Nectar glands possibly lessen herbivory on Yayo leaves and flowers

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Abstract

The Yayo tree, *Rehdera trinervis*, is a largely unstudied tree that produces foliar extrafloral nectar glands that are commonly exploited by a wide variety of insect species. These nectar glands are positively correlated with number of fruit, a commonly accepted metric of plant reproductive potential and fitness. Though there are many cases of ant-plant mutualistic systems, it is yet unclear if the Yayo tree represents one of those cases or if the nectar glands have a different adaptive significance. This paper studies the possibilities that nectar glands facilitate ant-plant mutualism and explores the Distraction Hypothesis stating that nectar glands distract herbivores away from more valuable floral nectaries and fruits, which both require higher energy investments due to their larger structure and can be rendered nonfunctional by minimal damage. Observations of exploitative ant species and predatory wasps foraging nectar glands provide mixed evidence of protection from herbivores by mutualistic insect species. Data that correlates number of nectar glands and number of fruit on a Yayo sprig appear to corroborate the Distraction Hypothesis. By collecting 218 leaf samples and categorizing them by level of damage from herbivory, strong trends emerge showing that herbivorous insects target foliar nectar glands at a statistically significant level. Leaves that are more damaged have far fewer nectar glands than one would expect based on the proportion of lost leaf tissue. Observed leaf surface scars caused by herbivory that were determined to be nectar glands by the presence of yellow puckered areas on the undersides of the leaf suggest that Yayo nectar glands distract herbivorous insects away from eating large areas of photosynthetic tissue, a less nutritious food source. This localizes damage and preserves the plant’s photosynthetic potential.

Resumen

El árbol de Yayo, *Rehdera trinervis*, es un árbol poco estudiado que produce glándulas de néctar extraflora foliares que son comúnmente explotadas por una amplia variedad de especies de insectos. Estas glándulas de néctar están positivamente correlacionadas con el número de frutos, una métrica comúnmente aceptada de potencial reproductivo de la planta y su éxito reproductivo. Aunque hay muchos casos de sistemas mutualistas de hormigas, todavía no está claro si el árbol de Yayo representa uno de esos casos o si las glándulas de néctar tienen una función adaptativa diferente. Aquí investigo la posibilidad de que las glándulas de néctar facilitan el mutualismo de hormigas y exploro la Hipótesis de Distracción, la cual afirmaría que las glándulas de néctar distraen a herbívoros de frutos y nectarios florales, los cuales requieren mayores inversiones energéticas debido a su estructura mayor. Si estas estructuras sufren aunque sea un daño mínimo, pueden ser no funcionales. Las observaciones de visitas a las glándulas por hormigas explotadoras y avispas depredadoras proporcionan pruebas mixtas de protección de los herbívoros por las especies mutualistas de insectos. Los datos que correlacionan el número de glándulas de néctar y el número de frutos en una rama de Yayo corroboran la Hipótesis de Distracción. Al recolectar 218 muestras de hojas y categorizarlas por el nivel de daño de la herbivoría, surgen fuertes tendencias que muestran que los insectos herbívoros se dirigen a las glándulas de néctar foliar a un nivel estadísticamente significativo. Las hojas que están más dañadas tienen mucho menos glándulas de néctar de lo que uno esperaría basándose en la
The Yayo tree (*Rehdera trinervis*), is a flowering tree that occurs in Central American tropical dry forests. It can be found on the mainland of Costa Rica and on islands in the Costa Rican Marine Protected Zone. Its morphology is interesting as most of its leaves contain foliar nectar glands. In many plants, nectar glands always appear in the same location. For example, many species of the Euphorbaceae family have extrafloral nectaries growing on the petiole at the base of the leaf. The structures in *Rehdera trinervis* do not grow in the same location on the leaf, which could be a result of variable induction of the nectar glands by insects or plasticity as a genetically controlled feature. This paper will refer to the structures on the Yayo tree foliar nectar glands, or simply nectar glands.

