

THE CORRELATION BETWEEN HERBIVORY AND MEDICINAL ACTIVITY IN *THESPESIA POPULNEA*, *HIBISCUS TILIACEUS* AND *HIBISCUS ROSA-SINENSIS* ON MO'OREA, FRENCH POLYNESIA

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Abstract. While secondary compounds are produced by plants in low abundance, these bioactive compounds are essential to human survival for their medicinal applications. These same compounds are crucial to plants, having evolved as defense mechanisms against herbivory. Chief among the theories of plant responses to herbivory, the Optimal Defense Theory (ODT) hypothesizes that plants will allocate defenses in direct proportion to the risk of a particular plant part to herbivory and the value of that part in terms of loss of fitness to the entire plant. Through insect damage assessments and antimicrobial assays, this study investigates the correlation between herbivory and medicinal activity and whether or not the within-plant ODT is followed in three ethnobotanically useful Malvaceae species, *Thespesia populnea*, *Hibiscus tiliaceus* and *Hibiscus rosa-sinensis*. All three plant species demonstrated an inverse relationship between herbivory and medicinal activity. Variation in secondary composition data from both insect damage assessment and antimicrobial tests supported the ODT.

Keywords: *Thespesia populnea*, *Hibiscus tiliaceus*, *Hibiscus rosa-sinensis*, *coevolution*, *secondary compounds*, *herbivory*, *Optimal Defense Theory (ODT)*, *antifungal*

INTRODUCTION

Since the beginning of human history, plants have played an integral role in human survival. However, while scientists have calculated that humans in their modern form speciated only approximately 200,000 years ago, primitive vascular plants originated in the Silurian period, roughly 430 million years ago. The oldest fossils identified definitively as angiosperms date from the early Cretaceous Period, around 127 million years ago (Raven and Johnson 1989). Thus the origin of medicinal activity in plants must have been in response to environmental, not human, pressures. There is a considerable amount of evidence that herbivory was a major selective force on the evolution of plant defenses

(Stamp 2003), which are typically used for medicinal applications.

The antagonistic arms race between plants and insects is evidenced by the diversification of insects and plants which occurred over the same approximate time periods, and many of these insect/ host plant relationships have been conserved (Farrell 1994). Today there are about 240,000 species of flowering plants, which greatly outnumber all other kinds of plants. As the flowering plants became more diverse, so did the insects whose feeding habits were closely linked with the characteristics of the flowering plants, supporting the idea that insects and flowering plants coevolved with one another (Raven and Johnson 1989). In response to attacks by herbivores, plants have evolved a variety of mechanisms for defense. Insects have in turn

countered with their own defenses (Stamp 2003) because once the members of a particular group acquired the ability to feed on a certain bioactive plant, these herbivores gained access to a new resource which they could exploit without competition from other herbivores (Raven and Johnson 1989). The defense mechanisms in plants that act as insect deterrents are known as “secondary” compounds since they do not contribute to any primary photosynthetic or metabolic activities to the plants (Stamp 2003). While secondary compounds are produced in low abundance, they are essential to human and plant use.

There are many possible ways plants can respond to herbivory. According to Stamp (2003), the within-plant Optimal Defense Theory (ODT) predicts that a plant will allocate its defenses in direct proportion to the risk of a particular plant part to herbivory and the value of that part in terms of loss of fitness to the entire plant. Further, there will be within-plant variation in the distribution of these bioactive compounds for defense; the plant will invest the greatest amount of defensive secondary compounds in its most reproductively active parts (flowers, fruits and young leaves) (Anderson, 2005). Anderson (2005) found support for this idea in a study focused on a model system using cotton (*Gossypium hirsutum*: Malvaceae) in which damage resulted in higher concentrations of secondary compounds in the young and/or reproductive tissue. This type of defense is known as an induced defense reaction because it involves new production or the translocation of secondary compounds within the plant in response to tissue damage (Anderson 2005). Anderson’s study further revealed that an induced response occurs after insect damage throughout the entire plant regardless of where plant damage originally occurred. Another major type of plant defense is constitutive defense, a defense always present in a plant. Anderson’s (2005) study documented the presence of both constitutive and induced defenses in cotton plants.

During this project I screened three species in the Malvaceae family (*Thespesia populnea*, *Hibiscus tiliaceus*, and *Hibiscus rosa-sinensis*), all with known bioactivity, for the purpose of assessing how the bioactivity of these species correlates with damage from herbivores. Specifically, I ask: (1) does the amount of herbivory damage on a given plant relate to the amount of insecticidal secondary compound in the plant; and (2) do these species allocate more secondary compounds to their younger and/or reproductively active parts as compared to their older parts, following the predictions of the ODT?

