I expected these responses to have a greater magnitude when *A. gracilipes* dominated local ant assemblages. In fact, these other arthropods were generally rare and present in low abundances on *M. citrifolia* plants. However, I did detect some plant-level responses to my experimental manipulations. Specifically, across all trophic groups, nectar availability on *M. citrifolia* plants influenced the morphospecies richness of other plant-visiting arthropods; however, this effect was only statistically significant at sites dominated by *A. gracilipes* (*A. gracilipes*-dominated sites: F=4.26, *P*=0.0147; Figure 4.5a; other sites: F=2.37, *P*=0.0951; Figure 4.5b). Interestingly, these effects of nectar on arthropod morphospecies richness were different for sites invaded by *A. gracilipes* versus other sites. At *A. gracilipes*-dominated sites, morphospecies richness peaked at ambient nectar levels and then declined when nectar was supplemented. In contrast, at sites dominated by other ant species, morphospecies richness was lowest at ambient nectar levels (although, as stated above, this was a non-significant trend). There was also a non-significant trend at sites dominated by *A. gracilipes* for the total abundance of all plant-visiting arthropods to increase as nectar levels increased when ants were permitted on plants, but to peak at ambient levels when ants were excluded (F=2.61, *P*=0.0749; Figure 6a).

Decomposing the community into trophic groups, I found strong effects of the experimental treatments on plant-visiting herbivores and detritivores, but only weak non-significant trends in terms of carnivore responses at the plant level (Table 4.1a). Furthermore, the only statistically significant responses occurred at sites dominated by *A. gracilipes.* Specifically, the total abundance and morphospecies richness of herbivores at these sites were both significantly lower when ants were permitted on *M. citrifolia* plants (Table 4.1a). Detritivores responded not to ant access to the plants, but to nectar availability, with peaks at ambient levels for both total abundance and morphospecies richness at *A. gracilipes*-dominated sites (Table 4.1a).

Plot-level: Because they would have a decreased likelihood of interacting with ants collecting nectar and/or tending *M. citrifolia* plants, I expected plot-wide arthropod communities to display weaker responses to my experimental manipulations than plant-visiting arthropod communities. However, nectar availability may influence overall activity or aggression rates, so I expected the general trends in arthropod communities to be similar at the plant and plot level. However, arthropod communities at the plot-level often displayed different responses to experimental manipulations of ant-*M. citrifolia* mutualisms that were, in some cases, even stronger than plant-level arthropod responses. Generally, significant arthropod community responses were stronger at sites dominated by *A. gracilipes* than at sites dominated by other ants. Across all trophic groups, the total abundance (P=0.0370; Figure 4.5c), morphospecies richness (P=0.0211), and morphospecies diversity (P=0.0323; Figure 4.5c) were all significantly influenced by the interaction between ant access to *M. citrifolia* plants and the nectar availability treatment at sites dominated by *A. gracilipes*. In all cases, arthropods responded positively or neutrally to increasing nectar levels when ants were allowed on plants, but sharply declined at the highest nectar levels when ants were excluded from plants (Figure 4.5c). At *A. gracilipes*-dominated sites, morphospecies evenness was significantly influenced by both the ant (P=0.0098) and nectar availability treatments (P=0.0262), but not their interaction. Arthropod responses to experimental manipulations of mutualisms between ants and *M. citrifolia* plants were much weaker at sites dominated by other ant species. Interestingly, morphospecies evenness was the only response that significantly changed across experimental treatments. In the presence of ants, morphospecies evenness was reduced at low nectar levels; however, at high nectar levels, the evenness of arthropods was equivalent across ant access treatments (P=0.0331, Figure 4.5d).

Trophic groups responded differently to our experimental treatments at the plot level than at the plant level. At the plot-level, the strongest responses occurred within carnivores, with weak responses in herbivore and detritivores guilds. At sites dominated by *A. gracilipes,* the interaction between the ant and nectar treatments strongly influenced the morphospecies richness (*P*=0.0005, Table 4.1b). At these sites, carnivores displayed a strong negative response to nectar, but only in the absence of ants. The abundance of detritivores was also significantly different across treatment groups (Ant x nectar treatment: *P*=0.0478; Table 4.1b). However, these responses were different. When ants were excluded from plants, detritivores were most abundant at ambient nectar levels; however, in the presence of ants, the lowest abundance of detritivores occurred at ambient nectar levels (Table 4.1b). There was also a non-significant response of herbivores at *A. gracilipes*-dominated sites, with a non-significant trend towards lower morphospecies evenness in the presence of ants (*P*=0.0597, Table 4.1b). Carnivores also responded to the interaction between the ant access and nectar availability treatments at sites dominated by other ant species. At these sites, the morphospecies richness of carnivores increased as nectar levels increased, but only when ants were allowed on *M. citrifolia* plants. There was also a significant effect of ants on carnivore diversity, with a higher diversity of carnivores in the presence of ants across all nectar treatments (*P*=0.0119; Table 4.1b). Herbivores and detritivores were unresponsive to experimental manipulations of the *M. citrifolia*-ant mutualism at sites dominated by non-*A. gracilipes* ants.

4.4 Discussion:

When exotic species invade naïve habitats, they often form facultative beneficial interactions with indigenous species. Recent studies have shown that these novel mutualisms can facilitate the progression and extent of species invasions (Richardson 2000). In this study I asked if such positive associations could additionally mediate the negative impacts of invasive species on recipient communities. I examined the effects of mutualisms between ants and a native, EFN-bearing shrub (*Morinda citrifolia*) on arthropod communities across sites dominated by the highly invasive ant species, *Anoplolepis gracilipes*, and those dominated by other ant species. Specifically, I manipulated ant access to *M. citrifolia* plants and nectar availability on the plants in a factorial design. One strength of this approach is that it pinpointed the association between interacting partners. Any community-wide effects of *A. gracilipes* or other ant species that were not associated with *M. citrifolia* plants were still present in exclusion plots. Similarly, effects of *M. citrifolia* plants on arthropod communities that were not linked to nectar resources persisted across nectar availability treatments. I found that these mutualisms did, in fact, have multiple significant effects on co-occurring arthropod communities. Furthermore, these influences of mutualisms on arthropod communities were strongest when *A. gracilipes* dominated local ant assemblages. Finally, we discovered that mutualisms between *A. gracilipes* and *M. citrifolia* influenced different trophic groups at the plant and plot level.