These nectar glands can be observed from the underside of the leaves as translucent yellow dots, but they only exude sweet-tasting nectar from the top side (personal observation). Multiple people have made observations of various ant species feeding on the nectar glands of the Yayo tree, a behavior that suggests facultative ant-plant mutualism. The relationship between nectar glands and insects is well studied and manifest in many different ways. Many plants (at least one species in more than a quarter of all plant families) provide nectar rewards to insects in exchange for protection from herbivores (Bronstein et al. 2006). The archetypal example of this type of mutualistic exchange occurs between ants in the genus *Pseudomyrmex* and the Ant-Acacia plants of the genus *Vachellia*. In this obligate mutualism, the plant’s nectar glands provide sugar-rich nectar and protein-rich Beltian bodies as food as well as hollow thorns which the ants inhabit. The ants in turn protect the Acacia from herbivores and chemically or physically remove sprouting competitor plants from the surrounding area (Janzen 1967). There are several case studies of facultative ant-plant mutualisms as well. Generalized facultative ant mutualists can influence the evolutionary trajectory of nectar glands by influencing a plant’s fitness based on the number of nectar glands that it possesses. Rudgers (2004) showed reduced ant visitation in wild cotton plants leads to increased leaf damage and decreased seed production and that number of nectar glands is positively correlated with ant visitation based both on studies of natural variation in the abundance of nectar glands and experimental removal of nectar glands. A strong correlation between number of foliar nectar glands and number of fruits found on sprigs of the Yayo tree with an $R^2$ value of 0.3006 was found in a study conducted on the Yayo trees on Isla San Jose (see Appendix). No cause and effect relationship was ever determined between these two variables. However, the trend found in this study matches one of the trends presented by Rudgers.

Plants often provide nectar to parasitic or herbivorous insects as well. Ants sometimes tend herbivorous hemipterans such as aphids in order to harvest their nutritious fluid secretions or honeydew. It is possible that some plant species provide nectar to ants in order to give them a non-herbivorous source of nectar that is superior to the honeydew produced by some, but not all, hemipterans (Becerra & Venable, 1989). The biomass of pseudococcid-tending ant colonies is independent of the number of nectar glands produced by their host plant, but the biomass of coccid-tending colonies depends heavily on nectar glands because the plant-produced nectar is either superior to coccid honeydew or abundant enough to significantly supplement the diet of
the ants (Gaume et al. 1998). This suggests that the host plant produces nectar whose cost of production is outweighed by the benefit of reduced coccid herbivory. However, it is not “economically” advantageous for the plant to produce nectar that deters pseudococcid herbivory. Under the Distraction Hypothesis originally proposed by Anton Kerner in 1878, nectar glands may provide nectar to deter ants from consuming a plant’s flowers, another nectar-producing structure that is much more costly to produce and has a much higher impact on the fitness of the plant. Ant visitation to floral nectaries is shown to be reduced when nectar glands are available on the same plant (Wagner & Kay, 2002).

During an initial observation period, I collected Yayo sprigs for further review. Most leaves (61/90) had at least one nectar gland. They also revealed that much of the herbivory does not start at the outside edges of the leaves. The sections of lost photosynthetic tissue typically appear as holes or scars indicating surface damage on the insides of the leaves. It is not possible to know what was present on the missing sections; however it is quite possible that these sections used to have nectar glands that were eaten by insects. Some nectar glands had also sustained minimal damage within their borders, indicated by black spots or brown ulcers on the gland surface.

I believe that the nectar glands are present on the leaves of the Yayo tree for one of two reasons: 1) They are an adaptation used to reward ant mutualists for protecting the plants from other insect species that would otherwise do damage to the plant’s leaves, or 2) They are present as a way to distract insects with nutritious nectar so that they are less likely to visit and damage flowers, thus preserving the plant’s ability to generate seeds and fruit. I expect that these nectar glands are not maladaptive to the tree; otherwise the adaptation would have been selected against. Nectar glands are most likely a beneficial or neutral trait for the tree. The function of the nectar glands can be explored under a central question: “How does the presence and number of nectar glands affect patterns of herbivory on a leaf?”

Materials and Methods

Study Site

In the town of Cuajiniquil and its surrounding area, which is historically a tropical dry forest, the Yayo tree can be found in relative abundance. Gaps in the dry forests of Costa Rica are an excellent habitat in which to study the nectar glands of the Yayo tree. The presence and abundance of ant-defense mechanisms in a plant likely reflect resource availability. This Resource-Limitation Hypothesis argues that sugar- or protein-rich rewards are much more cheaply produced by a plant when it receives a greater amount of a limiting resource, typically sunlight (Heil & McKey, 2003). When a plant receives sunlight in abundance, its leaves are flushed with photosynthate which both allows and necessitates that the plant produce herbivore defense mechanisms (McKey, 1989). The particular Yayo trees I studied grew on the side of a road and received abundant sunlight and were very likely to produce a large quantity of herbivore defenses, such as nectar glands. The trees I studied are located about 5km from Cuajiniquil on the main highway towards Liberia. They located around a telephone pole on the left side of the road where it curves gently to the right about 500 meters past a large gated entrance to a ranch. This provided an adequate sample size of trees that can be easily studied.
Methods

