AN INVESTIGATION OF MUTUALISTIC BEHAVIORS AND THE EVOLUTIONARY TRAIT OF EXTRAFLORAL NECTARIES ON *MORINDA CITRIFOLIA*

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Abstract. Extrafloral nectaries are one of many defense mechanisms that plants have evolved to counteract predators and insect herbivores. In addition, extrafloral nectaries (EFN's) is a type of nectar secreting organ which primarily function in plant defense. Often associated with the presence of aggressive ants, this evolutionary trait is known to be commonly found in tropical plant species like the commercially popular *Morinda citrifolia* tree. Thus, this study aims to investigate different factors that could potentially influence the interactions seen between insects and *M. citrifolia* on the island of Moorea, French Polynesia. Moreover, this study will look at how ant distribution and ant species composition will vary across time and space, and what these findings from this study can imply for future research. The variables observed in this study were ant counts, ant species composition, and leaf damage. These factors were looked at in response to 4 independent treatments: day vs. night, different developmental stages of the tree, various microhabitats, and fruit size. It was found that all dependent measures were highly variable amongst different microhabitats of the Noni tree rather than throughout different parts of the day. The biological implications that can be made within the confines of this report suggest that EFN's are indeed aiding in the Noni tree's fitness and survival on the island, and that this is important when trying to look for future solutions in combatting high amounts of herbivorous activities seen with climate change.

Key words: Morinda citrifolia*, plant-insect interactions, extra-floral nectaries,* Solenopsis geminata, Paratrechina longicornis*, canoe plants, cryptic herbivores*

INTRODUCTION

Spanning the South Pacific Ocean, Oceania and its islands can be divided into three parts: Micronesia, Melanesia, and Polynesia (Merlin 2000, Whistler 2009). Coming from volcanic activity that first occurred millions of years ago, these isolated archipelagos gave rise to hospitable environments and open niches in which many organisms and plant communities have been able to successfully colonize and thrive (Gillespie and Baldwin 2010). Mechanisms of transport for different species include natural means such as wind travel, river rafting, island hopping, and/or seed dispersal by birds (Gillespie and Baldwin 2010). In contrast, other organisms are introduced via anthropogenic means, as is particularly true for "canoe plants", or introduced plants that are considered to be of importance by Polynesian voyagers and placed in their canoes during their long journeys across the South Pacific Ocean (Whistler 2009).

Of the many canoe plants that Polynesians had taken on their voyages, 6 have been documented to be the most commonly carried by seafarers: *Aleurites moluccana, Morinda*

citrifolia, *Eugenia malaccensis, Solanum nigrum, Saccharum officinarum*, and *Ipomoea batata* (Abbot and Shimazu 1985). Although these plants have been carried around for centuries across Oceania, it was not until recently that one particular plant, *Morinda citrifolia*, has grown in popularity across the globe (McClatchey 2002, Dixon et. al 1999).

Morinda citrifolia and its varieties are part of the *Rubiaceae* plant family and originate from Southeast Asia (Singh et. al 2011, Razafimandimbison 2010). Commonly known as the "Noni" in French Polynesia, the plant is a small evergreen, monoecious tree that can grow up to 10 meters tall (Elevitch 2006). Its smooth margined leaves are arranged in a decussate-like pattern, with each simple and ovately shaped leaf attached to the stem and stipules using a petiole. Blades can grow up to about 45 cm long and 25 cm wide, and fruits up to 8 cm in diameter (Elevitch 2006). The plant can be identified by its green, immature, and aggregate fruits that become a yellow translucent color when ripe. The fruits contains seeds which are held in protective air chambers that allow the seeds to survive for months after being dispersed in ranges of habitats such as low-elevation lava flows, rocky coasts, and high saline soils (Elevitch 2006; Razafimandimbison 2010). Furthermore, the Noni plant can be identified by the types of insects it attracts and tissue damage that it accrues. This is particularly important because as Noni products gain popularity, they have been planted more widely across the globe (Elevitch 2006). With this increase in Noni cultivation, potentially invasive insects and related agricultural pests have often followed (Elevitch 2006).

The kind of pests and herbivory that the Noni plant attracts includes aphids, scale insects, leaf miners, weevils, whiteflies, caterpillars, thrips, and mites (Elevitch 2006). Interestingly, the plant also attracts ants, which are considered to be both beneficial and harmful for the Noni plant. This is especially true in disturbed habitats where ant-foraging behavior is relatively high (Bentley 1976). These ants tend to be associated with morphological features such as extrafloral nectaries found on the Noni tree and many other plant species (Keeler 1985, Savage and Rudgers 2013, Nelsen et al. 2018). Extrafloral nectaries (EFN's) are nectar-secreting organs found outside of the flower whose primary function is not involved in pollination, but in plant defense (Keeler 1985, Oliveira et. al 1999; Weber and Keeler 2013). This plant defense mechanism, commonly found in the tropics, had evolved during the mid-Cretaceous period and is often seen recruiting defender insects that increase plant fitness (Wilson and Holldobler 2005, Nelsen et. al 2018, Marazzi et. al 2013).

