OPTING FOR EXPIRATION: EFFICACY OF BIOACTIVE SECONDARY COMPOUNDS IN AFFECTING HERBIVORE FEEDING PREFERENCES OF MEDICINAL LEGUMINOSAE SPECIES IN MO'OREA, FRENCH POLYNESIA

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Abstract. Secondary compounds, often found in medicinal plants, are believed to have evolved as chemical defenses for many species. These bioactive chemicals have been shown to protect the plant from their numerous insect predators and pathogens through a wide variety of mechanisms. The purpose of this study is to examine the effect these secondary compounds have on the amount of predation towards medicinal species. Looking at the amount of leaf herbivory and an antifungal bioassay of six species of *Leguminosae*, the main objective of the study was to find a correlation between predation and medicinal properties. Herbivore feeding preference appeared averse to medicinal leaves, although no relationship between leaf damage and yeast inhibition was found. A feeding experiment conducted using common herbivores inadvertently demonstrated the antifungal properties of the medicinal species, and gave further indication of an herbivore aversion to leaves with bioactive secondary compounds.

Key words: secondary compounds; *medicinal*; Leguminosae; *feeding preference*; *herbivory*; *antifungal; bioassay*

INTRODUCTION

Plants are constantly exposed to predators, and with their usually sessile lifestyle comes an inability to physically evade any herbivores or pathogens that may prey on them. Thus, many plants have evolved mechanisms, often physical or chemical, through natural selection that can help deter their predators. One such biochemical mechanism is the synthesis of secondary compounds or metabolites, which are not a part of the main metabolic system of the plant but often act as "defensive agents" against predators (Mayer 2004). Such chemicals are believed to have originated in certain plants as metabolic waste or chemical mutation, then undergone selection towards individuals with better protection from predators, increasing the plant's livelihood (Seigler and Price 1976). The efficacy of these secondary compounds against plant herbivores of many types has been well documented, and their mechanism often functions by affecting the lifecycle of predatory organisms or deterring them from preying on the plant itself (Mayer 2004). Tannins, such as those found in grapes, can decrease the growth rate of herbivores, potentially subjecting them to increased predation themselves (Stamp 2003). On the other hand, chemicals such as terpenes and alkaloids can act as taste deterrents, sometimes even leading to a conditioned aversion to the compounds (Lawler et al. 1999). Some secondary metabolites have also been found to be effective against both herbivores and fungal pathogens, and vice versa (Mayer 2004), which could indicate some all-inclusive plant chemical defensive mechanism able to protect an individual from any number of potential predators.

The evolution of natural defenses within plants incurred a coevolution of the insects that prey on them, as natural selection favored those able to survive the effects of their prey's secondary metabolites. This arms race between insects and plants has often led to host-specificity for certain plants, where

predators able to cope with noxious secondary metabolites have a significant advantage over their conspecifics unable to do the same (Cornell and Hawkins 2003). Plants armed with chemical defenses will sometimes have certain groups of insects or pathogens as frequent predatory visitors. Some herbivores can adapt to these secondary metabolites, becoming specialists (Cornell and Hawkins 2003), often coevolving with the plant: as selection may tend towards more potent plant secondary compounds, the specialists will likely maintain their host preference by being selected towards chemical tolerance. Thus, even toxic plants may have some form of predator.

However, though secondary metabolites can be harmful to insect predators or pathogens, humans have historically used many plants for their medicinal qualities. Through our modern understanding of medical ethnobotany, these bioactive chemicals have exhibited a wide array of capabilities, such as antimicrobial or antifungal abilities, that support their traditional medicinal uses (Achrekar 1995). For instance, the beach hibiscus (*Hibiscus tiliaceus*) tested positive for antimicrobial properties (Achrekar 1995, Cox 2008) which seems to support its traditional Polynesian uses for treating fevers and infections. Many plant species have documented medicinal uses and activity (Whistler 1992) that may indicate the presence of bioactive secondary compounds, and a subsequent ability to deter some, but perhaps not all, predators.