METHODS

Study Sites

Mo’orea (GPS location: 06K 0192036 E, 8062786 N) is a volcanic, high island (maximum elevation 1207 m) that is part of the Society Islands archipelago in French Polynesia. Three species in the Malvaceae family were selected for study for two reasons: first, each species has documented medicinal activity (Whistler 1992) and second, Anderson’s (2005) study on the model Malvaceae species, cotton, found evidence for the presence of anti-herbivore defensive compounds. *Thespesia populnea*, *Hibiscus tiliaceus*, and *Hibiscus rosa-sinensis*, were collected from two sites on Mo’orea– site A along the coastline just south of Gump Station (06K 0199841 E, 8063792 N) and site B along the coastline on Mari Mari Kellum’s property (06K 0197422 E, 8061475 N) (Figure 1). All plant species collected were deposited into the University of California Herbarium, Berkeley.

Sample Species

Thespesia populnea, Soland ex. Correa 1807, of the Malvaceae family (Tahitian name “Miro”) is a native tree that is widespread from East Africa to Eastern Polynesia and is common both along beaches (Medicinal Plants in the South Pacific, 1998), and in the valleys

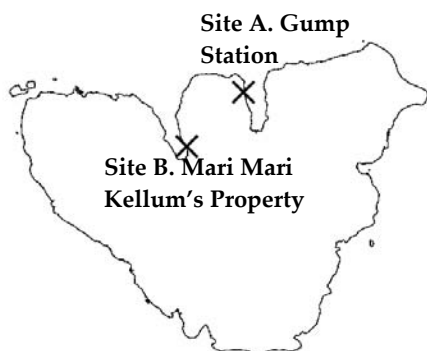


FIG. 1. Site locations on Mo'orea.

of Mo'orea (Chabouis, 1970). It is known to contain antibacterial, antifungal, anti-yeast, antiimplantation and antispasmodic properties (Medicinal Plants in the South Pacific 1998) and has a wide range of traditional uses throughout the South Pacific. These include the use of an infusion of the crushed seeds to soothe headaches and the use of sap from the fruit stalk to topically treat skin ailments such as centipede bites (Whistler, 1992), jellyfish stings and cuts (Tom Carlson, personal communication).

Hibiscus tiliaceus, Linnaeus 1753 (common name yellow or beach hibiscus or Tahitian name "Purau") is a native, medium-sized tree abundantly distributed throughout the tropics. It is found along coastlines worldwide and is common on beaches and thickets (Medicinal Plants in the South Pacific 1998), yet can spread up to the highest points on Mo'orea (Murdock, 2008). *H. tiliaceus* has traditionally been used by the Polynesians to treat a wide range of ailments including fractured bones, sprained muscles, gonorrhea, menstrual cramps, abrasions, and fever (Medicinal Plants in the South Pacific 1998). A student paper (Achrekar 1995) additionally found this plant to test positively for anti-yeast and antibacterial properties.

Hibiscus rosa-sinensis, Linnaeus 1753 (common name red hibiscus, Tahitian "'aute") is a non-native shrub commonly cultivated as a garden ornamental found from sea-level to

500 m elevation. *H. rosa-sinensis*, widely introduced throughout the South Pacific (Medicinal Plants in the South Pacific 1998) has been reported to have antioestrogenic, anti-cancer, analgesic, anti-inflammatory and antifungal properties (Medicinal Plants in the South Pacific 1998). Traditionally, *H. rosa-sinensis* has been used by Tahitians to induce abortion, ease menstrual cramps, assist in childbirth and to treat boils, sores and inflammations (Medicinal Plants in the South Pacific, 1998).

Plant Material Collection

At each site, plant material was collected from three individuals of each species ($n=18$). In order to assess if the amount of herbivory damage on a given plant correlated to the amount of insecticidal secondary compound in the plant, samples were collected from each individual, separating each tree into four quadrants: water-side left (A), water-side right (B), mountain-side left (C), and mountain-side right (D) (Figure 2). Each quadrant was separated into the highest and lowest four branches that could be reached. To determine if these species allocated more secondary compounds to their younger and/or reproductively active parts as compared to their older parts, four "young" and four "old" leaves were collected from each height within each quadrant from each individual ($n=1152$). "Young" leaves were categorized as the first sizable leaf at the tip of each branch and "old" leaves as the tenth leaf down on the same branch. Additionally, five haphazardly collected fruit samples for *T. populnea* and five flowers (if at least five were present) from all three plant species were collected from each individual. Fruit and flower samples were only collected from Site A; no viable reproductive structures were found on the trees present in Site B.

