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# INTERSPECIFIC INSECT INTERACTIONS AND MUTUALISM ON THE UNDERSIDE OF GUAVA *PSIDIUM GUAJAVA* LEAVES, MO'OREA, FRENCH POLYNESIA

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**Abstract.** Interspecific mutualisms between ant and scale insect species have been well documented as symbiotic relationships that merit resource acquisition in exchange for protection. In this study, insect removal experiments were performed on the branches of *Psidium guajava* in order to measure the effects that insect populations have on one another. Five ant removal, five scale removal and five control trees were treated. Data was taken daily over the course of 18 days. Ant activity was measured using a one-minute, unidirectional, point count and the number of scales per branch was determined by photographing a sample of five leaves per branch. Wilcoxon rank-sum tests found that ant populations differ significantly to that of controls in the absence of scale insects. Scale insect populations were not significantly affected by the removal of ants. Observational ant behavior data was also collected that supports possible tending behavior.

**Key words:** *Ants (Hymenoptera, Formicidae); scale insects; mealy bug; Nipaecoccus nipae; guava; Psidium guajava; insects; arthropods; mutualism; tending; Moorea, French Polynesia;*

## INTRODUCTION

Mutualisms are commonly found in arthropod communities. Social insects, such as ants (Hymenoptera: Formicidae), are well recognized as a component with a great amount of influence over arthropod communities (Morrison 1996a, Krushelnycky 2001). In tropical rainforests ants have been found to dominate canopy arthropod fauna (Davidson 1997). Ant herbivory of insect exudates is believed to be a leading cause for their overwhelming abundance. The ability to feed off insect exudates allows ants to benefit from the highly productive canopy foliage, (Davidson 2006).

There are roughly 7000 scale insect species worldwide, most of which have mutualisms with ants, (Eastwood 2004). Through this mutualism, ant tending provides scale insects with protection from predation, parasitoidism and reduces exudate associated fungal growth by removal of honeydew, overall enhancing scale insect survival rates. In turn, the honeydew of scale insects provides the ants with a stable source of energy, (Eastwood, 2004). Little nitrogen is acquired by ants through scavenging and predation, but rather through the processing of large amounts of carbohydrate rich, nitrogen poor insect exudates (Davidson 1997, Davidson et al. 2003).

Adaptations of particular ant species to feed on scale insect exudates lead to the dominance of few ant species in the canopy. High densities of exudate-feeding ants in the canopy can sustain large populations of the scale insects they tend (O'Dowd 2003). Those ant species that adapt to specialize for exudate-feeding have privileged access to

## Kellum Estate

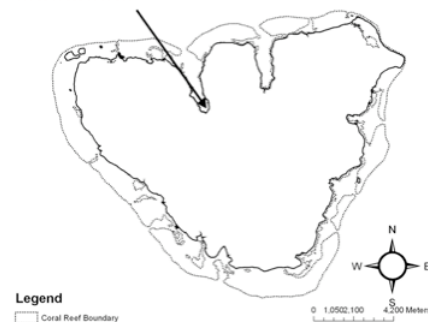


FIG. 1. Kellum Estate, Mo'orea, French Polynesia.

resources and an advantage over competing species, improving their success in the environment (Heckroth et al. 1998). Such specialization has the potential to threaten faunal heterogeneity (Wilson and Taylor 1967). The relationships that ants form with scale insects attract ants to form closer relationships with plants. These relationships play an important role to the evolution of myrmecophytes (Heckroth et al. 1998). Mechanisms relating to interspecific mutualisms are key to understanding the organization of community dynamics, evolution and the greater ecological system.

French Polynesia is hot spot whose biodiversity is being threatened due partially to human population and biological invasions. The ant fauna of French Polynesia are primarily dominated by tramp species whose increasing

immigration, eased by modern day technology, has increased Moorea's ant species diversity (Morrison 1996b, Gillespie and Roderick 2002, Wetterer 2002). In 1907, Moorea was found to have only 13 species of Formicidae (Wheeler 1908). Today there exists at least 39 naturalized species of Formicidae (Krushelnycky 2001, Ledoux et al. 2003). Despite the significance of scale insects to ecological systems, ant-plant studies have thus far paid little attention to associations with scale insects (Heckroth et al. 1998).