On the Noni plant, the EFN's are said to be located in the circular cavities of the fruit that may also contain regular nectaries and post-floral nectaries as well (Savage and Rudgers 2013, Keeler 1985). These nectary cavities are what secretes the sugars that have been seen to attract ants on the Noni tree for plant defense (Savage and Rudgers 2013, Keeler 1985). In contrast to this mutualism with plants, ants have also been categorized as "cryptic herbivores" by other scientists (Wilson and Holldobler 2005). In other words, ants are also attracted to honeydew-producing insects (HPI's) which destroy plant tissue. This evolutionary behavior amongst these two insects had evolved around the early Eocene period, and in one study has been shown to dramatically increase HPI abundance by 8200% (Wilson and Holldobler 2005, Styrsky and Eubanks 2007).

This study aims to investigate ant-plant mutualisms in response to insect herbivory as seen in the tropical evergreen tree - *Morinda citrifolia,* or the "Noni". More specifically, this study will focus on how insect herbivory may differ across time and space throughout a

species' life cycle, and how the Noni tree has adapted to utilize ants to their advantage among their different plant parts. This research will explore whether or not there is a difference in ant distribution during 24 hour periods, and to infer what this might say about the pest-host interactions between omnivorous insects and plants. My hypothesis is that there is a difference in ant activity between the day and night, and that this may implicate how strongly the two different ant-attractants are in comparison to one another. If my hypothesis is correct, does this infer anything more about insect herbivorous activity on the Noni? And if so, how does varying herbivory levels change throughout different developmental stages of the tree (between immature trees with no fruit vs. intermediate trees vs. older trees who bear fruit and EFN's)? Is there a correlation between fruit/EFN onset and percent herbivory found on this plant species? And if so, what might this say about the relationships between the plant species and insects through broader levels of time and space?

METHODS

Study site

This study was conducted on Moorea, French Polynesia; an island located in the Southern Pacific Ocean (17°32′ S, 149°50′W). In particular, data were gathered from individual *M. citrifolia* ("Noni") trees located at the Atitia Center, adjacent to the UC Gump Research Station. The Atitia Center is a public community space built in 2004 by local people on the island whose main goal was to preserve traditional knowledge and Polynesian biocultural heritage ("Gump station" 2018).

Study organisms

Based off a previous study also conducted on Moorea, French Polynesia, the species of ants that exist at the Atitia Center are *Solenopsis geminata* and *Paratrechina longicornis*. These two ant species are considered to be non-native and invasive "tramp ants" that have spread all around the globe and eventually reaching French Polynesia through human activity and commerce (Thomas 2010, Wetterer 2008)*. Solenopsis geminata* ("The Red Fire ant") are aggressive ants that have been documented to recruit food in higher numbers than any other ant species (Wetterer 2010). They also alter populations of other insects (Wetterer 2010). In comparison, *Paratrechina longicornis* ("Longhorn Crazy Ant")*,* is erratic in behavior and has been documented to be the most abundant and widespread "tramp ant" in the world (Banks and Williams 1989, Wetterer 2008). Both species of ants have been introduced to the island of Moorea, French Polynesia through anthropogenic means, and now exist in abundance in human-disturbed habitats such as the Atitia Center.

Tree & Microhabitat Classifications

I compared ant activity on nine separate individual *M. citrifolia* trees representing a range of ages: three young aged trees, three intermediate aged trees, and three adult sized trees. Trees were grouped by age and different groups were separated by at least ten meters. Only one of the three adult sized trees were analyzed for ants because of ease of physical access to the crown.

Ages of the trees were identified by whether or not they bore fruit: young trees were identified as trees with no fruit, intermediate trees were identified having only immature green fruit, and old trees were identified as bearing both immature and

mature fruit. At least three branches were analyzed per group (young, intermediate, old trees). Each branch was assigned a random number, and a random number generator application was used to select the branch to be analyzed for ant count (Version 1).

The microhabitats of each branch that were analyzed were: old leaves, young leaves, and fruits. To standardize for the amount of variation in the number of leaves per branch, two leaves from the first two nodes closest to the trunk were randomly selected for the "old leaves" category, and two leaves from the last two nodes furthest from the trunk were randomly selected for "young leaves" category.

Time

Field observations were collected at specific times of the day, across a set number of days. All day-time analyses were conducted between the hours of 10:00 and 17:00. All night time analyses were conducted between the hours of 20:00 and 00:30 of the following day. For every 24 hours, 15 randomly selected branches from 7 Noni trees were analyzed for the probability that ant distribution between the day (hour 10:00 to 17:00) versus the night (hour 20:00 to 00:30 of the following day) were the same. Pictures of all plant parts were also taken during day time periods using a white backdrop with three different scale bars: 1 inch, 1 cm, and 1 mm. These pictures were used to identify branches during the night to ensure that the same branches were analyzed as part of the day versus night comparisons.

Space & Insect Activity

At least 3 branches per tree age were randomly selected for analyses everyday. For each unit on a branch, I counted the number of ants for one minute. Ants were determined to be of different species in the field based on

color (red vs. black). In addition, qualitative observations upon behavior (aggressive vs. erratic) were informally noted to determine ant species. Aggressive behavior was defined as lunging or attacking other species and organisms. Erratic behavior was defined as avoidance when encountered by other species, including the other species of ant. All fruits present were also measured for their diameters using a ruler in the units of centimeters. To ensure that selections were not biased, branches and microhabitats were assigned a number, and a random number generator was used for selection (Version 1). Pictures taken during the day were used to calculate percent herbivory levels and leaf damage using Adobe Photoshop CC 2019 and an insect damage guide (Labandeira et al. 2007, Version 20.0.1). Data obtained from 3 leaves per branch for a total of 9 randomly selected leaves were used to test for differences seen in percent leaf damage across differently aged trees.