I documented the insects I observed on the leaves to get a better understanding of herbivores or potential mutualists associated with the Yayo tree. I observed the behavior and activity of the insects, paying close attention to all insects that visited the Yayo nectar glands. I have previously observed ants pausing at nectar glands while foraging Yayo trees on Isla San Jose as well as on the mainland. Based on initial observations on Isla San Jose, there were ants from multiple species visiting the foliar nectar glands and reports of a diverse array of insects that visit and feed from nectar glands including beetles and flies (F.Chinchilla). I took pictures of any insects I found and identified them.

I conducted an approximately ten day long study in which I marked certain leaves and tracked the changes in each leaf over the course of the study period. I started by using masking tape to mark an ample number of healthy, undamaged leaves with varying amounts of nectar glands and track herbivory of each leaf. Each masking tape flag was marked with a record-keeping number, such as L1, L2, L3 and so on, which corresponded with a section in my field notebook where I kept notes on that particular leaf. Leaf herbivory, the damage and consumption of a plant’s leaves by herbivores, can be categorized through ocular estimation by halves. Damaged leaves can be sorted into categories of ascending damage with category 0 representing undamaged or nearly undamaged leaves defined for the purposes of this study as leaves with up to 2% damaged or lost area, category 1 defined by 2%-6% leaf loss, category 2 defined by 7%-12%, category 3 defined by 13%-25%, category 4 defined by 26%-50% and category 5 defined by more than 50% leaf area. I also attempted to observe the growth of nectar glands on a younger leaf as it matures, or to observe the loss of nectar glands due to herbivory as insects possibly find and excise them. The labeled flags on the leaves allowed for easy comparison of the same leaf between different sample times. I returned to the study site to make observations of herbivory, nectar induction and insect activity daily, except when a passing hurricane (Hurricane Otto) made this impossible.

I collected 3 sprigs from 5 different trees, 15 sprigs in total, and I recorded the location of the sprig (high, middle or low on the tree), the relative age of the leaf, the number of nectar glands present and the damage category of each leaf. I entered this data into Excel for analysis. The average number of nectar glands in each category was calculated and compared to all other damage-category averages using t-tests. Irrespective of adaptive significance, one would expect that nectar gland loss is proportional to herbivory assuming that nectar glands appear and are lost randomly. Therefore, I propose the null hypothesis that if nectar glands are not preferentially targeted by insects, we expect that the number of nectar glands per leaf will be dictated by percent leaf loss or leaf-damage category. On average, Category 1 leaves should have up to 6% fewer nectar glands than Category 0 leaves, Category 2 leaves should have up to 12% fewer nectar glands than Category 0 leaves and so on. When comparing Categories 0 and 1 I reduced the Category 0 mean by 6% by applying a multiplier of 0.94 to the Category 0 mean. When comparing Categories 0 and 2 I reduced the Category 0 mean by 12% by applying a multiplier of 0.88 to the Category 0 mean. I will test the difference between Categories 1 and 2 to see if there is still an insect preference for nectar glands even when leaves become damaged and fewer nectar glands are present on the leaves. If the mean of Category 1 is expected to be 0.94 compared to that of Category 0 and the mean of Category 2 is expected to be 0.88 compared to that of Category 0, then we would expect the mean value of Category 2 to be 0.93617 compared to that of Category 1 based on the algebraic equation 0.94*x=0.88. Therefore, for this t-test the Category 1 leaf damage average will be modified by a multiplier of 0.93617, or a reduction of
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6.383%. A one-tailed test would be most appropriate because if the null hypothesis is not met, then nectar glands are preferentially eaten and we only expect to see a reduced, not an increased, mean number of nectar glands. I set a 0.05 confidence level for these tests.

While the central question of this study will attempt to relate presence and number of nectar glands with the incidence of herbivory, there are some other questions that can be addressed during the study period as well.