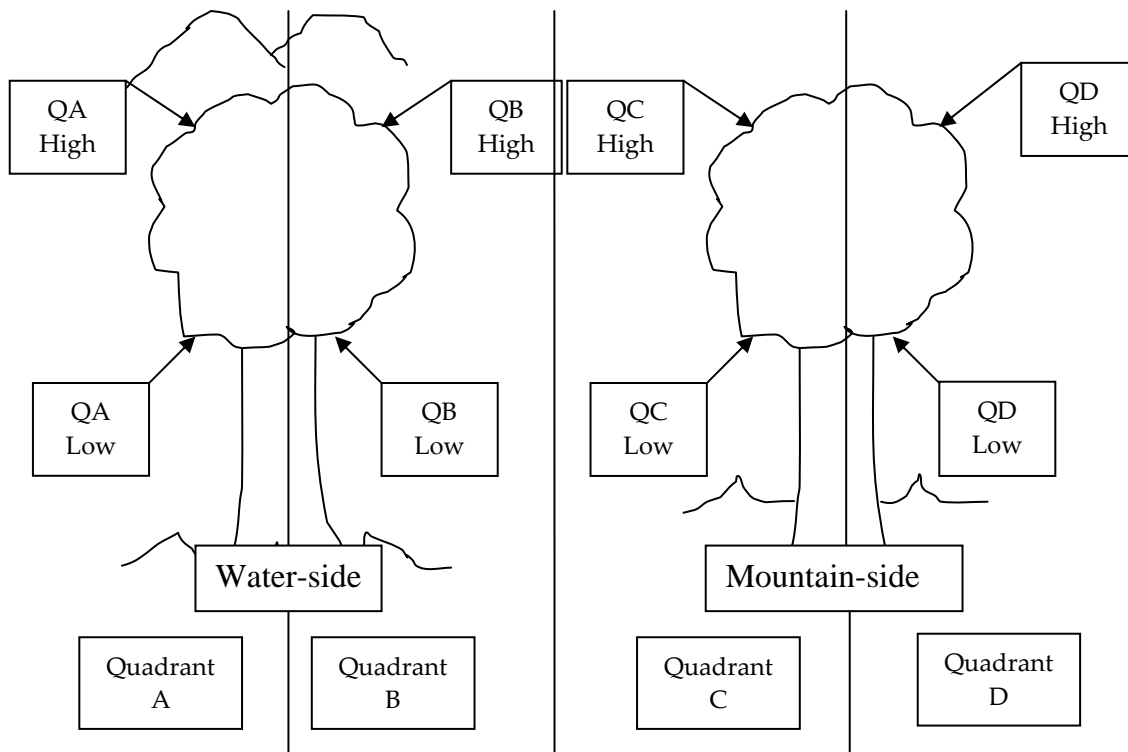


FIG 2. Diagram of plant material collection per individual tree. At each arrow, 8 total leaves per individual were collected, 4 “young” leaves and 4 “old” leaves. Therefore, 1152 leaves were collected overall from all individuals

Insect damage

Immediately after leaf collection, each leaf was photographed mounted on graph paper. Image J™ (1997) was used to determine the percentage of insect damage per leaf by dividing the amount of tissue removed by herbivores by the total area of the intact leaf. For all three species, insect damage was quantified as damage from leaf-chewing herbivores. However, an unidentified brown damage separately that was common to all *H. tiliaceus* individuals was also separately measured since it may or may not have been due to sucking insects. However, it could not be determined definitely that this damage was from insects and not from a deficiency in the plant or a fungal pathogen. Ultimately, only the damage done from leaf-chewing herbivores was used, as this was the one type of insect damage common to all three species.

Extract preparation

For each tree, 90% ethanol extracts were prepared from the leaf matter collected from quadrant A and filtered water extracts were prepared from leaf matter collected from quadrant B. Both types of extracts were chosen because each solvent may extract different compounds with varying levels of antifungal activity. Both 90% ethanol and filtered water extracts were made from the flowers and/or fruits collected from each tree. Five grams of fresh plant matter were weighed, sterilized by a Kim Wipe™ dipped in ethanol and then sliced into thin pieces using a razor blade. The plant matter was then added to either 30 mL of 90% ethanol or 30 mL of filtered water and then mixed in a blender until the mixture was as homogenous as possible. After blending, I transferred all extracts to vials. The water extracts were ready for use immediately, while the ethanol extracts were refrigerated at 7 degrees Celsius

and shaken daily for three days before they were ready for use.

Antimicrobial assay

The antimicrobial assay of all leaves, flowers and/or fruits of all three plant species was carried out using a culture of *Saccharomyces cerevisiae* or commercial, unicellular baking yeast. Yeast was chosen for its accessibility, similarity to fungi that affect humans, and the known antimicrobial activity of all three Malvaceae species. Following the methodology used by Trotter (2005), sugar-enriched agar medium was made by mixing 14g bacteria I grade agar powder with 100g white granulated sugar and 1L filtered tap water in a large Erlenmeyer flask. Then a pressure cooker was used to boil the mixture and pour approximately 0.5 mL into each Petri dish for a total of roughly 40 petri dishes per 1L batch. After the mixture solidified, which usually took between a couple of hours to overnight, each Petri dish was inverted to reduce condensation and refrigerated until use.