Statistical Analyses

First, day vs. night comparisons were made to address the possibility that the number of ants on trees of a particular age or tree parts of a particular stage were influenced by the time of day. For this comparison, I used a sign test to compare the number of ants to the variations of time throughout multiple 24 hour cycles. The sign test was used to test if there is a significant difference in the means of ants counted between the day vs. night, and what these interspecific interactions may reveal about how well extrafloral nectaries are doing to attract ants in comparison to honeydew secreting insects. No difference in counts by time of day was found between the two species (see Results). Therefore, I focused all analyses on numbers counted during the day for two reasons: (1) using counts from the night time yielded the same types of results,

and (2) using counts from one part of the day rather than both will eliminate the chances of committing pseudo replication and technical errors in statistical analyses.

To determine if total ant counts differed among trees of different ages (young, intermediate, old) and microhabitats (old leaves, young leaves, and fruits); two X^2 test for independences were employed: a 3x2 test that eliminated the fruit category, and a 2x3 test that eliminated the young tree category. Because errors were outputted using PAST when looking at individual species counts across 3x2 and 2x3 categories, the differently aged trees and microhabitats were separated into 3 different data sets to compare ant species distribution among differently aged trees and their microhabitats. To avoid pseudo replication again, averages for day time observations per tree age were used.

No significant difference was found between ant distribution and tree age (see results). Therefore, a further analysis was conducted to determine if different microhabitats on each of the individual branches are, in fact, what is driving the differences seen in ant activity and composition in the Noni tree. Here, I used a X^2 square analysis to test for goodness of fit, which tests to see if ants are evenly distributed on the different branch units or not.

Since the amount of ants found on different units of the branch may vary, then what is driving this influence? Is it the honeydew producing insects found on the leaves, or the nectaries found on the fruits? To answer this question, a regression analyses will be used. In particular, a regression analysis will be used to identify if there is a positive relationship between ant count and fruit size. Because the response variable is count of ants, I will use a Poisson regression with log link function to test the non-normally distributed data.

A kruskal-wallis test will be conducted on old and young leaves across differently aged trees to determine if the amount of leaf damage present varies with the different life stages of the plant.

All statistical analyses will be conducted within the Past Software Program, using alpha = 0.05 (Hammer et al. 2001**).**

RESULTS

Day vs. Night

A sign test was used to test for significant differences in the non-normally distributed ant counts between day versus night. Each microhabitat was treated independently every 24 hour period. P-values from the sign test suggest that there is no significant difference in ant count between day versus night for *S. geminata, P.longicornis,* and their total numbers added together (Table 1).

TABLE 1. Ant distribution differences between day (10:00 – 17:00) vs. night (20:00 – 00:30 of the following day) on independently sampled microhabitats*.*

24 Hour Period	Ant Samples	P-value
#1	All ants	1
#2	All ants	0.75523
#3	All ants	0.065994
#1	*S. geminata*	1
#2	*S. geminata*	0.5572
#3	*S. geminata*	0.22952
#1	*P. longicornis*	1
#2	*P. longicornis*	0.012939
#3	*P. longicornis*	0.70111

Notes: The left column lists each 24 hour cycle conducted throughout this study. The middle column represents the type of ants being analyzed on microhabitats. The right column lists P-values for the Sign Test which tests the probability that ants distribution is independent of the time of day. The test fails to reject the Null Hypothesis.

Two separate X^2 tests for independences were used in this analysis because young trees did not bear fruit (Fig. 1). When eliminating the fruit category, the average number of total ant counts were significantly independent of tree age and microhabitats ($X2 = 2.405$, df = 1, p-value= 0.29011,). Similarly when eliminating the young tree category, these two types of categorial variables were not significantly influencing the average number of total ant distribution ($X2$: 0.59328, df = 1, p-value: 0.74331,).

Further X^2 tests of independences reveal that *S. geminata* vs. *P. longicornis* were evenly distributed amongst young trees and its microhabitats (Fig. 2.1, $X2$: 1.3333, df = 1 p-value = 0.2481), but not intermediate aged trees (Fig. 2.2, , $X2 = 15.36$, df = 2, p-value = 0.000462) and old trees (Fig. 2.3, $X2$: 6.5185 , df = 2, p-value: 0.038417). When comparing figures 2.1, 2.2, and 2.3 it is also evident that black ant counts stay relatively the same across differently aged trees, and red ant counts comprise a majority of ants seen in the sampled population.
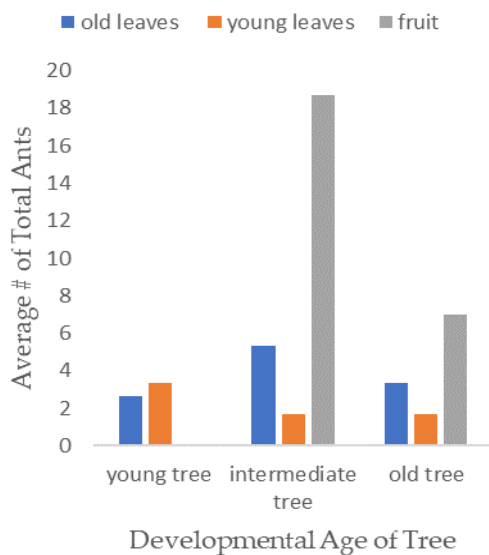