I extended a study previously conducted on Isla San Jose with Christina Lew. This entails measuring the number of nectar glands and fruit found on sprigs collected from the mainland Yayos to see if I observe the same positive correlation that we had observed on the island. This positive correlation relates the proportion of nectar glands divided by leaves found on a single sprig and the proportion of fruit divided by leaves per sprig. A higher proportion of nectar glands is assumed to be beneficial for the Yayo tree and the proportion of fruit is an accepted measure of fitness based on plant reproductive potential. The data points found during this collection have been added to an existing data set to see if the previously discovered trend on Isla San Jose is corroborated by data collected on the mainland (see Appendix).

I attempted to induce the growth of nectar glands on the leaves of the Yayo tree. Many plants do not produce rewards for mutualists until the ant mutualist induces their production or herbivory has already occurred (Agrawal & Rutter, 1998; Heil et al., 2001). I used a knife to puncture the surface of selected Yayo leaves on its yellow spots (possible pre-nectar glands) to see if sap collects at the point of disturbance and if the area develops into a nectar gland. As a paired control, I will also select a leaf which I will puncture on a green area. It has been shown that nectar glands are produced in response to leaf damage in *Vicia faba*. Experimentally removing one third of each leaf in a leaf pair resulted in a 106% increase in the number of extrafloral nectaries over a one-week period, indicating that extrafloral nectary production is an inducible response (Mondor & Addicott, 2003). This suggests that nectar glands are induced features. I hypothesize that the yellow spots on Yayo leaves could become nectar glands if properly treated. Over the course of a few days I observed the changes to see if a new nectar gland formed on the damaged areas which simulate biting by ants or other herbivores.
**Results**

Originally, I considered the possibility that the trees on the island were undergoing divergent adaptation based on observations made by a mentor and advisor of mine, Federico Chinchilla. He observed nectar glands on island trees, but not on mainland trees. Upon closer inspection of trees on the mainland, I confirmed the presence of nectar glands and closed the question of divergent adaptation.

![Figure 1: The effects of leaf age on nectar gland abundance. 1a. shows nectar gland frequency in young leaves up to one month old. 1b. shows nectar gland frequency in mid-aged leaves between one and three months old. 1c. shows nectar gland frequency in old leaves over three months old.](image)

Collected leaf specimens were split into three categories of relative age from youngest to oldest. The histograms in figure 1 display frequencies of leaves with 0-5 nectar glands for each age category. The data suggests that the number of nectar glands increases as the leaf ages. It is possible that leaves with more nectar glands live longer, however the lack of leaves with high numbers of nectar glands in this sample suggest otherwise. The mode by which nectar glands are induced remains unknown as the nectar gland induction by leaf cutting only produced scars but no foliar nectar glands. Foliar nectar glands development appears to take place mostly when young leaves less than about one month old develop into mature leaves up to 3 months old, but little development seems to take place when mature leaves age and become old leaves approaching senescence.
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A) Nectar Gland Overall Frequency

B) C0 Nectar Gland Frequency

C) C1 Nectar Gland Frequency

D) C2 Nectar Gland Frequency

E) C3 Nectar Gland Frequency
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Figure 2: Nectar gland frequency in several different damage categories. Number of nectar glands per leaf varied from zero to five. 2a. displays nectar gland frequency for all 218 leaves collected. 2b.-2f. display nectar gland frequencies for damage categories 0-4, respectively. Category 5 is not included because there were only two Category 5 leaves collected in this sample.

Figure 2 displays frequency in the amount of nectar glands per leaf where each histogram shows the data of a single damage category. The scale of each histogram is different, but these graphs are not concerned with the strict amount of nectar glands. Rather, figure 2 is a visual display of the proportion in amount of nectar glands per leaf across each damage category. In all histograms, there is a steady decline in the proportion of nectar glands per leaf from 2b. to 2f. with the exception of 2e. This is to be expected as progressively more leaf surface is removed. However, the decline appears to be visually greater than we would expect it to be if leaf surface is lost randomly. The difference between 2b. and 2c. is particularly interesting. In figure 2b, the most common number of nectar glands found on each leaf is two. In figure 2c, the number of leaves with two nectar glands is just over a third the number of leaves with no nectar glands.

Table 1: T-tests comparing foliar Nectar Gland averages between several categories of herbivory. 1a. tests the difference between mean values of Categories 0 and 1. 1b. tests the difference between mean values of Categories 0 and 2. 1c. tests the difference between mean values of Categories 1 and 2. All comparisons are significant at the 0.05 confidence level.