Afterwards, three grams of baking yeast was mixed with 50mL water and about 0.2mL was spread onto each plate, with care not to puncture the agar. Each dish was then sealed with Parafilm™ and incubated in an incubator constructed from two 60-watt desk lamps and a cardboard box with holes to attain a constant temperature throughout. Yeast was incubated for 24 hrs in order to attain a uniform lawn of fungi before the extracts were applied.

Next, a hole punch was used to create circular disks of filter paper 0.6 cm in diameter. For each vial of extract, ten disks were dipped three times each and placed evenly throughout the top of the recently cultured yeast layer. Filtered tap water and ethanol were both used as negative controls and two commercial anti-fungals, 2% miconazole nitrate and 1% econazole nitrate, mixed with either filtered water or ethanol were used as positive controls.

Finally, the plates were re-sealed with Parafilm™ and incubated in the incubator for three days. Inhibition of yeast growth under the extract-soaked filter paper was checked after this time period. Results were measured qualitatively by designating inhibition of yeast growth under each filter paper dot an inhibition rating of “0” for no inhibition, “1” for low inhibition, “2” for medium inhibition and “3” for high inhibition. Therefore, the total level of inhibition per each type of extract had a rating between 0-30, as each type of extract had used ten disks soaked in extract with a rating of 0-3 yeast inhibition per disk.

Statistical methods

First, the insect damage data was analyzed using an analysis of variance (ANOVA) test and Tukey-Kramer analysis for significant differences between extracts made from different tree species, individual trees, heights and leaf ages. Next, the inhibition data was analyzed using ANOVA and a Tukey-Kramer test to look for significant differences between extracts made from different tree species, individual tree, heights, ages, extract types and plant parts. Finally, inhibition data was compared with insect damage data using analysis of covariance (ANCOVA). All statistical tests were performed using JMP 7.0 (2008).

RESULTS

Insect damage

Initially, using ANOVA, it was discovered that for all three plant species, site (Site A vs. Site B) had a significant effect on the amount of insect damage ($p < .0001$, F ratio=18.76, $df=1$). Because of this, the two sites had to be treated separately in the analysis. Site A had a higher mean value of insect attack than Site B (Figure 3) (Site A: mean=3.961, $SD=0.233$; Site B: mean=2.134, $SD=0.265$). Next, it was determined using ANOVA that species (*T. populnea*, *H. tiliaceus*, or *H. rosa-sinensis*) had a

significant effect on the amount of insect damage ($p < .0001$, F ratio=36.38, $df=2$). *T. populnea* suffered the greatest amount of mean insect damage (mean=5.459, $SD=0.311$), followed by *H. rosa-sinensis* (mean=2.627, $SD=0.316$) and then *H. tiliaceus* (mean=1.527, $SD=0.288$) (Figure 3). Further, it was found using ANOVA that there was a significant interaction between site and species (site*species: $p < .0001$, F ratio=25.44, $df=2$), indicating that the magnitude of the effect of tree species on insect damage was different in different sites. It was also noted using ANOVA that there were different responses for individual trees ($n=18$) for insect damage (tree [species, site]: $p < .0001$, F value=5.69, $df=12$). Using a Tukey-Kramer test, it was discovered that there were some significant differences in insect attack between different species at different sites. The results from this test are indicated by the letters above the bars (Figure 3). Different letters indicate significant difference.

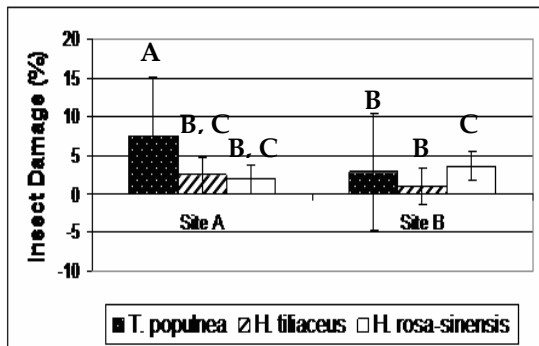


FIG 3. Average insect damage of each Malvaceae species by site. Results from Tukey-Kramer test are indicated by letter values above bars.

Using ANOVA, it was observed that age of leaves (young vs. old leaves) was important in the amount of insect damage documented ($p < .0001$, F ratio=103.89, $df=1$). Older leaves suffered higher overall mean levels of insect attack than younger leaves (Figure 4) (old: mean=4.845, $SD=0.246$; new: mean=1.422, $SD=0.246$). Next, it was found using ANOVA

that there was a significant interaction between species and age (species*age: $p = .0413$, F ratio=3.19, $df=2$), indicating that for each plant species, there was a different magnitude of the effect of age on insect damage. Using a Tukey-Kramer test, it was established that there were mainly significant differences in insect damage between different species of different leaf ages (Figure 4).