FIG. 1.   Number of total ant counts averaged among all observations made

between 10:00 to 17:00 on differently aged trees and their microhabitats. Two X^2 analyses reveal that the ant distribution is significantly independent of tree age and branch parts. ($X^2 = 2.405$, df = 2, p-value = 0.29011; $X^2 = 0.59328$, df = 2, p-value = 0.74331).
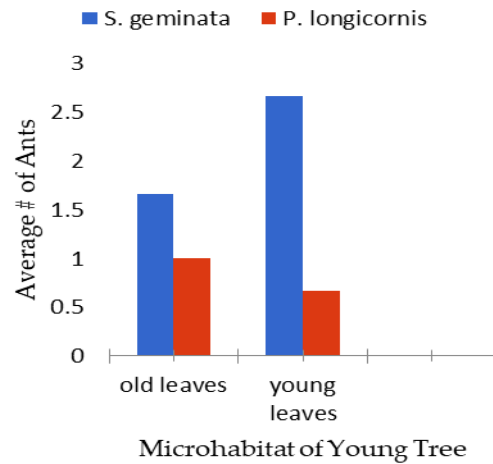

FIG. 2.1.   Ant species distribution averaged across daytime observations on young aged trees and their microhabitats ($X^2 = 1.3333$, df = 1, p-value = 0.2481).
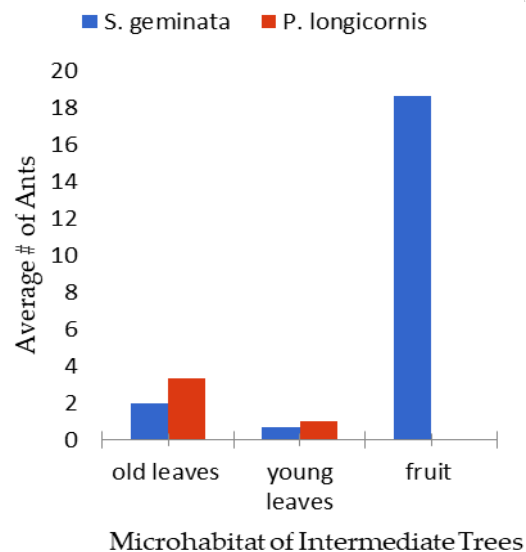

FIG. 2.2.   Ant species distribution averaged across daytime observations on intermediate   aged   trees   and   their microhabitats ($X^2 = 15.36$, df = 2, p-value = 0.000462,).
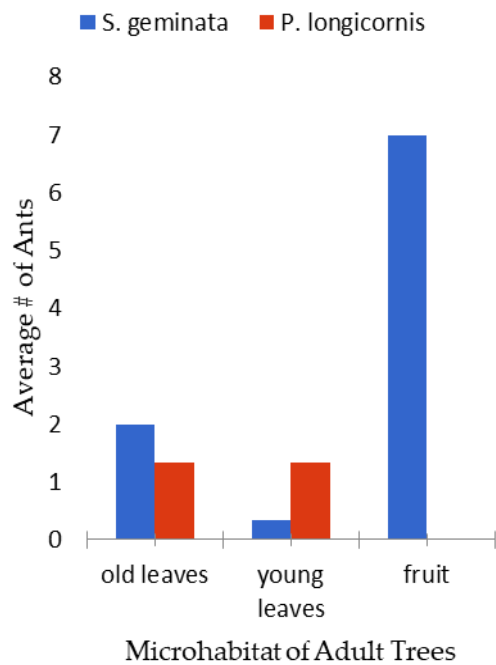
FIG. 2.3. Ant species distribution averaged across daytime observations on adult aged trees and their microhabitats (X^2 = 6.5185, df = 2, p-value = 0.038417).
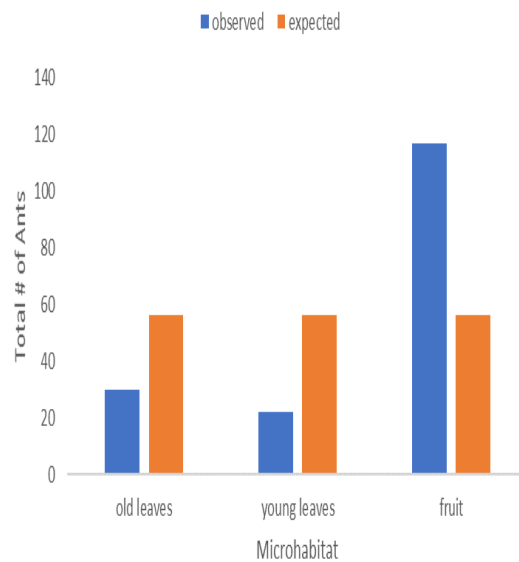
FIG. 3. Expected vs. observed values of total ant distribution compared upon 24 different and independently sampled Noni branches with both leaves and fruit (X^2 = 98.574, df = 2, p-value = 3.9351E-22).

Microhabitat

A total of 24 different and independently sampled branches with both leaves and fruits were used to test if the distribution of ants across a noni branch would be the same if all units of the branch were present (Fig. 3). According to the X^2 test for goodness of fit, total ant distribution was not independent of different microhabitats, and that this distribution is heavily skewed towards fruit (X^2 = 98.574, df = 2, p-value = 3.9351E-22).

