INSECTICIDAL PROPERTIES OF MEDICINAL PLANTS IN THE COASTAL STRANDS OF MOOREA, FRENCH POLYNESIA

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Abstract. Secondary metabolites, which are responsible for medicinal properties of ethnobotanical plants, originally evolved to provide protection against microorganisms and herbivores, including insects. These compounds affect various aspects of insect larval fitness, such as survivorship, growth, and development. Aqueous extractions of four medicinal plants *Hibiscus tilliaceus, Terminalia catappa, Thespesia populnea, Barringtonia asiatica,* and one non-medicinal plant *Fagraea berteriana* were studied for their effects on *Drosophila melanogaster* larval mortality and pupation. *Terminalia catappa* mimicked properties of commercial insecticide, with toxic effects on *D. melanogaster* larvae. *Fagraea berteriana* and *Hibiscus tiliaceus* inhibited pupation, with significantly less total pupae than negative control treatment. *Barringtonia asiatica* induced pupation earlier than water control.

Key words: *secondary compounds*; *medicinal plants*; *insecticide*; Fagraea berteriana; Hibiscus tiliaceus; Thespesia populnea; Barringtonia asiatica; Terminalia catappa; Drosophila melanogaster; *Moorea, French Polynesia*

INTRODUCTION

Secondary compounds are derived from a plant's primary metabolite system, but have no direct function in their primary metabolism (Fraenkel 1959). In nature, the roles of secondary compounds are shaped as a result of adapting to environmental stresses (Balandrin et al. 1985); plants use secondary compounds to attract pollinators or to defend against predators like microorganisms, insects, mammals or even other plants (Fraenkel 1959, Harborne 1993). Numerous studies have explored the effects of secondary metabolites on various herbivores and organisms, such as rats, tadpoles, and fish species (Applebaum and Birk 1979, Temmink et al. 1989, Dearing et al. 2002, Maerz 2005). However, the majority of research has focused on the most common plant herbivore: insects.

As plants evolve to strengthen their protection, herbivores co-evolve and specialize to be unaffected by the defense compounds (Erlich and Raven 1964). Insect herbivores build tolerance to chemicals that are produced by their normal host (Jaenike 1990, Bernays and Chapman 1994, Thompson 1988). Insects that specialize on one plant species ("specialists") perform better against chemicals they are adapted to than against novel chemicals (Cornell and Hawkins 2002). Insects that are flexible in choosing their host plants ("generalists") exhibit this pattern but to a lesser degree (Cornell and Hawkins 2002).

Secondary chemicals drive the arms race between the insects and the plants (Ehrlich and Raven 1964, Berenbaum 1983), influencing many aspects of larval fitness. The toxicities of secondary compounds have been measured by observing increases in larval mortality rates (Blaney et al. 1984, Chew 1985, Krischik et al. 1991, Dyer et al. 2003, Poykko and Hyvarinen 2003, Ghosh et al. 2008). Larval mortality rates rise dramatically when they are fed intact leaves, but when the secondary compounds are removed from the plants, the larval survival rates increase to 75-85% (Poykko et al. 2005).

The chemicals also affect other factors of larval development, such as development times and pupation. The effects vary by the type of secondary compound and the target organism. A single crucifer species cause varying developmental times for larvae of different insect species (Chew 1985). Amides, a category of secondary metabolites, have

been shown to decrease pupal weights and increase development times (Dyer et al. 2003). Secondary compounds have also been found to stop development, often inhibiting the larvae from pupation (Weissenberg et al. 1986).

Plants distribute secondary compounds to plant parts that are the most valuable for their efficiency at harvesting energy and increasing fitness. Younger leaves are more protected than older leaves because they provide more energy, which can be attributed to their efficient photosynthetic capability (van Dam et al. 1996). They often contain 50-190 times higher concentrations of secondary compounds than older leaves (van Dam et al. 1996). Developing leaves exhibit better resistance to feeding (Anderson and Agrell 2005). In one example, new Spruce needles caused 100% larval mortality rates (Jensen 1988), which the author attributed to the high concentration of the secondary compound, quinic acid.

While secondary compounds are used by plants as a means of defense against herbivory, the compounds can be beneficial for humans. The secondary metabolites are responsible for the medicinal property of ethnobotanical plants (Savithramma et al. 2011). These compounds are utilized in drugs of modern medicine (Balandrin et al. 1985, Balunas and Kinghorn 2005, Albuquerque et al. 2012).