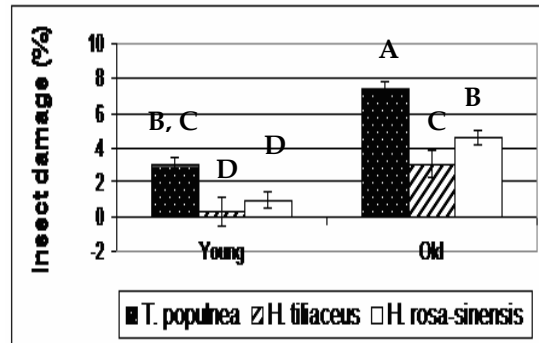


FIG. 4. Average insect damage of each Malvaceae species by age. Results from Tukey-Kramer test are indicated by letter values above bars.

Finally, using ANOVA it was concluded that height of collection (high vs. low) did not have a significant effect on insect damage ($p = 0.3355$, F ratio=.9278, $df=1$), indicating that these data could be grouped together for other analyses.

Antimicrobial assay

It was concluded first that the results from the antimicrobial assay were viable because both the water and ethanol negative controls demonstrated zero inhibition of the yeast both times the trial was run; and the 1% econazole nitrate positive control, extracted in both H_2O and EtOH, demonstrated full inhibition of the baking yeast. However, while the 2% miconazole nitrate positive control extracted in EtOH demonstrated essentially full inhibition, the same commercial anti-fungal extracted in H_2O only demonstrated about 63% inhibition of the baking yeast. When the

differences between the controls and the test extracts were analyzed using a Tukey-Kramer test, it was found that inhibition levels for both negative controls (EtOH and H₂O) were significantly different than inhibition levels for all extracts (Figure 5). Also, the four positive controls used (EtOH and H₂O with either 2% miconazole nitrate or with 1% econazole nitrate) were not significantly different from each other or significantly different than the inhibition levels for *H. rosa-sinensis* because the inhibition levels for *H. rosa-sinensis* were so high. Additionally, the 2% miconazole nitrate control was not significantly different than the inhibition levels for *T. populnea* (Figure 5).

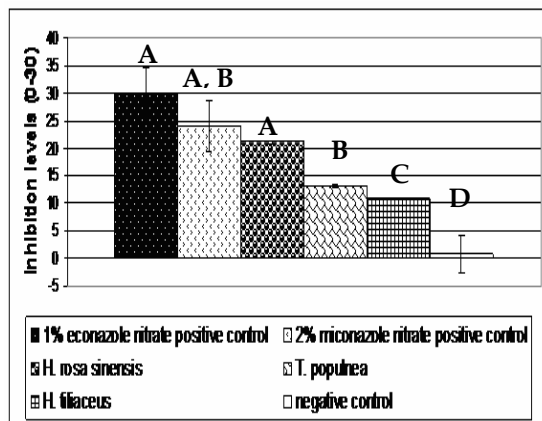


FIG. 5. Average inhibition levels of the positive and negative controls as compared to the average inhibition levels of each species. Results from Tukey-Kramer test indicated by letter values above bars.

Using ANOVA, it was established that antimicrobial activity or inhibition for all three plant species varied depending upon site ($p < .0001$, F ratio=46.35, df=1), which meant, as with insect damage, that each of the two sites had to be treated separately in the analysis. Site A had a greater overall mean inhibition level than Site B (Figure 6) (Site A: mean=15.504, SD=0.143; Site B: mean=13.457, SD=0.162). Next, it was discovered using ANOVA that species ($p < .0001$, F ratio=712.88, df=2) had a significant impact on the level of yeast inhibition. *H. rosa-sinensis* demonstrated highest overall mean inhibition (mean=21,

SD=0.194), followed by *T. populnea* (mean=12.9, SD=0.191 and then *H. tiliaceus* (mean=10.66, SD=0.177) (Figure 6). Further, there was a significant interaction between site and species (site*species: $p < .0001$, F ratio=273.3, df=2), meaning that there was a different magnitude for the effect of species on insect damage in each site. As with insect damage, the individual tree (n=18) also affected antimicrobial activity ($p < .0001$, F ratio=74.17, df=12). Next, using a Tukey-Kramer analysis, it was found that there was a significant difference in inhibition levels between species from different sites (Figure 6).