A previous study noted an inverse correlation between insect herbivory and medicinal activity in three species of plants used by traditional Tahitian healers in Mo'orea, French Polynesia (Cox 2008). I will investigate an expanded selection of both traditionally medicinal and non-medicinal plants in order to analyze this correlation with the inclusion of any microbial or fungal pathogens that may be preying on the plants. Looking at three species of medicinal and three species of non-medicinal plants of the Leguminosae family, I will see if the relative amount of leaf damage on medicinal species is different than on their non-medicinal counterparts. If having secondary metabolites deters leaf predation, do medicinal plants get preyed on less? Herbivore feeding preferences for medicinal or non-medicinal species will also be tested in the lab with natural insect predators of several of these species in order to determine if there is a difference in the level of predation between these two groups. I will compare these observations with an antimicrobial bioassay to see if having bioactive secondary metabolites actually helps deter plant predators.

METHODS

This study compared plants that are traditionally used by Tahitians medicinally and plants not used traditionally because they are not known to possess any medicinal qualities. Species from the *Leguminosae* family were chosen because of their prevalence on Mo'orea, French Polynesia, and to relate the two groups of plants (medicinal and non-medicinal) as closely as possible. Three species of plants were assigned to each group (Figure 1) based on records of their medicinal activity (e.g. used to treat inflammation, illness, etc.) or lack thereof (Whistler 1992):

Medicinal	**Non-medicinal**
Inocarpus fagifer	*Falcatoria moluccana*
Sophora tomentosa	*Leucaena leucocephala*
Vigna marina	*Mimosa pudica*

Fig. 1. Species belonging to medicinal and non-medicinal groups.

*For future reference, *V. marina*, *S. tomentosa*, and *I. fagifer* may be referred to as the "medicinal" species or group, and *F. moluccana*, *M. pudica*, and *L. leococephala* may be referred to as the "non-medicinal" species or group.

Study sites

Study sites (Figure 2) consisted of a variety of localities around Mo'orea and were chosen based on access and availability of sample species. Because distribution was not a focus of this study, sites included anywhere with at least three individuals of a species. Localities were sampled until ten individuals

per species were attained (minimum two localities). One site was on UC Berkeley Gump Station's property (17°29'28.58"S, 149°49'35.43"W), along the coast just south of the station. Another locality was Mari Mari Kellum's property (17°30'51.34"S, 149°50'53.42"W), specifically heading inland towards heavier vegetation. The non-medicinal species were also found near the Belvedere lookout (17°32'25.79"S, 149°49'36.08"W), and *I. fagifer* was found in the Mape forest along the trail leading towards Three Pines (17°32'23.55"S, 149°49'33.08"W). Individuals of *V. marina* were found near the Temae public beach (17°29'54.58"S, 149°45'42.50"W). *S. tomentosa* was collected at motu Teahora (17°29'19.26"S, 149°54'39.93"W) and on the main road just east of the Intercontinental Hotel (17°29'34.02"S, 149°53'26.83"W).

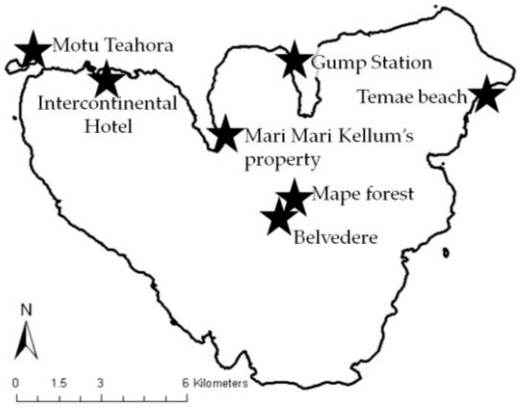

Fig. 2. Location of study sites on Mo'orea.

Plant material collection

For each species, ten individuals from a minimum of two localities were randomly selected for experimental sampling (n=60). Each individual had ten leaves (n=600) within three meters of the ground randomly excised and photographed, either in the field (specifically for mimosoid leaves, to reduce damage while in transport before photographing) or in lab for damage analysis. Immediately after collection, ethanol extracts of the leaves were prepared and stored for future use.

Leaf damage analysis

Each leaf sampled was photographed and scaled using ImageJ. Herbivory by predator or pathogen was quantified as percent leaf damage, which was calculated by dividing the area of damaged or eaten tissue by the total area of the intact leaf. These areas were digitized using ImageJ. Leaf photos were randomized during measurement to reduce bias. Predation was defined as any tissue seemingly removed by an insect herbivore or damaged (usually brown, visibly dead), possibly by some sort of fungal pathogen or leaf miner. Total area of large mimoisoid leaves were digitized by averaging the size of ten leaflets, then multiplying by the total number of leaflets.