*Effects of ant-*M. citrifolia *mutualisms on co-occurring arthropod communities*

Across all sites in this study, I found significant influences of mutualisms between ants and *M. citrifolia* plants on arthropod communities. While there was a great deal of variation in the degree of these effects across invasion status and trophic groups, community structure was consistently affected by experimental manipulations of this mutualism. Other recent studies have demonstrated that mutualisms can have strong influences on co-occurring communities and that these effects can be context dependent. For example, Matthews et al. (2009) manipulated the presence of EFN and ant access to peach plants in an agricultural setting. They found that the diversity of herbivore and predator communities was lower on EFN-bearing plants that were visited by ants, relative to plants without EFN or EFN-bearing plants with no ant access (at least in one year of the study). Other types of mutualism have also been shown to significantly affect arthropod communities. For example, Rudgers and Clay (2008) examined the influences of mutualism between a dominant grass species and a fungal symbiotic endophyte. When endophytes were experimentally removed from their hosts at the plot-level, arthropod abundance and diversity increased significantly. Mutualisms can also have positive influences on arthropod community structure. Katayama et al (2011) recently demonstrated that arthropod communities on legumes had higher abundance and diversity when the plants harbored nitrogen-fixing *Rhizobia* spp. bacteria in their roots. Thus, there is mounting evidence that mutualisms can have community-wide effects that rival the strength and importance of the effects of antagonistic interactions.

Community-wide effects of the mutualism were stronger when A. gracilipes *dominated local ant assemblages*

Whether examining aspects community structure for all arthropods or within guilds of carnivores, herbivores or detrivores, effects of mutualisms between *A. gracilipes* and *M. citrifolia* were stronger than the effects of mutualisms between *M. citrifolia* and other ant species. These data suggest that the highly invasive species, *A. gracilipes* responds differently than co-occurring ants to mutualist-derived, carbohydrate-rich nectar resources. This corresponds with other recent work on invasive ants and carbohydrate-rich resources. For example, when *A. gracilipes* were introduced to Christmas Island, they remained at low population densities during a lag phase that lasted >70 years (O'Dowd et al. 2003). In the 1990's, this species began displaying invasive characteristics. Within ~10 years, *A. gracilipes* had killed up to one-third of the island's endemic red land crabs (*Gecarcoidea natalis*). Furthermore, *A. gracilipes* actively tended scale insects, and correlative evidence suggests that the combination of increased carbohydrate resources for the ants and population expansion of scale insects led to the death of native canopy trees (O'Dowd et al. 2003). Additionally, Lach (2005) examined the relative responses of *A. gracilipes* and two other invasive ants to floral nectar and found that *A. gracilipes* was more likely to be found in flowers, collected more nectar and was less likely to abandon floral resources following perturbation than the other ant species (*Linepithema humile* and *Pheidole megacephala*). I also recently experimentally demonstrated that over a gradient of nectar availability on *M. citrifolia* plants, *A. gracilipes* both recruited strongly to nectar resources and intensified aggressive encounters with other arthropods as nectar levels increased (Savage and Whitney *In review*). These behavioral responses of *A. gracilipes* to nectar resources may provide a viable mechanism for the differences that we detected in some of the arthropod community responses to ant-*M. citrifolia* mutualisms when *A. gracilipes* displaced other ants as a mutualist partner (see below).

Trophic groups responded differently to M. citrifolia-A. gracilipes *mutualisms*

Although herbivores, carnivores and detritivores all responded significantly to mutualisms between *M. citrifolia* and *A. gracilipes,* these responses were not consistent among groups. Generally, these differences may be critically important because different trophic groups often exert variable influences on ecosystem processes. For example, we may expect changes to herbivores to affect levels of primary productivity and changes to detritivores to modify rates of decomposition. On *M. citrifolia* plants, herbivore communities responded to the presence of ants on plants, with increases in both morphospecies richness and total abundance of herbivores in plots with ants excluded from *M. citrifolia* plants. This is likely due to the aggressive behaviors of ants tending EFN. However, the level of nectar did not influence this effect, which is surprising since I previously documented that *A. gracilipes* intensifies aggression towards other arthropods when nectar levels increase (Savage and Whitney *In review*). In contrast, detritivores on *M. citrifolia* responded more strongly to nectar availability than ant access, but only at sites dominated by *A. gracilipes.* There were more detritivores on plants as long as any nectar was available (in the ambient and supplemented nectar treatments). One possible explanation is that detritivores are feeding on fungi that are associated with nectar (Herrera et al. 2008). Because *A. gracilipes* displays strong responses to nectar (Savage et al. *In press, in review*), the plants may produce more nectar at sites dominated by this species. Detritivores also responded to our manipulations at the plot level, with the greatest number of detritivores in plots where ants had access to *M. citrifolia* plants and nectar was ambient. This may be an effect of resource availability for detritivores. When nectar levels are low, there could be reduced accumulation of fungus and other detritus. When nectar levels are high, increased activities of *A. gracilipes* workers in response to high nectar levels could lead to a similar reduction of detritus at high nectar levels. If these dynamics are occurring, then they would lead to these observed patterns in the abundance of detritivores.

In contrast to other trophic groups, carnivores displayed weak and non-significant responses to the mutualism at the plant level, but strong responses at the plot level. In fact, across all trophic groups, the strongest responses were these plot level responses of carnivores, in which their abundance and diversity sharply declined at the highest nectar levels when ants were excluded from *M. citrifolia* plants. This response at the plot level may be due to a response of carnivores to nectar, rather than ants. When ants are allowed on plants, the likelihood that other arthropods are able to access the nectar is low. However, when ants are excluded, this likelihood increases. Therefore, if there are any negative consequences of nectar for carnivores (such as parasitoids or jumping spiders), then they would only be evident when ants are excluded from *M. citrifolia* plants.

Community responses to the mutualism differed at the plant and plot level

We examined arthropod community responses both on *M. citrifolia* plants and more broadly, across entire plots with the expectation that there would be similar effects at both scales, but greater magnitude in arthropod responses at the plant level. However, this prediction was not supported by our experiments. In fact, not only did responses of local arthropod communities to *A. gracilipes-M. citrifolia* mutualisms differ among these different spatial scales, they were actually stronger at the plot level. As described above, the strongest response in the experiment was a carnivore-driven crash in abundance and diversity at the highest nectar levels when ants were excluded from plants at *A. gracilipes*-dominated sites. Most studies of ant-plant mutualisms focus on plant-associated arthropod communities (Wimp and Whitham 2001, Mody and Linesmair 2004, Rudgers et al. 2010). However, this is the first study that I am aware of that has examined broader plot-wide community responses to these mutualisms. Thus, we may be underestimating the effects of ant-plant mutualisms on broader, community-wide patterns of species co-occurrence and relative abundances.