The objective of this study is to collect evidence relating to interspecific mutualism and tending behavior on the tropical island environment of Moorea, French Polynesia. Populations of the scale insect *Nipaecoccus nipae*? Maskell, 1983, in the family Pseudococcidae will be measured in their response to removals of an unidentified species in the family Formicidae. Scale insect removal experiments will also be performed to investigate the effect *N. nipae* populations have on associated Formicidae. In order to establish results, experimental data will then be compared to data gathered from control trees where no insect removals were performed. In order to draw conclusions about tending behavior, an observational study will examine the behavior and movement of Formicidae in the presence and absence of *N. nipae*. The interaction of interest will be studied exclusively on the underside of leaves from guava *Psidium guajava* Linn in the family Myrtaceae.

## METHODS

### *Study site*

The study took place at the Kellum Estate on the island of Moorea, in French Polynesia (149° 50' West longitude, 17° 32' South latitude). The Kellum Estate is located approximately 15 meters above sea level (Fig. 1), and is adjacent to Mo'orea's main highway. The study site was located approximately 100 yards from the ocean. This site was selected because of the high prevalence of *P. guajava* that existed in a single area of relatively uniform elevation and habitat. The study site was once used as grazing land and is now partially cultivated (Marimari Kellum, personal communication). The study took place over the course of 18 days, during the months of October and November in 2006. A total of 15 trees were selected for treatment based on the criteria that a) the entirety of the tree was easily accessible (mainly determined by height), b) the interaction of interest between Formicidae and *N. nipae* was present on at least one branch of the tree, and c) the interaction took place on a branch that was isolated

from adjacent branches, for the purpose of accomplishing complete insect removal without interference.

### *Experimental design*

In order to investigate the effects that Formicidae and *N. nipae* populations have on each other, insect removal experiments were performed. Ants were removed from a single branch on each of five trees. *Nipaecoccus nipae* and larvae were removed from a single branch on each of five trees. In addition, five trees were treated as controls from which no insects were removed.

Ants were removed from branches by applying a Tanglefoot equivalent to the base of each branch (Eastwood 2004). Tanglefoot is a sticky, paste barrier designed to prevent crawling insects from reaching the tops of trees, and is traditionally used for pest management. Within one to two days ants were found to be entirely absent from the branch. *Nipaecoccus nipae* and larvae were removed from each leaf by hand with the use of a small paintbrush. Control trees were treated by using a paintbrush to brush each leaf with the intention of not removing insects. The control treatment performed was chosen in order to compensate for possible confounding factors that leaf brushing might have on ant prevalence, by either indirectly influencing ants to leave or by removing natural plant produced chemicals that attract the ants.

### *Sampling*

In order to determine ant activity in the absence of *N. nipae*, ant activity, unidirectional, point measurements were performed. On each scale removal tree a single point at the base of each treated branch was chosen. Ants walking on to the branch were counted over the course of one minute. Ants were counted moving in only one direction for consistency and in order to avoid multiple counting of Formicidae individuals.

In order to measure *N. nipae* presence on leaves where ants were no longer present, five leaves from each ant removal branch were selected randomly and photographed. Scale insects were later counted using Photoshop. Control trees were measured for both ant activity and scale presence. All measurements were taken on a daily basis.

### *Observational study design*

In order to investigate the possible presence of ant-scale tending behavior, an observational component was included in this study that examined ant behavior on leaves of *P. guajava*. Two leaves were selected from each study tree, one

of which had both Formicidae and *N. nipae* interacting and the other of which had only Formicidae present in the absence of *N. nipae*, for a total sample size of 30 leaves. No leaves occurring on treated branches were included in the observational study.

Data were recorded once every minute for a total of five minutes at each leaf. At the start of every minute each Formicidae individual was recorded as doing one of six activities, 1) sitting on scales (SS), 2) running towards the edge of the leaf (RE), 3) running towards the branch of the leaf (RB), 4) sitting on the leaf where there was no scale (SL), 5) interacting with another ant (I), or 6) performing another activity (other). Ants whose behaviors were categorized as “other” were generally engaging in rapid movement without discernible direction and without leaving the leaf.

Leaves where ants occurred without the presence of scale insects were rare. Methods for their observation were altered slightly so that the observation time began the moment an ant was found, regardless of the duration of its presence on the leaf.