Anti-microbial activity has been found in medicinal plants all around the world (Taylor et al. 1995, Samy and Ignacimuthu 1998, Adamu et al. 2005, Duraipandiyan et al. 2006, Araujo et al. 2008, Arnason and Benson 2010). It has been suggested that the toxic property responsible for anti-microbial activity is also responsible for anti-herbivory activity (Cornell and Hawkins 2002). Medicinal weeds have been found to have anti-herbivory properties (Stepp 2004), and insecticidal properties have also been found in various medicinal plants around the world (Chariandy et al. 1999, Xu et al. 2003, Pavela 2004, Jbilou et al. 2006, Palacios et al. 2009).

This study was conducted to investigate the effects of secondary compounds of Moorean medicinal plants by observing their insecticidal potential against an herbivorous insect, *Drosophila melanogaster*. Four species of medicinal plants were compared: *Barringtonia asiatica*, *Hibiscus tiliaceus*, *Thespesia populnea*, and *Terminalia catappa*. A non-medicinal plant *Fagraea berteriana* was also included as a treatment to serve as negative control. *Hibiscus tiliaceus* and *Thespesia populnea* were found to have inverse relationship between herbivory and medicinal activity (Cox 2008). Additionally, Chan (2009) observed the inverse relationship with six species of the medicinal plant family *Leguminosae*.

While previous studies explored how the defense system protects the plants, the current study investigated how the toxic compounds affect the herbivores. Specifically, this study addressed the following questions: (1) Do medicinal plants contain toxic compounds that raise insect mortality rates? (2) Which plants are most effective at raising insect mortality rates, inhibiting larval progression to adulthood? (3) Do the secondary compounds affect other factors of larval growth and development, such as pupation?

METHODS

Study sites

The study was conducted in Moorea, French Polynesia (Fig. 1). Five plant species were observed and collected: four medicinal plants, *Hibiscus tiliaceus*, *Terminalia catappa*, *Thespesia populnea*, *Barringtonia asiatica*, and one non-medicinal plant *Fagraea berteriana*. Specimens were collected at two sites along the island coast: the coast surrounding the

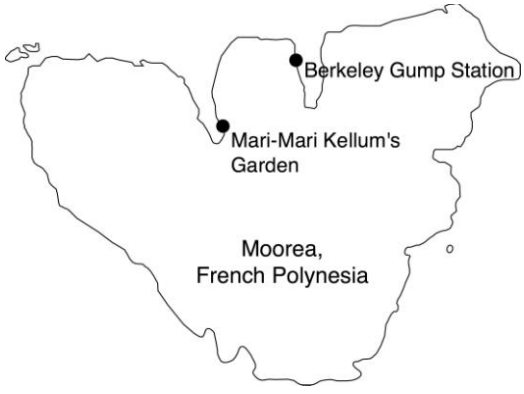

FIG. 1. Study sites on Moorea, French Polynesia.

Gump Station (17°29′28.12″S, 149°49′34.32″W) and Mari-Mari Kellum's property (17°30′51.34″S, 149°50′53.42″W).

Field survey and plant collection

Eight specimens of each plant species were collected throughout the two sites (n=40). In order to collect younger leaves, the third leaf from the apex was picked from branches chosen randomly throughout the specimen. The samples were stored in the -80° C freezer until use.

A general survey of insects and herbivory was conducted on each specimen. Five minutes were spent observing each plant, identifying the insect species present and the type of leaf damage present on each individual specimen. The data on herbivory damage were compiled for each species.

Plant extracts and gelatin plates

Prior to extraction, collected plants were removed from the -80° C freezer and defrosted at room temperature for thirty minutes. Plant specimens were shredded in a food processor and two grams of plant tissue was added to 40ml of distilled water. This preparation was maintained at room temperature for three hours to make the plant extracts.

Ten gel plates were made at a time, using six grams of gelatin, 50 ml of microwaved mango juice, and 70 ml of distilled water. They were left to solidify overnight and stored in a refrigerator until use.

A total of 56 plates were made, with eight replicates for each treatment. The treatments were insecticide (positive control), distilled water (negative control), and the five plant extracts (four medicinal, one non-medicinal).

One-half ml of the plant extract, water, or 1.5% Bayer Jardin™ insecticide v/v in water was pipetted onto a gel plate. The plates were stored at room temperature for two hours before use.

