

HOST PLANT EFFECTS ON DEFENSIVE BEHAVIORS OF WHITE PEACOCK
(*ANARTIA JATROPHAE*) CATERPILLARS

by

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Host plant effects on defensive behaviors of White Peacock (*Anartia jatrophae*)
caterpillars

Thesis directed by Professor Deane Bowers

The White Peacock (*Anartia jatrophae*) is a common butterfly of the Southeastern United States that has recently shifted to a novel host plant, Narrowleaf Plantain (*Plantago lanceolata*). The impact of host plant species on the White Peacock caterpillar defensive behavior is explored in this study. The effect of host plant and defense on the development of caterpillars was also examined. It was found that White Peacock caterpillar defenses are mainly influenced by caterpillar age and not by host plant. Regurgitation, the defense of interest, was found to decrease the pupal weight of caterpillars reared on both host species. Caterpillars reared on *Plantago* had longer development time and higher pupal weights than those reared on the normal host Water Hyssop (*Bacopa monnieri*). Regurgitant collected from caterpillar reared on *Plantago* was found to contain low levels of iridoid glycosides.

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CHAPTER I

INTRODUCTION

Insect defenses vary, ranging from behavioral responses to physical and chemical mechanisms (Greeney et al., 2002). These defenses fall into two categories, classified as primary and secondary defenses (Gentry & Dyer, 2002). Primary defenses, such as camouflage and defensive coloration, prevent predators from encountering insects (Gentry & Dyer, 2002). Secondary defenses are activated upon predator interaction with an insect, such as thrashing, biting, and regurgitation, or unpalatable chemicals that are tasted by predators upon encountering the insect (Gentry & Dyer, 2002). These defenses are manufactured by the insect or sequestered from the plants on which it feeds (Gentry & Dyer, 2002).

For herbivorous insects that have the ability to sequester chemical compounds from their host plants, their defenses can be affected by their host plant chemistry. For example, different larval host plants can determine adult palatability to predators (Bowers, 1980). Sequestration of chemicals from plants has been widely studied in insects, particularly butterflies and moths (Nishida, 2002). For example, monarch caterpillars are unpalatable due to the sequestration of toxic cardiac glycosides from their milkweed host plants (Jones & Agrawal, 2019). Insects that shift to novel host plants may gain new defenses due to novel host plant chemistry (Graves & Shapiro, 2003; Dyer, 1997; Knerl & Bowers, 2013). For example, larvae of the Common Buckeye (*Junonia coenia*) are unpalatable when

feeding on Narrowleaf Plantain (*Plantago lanceolata*, Plantaginaceae), a novel host plant (Knerl & Bowers, 2013).

Studies on the effects of host plant species on behavioral defenses of herbivorous insects are relatively uncommon; however, those few show that host plant may, indeed, influence defensive behavior. For example, in adult chrysomelid beetles, the foraging and dispersal behavior of the adults has been shown to be influenced by the host plant on which they feed (Muller & Muller, 2017). Beetles which were reared on cabbage instead of watercress, were more active in foraging and searching for mates (Muller & Muller, 2017). However, Muller & Muller (2017) found that the defensive behaviors of these beetles was influenced by the age and sex of the beetles more so than the host plant on which they fed. Another study that examined foraging behavior and host plant identity, found that when moth caterpillars are harassed in a simulated predator attack, they were more likely to forage at night and move to a different host plant individual to avoid predator detection (Stamp, 1997). However, Stamp (1997) found the change in behavior was induced by predation and not the host plant itself.

Outside of insect defenses, host plants also play an important role in survival and distribution of insect species. In order to shift to a new host plant, an insect herbivore must be able to overcome the plant defenses and possible nutritional differences (Yoon & Read, 2016). Most novel host plants are phylogenetically related to the original host plants, have a similar phytochemical makeup, or are used by closely related lepidopteran species (Yoon & Read, 2016). Generalist lepidopteran

species have been shown to be able to more easily shift to novel host plants due to their broad diet breadth (Jahner et al., 2011; Dyer, 1997). Species with large geographic ranges are also able to more easily shift to new hosts since they have more probability of encountering plants similar to their original host (Jahner et al., 2011; Cogni, 2010; Yoon & Read, 2016). Range expansions can occur more easily in species with a wide diet breadth since they can more easily adapt to novel host plants (Graves & Shapiro, 2003; Jahner et al., 2011).