Conclusions:

This study adds to a growing body of literature that suggests that mutualisms and other positive species interaction may rival antagonistic interactions in terms of their role as structuring forces for ecological communities. Furthermore, we have demonstrated that the community-wide influences of mutualisms can change in the context of species invasions and suggest that mutualisms can mediate the negative impacts of invasive species on co-occurring community members. If these findings remain consistent across different types of mutualism and invasion, then understanding novel mutualisms may prove critical in understanding and predicting the progression and impacts of species invasions. Furthermore the results of this study show that community-wide responses can vary strongly by trophic group and spatial scale. Therefore, we need studies that examine groups both collectively and individually and that examine effects of mutualisms across multiple spatial scales.

Acknowledgements

Many thanks to private landowners in Savaii for allowing me to access their property for my experiments. Thanks also to Sally Johnson, Julie Goldsbury, Jeremy Caves, Olivia Barnes, Kevin

Dougherty, Rebecca Sandidge, and Afele Faiilagi for critical field assistance. Thank you to Jennifer Rudgers and Ken Whitney for logistical support of many kinds. This research was funded by a National Geographic Society Research and Exploration grant, no. 8237-07 and a Rice University Wray-Todd fellowship.

References

Abbott, K. L. 2006. Spatial dynamics of supercolonies of the invasive yellow crazy ant, *Anoplolepis gracilipes* on Christmas Island, Indian Ocean. Diversity and Distributions 12: 101-110.

Bronstein, J. L. 2004. Our current understanding of mutualism. Quarterly Review of Biology 69: 31-51.

Bruno, J. F., Stachowicz, J. J., and Bertness, M. D. 2003. Inclusion of facilitation into ecological theory. Trends in Ecology and Evolutionary Biology 18: 119-125.

Callaway, R.M. & Moran, J.L. (2006) What have exotic plant invasions taught us over the past 20 years? Trends in Ecology and Evolution, 21, 369–375

Colautti, R. I., Eckert, C. G., and Barrett, S. C. H. 2010. Evolutionary constraints on adaptive evolution during range expansion in an invasive plant. Proceedings of the Royal Society, Series B 277: 1799-1806.

DeWalt, S. J., Denslow, J. S., and Ickes, K. 2004. Natural enemy release facilitates habitat expansion of the invasive tropical shrub *Clidemia hirta.* Ecology 85: 471-483.

Freeman C. E., Worthington R. D. & Jackson M. S. 1991. Floral nectar sugar compositions of some south and southeast Asian species. Biotropica, 23: 568–74.

Hay, M. E., Parker, J. D., Burkepile, D. E., Caudill, C. C., Wilson, A. E., Hallinan, Z. P., and Chequer, A.D. 2004. Mutualisms and aquatic community structure: the enemy of my enemy is my friend. Annual Review of Ecology, Evolution and Systematics 35: 175-197.

Herrera, C. M., Garcia, I.M., and Perez, R. 2008. Invisible floral larcenies: microbial communities degrade floral nectar of bumble beee-pollinated plants. Ecology 89: 2369-2376.

Hofsetter, R. W., Cronin, J. T., Klepzig, K. D., Moser, J. C., and Ayres, M. P. 2006. Antagonisms, mutualisms, and commensalisms affect outbreak dynamics of the southern pine beetle. Oecologia 147: 679-691.

Holway, D.A., Lach, L., Suarez, A.V., Tsutsui, N.D. & Case, T.J. 2002. The ecological causes and consequences of ant invasions. Annual Review of Ecology and Systematics, 33, 181–233.

Jongejans, E., Sheppard, A. W., and Shea, K. 2006. What controls the population dynamics of the invasive thistle *Carduus nutans* in its native range. Journal of Applied Ecology 43: 877-886.

Katayama, N., Zhang, Z. Q. and Ohgushi, T. 2011, Community-wide effects of belowground rhizobia on above-ground arthropods. Ecological Entomology, 36: 43–51.

Lach, L. 2003. Invasive ants: unwanted partners in ant-plant interactions? Annals of the Missouri Botanical Garden 90: 91-108.

Lach, L. 2005. Exploitation and interference competition in the nectar-thieving behavior of three invasive ant species. Insectes Sociaux **52**: 257-262.

Lach, L. 2008. Argentine ants displace floral arthropods in a biodiversity hotspot. Diversity and Distributions 14: 281-290.

Lau, J. A., and Strauss, S. Y. 2005. Insect herbivores drive important indirect effects of exotic plants on native communities. Ecology 86: 2990-2997.

Lester P. J. & Tavite A. 2004. Long-legged ants, *Anoplolepis gracilipes* (Hymenoptera: Formicidae), have invaded Tokelau, changing composition and dynamics of ant and invertebrate communities. Pacific Science. 58, 391–401.

Manly, B.F.J. (1991) Randomization and Monte Carlo methods in biology. Chapman and Hall, London.

Matthews, C. R., Bottrell, D. G., and Brown, M. W. 2009. Extrafloral nectaries alterarthropod community structure and mediate peach (*Prunus persica*) plant defense. Ecological Applications, 19: 722-730.

Menke, S. B., and Holway, D. A. 2002. Abiotic factors control invasion by Argentine ants a the community scale. Animal Ecology 75: 368-376.

Morris, W. F., Hufbauer, R. A., Agrawal, A. A., Bever, J. D., Borowicz, V. A., Gilbert, G. S., Maron, J. L., Mitchell, C. E., Parker, I.M., Power, A. G., Torchin, M. E., and Vazquez, D. P. Direct and interactive effects of enemies and mutualists on plant performance: a meta-analysis. Ecology 88: 1021-1029.

Nelson, S. C. 2006. *Morinda citrifolia* (Noni), v.4. *In* Elevitch, C. R. (ed.), Species profiles for Pacific Island forestry. Permanent Agricultural Resources (PAR), Holualoa, HI

O'Dowd, D. J, P. T. Green, and P. S. Lake. 2003. Invasional 'meltdown' on an oceanic island. Ecology Letters **6**: 812-817.

Ness, J.H. & Bronstein, J.L. 2004. The effects of invasive ants on prospective ant mutualists. Biological Invasions, 6, 445–461.

Pangle, K. L., Peacor, S. D., and Johannsson, O. E. 2007. Large non-lethal effects of an invasive invertebrate predator on zooplankton population growth rate. Ecology 88: 402-412.

Parker, I. M. 1997. Pollinator limitation of *Cystius scoparius* (Scotch Broom), an invasive shrub. Ecology 78: 1457-1470.

Rudgers, J. A. and Clay, K. 2008 An invasive plant-fungal mutualism reduces arthropod diversity. Ecology Letters, 11: 831-840.