Extract preparation

For each individual, due to time constraints, only one ethanol extract was prepared. Approximately 5g of leaf matter collected that day were wiped clean of debris using Kim Wipes dipped in ethanol and cut into smaller pieces, which were then mixed in a kitchen blender with 20mL of 90% ethanol. The mixture was blended until all leaf tissue was sufficiently macerated and the mixture homogeneous. The resulting liquid, green in color, was transferred to Falcon tube vials and refrigerated, being shaken intermittently until use (Cox 2008).

Antimicrobial bioassay

Using the ethanol extract, an antimicrobial bioassay was conducted to test the individual plant extract's ability to combat microbial growth in order to simulate the individual's effectiveness against pathogenic predators. The microbe used for the bioassay was commercial baking yeast, *Saccharomyces cerevisiae*, because of its availability (Cox 2008). A 1.5% malt extract agar was prepared using agar powder, malt extract, and filtered water, and amended with 100mg/mL Streptomycin sulfate, an antibiotic to prohibit any bacterial growth and ensure only fungal growth. The solution was boiled/autoclaved and distributed into medium-sized Petri dishes

(n=60). The medium was allowed to solidify overnight, then inverted and stored until use.

Once the medium was prepared, 3.0g of yeast (*S. cerevisiae*) was mixed with 50mL distilled water and was added to the medium, with each Petri dish receiving approximately 0.2mL of yeast solution. The solution was streaked around the plate using a micropipette tip, making sure to not puncture the agar. *S. cerevisiae* was incubated and allowed to establish on the medium for 24 hours, growing into a uniform spread over the agar medium.

Using a hole punch, filter paper discs approximately .6cm (need exact measurement) in diameter were created and used as the standardized vessel for applying the ethanol extracts. Each individual vial of plant extract had ten filter paper discs dipped in the extract three times each, and placed on top of the yeast layer equal distance from the others within one Petri dish. An antifungal cream (Ketone 2%) that was prescribed for general topical treatment was mixed into 90% ethanol and used as a positive control, while 90% ethanol was used as a negative control.

Once inoculated with plant extract, the Petri dishes were sealed and incubated for three days at 30 degrees Celsius before being removed for analysis. Effectiveness of individual plant extract was measured qualitatively, with inhibition under and around each filter paper disc designated a rating of "0" for no inhibition, "1" for low inhibition, "2" for medium inhibition, and "3" for high inhibition. The inhibition rating was averaged amongst the ten replicates per individual.

Feeding experiment

Using herbivores found near the study species and around other vegetation, prey preference of captured insect herbivores was examined using a cage experiment. Insect sweeps were conducted near the Gump station and sampling sites every day to gather insect herbivores, which were put into a closed cage with five pristine leaves of each species that were photographed beforehand. The leaves were checked every day, and left in the cage for an arbitrary amount of time, after which all leaves were to be removed and photographed again to calculate percent leaf damage. Differences within group and between groups were compared.

Statistical analysis

For analysis of the leaf damage data, an analysis of variance (ANOVA) using JMP statistical software was conducted to compare the means between species to determine if there was a difference in percent leaf damage. Replicates were averaged to the individual (n=10 per species) and an ANOVA of the two groups was used to show the difference between the two groups, medicinal and non-medicinal. A Tukey-Kramer multiple comparisons test was used to show where the difference was by showing statistically similarity between each species by pair. The same process was utilized for comparing differences in yeast inhibition rating for the antimicrobial bioassay. A correlation was tested using a multivariate analysis comparing leaf damage and yeast inhibition rating. A nonparametric Spearman's Rank Correlation analysis calculated a p-value to determine if there was a significant correlation in the data.

RESULTS

Leaf Damage

Using a one-way ANOVA to compare the mean percent leaf damage between the six species (*F. moluccana, I. fagifer, L. leococephala, M. pudica, S. tomentosa, V. marina*), the result showed a statistically significant difference between groups ($p<0.001$, F ratio=7.3797, df=5). This indicated that species had a significant effect on the level of leaf damage. Two non-medicinal species, *F. moluccana* (mean=3.863) and *L. leococephala* (mean=3.923), had the most mean leaf damage, while two medicinal species, *S. tomentosa* (mean=0.975) and *V. marina* (mean=1.216), had the lowest mean leaf damage; within that range were the last two species, *I. fagifer* (mean=2.227) and *M. pudica* (mean=2.109), a medicinal and non-medicinal species, respectively. Standard error for analysis was 0.466.