Insect bioassay

Twenty fly bait traps were set out in a shady area away from weather and disturbance. Overripe bananas were used to attract the flies. The traps were checked every day for oviposition and larval development. The larvae were reared to the third instar stage.

Ten *Drosophila melanogaster* larvae were obtained from the bait traps and added to each gel plate. Plates were checked at hour six, twenty-four, forty-eight, and seventy-two after placement, and the larvae were categorized into one of the four states: moving, pupal, pre-pupal, or dead. Moving larva indicated that the larva was still alive. Pre-pupal stage indicated that the larva was in transition to pupation. Pupal stage indicated that the larva had pupated. Dead individuals were stationary, and by hour seventy-two, these individuals had yeast growth and evidence of decomposition.

Statistical methods

The effect of treatments on the larval mortality was analyzed using a one-way ANOVA, comparing the number of larval deaths by hour seventy-two. The Tukey HSD post-hoc test was conducted to determine the groups between which there were significant differences.

The effect of treatments on pupation was analyzed using a one-way ANOVA, comparing the number of larvae that progressed to the pupal stage by hour seventy-two. The Tukey HSD post-hoc test was conducted to determine the groups between which there were significant differences.

The effect of treatments on the pupation rate was analyzed by calculating the rates for each replicate, then using a one-way ANOVA to compare these rates with each other. Pupation rates were determined for each replicate of the treatments at four intervals: hour zero to six, hour six to twenty-four, hour twenty-four to forty-eight, and hour forty-eight to seventy-two. Using the one-way ANOVA, the pupation rates for each treatment were compared at each time interval.

All statistical tests were performed using R, with an additional package "ggplot2" (Wickham 2009).

RESULTS

Field surveys

Evidence of herbivory were present on all of the plants observed. There were two main forms of insect herbivores: sap-suckers and leaf-chewers. There were also other types of insects, including scavengers and those that prey on other insects. A complete list of herbivores and herbivore damage for each plant species has been summarized in Appendix A.

Final death count

The effects of the treatments on mortality were compared by counting the number of dead larvae in each replicate by hour seventy-two (Fig. 2). ANOVA revealed that the effects of the treatments were significantly different from each other ($F(6,49)=8.0$, $p<0.001$).

The plant treatments were compared to water, the negative control, and insecticide, the positive control. *T. catappa* ($p<0.001$) raised mortality rates significantly more than negative control. Insecticide (mean=5.9 deaths) and *T. catappa* (mean=5.9 deaths) had the highest average number of larval deaths, while *B. asiatica* (mean=1.6 deaths) and water (mean=1.8 deaths) had the lowest average number of larval deaths. Larvae treated with insecticide or *T. catappa* had three times the death count compared to larvae treated with *B. asiatica* or water. In comparison to positive control, *B. asiatica* ($p<0.001$) and *T. populnea* ($p<0.05$) were significantly less toxic.

Final pupa count

The effects of the treatments on pupation were compared by counting the number of developed pupae in each replicate by hour seventy-two (Fig. 3). ANOVA revealed that the effects of the treatments were significantly different from each other ($F(6,49)=15.1$, $p<0.001$).