Rudgers, J. A., Holah, J., Orr, S. P, and Clay, K. 2007. Forest succession suppressed by an introduced plant-fungal symbiosis. Ecology 88: 18-25.

Rudgers, J. A., Savage, A. M. and M. A. Rua. 2010. Geographic variation in a facultative mutualism: consequences for local arthropod composition and diversity. Oecologia 163: 985-996.

Sakai, A. K., Allendorf, F. W., Holt, J. S., Lodge, D. M., Molofsky, J., With, K. A., Baughman, S., Cabin, R. J., Cohen, J. E., Ellstrand, N. C., McCauley, O'Neil, P., Parker, I. M., Thompson, J. N., and Weller, S. G. 2001. Population Biology of invasive species. Annual Review of Ecology and Systematics 32: 305-332.

SAS Institute. 2003. *SAS version 9.1.3*. SAS Institute, Cary, North Carolina, USA

Savage, A. M. and Whitney, K. D. *In review.* Mutualistic, trait-mediated indirect interactions in invasions: a highly invasive ant has unique behavioral responses to plant nectar. Functional Ecology.

Savage, A. M. and Peterson, M. A. 2007. Mutualism in a community context: the positive feedback between an ant-aphid mutualism and a gall-making midge. Oecologia 151: 280-291.

Savage, A. M., Rudgers, J. A., Whitney, K. D. 2009. Elevated dominance of extrafloral nectary bearing plants is associated with increased abundances of an invasive ant and reduced native ant richness. Diversity and Distributions 15: 751-761.

Savage, A. M., Johnson, S. D., Whitney, K. D., and Rudgers, J. A. *In press.* Do invasive ants respond more strongly to carbohydrate availability than co-occurring non-invasive ants? A test along an active *Anoplolepis gracilipes* invasion front. Austral Ecology.

Shattuck, S. O. 1999. Australian Ants: Their biology and identification. Monographs on invertebrate taxonomy,vol. 3. CSIRO Publishing, Collingswood, Victoria, Australia.

Shinen, J. S., and Morgan, S. G. 2009. Mechanisms of invasion resistance: Competition among intertidal mussels promotes establishment of invasive species and displacement of native species. Marine Ecology Progress Series 383: 187-197.

Snyder, W.E. & Allendorf, F.W. 2006. Ecological effects of invasive arthropod generalist predators. Annual Review of Ecology Evolution and Systematics, 37, 95–122.

Stachowicz, J.L. 2001. Mutualism, facilitation, and the structure of ecological communities. Bioscience 51: 235-246.

Veit, R.R., and M. A. Lewis. 1996. Population growth and the allee effect: dynamics of the house finch invasion of North America. American Naturalist 148: 255-274.

Wetterer J. K. 2005. Worldwide distribution and potential spread of the long-legged ant, *Anoplolepis gracilipes* (Hymenoptera: Formicidae). Sociobiology **45,** 77–96.

Whistler W. A. 1993. Flowers of the Pacific Island Seashore: A Guide to the Littoral Plants of Hawaii, Tahiti, Samoa, Tonga, Cook Islands, Fiji and Micronesia. University of Hawaii Press, Honolulu.

Wilson E. O. & Taylor R. W. 1967. The ants of Polynesia (Hymenoptera: Formicidae). Pacific Insects Monograph. **14,** 1–10.4.

4. 7 Tables:

Table 4.1a: Summary of significant responses of plant-foraging arthropod communities at sites dominated by *A. gracilipes* and sites dominated by other ant species to experimental manipulations of ant access and nectar level of *M. citrifolia* plants. Least squares means were calculated from models with plant size and the number of nectary bodies per plant as covariates and calculated from cumulative abundances per plant part per survey (see text for a more detailed explanation).

			Least Squares Means ± Standard Error						**$P_{permutation}$**		
Dominant ant species	**Trophic group**	**Significant response**	**Ant (+) Nectar (-)**	**Ant (+) Nectar (+)**	**Ant (+) Nectar (++)**	**Ant (-) Nectar (-)**	**Ant (-) Nectar (+)**	**Ant (-) Nectar (++)**	**Ant Access**	**Nectar Level**	**Ant access x Nectar Level**
A. gracilipes	Herbivores	Abundance	0.0116 ± 0.01	0.1076 ± 0.009	0.0131 ± 0.01	0.0567 ± 0.01	0.0330 ± 0.01	0.0144 ± 0.01	**0.0074**	0.2411	0.2033
		Richness	0.0791 ± 0.04	0.0718 ± 0.03	0.0931 ± 0.04	0.1892 ± 0.04	0.1869 ± 0.04	0.1324 ± 0.04	**0.0020**	0.7723	0.6390
	Detritivores	Abundance	0.0079 ± 0.02	0.0433 ± 0.02	0.0113 ± 0.02	0.0090 ± 0.02	0.0584 ± 0.02	0.0084 ± 0.02	0.5738	**0.0024**	0.6914
		Richness	0.0296 ± 0.05	0.2250 ± 0.04	0.0.65 ± 0.04	0.0370 ± 0.05	0.0609 ± 0.05	0.0213 ± 0.05	0.1197	**0.0113**	0.1023
Other ants	Herbivores	*Richness*	0.2076 ± 0.07	0.1019 ± 0.05	0.2864 ± 0.07	0.2407 ± 0.08	0.1857 ± 0.07	0.0213 ± 0.08	0.5481	0.4200	*0.0585*

Table 4.1b: Summary of significant responses of plot-wide arthropod communities at sites dominated by *A. gracilipes* and sites dominated by other ant species to experimental manipulations of ant access and nectar level of *M. citrifolia* plants. Least squares means were calculated from models with average plant size, average nectary bodies per plant, and density of *M. citrifolia* plants per plot.