#### Statistical analysis

To test for differences in the number of insect individuals according to experimental treatment, the total numbers of insect individuals were calculated. Because the data found were not normally distributed, the data was used as the dependent variables in Wilcoxon rank-sum tests, conducted using JMP statistical analysis software. For all observational data, numbers of Formicidae individuals performing each behavior over five minutes intervals were converted into averages, as were the total number of Formicidae individuals, in order to summarize ant behavior. These values were used as the dependent variables in Wilcoxon rank-sum tests.

## RESULTS

### Experimental data

#### 1) Influence of ant numbers on scale insect populations

Scale insect populations on branches where ants were removed were found not to differ from those where ants were not removed (Fig. 2), indicating that scale presence is likely not dependent on ant presence.

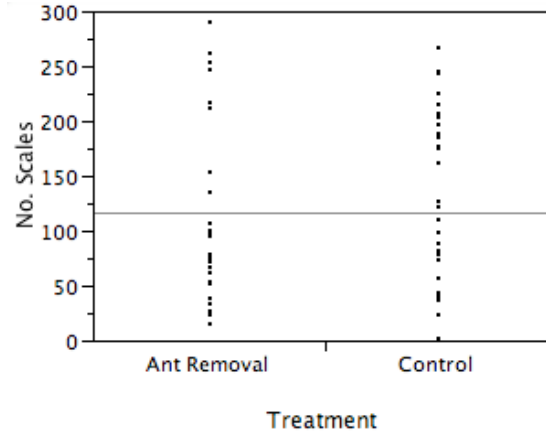


Fig. 2 Wilcoxon rank-sum test. Control showed no significant difference of number of scales from ant removal.  $P = 0.1966$ .

#### 2) Influence of scale insect numbers on ant populations

Ant populations on branches where scales were removed were found to differ significantly from those where scales were not removed (Fig. 3). Fewer ants were found on branches where scale insects were removed, indicating that there is a high degree of probability that ant presence is dependent on scale presence

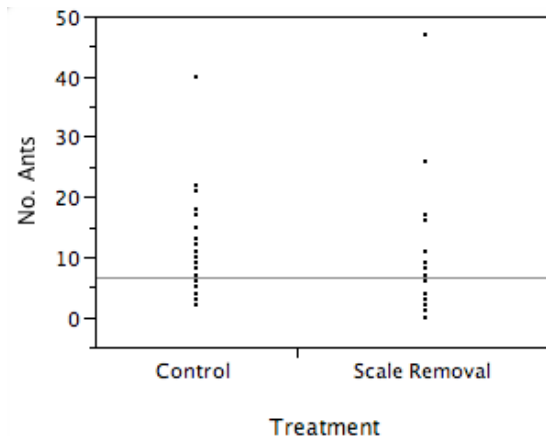


Fig. 3 Wilcoxon rank-sum test. Control showed significantly more ants than ant removal.  $P < 0.001$ .

### Observational data

#### 1) Influence of scale insects on ant populations

Total number of ants on leaves where scales were present was found to differ significantly from those where scales were not present. More ants were present on those leaves where scales were present. Very few ants were found on leaves without scales (Fig. 5). Upon multiple

inspections, no control leaf could be found for tree 3, and the sample was excluded from analysis.

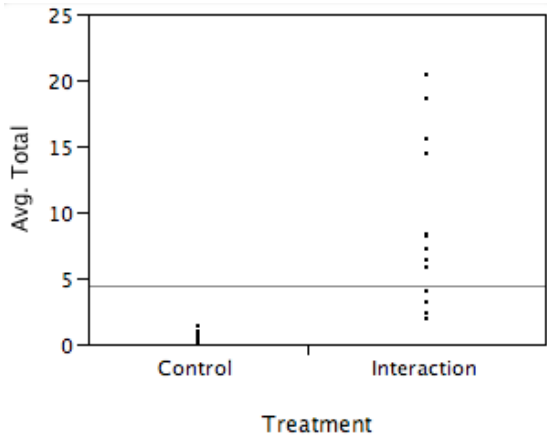


Fig. 5 Wilcoxon rank-sum test. Control showed significantly less ants than interaction.  $P < 0.001$ .

2) Influence of scale insects on ant movement

Ant sitting behavior differs significantly on leaves with scales than from those without. Ants are found to move more on leaves without scales and to remain stationary more on leaves with scales (Fig. 6).