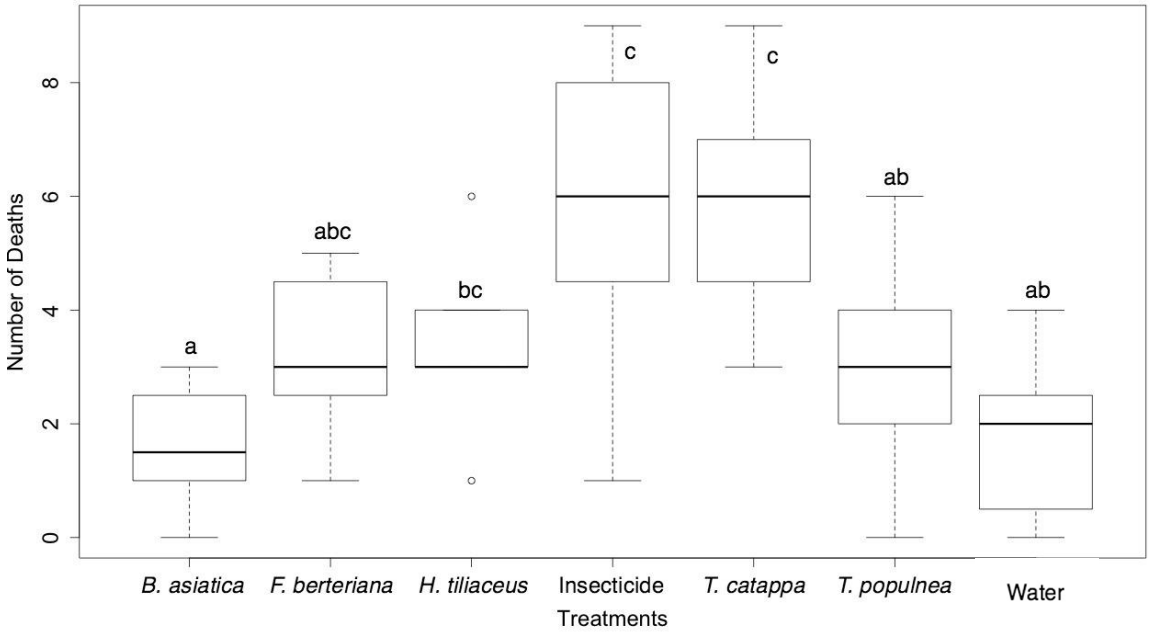

FIG. 2. Boxplot comparing the effects of treatments on the total number of deaths by hour seventy-two. The range indicates the maximum and minimum number of deaths observed from replicates. The bolded black lines denote average number of deaths by treatment. The box encompasses the interquartile range. Letters a-c show treatments with significantly different results, as determined by TukeyHSD post-hoc test.

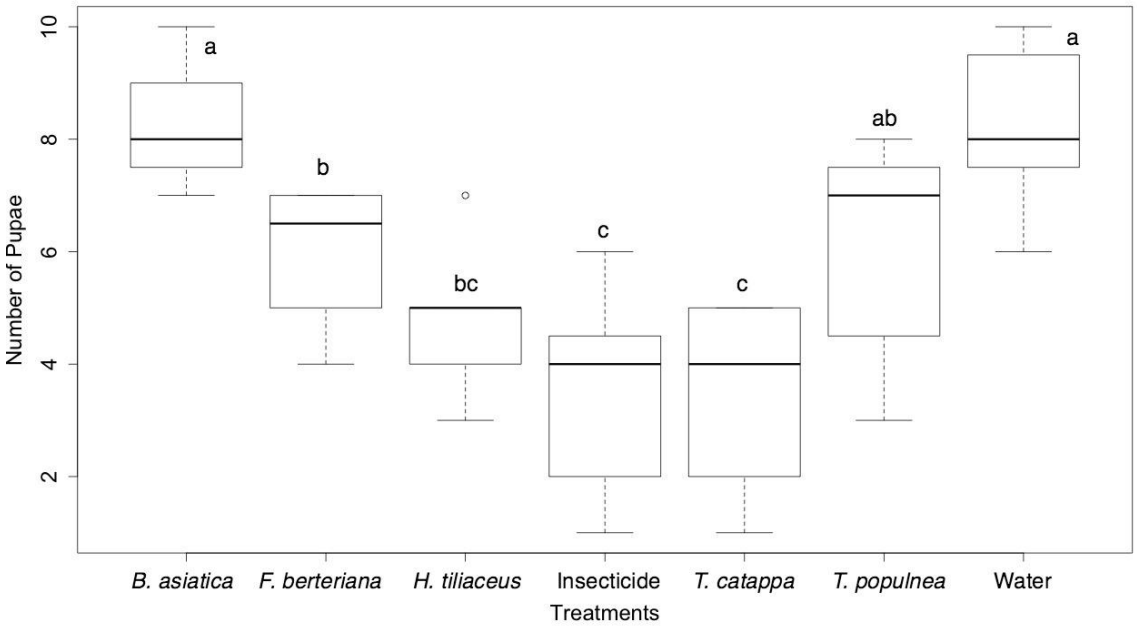

FIG. 3. Boxplot comparing the effects of treatments on the total number of total pupae by hour seventy-two. The range indicates the maximum and minimum number of pupae observed from replicates. The bolded black lines denote average number of pupae by treatment. The box encompasses the interquartile range. Letters a-c show treatments with significantly different results, as determined by TukeyHSD post-hoc test.