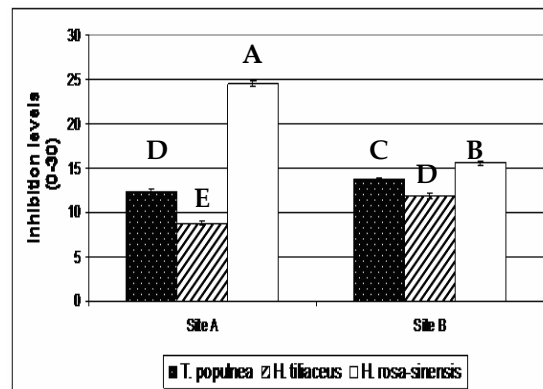


FIG. 6. Average inhibition level of each Malvaceae species by site. Results from Tukey-Kramer test indicated by letter values above bars.

It was concluded using ANOVA that leaf age affected levels of yeast inhibition or medicinal activity ($p < .0001$, F ratio=163.94, df=2). Younger leaves had a higher overall mean yeast inhibition level than older leaves (Figure 7) (young: mean=15.915, SD=0.151; old: mean =13.210, SD=0.151). However, unlike with insect damage, it was established using ANOVA that there was no significant interaction between species and age ($p = 0.2533$, F ratio=1.37, df=2), meaning that for all species there was the same effect of age on yeast inhibition levels. Additionally, it was noted using a Tukey-Kramer test that there was a

significant difference between inhibition levels between species of different ages (Figure 7).

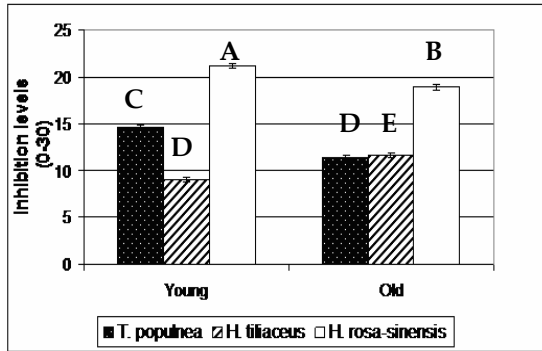


FIG. 7. Average inhibition level of each Malvaceae species by age. Results from Tukey-Kramer test indicated by letter values above bars.

Unlike with the insect damage data, it was concluded using ANOVA that the collection height ($p < .0001$, F ratio=57.42, $df=1$) factored in significantly when determining inhibition levels. There were greater mean inhibition levels at a lower collection height than at a higher collection height (Figure 8) (low: mean=15.571, $SD=0.251$; high: mean=13.664, $SD=0.238$).

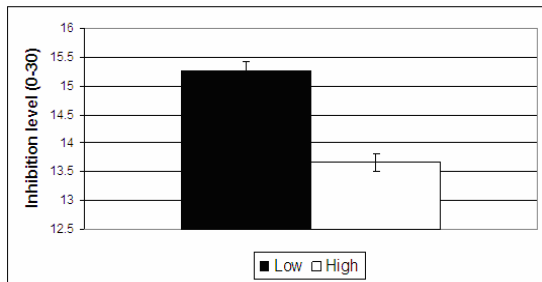


FIG. 8. A comparison of overall average inhibition levels by collection height

Extract type used (EtOH vs. H₂O extracts) also was a significant factor in the level of medicinal activity ($p < .0001$, F ratio=20.0504, $df=2$). There was a higher overall mean inhibition level for H₂O extracts as opposed to EtOH extracts (Figure 9) (H₂O: mean=15.550, $SD=0.244$; EtOH: mean=13.588, $SD=0.244$).

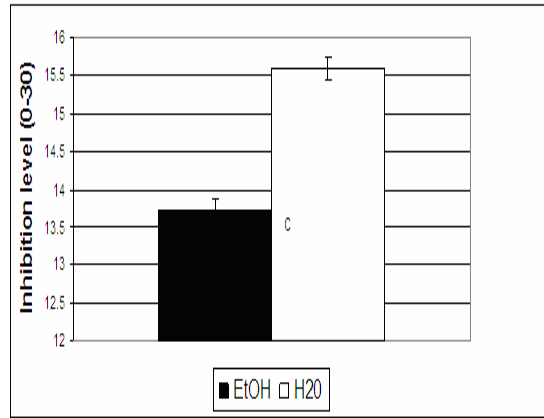


FIG. 9. A comparison of overall average inhibition level by extract type

Additionally, it was determined using ANOVA that the extracts of the reproductive plant parts (fruits and flowers) had different levels of inhibition than the extracts of the leaves ($p < .0001$, F ratio=9.2822, $df=2$). Furthermore, when analyzing inhibition levels exclusively for extracts of fruits and flowers individually, it was concluded using ANOVA that inhibition varied significantly between species ($p=0.0175$, F ratio=5.4801, $df=2$), but extract type ($p=0.6710$, F ratio=0.1882, $df=1$) and individual tree ($p=0.0684$, F ratio=2.4483, $df=8$) did not have significant effects on the amount of antimicrobial activity. The highest mean inhibition levels occurred in flowers (mean=20.952, $SD=1.689$), followed by in fruits (mean=20.583, $SD=3.160$), followed by in young leaves (mean value =15.9151, $SD=0.243$) and finally in old leaves (mean=13.2096, $SD=0.242$) (Figure 10). Using a Tukey-Kramer test, it was found that yeast inhibition levels for flowers were not significantly different from those for fruits, but were significantly different than inhibition levels for young and old leaves. Fruits were not significantly different from any other plant part probably due to their very low sample size ($n=5$). Finally, young and old leaves were significantly different from each other (Figure 10).