Using a Tukey-Kramer multiple comparisons test, the specific area of difference was determined in the paired-comparisons between *L. leococephala*/*F. moluccana* and *S. tomentosa*/*V. marina*. These two pairings showed a significant difference between one another, indicating a lack of statistical similarity ($p<0.001$ for all four pairings), with the latter pair having a significantly lower amount of leaf damage. *I. fagifer* and *M. pudica*, however, showed a significant similarity to all groups ($p>0.05$) and each other ($p=1.00$).

A one-way ANOVA (Figure 3) between all medicinal species and all non-medicinal species further emphasized the significant difference between the two groups ($p<0.0001$, F ratio=19.701, df=1), with the medicinal species (mean=1.472) exhibiting a lower mean percent leaf damage than the non-medicinal species (mean=3.298). Standard error for the analysis was 0.291.

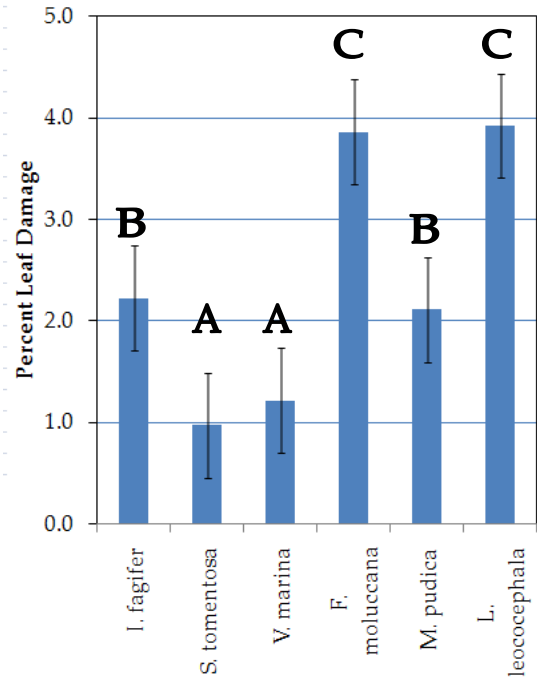

Fig. 3. Mean percent leaf damage for all six species. A one-way ANOVA showed a significant difference ($p<0.0001$) between species. A Tukey-Kramer multiple comparison test showed 3 groups of statistical similarity indicated by letters above data. Error bars denote standard error.

Antimicrobial assay

A one-way ANOVA between all species and the positive and negative control shows a significant difference between the six species and controls ($p<0.0001$, F ratio=16.857, df=7). The results from this experiment seem viable because of the results from the controls, where the negative control (ethanol) showed zero inhibition and the positive control (Ketoderm antifungal cream) exhibited the highest inhibition rating. Using a Tukey-Kramer multiple comparisons test, Ketoderm is shown to be significantly different from all other pairings ($p<0.0001$). Though all six species are statistically different, the Tukey-Kramer test shows statistical similarity between all species ($p>0.05$) and ethanol, with only *V. marina* being significantly different from the negative control ($p=0.001$). This can be seen in the data, with *V. marina* having the highest mean rating (mean=0.67). Curiously, the non-medicinal *F. moluccana* and *L. leococephala* (means=0.47, 0.45 respectively) had higher mean inhibition ratings than *S. tomentosa* and *I. fagifer* (means=0.41, 0.34 respectively), two medicinal plants. *M. pudica* registered the lowest mean inhibition rating (mean=0.25). Standard error for the analysis was 0.108.

Using a one-way ANOVA (Figure 4) between all medicinal species versus all non-medicinal species excluding the controls, the data shows no significant difference between the two groups ($p=0.2668$, F ratio=1.257, df=1), though the mean of the medicinal group is higher (mean=0.47) compared to the non-medicinal species (mean=0.39).