The plant treatments were compared to water, the negative control, and insecticide, the positive control. *F. berteriana* ($p<0.05$), *H. tiliaceus* ($p<0.001$), and *T. catappa* ($p<0.001$) had significantly less pupae than negative control. *B. asiatica* ($p<0.001$), *F. berteriana* ($p<0.05$), and *T. populnea* ($p<0.05$) had significantly more pupae than positive control. Water (mean=8.3 pupae) and *B. asiatica* (mean=8.3 pupae) had the highest average number of pupae, more than twice the number of pupae treated with *T. catappa* (mean=3.5 pupae) or insecticide (mean=3.5 pupae).

Pupation rates

There were significant differences among the treatments in pupation rates in interval one ($F(6,49)=7.2$, $p<0.001$) and interval two ($F(6,49)=6.9$, $p<0.001$), but no significant differences in interval three and interval four (Fig. 4). Tukey HSD post-hoc test was used to determine the treatments with the significant differences from water, the negative control and insecticide, the positive control.

For interval one, water (mean=0.17 pupae/hour) and insecticide (mean=0.13 pupae/hour) caused very similar pupation rates. *T. catappa* (mean=0.15 pupae/hour) also caused a very similar pupation rate to insecticide and water. Larvae treated with *B. asiatica* (mean=0.77 pupae/hour) had significantly higher average pupation rate than water ($p<0.001$) and insecticide ($p<0.001$). The average rates for *F. berteriana*, *H. tiliaceus*, and *T. populnea* are higher than the average rate for water treatment, but to a lesser degree than *B. asiatica*.

For interval two, larvae treated with water (mean=0.38 pupae/hour) and insecticide (mean=0.15 pupae/hour) had significantly different pupation rates ($p<0.001$). All of the plant treatments caused significantly lower pupation rates than water: *B. asiatica* (mean=0.19 pupae/hour, $p<0.01$), *F. berteriana* (mean=0.17 pupae/hour, $p<0.001$), *H. tiliaceus* (mean=0.11 pupae/hour, $p<0.001$), *T. catappa* (mean=0.13 pupae/hour, $p<0.001$), and *T. populnea* (mean=0.15 pupae/hour, $p<0.001$). None of the plant treatments had significant differences from insecticide.