DISCUSSION

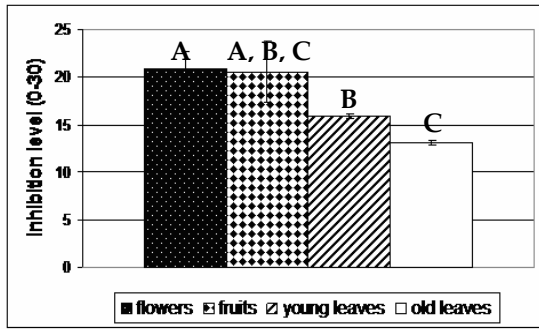


FIG. 10. A comparison between average inhibition levels of all species by plant part type. Results from Tukey-Kramer test indicated by letter values above bars.

Relating Insect damage to Medicinal activity

Using analysis of covariance (ANCOVA), it was concluded that there was an inverse relationship between herbivory and medicinal activity ($p=0.0022$, F ratio=6.1558, $df=2$) in which the amount of antimicrobial activity decreased with increasing insect damage. Further, the magnitude of this effect varied with species. As demonstrated in the slopes of the lines, the greatest inverse relationship existed in the *H. rosa-sinensis*, followed by *H. tiliaceus*, and finally *T. populnea*, which appeared to have had a very weak inverse relationship between the two variables (Figure 11).

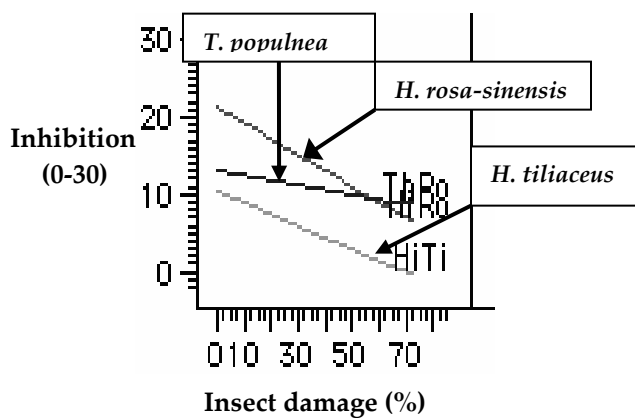


FIG. 11. Correlation between inhibition and insect damage for all three species

The insect damage assessment and antimicrobial assay were both preliminary indications of potential variation in secondary composition. As indicated by Stamp (2003), the defense mechanisms in plants that act as insect deterrents are found in a plant's "secondary" compounds, which are often useful for medicinal applications as well. The results of this study indeed demonstrated that insect damage and antimicrobial activity were both dependent on tree species, a first indication that a particular plant species' secondary compounds may have an effect on insect damage. However, it hadn't been expected for site to have a significant effect on insect damage or inhibition, which would not be attributed to a plant's secondary composition. This effect of location was probably due to other environmental factors, such as a greater abundance of herbivores or different types of herbivores in a given location as compared to another.

As hypothesized, leaf age also had a significant effect on insect damage and yeast inhibition, varying by species. These results followed Anderson's study (2005), the ODT (Stamp 2003) and supported my initial hypothesis that there would be within-plant variation in all three Malvaceae species in their allocation of bioactive secondary compounds used for defense. According to Stamp (2003), when examining the within-plant ODT one must look at three factors: value of plant part, benefit of defense, and probability of attack. Older leaves had the lowest value to the plant, had a high probability of attack, and were easily accessible to herbivores. Therefore, since older leaves had a low benefit of defense, plants allocated less defensive secondary compounds to these less important parts. However, older leaves could have had a greater amount of insect damage simply because they had been in existence for a longer time period. This is something that would require a direct experiment to tease apart. Along with age,

reproductive organs (flowers and fruits) demonstrated even higher levels of antimicrobial activity than leaves. This again supported Stamp's views (2003) that reproductive parts have a greater value to the entire plant's fitness than nonreproductive parts.