However, incorporation of novel host plants may also have negative impacts. Novel host plants which are introduced species can outcompete and potentially extirpate native hosts (Graves & Shapiro, 2003; Yoon & Read, 2016). This leads to consequences for specialist species that are less able to shift to a novel host due to narrow diet breadth or limited range (Graves & Shapiro, 2003; Dyer, 1997; Yoon & Read et al., 2016). Furthermore, introduced species that are not suitable host plants, although they may have similar chemical signatures to related, suitable host plants, can result in oviposition by females, but with lethal effects on the offspring (Chew, 1975; Chew, 1977; Graves & Shapiro, 2003). In some cases, species will show a preference for the novel host plant over the original host plant (e.g., Cogni, 2010; Thomas et al., 1987; Graves & Shapiro, 2003; Yoon & Read, 2016), even if these novel host plants are toxic to the larvae. Overall, the use of introduced host plants has been shown to potentially decrease the diversity of resident lepidopteran species; this may then have serious consequences for the diversity of organisms that prey upon caterpillars (Yoon & Read, 2016).

In order to investigate the effects of host plant species on the defensive behaviors of a native insect herbivore, we focused on the White Peacock (*Anartia jatrophae* L., Nymphalidae), a common butterfly of the southeastern United States. The behavior of White Peacock caterpillars is not well studied (Knerl & Bowers, 2013; Rawson, 1976). The White Peacock is of particular interest since this species was recently recorded feeding on Narrowleaf Plantain (Plantaginaceae: *Plantago lanceolata*), a novel, introduced host plant (Knerl & Bowers, 2013). *Plantago lanceolata* (hereafter *Plantago*) contains iridoid glycosides and has been added to the dietary repertoire of other native North American lepidopterans (Knerl & Bowers, 2013).

The most commonly used native host plant for White Peacocks in North America, Water Hyssop (*Bacopa monnieri*, family Plantaginaceae), does not contain iridoid glycosides or other chemicals that can be sequestered and used as potential defenses, as far as is known (Knerl & Bowers, 2012; Rawson, 1976). White peacock caterpillars reared on *Plantago* may be unpalatable, since it has been found they are able to sequester iridoid glycosides (Knerl & Bowers, 2013), a class of terpenoid compounds that, when sequestered, render insects containing them unpalatable (Bowers 1991; Nishida 2002). Sequestration of these defense compounds may impact the behavioral defenses of the caterpillars, due to the potential new chemical defense. The use of *Plantago* may also have developmental tradeoffs which could impact defenses, since previous research showed that caterpillars reared on

Plantago had higher pupal weights but slower development time (Knerl & Bowers 2013), compared to those reared on the host plant, *Bacopa*.

In preliminary experiments, White Peacock caterpillars were observed to demonstrate several behaviors that could serve as defenses against enemies (Barbeau, unpublished data): thrashing, biting, regurgitation, and escape attempts. These defenses can be categorized as "aggressive" or "evasive" behaviors (Greeney et al., 2012). Biting and regurgitation are considered aggressive since they are meant to repel or remove attackers (Greeney et al., 2012). Thrashing can be both aggressive and evasive since it can be used to repel predators but also allows for escape (Greeney et al., 2012). Regurgitation is of most interest to this study since it is the disgorgement of gut contents (regurgitant) (Rhains et al., 2011) and preliminary observations of defense behaviors showed that White Peacock caterpillars often regurgitate when disturbed (Bowers, personal observation). Regurgitant may contain noxious chemicals that are either sequestered from a plant host or manufactured by the insect *de novo* as a predator repellent (Peterson, Johnson, & LeGuyader, 1987; Smedley, Ehrhardt, & Eisner, 1993; Zvereva et al., 2017). Because White Peacock caterpillars can sequester iridoid glycosides (Knerl & Bowers, 2013), it is also possible that their regurgitant may contain these compounds; a hypothesis that is tested here.