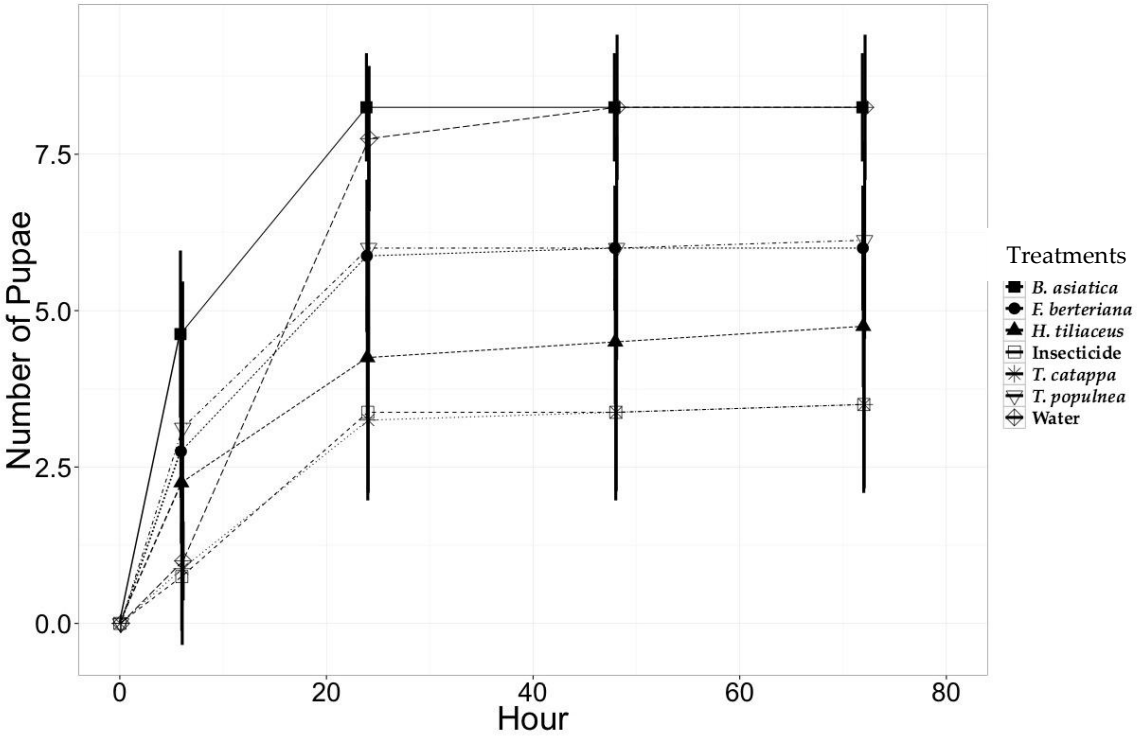

FIG. 4. Comparison of the effects of treatments on pupation rates. Interval one is from hour six to twenty-four. Interval two is from hour six to twenty-four. Interval three is from hour twenty-four to forty-eight. Interval four is from hour forty-eight to seventy-two.

DISCUSSION

There are numerous examples in the literature of plants producing insecticidal compounds (Miller and Feeny 1983, Lindroth and Peterson 1988, Poykko et al. 2005). Because tropical plants contain more toxic alkaloids than temperate plants (Levin and York 1978), Moorean plants were expected to contain potent chemicals. However, *Terminalia catappa* was the only plant that raised mortality rates of *D. melanogaster* larvae. *Terminalia catappa* also consistently mimicked commercial insecticide in pupation rates throughout all four intervals.

Chemical analyses show that *T. catappa* has a wide range of metabolites, including alkaloids, reducing sugars, saponins, tannins, resins, steroids, flavonoids and glycosides (Muhammad and Mudi 2011, Packirisamy and Krishnamorthi 2012). Alkaloids, flavonoids, and tannins are known for their anti-microbial properties (Muhammad and Mudi 2011), and *T. catappa*'s anti-microbial activity has been documented (Akharaiyi et al. 2011). Tannins are also known to reduce herbivory and decrease larval growth (Feeny 1976). Since the same toxic properties are responsible for anti-microbial activity and anti-herbivory (Cornell and Hawkins 2002), it is reasonable to infer that *T. catappa*'s toxicity is related to its insecticidal properties.

Barringtonia asiatica, F. berteriana, T. populnea, and *H. tiliaceus* treatments did not result in significantly higher mortality compared to water treatment. In 1988, Lindroth and Peterson tested eight different secondary compounds, and found that only a specific concentration of the compound rutin caused an increase in mortality rates in insects. This suggests that not all secondary compounds are produced for the purpose of direct defense against insects. In a study by Usher and Feeny (1983) the secondary compounds had no effect on larval mortality or development. It is possible that these four plants lack the secondary compounds that are toxic enough to have significantly different

results from negative control. This study only used one generalist insect and thus it is possible that secondary compounds in these plants may have specifically evolved for specialist insects (Ghosh et al. 2008).

It is important to note that for the purposes of this study, a "death" event was defined as death taking place some time before pupation. It is possible that the secondary compounds affected death post-pupation, which is indicated by the pupa's failure to reach adulthood.

Fagraea berteriana, a non-medicinal plant, was included in the study as a negative control, a point of comparison for the medicinal plants. However, *F. berteriana* treatments performed similarly to the medicinal plant treatments. The results show that *F. berteriana* was more toxic than *B. asiatica*, and inhibited pupation more than *B. asiatica* and *T. populnea*. This suggests that medicinal plants are not necessarily superior to non-medicinal plants in their effects on larval mortality and development. Further research is needed for a more definitive conclusion.

The *F. berteriana*, *H. tiliaceus*, and *T. catappa* treatments resulted in significantly lower final pupa count in comparison to water treatment, the negative control. Toxicity can explain *T. catappa*'s low pupa count. The literature offers little information on the chemical composition of *F. berteriana* and *H. tiliaceus*. Neither of these two species were toxic enough to have significantly different effect from the water treatment. It is possible that *F. berteriana* and *H. tiliaceus* are composed of secondary compounds that affect the larvae in other ways besides mortality.

Plant defense can come from other indirect methods that weaken the larvae. Secondary compounds may cause a decrease in growth and leaf consumption, and it may even change feeding and settlement behavior without having an effect on mortality (Slansky 1979, Blewitt and Cooper-Driver 1990, Peneder and Koschler 2011). These developmental factors were not observed in this study, but future research could incorporate these factors.