			Least Squares Means ± Standard Error						**$P_{permutation}$**		
Dominant ant species	**Trophic group**	**Significant response**	**Ant (+) Nectar (-)**	**Ant (+) Nectar (+)**	**Ant (+) Nectar (+ +)**	**Ant (-) Nectar (-)**	**Ant (-) Nectar (+)**	**Ant (-) Nectar (+ +)**	**Ant Access**	**Nectar Level**	**Ant access x Nectar Level**
A. gracilipes	Herbivores	*Evenness*	0.0441 ± 0.18	0.2093 ± 0.12	0.3026 ± 0.18	0.5022 ± 0.19	0.6225 ± 0.19	0.8702 ± 0.18	***0.0597***	*0.0968*	0.8303
	Carnivores	Abundance	1.7184 ± 0.84	1.7355 ± 0.58	1.6138 ± 0.89	3.5354 ± 0.92	2.0663 ± 0.89	0.7619 ± 0.87	*0.0826*	***0.0567***	*0.0947*
		Richness	1.5578 ± 0.63	1.4852 ± 0.43	1.6255 ± 0.66	3.0421 ± 0.68	1.5511 ± 0.66	0.4197 ± 0.65	0.5549	**0.0334**	**0.0005**
	Detritivores	Abundance	1.0863 ± 0.42	0.8328 ± 0.29	1.4638 ± 0.45	0.2608 ± 0.46	1.6467 ± 0.45	0.7101 ± 0.44	0.4684	**0.3318**	**0.0478**
Other ants	Carnivores	Richness	1.8400 ± 0.84	2.6554 ± 0.56	2.8693 ± 0.79	1.8367 ± 0.79	0.9801 ± 0.79	1.8644 ± 0.82	**0.0020**	**0.0361**	**0.0338**
		Diversity	0.5583 ± 0.29	0.8710 ± 0.20	0.8655 ± 0.27	0.6031 ± 0.28	0.1669 ± 0.28	0.3973 ± 0.29	**0.0119**	0.1260	0.3159

4.8: Figures:

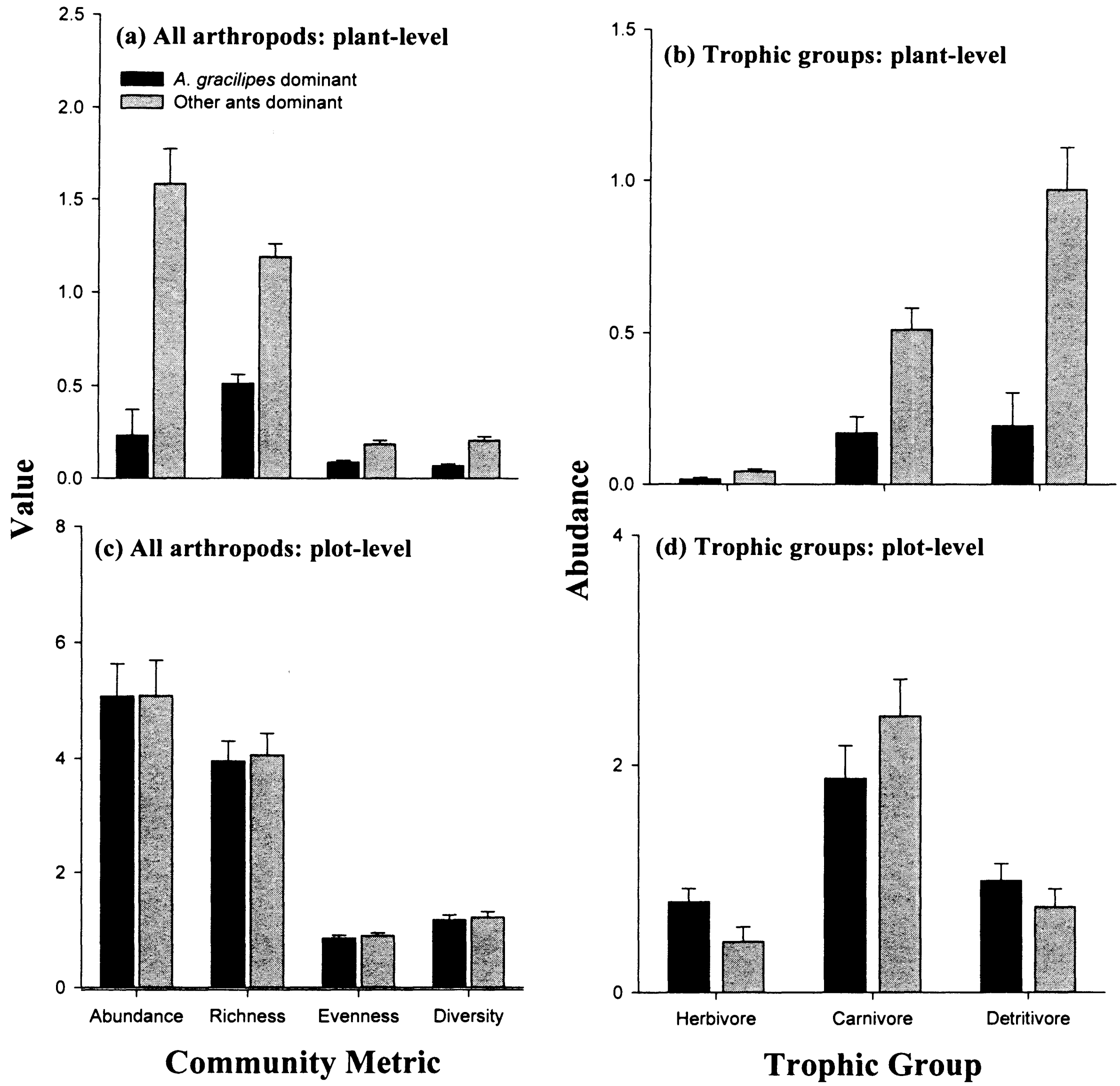


Figure 4.1: Summary of (a) plant-level arthropod community diversity metrics, (b) relative abundances of plant-visiting arthropods by trophic group, (c) plot-wide arthropod community diversity metrics and (d) plot-wide relative abundances of arthropods by trophic group across sites dominated by *A. gracilipes* and those dominated by other ant species.