Correlation

Data for all species for percent leaf damage and inhibition ratings were used to determine if there was a correlation between the amount of herbivory and the level of yeast inhibition (Figure 5). A multivariate analysis of the data using a nonparametric Spearman's Rank Correlation test showed there was no significant correlation ($p=0.785$) between leaf damage and inhibition rating for all six species. Looking by group, there was still no significant correlation for the medicinal ($p=0.914$) and non-medicinal ($p=0.585$) species.

None of the leaf damage and inhibition ratings for each individual species showed a significant correlation either ($p>0.05$). From this data, there appears to be no statistically significant relationship between leaf damage and yeast inhibition.

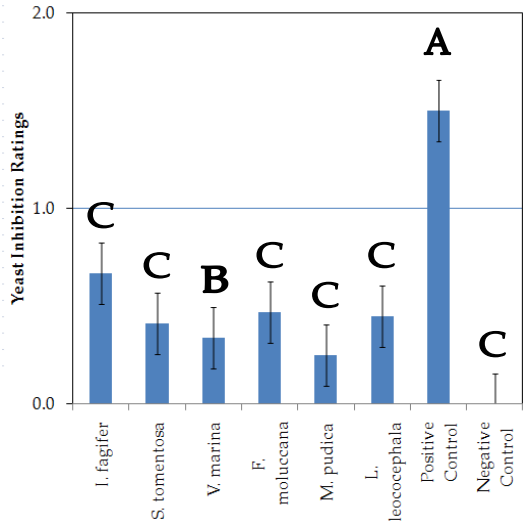

Fig. 4. Mean yeast inhibition rating for all six species. A one-way ANOVA showed a significant difference ($p<0.0001$) between species. A Tukey-Kramer multiple comparison test showed all species to be statistically similar, with only *V. marina* being significantly different than the negative control. Statistical similarity indicated by letters above data. Error bars denote standard error.

Feeding experiment

The original goal of this experiment was to have percent leaf damage before and after the experiment, in order to compare the data to see if there was a quantifiable change over time in the amount of leaf damage per species. This data would have been compared across species to see if there was a difference in herbivore feeding preference between the medicinal and non-medicinal groups. However, only data for the leaves of the three medicinal species (*I. fagifer*, *S. tomentosa*, *V. marina*) were available. Percent leaf damage was calculated for all leaves as virtually 0%, with no sign of herbivory. Each leaf used for this experiment from these three species was seemingly as pristine as it had started.

The damage on the non-medicinal leaves was not quantified because the leaves became moldy. Herbivores were observed feeding every day of the experiment, and notes taken during the experiment show that by Day 5, the non-medicinal leaves had begun changing color and looking moldy. Herbivores had begun dying, though additional herbivores had been continuously added. The experiment was ended on Day 7, when most herbivores in the cage were dead and the non-medicinal leaves had visibly molded.

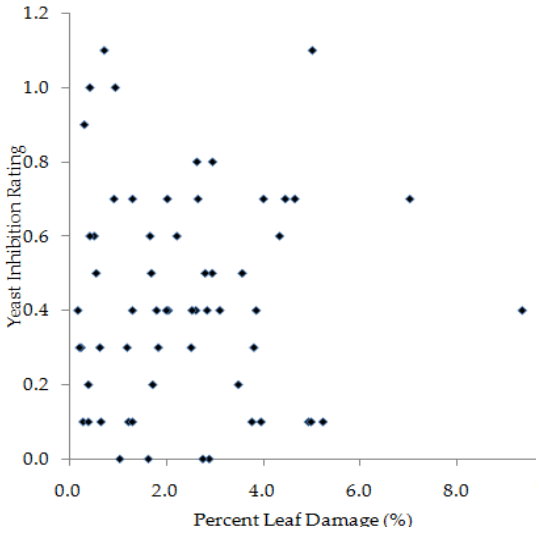

Fig. 5. Leaf damage versus yeast inhibition ratings. The data points show no clear correlation. Spearman's Rank Correlation showed no significance ($p=0.785$).