Fruit Size

Using the Poisson distribution and log link function to model the non-normally distributed data of fruit size and total ant count, the probability that ants were randomly and independently distributed among different fruit sizes resulted in a p-value of 0.010884. This p-value is representative of the shape of the line that best fits the spread of data (Fig. 4). From the spread of the data, it is assumed that there is no correlation shown between fruit size and total ant count.
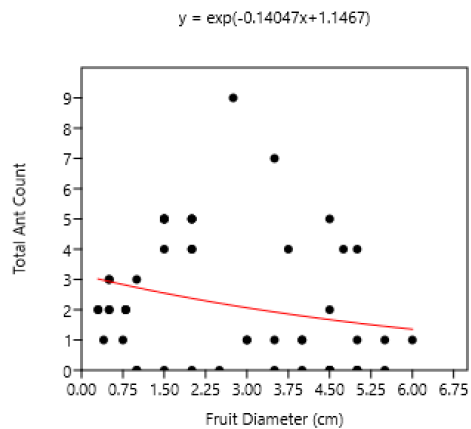
FIG. 4. Analysis of Fruit Diameter (cm) vs. Total Ant Count modeled by the Poisson Distribution and log link function. The model describes the relationship which exponentiates the linear predictors to the mean of the distribution function (y=exp(-0.14047x+1.1467). A p-value of 0.010884 was given for the line that best fits the data.

FIG. 5. Percent leaf damage across differently aged Noni trees (p-value for young leaves = 0.5501, p-value for old leaves = 0.02732)

Percent Herbivory

A Kruskal Wallis test shows that the percent damage of young leaves across differently aged trees is significantly the same (p-value= 0.5501). However, amongst old leaves, percent damage is significantly different across differently aged trees (p-value= 0.02732) (Figure 5).
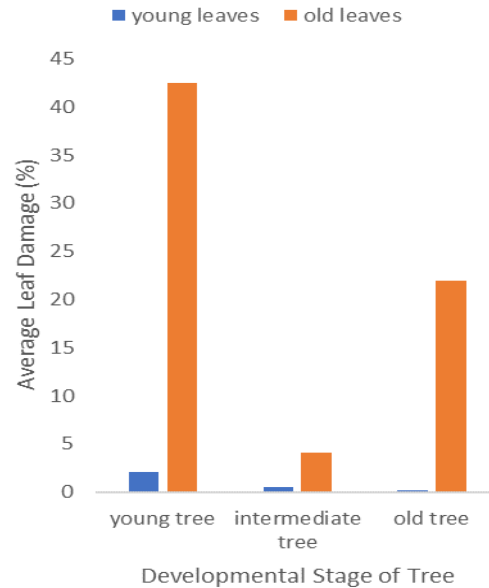
DISCUSSION

The main purposes of this study were to investigate how different factors may affect ant activity and insect herbivory across time and space within differently aged *Morinda citrifolia* ("Noni") trees and their different microhabitats. The ant species that were observed in this study were *Solenopsis geminata* ("The Red Fire Ant") and *Paratrechina longicornis* ("The Longhorn Crazy Ant"). Several factors that may have affected their total distribution patterns were described in this study: the time of day, the developmental age of the tree, various microhabitats, fruit size, and insect herbivory. Results from statistical analyses revealed that, among these different factors, the presence of fruit significantly influenced the distribution and species composition of the ants, whereas the developmental age of the Noni trees may only play a minimum role or none at all when it comes to changing the same response variables. In addition, herbivorous activity

and percent leaf damage seen on the leaves were recorded. Again however, the effects of the developmental life stages upon the damage seen on the leaves remain unclear.

First, day versus night time observations were assessed to avoid pseudo replication in later analyses. Statistical tests revealed that independently sampled microhabitats at multiple 24 hour cycles were statistically insignificant, and that ant distribution and ant species composition were not uniquely different between the two time periods. This alludes to the implications that foraging times for ants may be dependent upon species type and temperature tolerances rather than the existence of light throughout different times of the day. This claim is especially evident in other studies that have taken place in different environments where temperatures are seen to vary more greatly between day versus night (Cros et al. 1997, Alsina et al. 1988, Diaz-Castelazo et al. 2004, Lessard et al. 2008, Spotti et al. 2014, Botnevik et al. 2016, Lange et al. 2017). On average, ants can withstand up to 40°C and have peak levels of activity around 32°C (Holldobler and Wilson 1990). Moreover, *S.geminata* and *P. longicornis* have similar temperature tolerance levels ranging from 22°C to 40°C (+/- 3°C) in tropical climates (Harris and Barker 2007, Lee 2002). This may explain why the two non-native ant species seen in this study at this particular site and time of the year were not influenced by times of day, but rather the temperature that may not have varied much during the recordings and location of my study. Future studies may further investigate this claim by taking formal temperature recordings in the field and performing controlled lab experiments that manipulate ant behavior with different temperatures ranges. Furthermore, future studies may also want to consider larger time scales, in which case they can account for greater and more definitive temperature variations that may change with seasonality,

or with deep time when studying effects of climate change on ants and insect activity (Andersen 1983, Cros et al. 1997, Fergnani et al. 2008).