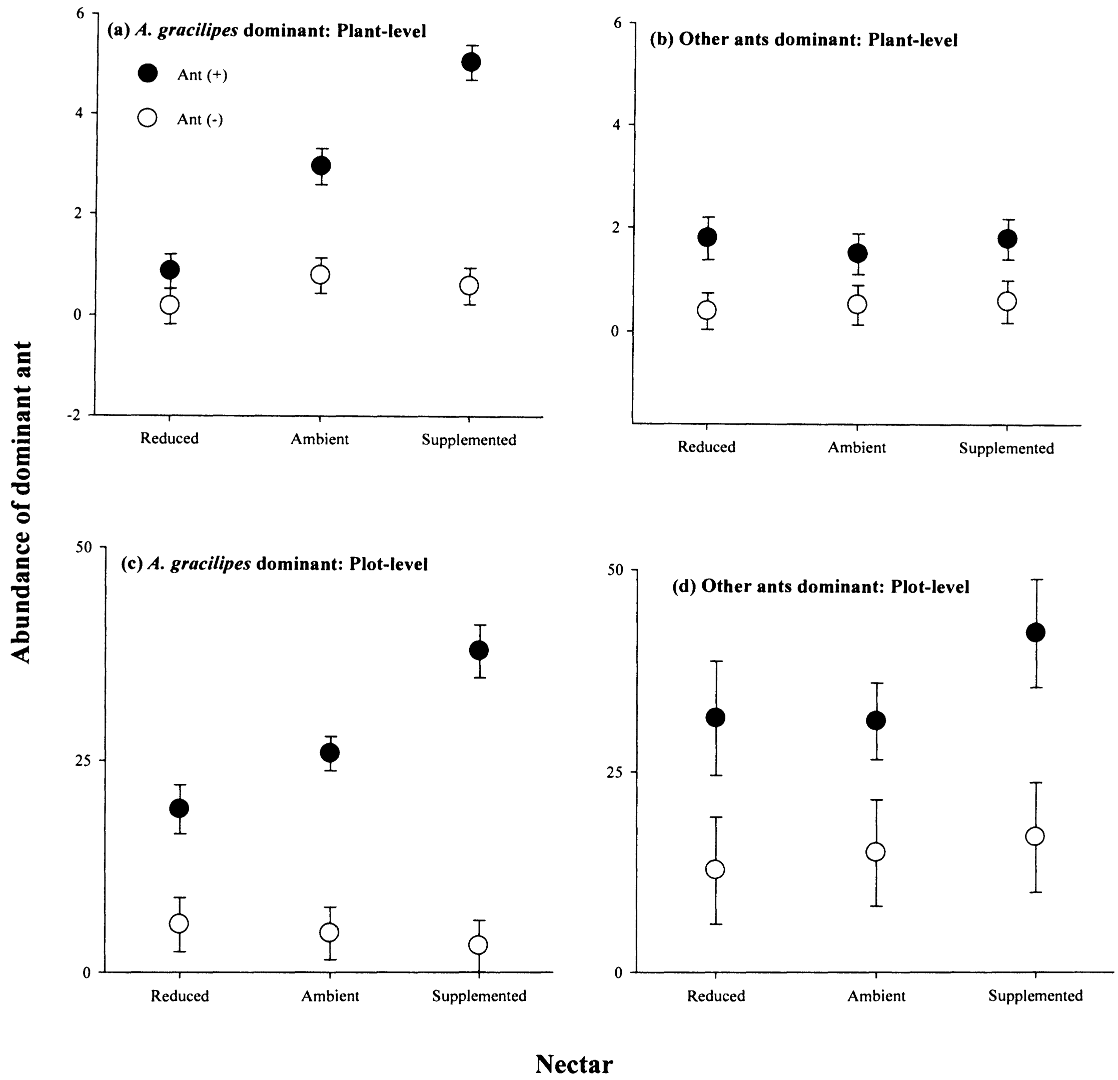


Figure 4.2: Average (a) abundances of *A. gracilipes* on plants, (b) abundances other dominant ants on plants, (c) plot-wide abundances of *A. gracilipes*, and (d) plot-wide abundances of other dominant ants plot-wide across all treatment combinations.

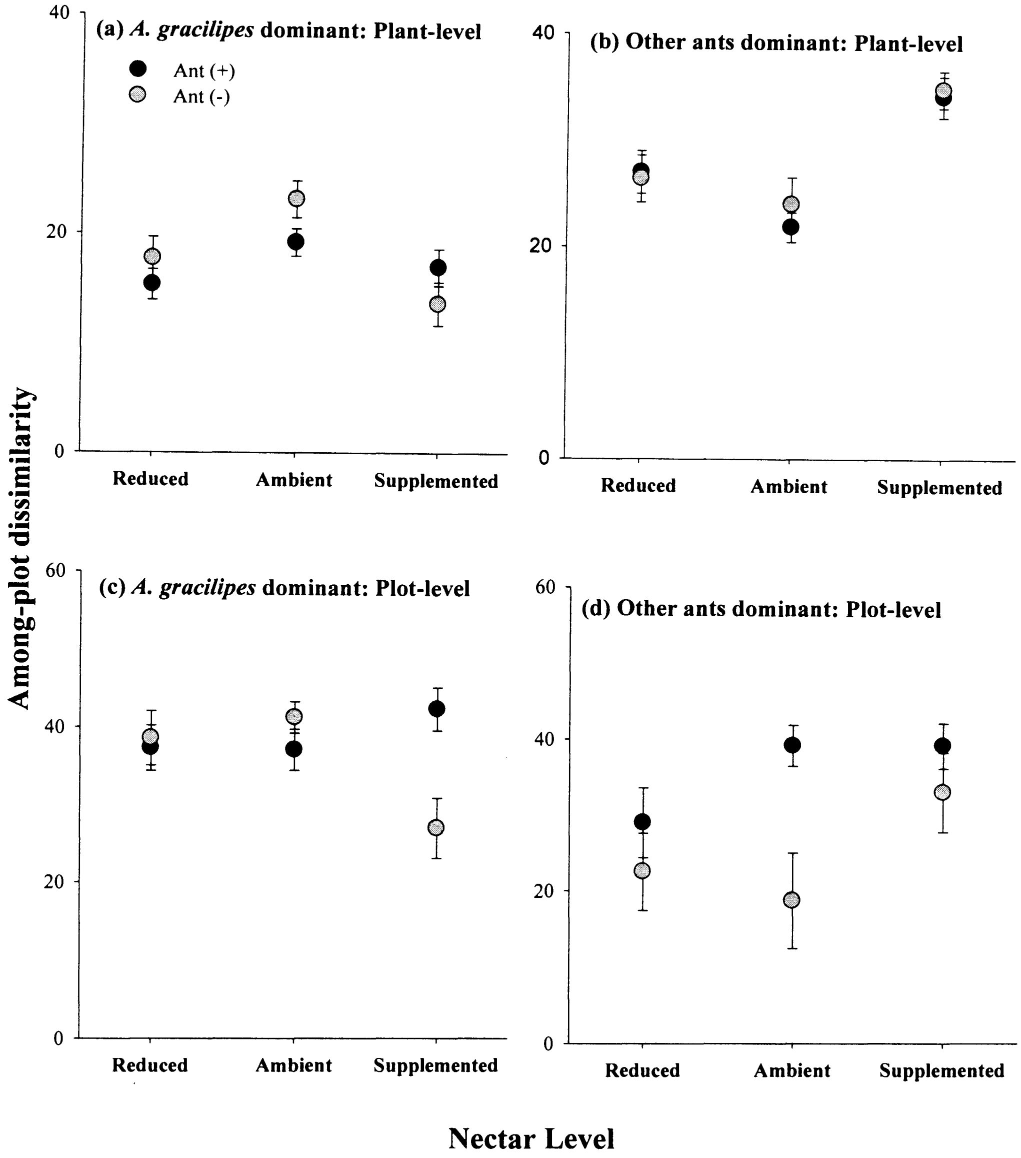


Figure 4.3: Relative among-site dissimilarity in arthropod morphospecies composition across ant access and nectar availability treatments for (a) *M. citrifolia* plants at sites dominated by *A. gracilipes;* (b) *M. ctirifolia* plants at sites dominated by other ant species; (c) entire plots at sites dominated by *A. gracilipes;* and (d) entire plots at sites dominated by other ant species. Data were analyzed using the PERMDISP procedure.