DISCUSSION

Leaf Damage – The results from the leaf damage analysis show the non-medicinal group to have significantly higher leaf damage than the medicinal species, which could indicate a trend in predation away from plants that reportedly have secondary compounds. The data from the Tukey-Kramer test appears to follow my hypothesis that medicinal plants will exhibit less leaf damage, with *S. tomentosa* and *V. marina* having the least percent leaf damage, and *F. moluccana* and *L. leococephala* showing the most percent leaf damage. It is

interesting to note that *I. fagifer* and *M. pudica* are statistically similar, with their mean leaf damage sandwiched evenly between the means of the other two pairings. This seems to agree with my hypothesis for *I. fagifer*, a medicinal plant, but *M. pudica* was not thought to have any secondary compounds yet exhibits the same level of leaf damage. This could be explained by *M. pudica*'s commonly known non-chemical defense, the rapid seismonastic movement that results in the contraction of the leaflets (Allen 1969). Such reaction to stimuli may play a role in deterring insect herbivores and reducing the level of leaf damage sustained by the plant.

However, the relatively low leaf damage in *M. pudica* compared to the other two non-medicinal species could also be an indication of the presence of secondary compounds. One study conducted at the University of the West Indies shows *M. pudica* to have medicinal uses as an antihelminthic, an agent used to expel or kill worms (Mitchell and Ahmad, 2006). Another study reported the root of *M. pudica* to help induce wound healing activity possibly due to the presence of tannins and mimosine, an alkaloid chemical (Kokane et al. 2009). Though *M. pudica* is not traditionally used by Tahitians for medicinal purposes, some level of bioactivity may be present within the plant which, in combination with its seismonastic reflexes, may explain its relatively small amount of leaf damage.

Although the leaf damage results do not directly indicate the presence of secondary compounds, there is still evidence of a significant difference in herbivore preference toward non-medicinal species and away from those that may contain bioactive secondary metabolites. Nevertheless, the trends in relative leaf damage should be acknowledged along with the possibility of other confounding factors. Given the wide array of physical defenses a plant may have (e.g. leaf thickness, waxy cuticle, movement) and the complexities of interspecies relationships (e.g. Enemy Release Hypothesis), any number of factors can contribute to a plant's defense against herbivores.

Antimicrobial assay – The results of the yeast inhibition assay failed to shed any light on the presence or absence of bioactive secondary compounds as I had hoped due to the lack of a significant difference between the medicinal and non-medicinal group. There was no clear trend as I had hypothesized, with two medicinal species registering lower mean inhibition ratings than two non-medicinal species. Such results point to several possibilities: the medicinal species may not contain bioactive chemicals as hypothesized and the non-medicinal species may have some chemical defenses, or the bioassay as performed did not accurately represent chemical activity.

The latter seems more likely, as studies conducted on the three medicinal species show the presence of chemical compounds, validating their traditional medicinal use. *S. tomentosa* is known to contain an alkaloid chemical called cytisine, which can induce emesis (vomiting) and is a known purgative (Gilman 1999), as well as a host of other chemicals such as prenylflavanones. These chemicals have exhibited cytotoxic and antimicrobial activity, making *S. tomentosa* a very bioactive species (Shirataki et al. 2001). A study conducted in 2000 used UV spectroscopy and chromatography to show the presence of antibiotic flavonoid compounds in the leaves of *V. marina* that exhibited an inhibitory effect on their aphid predators (Lattanzio et al. 2000). No studies were found showing the presence of secondary compounds in *I. fagifer*, but the plant's bark is known to be used for treating various ailments such as burns and diarrhea (Whistler 1993).

In contrast, the non-medicinal species have been shown to have some chemical activity or potential, but do not appear to be as bioactive. A study published in 2007 showed the cytotoxic and anticancer capabilities of certain polysaccharides isolated from *L. leococephala* after chemical modification, though the "unmodified crude extract" was not chemically active (Gamal-Eldeen et al. 2007). As reported above, *M. pudica* was shown to have specialized medicinal properties due to the presence of tannins and mimosine, but this study was performed using the root of the plant, whereas my experiment was only concerned with the leaves. No

studies were found that identified any secondary metabolites in *F. moluccana*.

Due to the studies demonstrating the presence of secondary compounds in the medicinal species, it seems likely my experiment was not indicative of their true chemical activity. This could have been due to the antimicrobial assay experimental procedure. My protocol was replicated from two previous studies conducted in Mo'orea in 2005 and 2008; in one study, the protocol produced significant results of yeast inhibition (Cox 2008) while the other study was unable to produce significant results because the positive control failed, which was attributed to inadequate assay design (Trotter 2005). The effectiveness of my experiment was somewhere in between these two studies, where my controls worked and there was inhibition of yeast, but the results were not as defined as I had anticipated. This lack of consistency over the span of three studies is not desirable and leaves much room for improvement. Future studies may be wise to allow the yeast more time to grow after the application of the extracts via filter paper disc, which in my case may have made the difference in yeast inhibition more apparent. Commercial baking yeast may also have not been a good model-microbe to use for this experiment, but due to a lack of time and supplies no other fungal or bacterial colonies could be cultured. The medicinal species may be inhibitory towards microbes other than *S. cerevisiae*; future studies using similar broad-spectrum bioassays should test inhibition of other common microbes such as mold fungi or *Escherichia coli*.