<table>
<thead>
<tr>
<th>a) t-Test: Two-Sample</th>
<th>Category 0</th>
<th>Category 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>1.387</td>
<td>0.969</td>
</tr>
<tr>
<td>Variance</td>
<td>1.314</td>
<td>1.061</td>
</tr>
<tr>
<td>Observations</td>
<td>61</td>
<td>98</td>
</tr>
<tr>
<td>P(T&lt;=t) one-tail</td>
<td>0.011</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>b) t-Test: Two-Sample</th>
<th>Category 0</th>
<th>Category 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>1.298</td>
<td>0.304</td>
</tr>
<tr>
<td>Variance</td>
<td>1.151</td>
<td>0.312</td>
</tr>
<tr>
<td>Observations</td>
<td>61</td>
<td>23</td>
</tr>
</tbody>
</table>
In these tables, the data for three different t-tests are displayed. The first, seen as the left half of the upper table, is a test between the Category 0 mean value reduced by 6% and the Category 1 mean value. The second, seen as the right half of the top table, is a test between the Category 0 mean value reduced by 12% and the Category 2 mean value. The third table tests the difference between the Category 1 mean value reduced by 6.383% and Category the 2 mean value in order to see if herbivores tend to prefer consuming nectar glands even when the number of nectar glands has already been significantly reduced. At a confidence level of 0.05, all three tests show that the mean values are significantly different.

Discussion

The 10 day long study intending to measure herbivory over time did not pan out due to unforeseen weather events (Hurricane Otto). Insect activity was greatly reduced during my study period and not enough herbivory took place for any meaningful statistical data to be generated in this respect.

On the mainland, I collected data to extend my previous Isla San Jose study relating number of foliar nectar glands with number of fruit per sprig on the Yayo tree. I was able to collect and average data across 8 trees and found a relationship with an $R^2$ value of 0.073. When compiled with the previously collected data to generate a set with 27 data points, an $R^2$ value of 0.2626 was generated (see Appendix). While this is a weaker correlation than that of Isla San Jose Yayo trees alone ($R^2=0.3006$), it is still quite strong as biological systems go.

During the course of the study period, I did not observe any induction of nectar glands on any of the marked leaves at all. There was little change of any kind on the leaves I had marked for the herbivory study and no foliar nectar glands were induced after leaves were experimentally punctured by a knife. The puncture wounds resulted in leaf scars similar in appearance to typical herbivory observed on the leaves. The origins of the nectar glands are still inconclusive, though it seems that more nectar glands appear as leaves age. There does appear to be a link between damage to leaves and nectar glands as it is shown that natural herbivory, artificial damage and artificial application of the plant hormone jasmonic acid to leaves in the $M. tanarius$ tree all increase production of extraloral nectar on preexisting extraloral nectar glands (Heil et al. 2001). In some plant speices, nectar glands are produced in response to leaf damage. (Mondor & Addicott, 2003; Wooley et al., 2007). The purpose of this response in $Vicia faba$ is to attract predatory arthropod mutualists, which may or may not be the adaptive significance of Yayo nectar glands. Herbivory is correlated with significantly fewer nectar glands in Yayo and nectar glands are more commonly observed on undamaged leaves, suggesting that herbivory does not lead to production of more nectar glands. It is still possible that the nectar glands are induced by some other factor, such as an ant-secreted chemical, although further study involving ant-
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excluding sticky traps around the base of a tree is needed in order to address this question properly.

The statistical tests presented in Table 1 show significant differences between expected and observed nectar gland frequencies between damage categories. Therefore, we reject the null hypothesis that insects do not preferentially consume nectar glands in favor of the alternate hypothesis that herbivorous insects preferentially consume nectar glands.