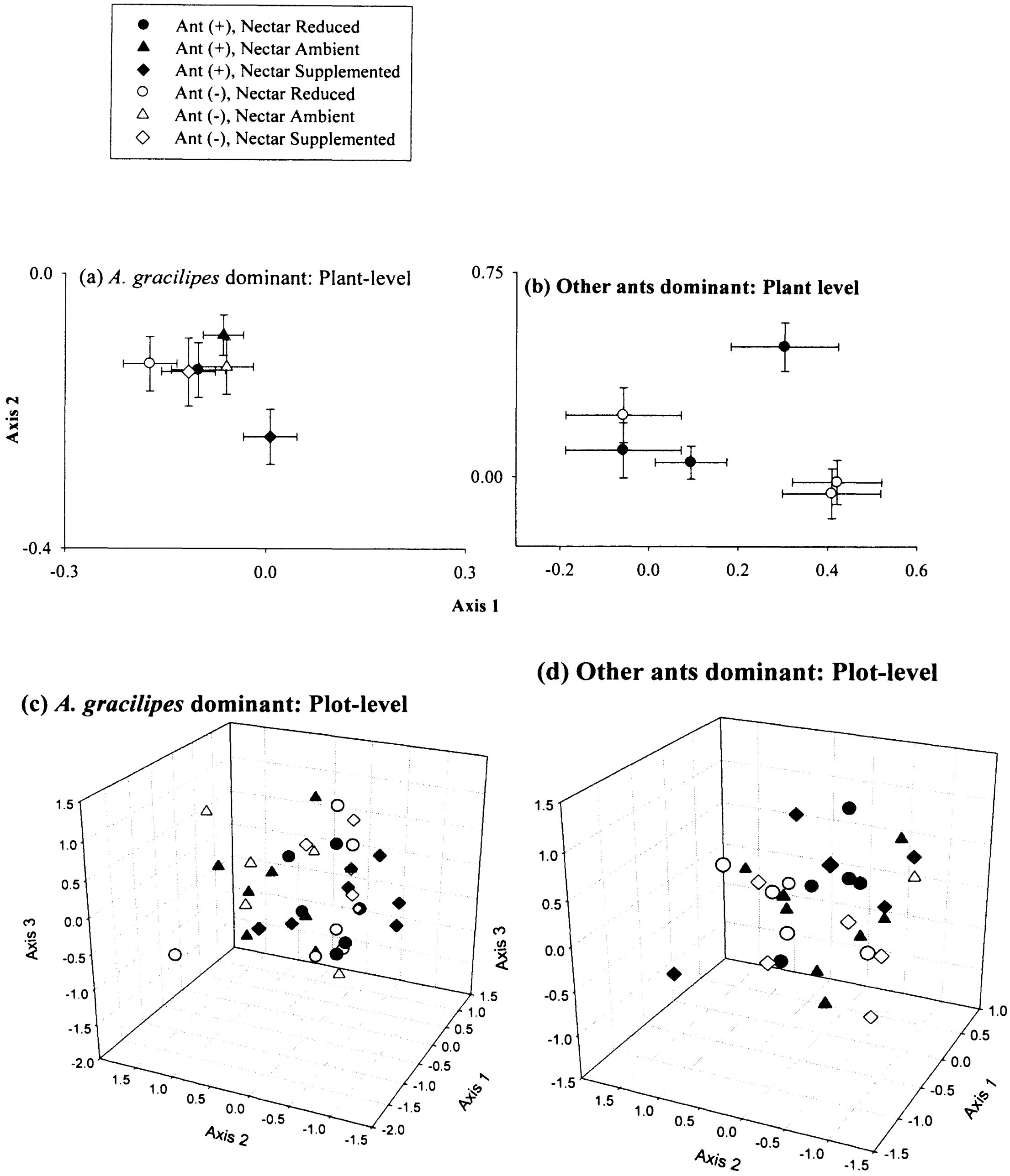
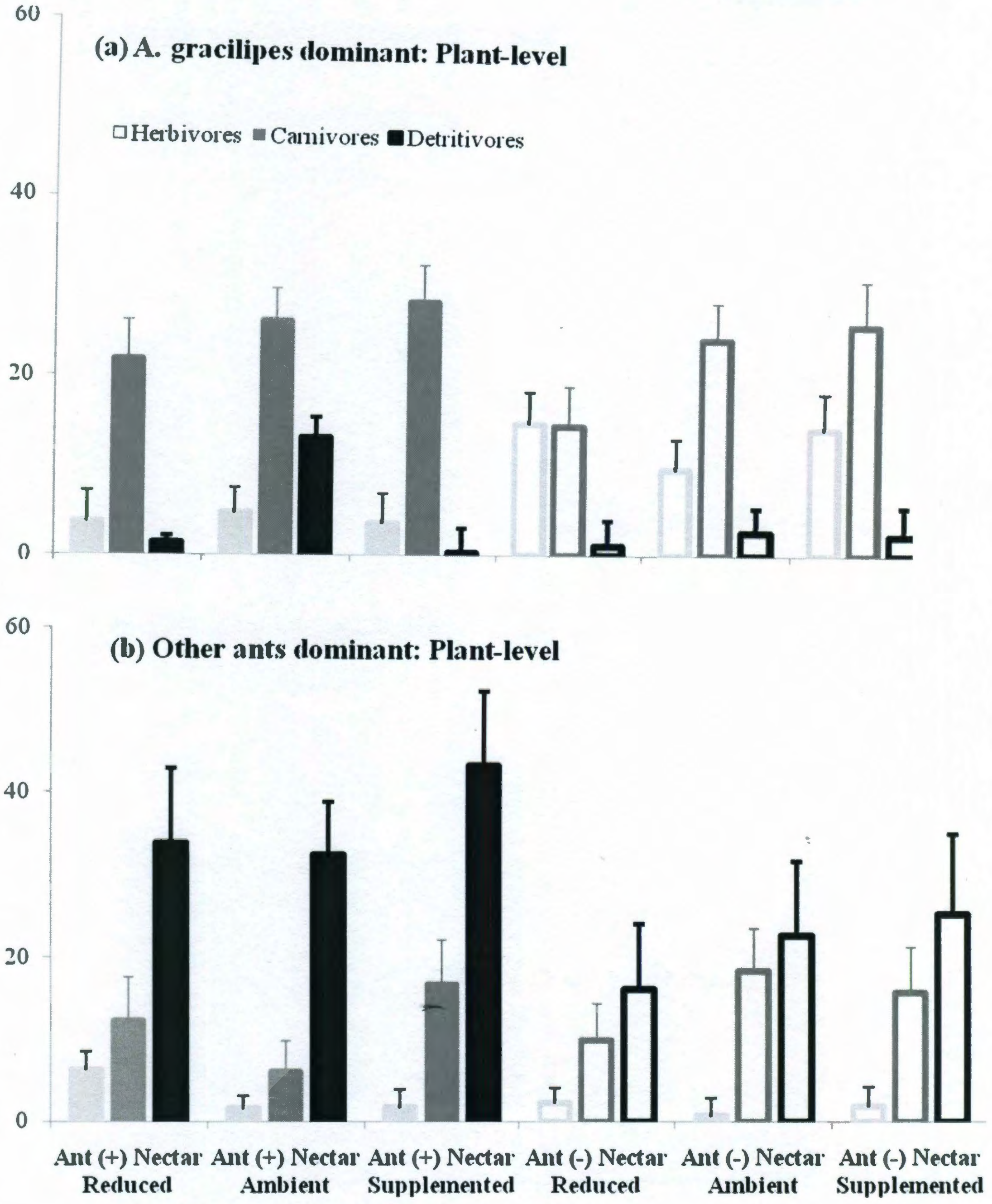