Secondly, the total number of ants was averaged across all day time observations and was tested against differently aged trees and individual branch parts. This revealed that there were significant differences in ant species counts, and that this difference was only present on microhabitats of intermediate and old aged trees. In contrast, when disregarding developmental age of the tree, total ant distribution was significantly skewed towards fruits on independently sampled branches that contained all 3 types of microhabitats analyzed in this study. These findings suggest that the developmental age of the tree may be limited in its role to control for the amount of ants seen on the trees. Rather, fruit onset may account for the differences in ant distribution and species composition seen on only the intermediate and old aged trees since they only bear fruit. This makes sense as we see that the total number of ants counted in this study was mostly comprised of *S.geminata*, and that all types of floral nectaries on the Noni only exist on the drupelets of fruits (Savage and Rudgers 2013, Keeler 1985). Higher levels of sugar content in either the nectaries or honey-dew are seen to be associated with more aggressive ants (Wackers et al. 2005). Similarly, one study discovered that ants tended towards higher sugar content on different plant species and areas (Lee 2002). In line with other studies, my findings suggest that the fruits on Noni trees may be producing higher concentrations of sugar content that attracts ants towards their floral nectary cavities rather than the sugar secreted by honeydew-producing insects . This assumption is likely to be independent of tree age. To further test this claim and make it clear as to what extent tree ontogeny has on insect activity, future studies can incorporate

the use of artificial sugars and ant attractants on trees bearing no fruit (i.e. young trees). In contrast, these same future studies can also use tanglefoot product on intermediate and old aged trees to see where ants would go when access to fruits are blocked.

Thirdly, although fruit onset seemed to matter more when it came to changing the total distribution of ant counts for a particular species of ants, the diameter of the fruit did not. In this case, it may be helpful to consider other factors that may play a role in influencing the distribution of ants and ant species on already existing fruits and EFN's. Such factors are: relative humidity, distance of fruits from ant nesting areas, ratios of fruit surface area to sugar concentration levels, availability of other food sources around the fruit for ants, shaded versus non-shaded fruits, precipitation, etc. (Chong and Lee 2006, Lange et al. 2017, Fergnani et al. 2008). I recommend that future studies try to incorporate these several factors into their own experiments to further grow an understanding of the mechanisms underlying ant recruitment to EFN's and/or HPI's.

Lastly, my study observations also looked at how differently aged trees influenced percent herbivory levels, and how leaf damage can hint at the causes for differences in insect activity seen on the Noni plant. As seen in my results, tissue damage was only significantly different in old leaves, but not young leaves across differently aged trees. One possible explanation for why old leaves may have more herbivorous activity compared to young leaves is that old leaves have had relatively longer exposure times to herbivorous insects within their environment (Boege and Marquis 2005). Furthermore, the old leaves of the young trees were seen to have the most herbivorous activity while the old leaves of intermediate trees were seen to have the least. This, again, implies that the developmental age of the tree may only matters to a certain extent. Rather, tree age may be disregarded in this case and the amount of damage an individual tree accrues is thought to occur at any given moment and/or location so long as herbivorous pests are present upon any particular tree it chooses to infiltrate (Boege and Marquis 2005). Another possible reason why old leaves held a disproportionate amount of leaf damage compared to young ones is that this plant species, and many others, have other modes of defense mechanisms employed by their genes (War et al. 2012, Maron and Crone 2006). Rather than just ants, it is well known that plants can have multiple defense strategies being used at the same time, and that this pertains to the plant's morphological and chemical features (Agrawal and Rutter 1998, Gatehouse 2002, Wackers et al. 2005, Becklin 2008). Although my study had only addressed one type of morphological defense trait, future researchers may try to incorporate other plant defense mechanisms into their studies. In addition trees at a young life stage have not had enough time nor resources to invest into growing morphological defensive traits that may be energetically costly to build. In these cases it may be best to compare morphological defense traits to chemical ones in the future (Boege et al. 2011).

In conclusion, ant-plant mutualisms in response to insect herbivory on tropical evergreen Noni trees seem to be heavily influenced by several factors that were investigated in this study. It is important to note that the results had shown the highest amounts of herbivorous activities to be found in the trees with the least amount of ants on fruits. In contrast, the group of trees with the lowest amounts of herbivorous activity were seen to have the highest amount of ants skewed towards fruit, and thus causing an increase in sample size that was mainly comprised of the more aggressive ant species. The biological implications that come with this

phenomenon suggests that, in this particular environment at this particular time, EFN's and other nectaries on the fruiting Noni trees are outcompeting HPI's found on vegetative parts. By using EFN's and other morphological features, tropical plants like *M.citrifolia* have adapted to defend themselves by forming mutualistic partnerships with its ant counterparts against insect enemies (War et al. 2012). With climate change getting worse, it is imperative that we understand these relationships, as percent herbivory levels have been seen to dramatically increase throughout geological time scales (Wilf 2001; Currano 2008). Future scientists may be able to help us further investigate these relationships by sampling from different field sites and using a greater number of individual trees to avoid risks of pseudo replication. Although not recommended, choosing to do certain statistical analyses will also help to avoid running into such a risk when experimenting with a small sample size as seen in my study.