In the experimental study using artificial flowers and two species of ants conducted by Wagner and Kay (2002), the ants were shown to be less likely to visit primary nectaries (flowers) when secondary nectaries (i.e., foliar nectar glands) are present. When there are more nectaries for the ants to visit, the chance of an ant visiting any one nectary is reduced by dilution. Their study also shows that ant colonies do not recruit more workers to the plant if it has more nectar sources, even though the artificial flowers were set up approximately a meter away from the ant colonies. They note that number of ants recruited to nectar glands is “slightly increased, although not significantly so” if their amino acid content is higher than in other surrounding nectar sources. It is likely that nectar glands that exude nectar with high amino acid concentrations are designed to attract mutualistic ant species whose presence confers a fitness advantage to the plant via defense against herbivores. Future research into the nectar glands of the Yayo tree should investigate and compare the nutrient makeup of foliar nectar glands and floral nectar. If foliar nectar glands have a higher concentration of amino acids, it might suggest that they may be adapted to attract mutualistic ant species; if they have a lower or similar amino acid concentration compared to that of the floral nectaries, it would support the Distraction Hypothesis and fit the paradigm set forth by the results of the experiment conducted by Wagner and Kay (2002).

The Distraction Hypothesis is further supported by other studies and experiments. There is quite a bit of selective pressure for many plant species to keep ants away from their flowers. Some ants can produce antimicrobial compounds that render pollen grains inviable upon contact (Beattie et al. 1984), and while mutualistic ants have been shown to deter herbivorous insects, parasitic species that exploit floral nectar glands have been shown to deter pollinators from visiting flowers (Norment, 1988) or destroy floral tissue altogether (Galen, 1999). A paper written by Yu & Pierce in 1998 titled *A castration parasite of an ant-plant mutualism* explores an ant symbiont that “castrates” its host plant by destroying flowers and “reducing fruit production to zero in most host plants.” Furthermore, the nectar glands on many plant species only secrete nectar when flowers are producing pollen, suggesting that nectar glands actively provide a distraction specifically to offer an alternative to floral visitation by insects (Bentley, 1977).

It is possible that the distraction provided by the nectar glands is twofold; they may simultaneously distract ants from foraging for nectar at floral nectar glands and provide localized areas for other herbivores to consume. While recording the raw data for these graphs, I began to notice many spots of surface damage on the leaves that were the size and shape of typical nectar glands. By checking the underside of the leaf, I found evidence that nectar glands had indeed been present in those spots. Nectar glands are always on the top side of the leaf (this is exclusively where they exude nectar), but the underside of the Yayo nectar gland is a yellow spot that is more faint than the topside of a nectar gland and looks similar to a scar or a puckered convergence of small leaf veins. I recorded many Category 1 leaves (12 based on photographs I took during the study) that sustained surface damage exclusively to areas that used to host nectar glands. This indicates that herbivores prefer to eat the nectar glands on these leaves. This
observation along with the sharp proportional decline in nectar gland presence suggest that insect herbivores do in fact preferentially eat nectar glands and supports the hypothesis that nectar glands localize damage and therefore minimize damage to photosynthetic leaf surfaces as well as distract herbivores away from flowers and fruit.

It is unlikely that the surface scars are produced by ants as they feed only by drinking the dewlike drops of nectar on the surface of the nectar glands but do not appear to damage the nectar glands themselves. I have not observed any herbivory on the leaves of the Yayo tree firsthand, unfortunately, although I have observed flies, beetles, hemipterans, three ant species and Polistes wasps visiting the leaves or nectar glands of the tree. The damage could be done by some of these insects, a combination thereof, or other species that I did not observe during the study period. The results, specifically the statistically significant t-tests, of this study indicate that insects preferentially consume nectar glands. I believe that this is ultimately beneficial for the plant as the extra cost of producing nectar glands is likely minimal. Nectar gland tissue usually consists of an epidermis covered in modified nectar-secreting stomata and a nectary parenchyma that collects fluid from phloem tissues and stores pre-nectar. The nectary parenchymal cells contain a higher concentration of ribosomes and mitochondria, a complex endoplasmic reticulum and vesicles used to transport nutrients. These features increase the cost of producing nectary cells. However, nectary cells also contain a very large vacuole, a structure that consists of a phospholipid bilayer and not much else, and very few chloroplasts as they do not actively produce nectar (Escalante-Perez & Heil 2012). These factors reduce the overall costs of production. Rather, the cost lies in producing the nectar itself. Nectar production, like the production of all parts of most plants, is fueled primarily by photosynthesis. The yellow color of the nectar glands suggests that they lack chlorophyll which is the most efficient photosynthetic pigment that plants possess. Therefore, the loss of a nectar gland does not represent a significant loss of photosynthetic potential compared to losing the same amount of area of green leaf surface, nor does it represent a significant loss due to production costs, as mentioned above. The insects that consume the nectaries themselves are likely exploiting the sugars and pre-nectar stored inside the structure. This sugar has already been produced with the final goal of feeding an insect, so the loss has already been “accounted for” by the plant. The loss of the foliar nectary structure itself is probably not a significantly greater loss to the plant compared to typical photosynthetic tissue and is likely a much smaller loss compared to an insect damaging a flower or fruit. If insects have learned to specifically target nectar glands and move on in order to optimize foraging efficiency.