Correlation – The non-correlation calculated using the percent leaf damage and yeast inhibition ratings was most likely a reflection of the unclear results of the antimicrobial assay. My data clearly does not show a relationship, which is inconsistent with my original hypothesis based on Cox's 2008 findings of a negative correlation between herbivory and yeast inhibition (Cox 2008). However, if the antifungal bioassay was to be refined and the study repeated, there may indeed be a relationship between level of herbivory and the presence of antimicrobial secondary compounds. Though my yeast inhibition assay did not provide any clear-cut evidence of bioactive chemicals, observations made during my feeding experiment seem to support the literature and my hypothesis about the presence of secondary compounds in *I. fagifer, S. tomentosa, V. marina* species.

The feeding experiment provided interesting observations that support my belief that medicinal species possess secondary metabolites and seem to indicate a trend in the feeding preferences of herbivores. While the non-medicinal plants developed extreme fungal growth (*M. pudica* less so), the medicinal plants came out with zero mold, which appears to indicate the presence of antifungal chemicals in the leaves of the medicinal plants that prevented mold colonies from establishing. By contrast, the absence of antifungal chemicals in the non-medicinal species allowed for complete fungal takeover. *M. pudica* was clearly in better shape than *L. leococephala* and *F. moluccana*, which might confirm the presence of chemicals in *M. pudica* as shown in the literature. Nevertheless, all three non-medicinal species did not fare well. Because the medicinal plants were pristine after the seven day experiment, during which time the herbivores were observed eating, it seems the herbivores were feeding exclusively on *L. leococephala*, *F. moluccana*, *M. pudica*. A point of interest is on Day 6 and 7, just one day after the non-medicinal leaves became moldy, when herbivores were observed to be dead. The two events seem correlated, in that once the non-medicinal leaves became inedible, the herbivores opted for expiration.

Though the exact mechanism of this aversion to medicinal leaves cannot be known through this experiment, there is a definite tendency for the herbivores to avoid eating secondary compounds. Determining the cause of this feeding partiality is the difficult part. The herbivores may be taste sensitive to the plants, or have some other negative sensory response to the leaves. As with the leaf damage analysis, the cause might not even be chemical and could be attributable to physical factors such as leaf thickness or a waxy cuticle. Whatever the reason, two general interpretations can be made from this experiment: medicinal species were able to fend off mold while the non-medicinal species

could not, and the herbivores chose not to prey on medicinal species' leaves.

The findings presented by my study were unable to show a relationship between herbivory and the presence of secondary compounds as I had theorized, but the trends shown are supportive of my original hypotheses that the medicinal species contained antimicrobial chemicals and would experience less predation. The feeding experiment inadvertently showed the antifungal properties of the medicinal group's leaves in action, especially when compared to their non-medicinal counterparts. This observation was not represented well in the yeast inhibition bioassay, which I believe warrants a future refinement of the protocol to ensure accurate, consistent results. Studies that wish to look at specific chemical composition are difficult due to the lack of supplies and equipment on Mo'orea, so the continued refinement of general bioassays is essential to performing future studies on chemical activity. One modification to this procedure could be to determine if there is a difference between extracting plant compounds in ethanol, methanol (another common extractant), or some combination of the two to see which is most effective. An interesting future study utilizing these same bioassays would be to look at the preparation of traditional Tahitian treatments using medicinal species, and determine the functional bioactivity of the actual remedies rather than just the raw plant material.

Overall, based on the leaf damage analysis and feeding experiment, there is strong evidence in the field and in lab that common herbivores on Mo'orea have an aversion to leaves with secondary compounds. Future research into these species and other medicinal plants may indicate more about their chemical defenses and, in general, reveal more about the evolution of plants and their co-evolution with their predators.

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