On another note, herbivorous activities on many plant species around the globe have been seen to dramatically increase throughout geological time scales with climate change (Wilf et al. 2001, Currano 2008). It still remains unclear whether or not the developmental age of trees or the presence of EFN's mattered more in affecting what was observed in my study. To address this note, future scientists may want to consider other variables and recommendations mentioned earlier. Although only one type of defense mechanism was studied in my work, future potential studies may want to consider other possibilities that may affect insect activity on plants (Chong and Lee 2006, Lange et al. 2017, Fergnani et al. 2008). Researching about additional plant defense traits, such as chemically induced responses to insect herbivory, may not only help us better understand how to combat climate change affecting important crop species, but may also give us further insight as to what may be contributing to the high demands and popularity claims for Noni's "natural healing powers" (Kavitha and Umadevi 2016, Bennett et al. 2006, Potterat and Hamburger 2015). With such complex systems, it is no wonder on-going research upon the topic of plant-insect interactions still needs to continue. If we are seeing a decline in crop yield due to increased herbivorous damage then it is important that we review these findings and other literature upon the basis of what is exactly effecting insect-plant relationships, and what is exactly affecting important food crops that people have been using and carrying around for past generations and years to come (Wilf et al. 2001, Whistler 2009, Currano 2008, Labandeira 2007).

LITERATURE CITED

Abbott, I. A., and C. Shimazu. 1985. The geographic origin of the plants most commonly used for medicine by Hawaiians. Journal of Ethnopharmacology 14:213-222.

Agrawal, A. A., and M. T. Rutter. 1998. Dynamic anti-herbivore defense in ant-plants: the role of induced responses. Oikos 83:227-236.

Alsina, A., X. Cerda, J. Retana, and J. Bosch. 1988. Foraging ecology of the aphid-tending ant Camponotus cruentatus (Hymenoptera, Formicidae) in a savanna-like grassland. Miscellania Zoologica. Barcelona 12:195-204.

Andersen, A. N. 1983. Species diversity and temporal distribution of ants in the semi-arid mallee region of northwestern Victoria. Australian Journal of Ecology. Oxford 8:127-137.

Banks, W., and D. Williams. 1989. Competitive displacement of Paratrechina longicornis (Laterille) (Hymenoptera: Formicidae) from baits by fire ants in Mato Grosso, Brazil. Journal of Entomological Science 24:381-391.

Becklin, K. M. 2008. A coevolutionary arms Race: Understanding plant-herbivore interactions. The American Biology Teacher 70:288-292.

Bennett, R. N., E. A. S. Rosa, F. A. Mellon, and P. A. Kroon. 2006. Ontogenic profiling of glucosinolates, flavonoids, and other secondary metabolites in Eruca sativa (Salad Rocket), Diplotaxis erucoides(Wall Rocket), Diplotaxis tenuifolia (Wild Rocket), and Bunias orientalis (Turkish Rocket). Journal of Agricultural and Food Chemistry 54:4005-4015.

Bentley, B. 1976. Plants bearing extrafloral nectaries and the associated ant community: interhabitat differences in the reduction of herbivore damage. Ecology 57:815-820.

Boege, K., and R. J. Marquis. 2005. Facing herbivory as you grow up: the ontogeny of resistance in plants. Trends in Ecology & Evolution 20:441-448.

Boege, K., K. Barton, and R. Dirzo. 2011. Influence of tree ontogeny on plant-herbivore interactions. In anonymous size- and age-related changes in tree structure and function. Springer. Pages 193-214

Botnevik, C. F., J. Malagocka, A. B. Jensen, and B. L. Fredensborg. 2016. Relative effects of temperature, light, and humidity on clinging behavior of Metaceracariae-infected ants. The Journal of Parasitology 102:495-500.

Chong, K., and C. Lee. 2006. Food preferences and foraging activity of field populations of a pest ant, Tapinoma indicum (Hymenoptera: Formicidae). Sociobiology 48:875-884.

Ceos, S., X. Cerda, and J. Retana. 1997. Spatial and temporal variations in the activity patterns of Mediterranean ant communities. Écoscience 4:269-278.

Diaz-castelazo, C., V. Rico-Gray, S. O. Paulo, and C. Mariana. 2004. Extrafloral nectary-mediated ant-plant interactions in the coastal vegetation of Veracruz, Mexico: Richness, occurrence, seasonality, and ant foraging patterns. Écoscience 11:472-481.

Dixon, A., H. McMillen, and N. Etkin. 1999. Ferment this: the transformation of Noni, a traditional Polynesian medicine (Morinda citrifolia, Rubiaceae). Economic Botany 53:51-68.

Elevitch, C. R. 2006. Traditional trees of Pacific Islands: their culture, environment, and use. PAR, http://www.agroforestry.org/projects/traditional-tree-initiative.

Currano, E. D., P. Wilf, S.L. Wing, C. C. Labandeira, E. C. Lovelock, and D. L. Royer. 2008. Sharply increased insect herbivory during the Paleocene–Eocene thermal maximum. Proceedings of the

National Academy of Sciences **105**:1960-1964.

Fergnani, P., P. Sackmann, and F. Cuezzo. 2008. Environmental determinants of the distribution and abundance of the ants, Lasiophanes picinus and L. valdiviensis, in Argentina. Journal of Insect Science **8**:1-16.

Gatehouse, J. A. 2002. Plant resistance towards insect herbivores: a dynamic interaction. New Phytologist **156**:145-169.

Gillespie, R. G., and B. G. Baldwin. 2010. Island biogeography of remote archipelagoes. Page 358-387 *In* Losos, J. B., and R. E. Ricklefs, editors. The theory of island biogeography revisited.

Gump Station. 2018. https://www.moorea.berkeley.edu/facilities/atitia-center.

Hammer, Ø., Harper, D. A. T., and P. D. Ryan. 2001. PAST: Paleontological statistics software package for education and data analysis. Palaeontologia Electronica **4**(1): 9.