The greatest level of inhibition was found in the *H. rosa-sinensis*. According to my prediction, *H. tiliaceus* should have had the highest antimicrobial activity as it suffered the least insect attack, but there were other factors involved here. *H. rosa-sinensis* probably had the greatest antimicrobial activity because it was the only non-native, ornamental species and therefore could be artificially selected for highest amount of secondary compounds to decrease insect attack. Also, only one type of insect damage was taken into account in this study, damage from leaf-chewing herbivores. *H. tiliaceus* consistently had an unidentified brown damage that may or may not have been caused by xylem-sucking insects. If this damage had been taken into account, *H. tiliaceus* may have had the highest levels of insect damage. In fact, a past student, Prado (2006), found that *H. tiliaceus* did indeed demonstrate the greatest amount of insect damage of any plant species in her study, which would indicate a low level of defensive secondary compounds. This would follow the result in this study that *H. tiliaceus* demonstrated the lowest overall levels of antimicrobial activity.

Extract type was another significant unexpected factor in inhibition levels. In a study conducted by Jesudass (2003), it was determined that out of 15 species of pteridophytes analyzed, ethanol extracted the most secondary compounds. However, the opposite effect was demonstrated in the results of this study in which water extracts yielded the highest overall levels of inhibition. When making the solutions, the EtOH extracts appeared much more homogeneous than the H₂O extracts. It is possible that there was an uneven distribution of secondary compounds for the water extracts and that the results

indicated an artificially high level of inhibition. The ethanol could have also potentially killed off some of the bioactive secondary compounds in the plant matter. The variation in inhibition among extract types could additionally indicate that each extract may have extracted different compounds that affected the yeast differently. The ethanol extracts were probably a more accurate measure of the true amount of secondary compounds present in each plant part. Future experiments would benefit from filtering the extracts to separate out the liquid from the leaf pieces to minimize this problem.

The negative controls and both 1% econazole nitrate positive controls for this study proved effective. However, the 2% miconazole nitrate water based control only demonstrated 63% inhibition. Baking yeast was chosen as the study organism because that was what had been accessible. However, 2% miconazole nitrate may not have been an effective anti-fungal against baking yeast. If accessible, it may have been more effective to use study organisms more closely related to microbes that affect humans such as *Candida albicans* for antifungal studies and *E. coli* for antibacterial studies. Yet, for most purposes, baking yeast is a suitable model study organism in antifungal studies due its similarity to yeast that might afflict humans.

Using an analysis of covariance (ANCOVA) on the interaction between inhibition and damage by species, it was found that there was an inverse relationship between damage and inhibition. The less a plant produces its own insecticidal secondary compounds, the greater the effect from insect attack on that particular plant. Further, it is these secondary compounds which contain medicinal activity for human use. In order to definitively determine that this medicinal activity is due to secondary chemistry, it would be necessary using correct laboratory equipment to perform a phytochemical screening of the plant extracts to determine the exact compounds present.

To further explore the secondary

composition of these plant species, it would also be useful for a future study to perform an insecticidal test to test the variation in secondary compounds between and within plant species through the variation in insecticidal potential. It was not possible to complete this portion of the assessment due to time constraints and to an insufficient abundance of one particular insect species to analyze. However, one herbivorous caterpillar was found and an insect choice test was initiated in which the caterpillar was caged with squares of the same size of the four different leaf categories (young-low, old-low, young-high, old-high). The caterpillar did indeed eat the old-high leaf first and then both young leaves. However, further tests were not possible as the test subject began molting before running trials were complete. This preliminary result from this trial suggests fruitful lines of future research.

It was not possible to deduce from these results the presence of induced vs. constitutive responses in these Malvaceae species like those demonstrated by Anderson (2005) in the closely related Malvaceae species, cotton. In an induced response, insect attack causes many of the defensive mechanisms in plants to change, while constitutive responses change over evolutionary time or during the time period of the maturation of an individual plant, but constitutive responses function independently of damage (Karban and Baldwin, 2007). However, it was not possible to determine if there was a change in the plants' defenses due to insect attack, as it was not possible to know when the attacks had occurred or the amount of antimicrobial activity before initial insect attack. To determine this, a study would have to be performed with sufficient time to grow plants from seedlings, be able to introduce herbivores, and then measure changes in secondary compounds before and after incursion of insect damage.

Overall, this study supported the conclusion that a correlation between herbivory and medicinal activity exists in

these three plant species and that these Malvaceae species, like cotton, allocate the greatest amount of defensive, bioactive secondary compounds to their most valuable plant parts (fruits, flowers and young leaves) in response to this herbivory. This correlation and the abundance of secondary compounds for insect defense found in the antimicrobial assay support the greater theory of coevolution between angiosperms and insects. Flowering plants have evolved their defensive compounds under the constraints of attack by insects and insects have responded with their own defenses. In conclusion, the origin of antimicrobial activity in plants crucial to human medicinal use can in fact be attributed to the same creepy, crawly critters that we so often dismiss as household pests.

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