The comparison of pupation rates shows that the larvae treated with water, insecticide and *T. catappa* extract does not pupate in interval one. The toxicities in *T. catappa* and insecticide can explain why there is minimal pupation event in interval one. *Barringtonia asiatica* was the only plant treatment that caused significantly higher pupation rate than water treatment, but all of the plant treatments (except *T. catappa*) induced pupation earlier than the water treatment. By interval two, pupation rates rose in larvae treated with water, to the point where water treatment caused significantly higher pupation rates than the other six treatments. In Colorado, crucifer plant treatments affected development rates of *Pieri* butterfly populations Chew (1975); it was speculated that rapid development would be beneficial to larvae because it reduces the time exposed to predators and parasites. The stresses from plant treatments may have induced an earlier pupation event in the larvae.

One possible mechanism for affecting developmental timing involves phyto-ecdysones. Ecdysone is a naturally occurring compound in *Drosophila* that controls the transition between developmental stages (Riddiford et al. 2000, Thummel 2001). Gymnosperms and ferns are found to produce phyto-ecdysones, which mimic the insect ecdysone and have a potent molt-inducing effect (Stenersen 2004). Like secondary compounds, phyto-ecdysones are beneficial for human use; some usages include protein synthesis for AIDS patients, antidepressants, disease prevention and sexual performance. Medicinal plants have been found to have phyto-ecdysones as well, with one example being the Siberian medicinal plant *Leuzea carthamoide*s (Zeleny et al. 1997). However, not all medicinal plants contain the compounds. For example, *Asparagus filicinues* is rich in phyto-ecdysones, but its relatives *A. cochinchinensis*, *A. officinalis*, and *A. setaceus* do not contain high levels of the compounds (Wu et al. 2009).

In the field of entomotoxicology, fly larvae are used to study causes of human death. Maggots were found to exhibit accelerated development when they feed on lethal dose of cocaine (Introna et al. 2001). A study in 1993 also showed that cocaine in coca leaves act as natural insecticide (Nathanson et al. 1993).

Heroine also causes accelerated larval growth, but a decrease in the development rate at pupal stage (Murthy and Mohanty 2010). A similar process may be occurring with the Moorean medicinal plant treatments on larvae.

An area for future research would be comparing how reproductive parts may compare with the leaves in their effect on insect larvae. A study by Nahrstedt (1985) shows that reproductive parts may be armed with a stronger defense system than non-reproductive parts. However, because the plants also rely on herbivores and frugivores to reproduce, plants may vary the level of toxicities of their fruits depending on the rate of fruit removal rate (Cazetta et al. 2008). It would be interesting to see how the varying toxicity levels affect larval development.

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APPENDIX A

Plant	Leaf Damage Present	Insects Present	
		Insect Type	**Species Present**
F. berteriana	Margin feeding Hole feeding Leaf mining Fungal growth Necrotic spots	Sap-suckers	*Plataspidae brachyplatys capito* *Tropoduchidae kallitaxila* Unidentified scale-feeding insects
		Leaf-chewers	None
		Scavengers/ Predators	*Polistes olivaceus* *Technimyrmex albipes*
H. tiliaceus	Hole feeding Margin feeding Necrotic spots	Sap-suckers	*Pseudococcidae* *Tropoduchidae kalli* nymph *Lygaeidae*
		Leaf-chewers	None
		Scavengers/ Predators	*Polistes olivaceus* *Anthocoridae* *Scholastes lonchifer* Unidentified ant species
B. asiatica	Hole feeding Margin feeding Surface feeding Leaf mining Fungal growth Necrotic spots	Sap-suckers	*Psyllidae* *Fulgoridae* *Drosophila melanogaster* *Tropoduchidae kalli* nymph
		Leaf-chewers	*Gryllidae*
		Scavengers/ Predators	*Polistes olivaceus* *Tapinoma melanocephalum* *Chrysopidae*
T. populnea	Hole feeding Margin feeding Necrotic spots	Sap-suckers	*Tropoduchidae kallitaxila* *Melormenis basalis*
		Leaf-chewers	None
		Scavengers/ Predators	*Polistes olivaceus* *Anoplolepis gracilipes* *Tapinoma melanocephalum*
T. catappa	Surface feeding Leaf mining Margin feeding Hole feeding Necrotic spots	Sap-suckers	None
		Leaf-chewers	*Cosmopterigidae* Unidentified caterpillar species
		Scavengers/ Predators	*Polistes olivaceus* *Technimyrmex albipes*