Figure 4.4: Relative within-site diversity of arthropod morphospecies compositon across ant access and nectar availability treatments for (a) *M. citrifolia* plants at sites dominated by *A. gracilipes;* (b) *M. ctirifolia* plants at sites dominated by other ant species; (c) entire plots at sites dominated by *A. gracilipes;* and (d) entire plots at sites dominated by other ant species. Plant-level plots include centroids ± 1SE.


60
(a) A. gracilipes dominant: Plant-level
□Herbivores ■Carnivores ■Detritivores
40
20
0
60
(b) Other ants dominant: Plant-level
40
20
0
Ant (+) Nectar Reduced
Ant (+) Nectar Ambient
Ant (+) Nectar Supplemented
Ant (-) Nectar Reduced
Ant (-) Nectar Ambient
Ant (-) Nectar Supplemented

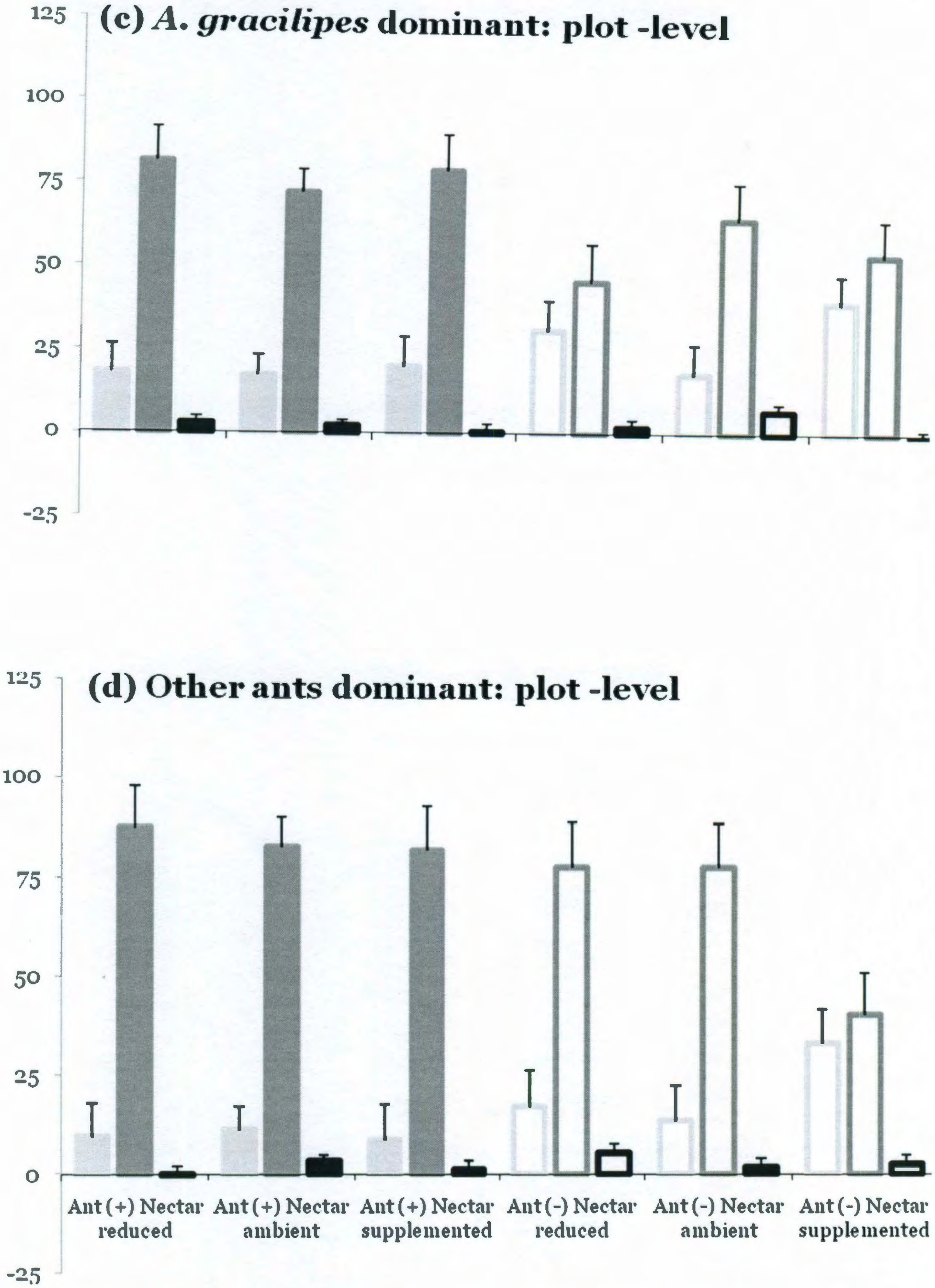


Figure 4.5: Percentages of herbivores, carnivores and detritivores across all treatment combinations at the plant (a-b) and plot (c-d) level.