The species of ant that was observed foraging on the leaves of the Yayo tree in the study system the most is Pseudomyrmex gracilis, which is an opportunistic generalist exploiter that prefers sucrose-rich nectar solutions (Kautz et al., 2009). I observed that one of my study leaves had dewlike drops of nectar on 4 of 5 of its glands. I found a nearby P. gracilis ant and artificially placed it onto the leaf and watched as it fed on the nectar produced on the yellow glands. The ant didn’t fully drain any of the nectar glands, though it almost finished off the nectar supply at the first gland it visited before briefly drinking from two of the other glands. P. gracilis has also been observed visiting flowers of the O. spectabilis tree (Byk & Del-Claro, 2010), which is likely bad for the tree due to the opportunistic nature of the ant as well as the previously cited research that ants near flowers are bad for a plant for several reasons. This ant was also observed to be living in the hollow thorns of a nearby Acacia species tree where it cheats the typical system of ant-plant mutualism by feeding on food sources provided by the tree without actively defending it. P. gracilis likely forage on Yayo trees near to their home Acacia
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plant opportunistically. This supports the idea that the foliar nectar glands of the Yayo tree are used to distract ants away from flowers and fruit.

The findings of this paper appear to suggest that the foliar nectar glands on the Yayo tree tend to feed non-mutualist exploiter ants in order to divert their attention away from fruits and flowers, corroborating the study conducted by Wagner and Kay (2002). The data indicate that nectar glands are eaten preferentially by herbivores at a statistically significant level. This could possibly be an adaptation to reduce herbivory by localizing damage to areas with less photosynthetic potential suggested by observations of surface scars indicating a nectar gland that used to occupy the area.

Acknowledgements

This research project was originally inspired by Federico Chinchilla, who originally showed me the Yayo tree while he led me and a group of students on a hike on Isla San Jose. His insights into the nectar glands’ exploitation by various insect species and the enigmatic adaptive significance of the structures filled my head with questions that I could not leave unanswered.

I would also like to mention Christina Lew, friend and classmate with whom I first studied this fascinating system. Our research project conducted on Isla San Jose showed me how the system could yield intriguing results. The data we collected even makes an appearance in this paper.

My initial observations were aided by the expertise and guidance of Rafael Lara, a Cuajiniquil local who helped me identify Yayo trees whose morphology on the mainland differed from that of the Isla variety, and Minor Lara Jr., who guided me through the town of Cuajiniquil and showed me that there were indeed enough Yayo trees in the area to be found.

I would also like to mention Professor John T. (Jack) Longino at the University of Utah, Salt Lake City, whose expertise in ant taxonomy was invaluable in identifying the ants I observed foraging on the Yayo trees.

This research would not have been possible without Frank Joyce Jr., director to my study abroad program in Costa Rica, invaluable teacher and primary advisor to this research project. His assistance ranged from helping me find an ideal study site, providing a mode of transportation to reach said site, challenging me to think more deeply about a unique system, suggesting several possible variables to consider and questioning the logical soundness of claims that were, at the time, dubiously supported.
Nectar glands possibly lessen herbivory on Yayo leaves and flowers

Sources Cited
Nectar glands possibly lessen herbivory on Yayo leaves and flowers


Appendix

**Flowers by Nectar Glands (Isla San Jose)**

![Graph showing the relationship between Nectar Glands and Flowers](image)

\[ R^2 = 0.30059 \]
Nectar glands possibly lessen herbivory on Yayo leaves and flowers

These figures show the data gathered on Isla San Jose that related the proportion of flowers over leaves on a single sprig (y-axis) vs. the proportion of nectar glands over leaves on a single sprig. Each data point is an average across one tree of at least eight sprigs. The “Isla San Jose” scatter-plot shows data collected on Isla San Jose in October 2016 while the “Aggregated” scatter-plot includes the data gathered on the mainland in late November 2016.

![Flowers by Nectar Glands (Aggregated)](image)

\[ R^2 = 0.2626 \]