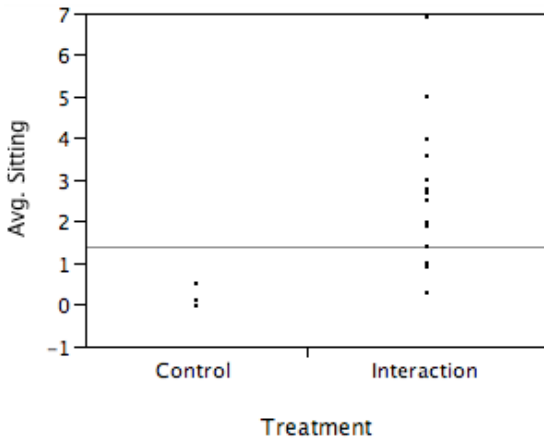


Fig. 6 Wilcoxon rank-sum test. Control showed significantly less sitting than interaction.  $P < 0.001$ .

3) Ant Behavior

Ant behavior was divided into six categories. Ant behavior was found to differ dramatically by treatment. The primary behavior that ants displayed on leaves with scales were to sit on those scales, the second most common behavior being to perform another movement activity that did not result in their leaving the leaf (Fig. 7).

Ant behaviors found on leaves without scales appeared to be more evenly distributed, with the

dominant behavior being running towards the edge of the leaf (Fig. 8). Tree 13 was rare in that it was a control that was found with five ants on it that remained stationary for the entire five minutes of observation.

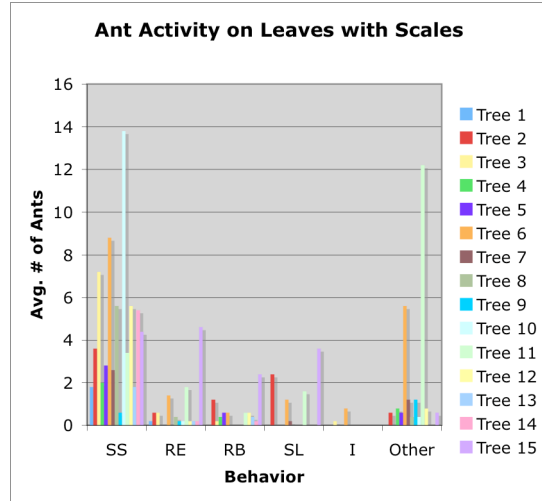


Fig. 7 Ant behavior for interaction leaves

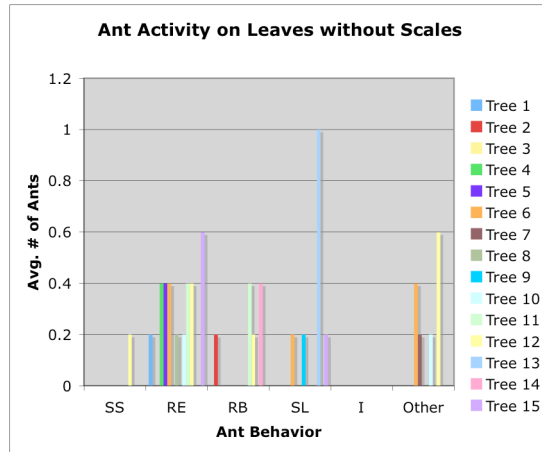


Fig. 8 Ant behavior for control leaves

DISCUSSION

Results support that while Formicidae populations on leaves show a dependence on scale presence, *N. nipae* populations do not fluctuate significantly according to ant presence. Evidence found in this study supports the occurrence of commensalisms between ant and scale insects, where ants benefit from the resources provided by scale insects while having no effect on scale insect populations.

*Experimental data*

1) Influence of ant numbers on scale insect populations

By removing ants from the branches of leaves where scales existed, one is able to see whether *N. nipae* populations were dependent on Formicidae. Results showed that *N. nipae* populations were not significantly altered after the removal of ants.

The degree of this dependence unknown but these results provide evidence that the scale's relationship to the ant in this instance appears to be facultative rather than an obligate one, as the scale numbers are not affected by the absence of ants (Heckroth et al. 1998, Eastwood 2004). However, the brevity of the experiment may have had an effect on this result seeing as how it may take longer than 18 days to see changes in herbivory, parasitoidism or fungal growth.