Harris, R. J., and G. Barker. 2007. Relative risk of invasive ants (Hymenoptera: Formicidae) establishing in New Zealand. New Zealand Journal of Zoology **34**:161-178.

Holldobler, B., and E. O. Wilson. 1990. The ants. Cambridge: Harvard University Press

Maron, J. L., and E. Crone. 2006. Herbivory: effects on plant abundance, distribution and population growth. Proceedings of the Royal Society B: Biological Sciences **273**:2575-2584.

Kavitha, P. G., and M. Umadevi. 2016. Medicinal properties & pests & diseases of Noni a review. Research Journal of Pharmacognosy and Phytochemistry **8**:41-48.

Keeler, K. 1985. Extrafloral nectaries on plant communities without ants: Hawaii. Nordic Society Oikos **44**:407-414.

Labandeira, C., K. Johnson, and F. Marsh. 2007. Guide to insect (and other) damage types on compressed plant fossils. Version 3.0.

Lange, D., K. Del-Claro, and E. S. Calixto. 2017. Variation in extrafloral nectary productivity influences the ant foraging. PLoS One **12**:e0169492.

Lee, C. Y. 2002. Household ants: pest status, species diversity, foraging behavior, and baiting studies. Universiti Sains Malaysia, 11800 Penang, Malaysia

Lessard, J., R. R. Dunn, and N. J. Sanders. 2009. Temperature-mediated coexistence in temperate forest ant communities. Insectes Sociaux **56**:149-156.

Marazzi, B., J. L. Bronstein, and S. Koptur. 2013. The diversity, ecology and evolution of extrafloral nectaries: current perspectives and future challenges. Annals of botany **111**:1243-1250.

McClatchey, W. 2002. From Polynesian healers to health food stores: changing perspectives of Morinda citrifolia (Rubiaceae). Integrative Cancer Therapies **1**:110-120.

Merlin, M. D. 2000. A history of ethnobotany in remote Oceania. Pacific Science **54**:275-287.

Nelsen, M., R. Ree, and C. Moreau. 2018. Ant–plant interactions evolved through increasing interdependence. Proceedings of the National Academy of Sciences :201719794.

Oliveira, P. S., V. Rico-Gray, C. Diaz-Castelazo, and C. Castillo-Guevara. 1999. Interaction between ants, extrafloral nectaries and insect herbivores in neotropical coastal sand dunes: herbivore deterrence by visiting ants increases fruit set in Opuntia stricta (Cactaceae). Functional Ecology **13**:623-631.

Razafimandimbison, S., T. D. McDowell, D. Halford, B. Bremer, and P. Ladiges. 2010. Origin of the pantropical and nutriceutical

Morinda citrifolia L. (Rubiaceae): comments on its distribution range and circumscription. Journal of Biogeography **37**:520-529.

Savage, A. M., and J. A. Rudgers. 2013. Non-additive benefit or cost? disentangling the indirect effects that occur when plants bearing extrafloral nectaries and honeydew-producing insects share exotic ant mutualists. Annals of botany 111:1295-1307.

Singh, A. K., K. Singh, and P. I. Peter. 2011. Revisiting the origin of the domestication of noni (Morinda citrifolia L.). Plant Genetic Resources **9**:549-556.

Spotti, F. A., C. Castracani, D. A. Grasso, and A. Mori. 2014. Daily activity patterns and food preferences in an alpine ant community. Ethology Ecology & Evolution 27:306-324.

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

Thomas, E. A. (2010). Interspecific competition and relative distribution of the co-occurring invasive ants paratrechina longicornis and solenopsis geminata (Hymenoptera: formicidae) on Mo'orea, French Polynesia. "Biology and Geomorphology of Tropical Islands" 2010, University of California, Berkeley: Student Papers [https://berkeley.app.box.com/file/21910 6566476]

Wackers, F., P. C. J. Rijin, and J. Bruin. (2005)

Plant provided food for carnivorous insects a protective mutualism and its applications. Cambridge University Press, Netherlands Institute of Ecology; University of Amsterdam.

War, A. R., M. G. Paulraj, T. Ahmad, A. A. Buhroo, B. Hussain, S. Ignacimuthu, and H. C. Sharma. 2012. Mechanisms of plant defense against insect herbivores. Plant Signaling & Behavior **7**:1306-1320.

Weber, M. G., and K. H. Keeler. 2013. The phylogenetic distribution of extrafloral nectaries in plants. Annals of botany **111**:1251-1261.

Wetterer, J. 2010. Worldwide spread of the tropical fire ant, Solenopsis geminata (Hymenoptera: Formicidae). Myrmecological News. Vienna. **14**:21-35

Wetterer, J. 2008. Worldwide spread of the longhorn crazy ant, Paratrechina longicornis (Hymenoptera: Formicidae). Mrymecological News. Vienna. **11**:137-149

Whistler, W. A. 2009. Plants of the canoe people: an ethnobotanical voyage through Polynesia. Lawai, Kaua'i, Hawai'i : National Tropical Botanical Garden, Lawai, Kaua'i, Hawai'i.

Wilf, P., C. C. Labandeira, K.R. Johnson, P. D. Coley, and A. D. Cutter. 2001. Insect herbivory, plant defense, and early cenozoic climate change. Proceedings of the National Academy of Science. **98**(11): 6221-6226.

Wilson, E., and B. Hölldobler. 2005. The rise of the ants: a phylogenetic and ecological explanation. Proceedings of the National Academy of Sciences of the United States of America **102**:7411-741