These ant exclusion experiments also suggest that ants do not have an effect on scale survival rates, therefore lessening the likelihood of a mutualism. Studies researching protection behavior would be of use in order to further explore this possibility.

Seasonal changes relating to breeding may also influence ant effects on scale survival rates. Ant-aphid mutualisms have shown attendant ants to provide aphid eggs with protection, grooming and transport to nests, all shown to increase their rates of survival (Matsuura and Toshihisa, 2006).

## 2) Influence of scale insect numbers on ant populations

The numbers of ants found on leaves were found to be highly dependent on the presence of scale insects. Ants did not have an attraction to leaves where scales were not present, suggesting that scales serve as a resource to the ants, evidence also supported by the observance of tending behavior.

### Observational data

#### 1) Influence of scale insects on ant populations

Observational studies counted more Formicidae individuals overall on leaves where they interacted *N. nipae*. When ants were removed from tree branches they succeeded in building bridges made of dirt across the tangle foot on trees 6 and 14. The benefit of these the building of the bridges can be assumed to be worth the energy they require. These observations provide evidence that the ants present on *P. guajava* leaves are interested in obtaining resources from *N. nipae*.

#### 2) Influence of scale insects on ant movement

Sitting behavior for ants (both on scales and on leaf without scales) were combined and those numbers were averaged for both control and interaction trees. When compared, it was found that those leaves where the interaction occurred had

significantly less movement overall. This high occurrence of Formicidae sitting still on those leaves with scales, when they were other wise moving on those leaves without scales, provides behavioral evidence that tending behavior is occurring.

## 3) Ant Behavior

The individual behaviors that took place on both classes of leaves differed greatly. On leaves where the interspecific interaction took place, ants were mainly observed sitting on scale insects or placed in the category "other". "Other" ants were mostly observed moving quickly, with a constant change of direction, occasionally pausing before resuming movement. These ants were typically found to stop moving once they found a scale insect on which to sit. The "other" is most likely a category for ants that are in the process of selecting a scale on which to sit.

Ants on leaves where scale insects were not present were mostly observed running towards the edge, eventually leaving the leaf. One explanation for this had to do with the sampling technique that was used. Control leaves with ants and no scales were exceptionally hard to find. As a result, methods for observing control leaves were changed. Because control leaves were difficult to find, upon the immediate discovery of an ant on a leaf without scales the timed observation began regardless of the amount of time the ant was present. This mainly resulted in ants being caught during a quick visit to a leaf where nothing was of interest, and then retreating to the edge of the leaf.

Sample trees were selected randomly. Coincidentally, control trees were found to be disproportionately located in areas of partial cultivation where there was a greater degree of sun exposure (Fig. 9). Whether or not this factor had an affect on the results found in this study is unknown. Further research related to interspecific insect interactions in relation to cultivation or sun exposure would of great interest and value to many.

### Tree Location

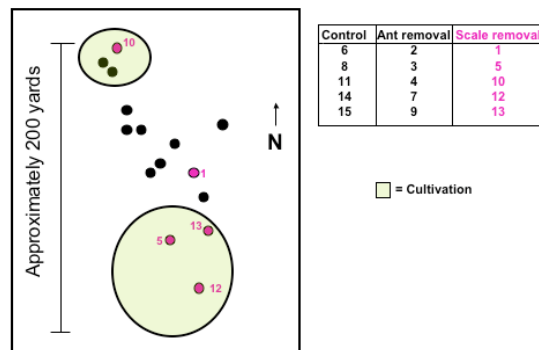


FIG. 9. Tree location of control trees in relations to areas of cultivation.

#### CONCLUSION

The sitting behavior on leaves where scales were present, high abundance of ants on the same leaves and the high degree of difficulty finding leaves where ants existed without scale insects are all factors that strongly support tending behavior of ants of scale insects on the leaves of *P. guajava* in French Polynesia.

A linear regression analysis of ant and scale insects in their natural populations would likely reveal a positive correlation, seeing as how leaves with Formicidae and without *N. nipae* were exceptionally hard to find. A positive correlation would provide evidence supporting the occurrence of a symbiotic relationship between Formicidae and *N. nipae*. Evidence provided from this study specifies that the likelihood of that relationship would be one of commensalism rather than mutualism.

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