The gut of caterpillars which regularly regurgitate have larger crops and smaller midguts than non-regurgitating caterpillars (Grant, 2006). This suggests that regurgitation may be a costly defense, since most nutrient absorption in insects

occurs in the midgut (Grant, 2006). Regurgitation has been shown to negatively impact the development of some lepidopteran species (e.g., Bowers, 2003; Higginson et al., 2011). In *Ceratomia catalpae* (Lepidoptera: Sphingidae), caterpillars which regurgitated regularly had lower pupal weights, a measure that is correlated with fitness (Bowers, 2003). In *Pieris brassicae* (Lepidoptera: Pieridae), regular regurgitation led to decreased pupal weight, adult size, and adult fecundity (Higginson et al., 2011). Regurgitation often is accompanied by other defenses, further suggesting the higher potential cost due to the loss of gut contents (Grant, 2006; Dyer, 1997). For example, in *Langia zenzeroides* (Lepidoptera: Sphingidae), regurgitation was observed to accompany sound production and was not seen to occur by itself (Sugiura & Takanashi, 2018; Rhainds et al., 2011). Species with higher rates of regurgitation have been shown to reimbibe regurgitant, perhaps mitigating potential nutritional loss (Grant, 2006). Furthermore, regurgitation may have a benefit, since it has been shown that caterpillars which regurgitated in defense survived to adulthood at higher rates (Higginson et al., 2011).

In this study, we investigated the effects of incorporating a novel host plant, *Plantago lanceolata*, on the defense behaviors shown by larvae of the White Peacock, and how this might change over larval development. We addressed several questions in this study.

1. How do host plant and caterpillar developmental stage (instar) affect larval defensive behaviors?

2. How does regurgitation affect caterpillar development and does this change with the host plant on which caterpillars have fed?
3. Does the regurgitant of the caterpillars reared on *Plantago* contain iridoid glycosides?
4. How does a model predator react to regurgitation by these larvae?

CHAPTER II

METHODS

Study System

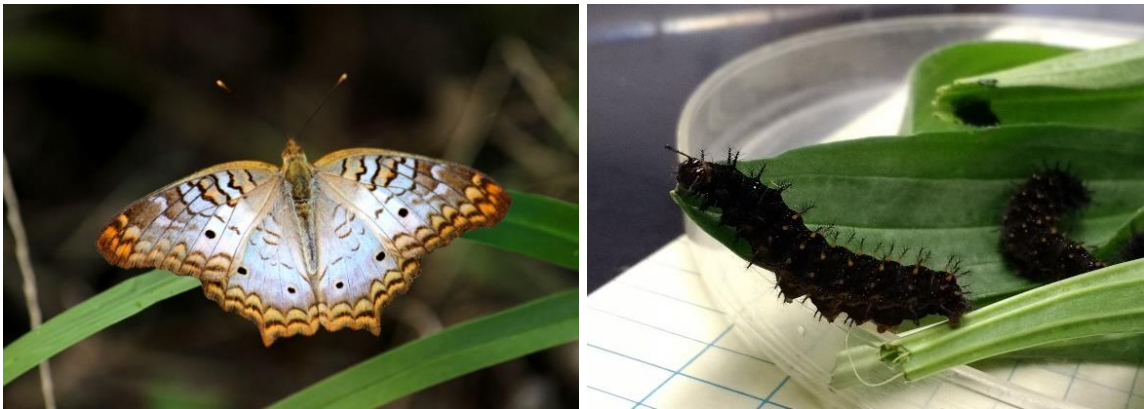
White Peacock (*Anartia jatrophae*)

The White Peacock (Nymphalidae: *Anartia jatrophae*) (Figure 1) is a common, medium sized butterfly of the new world tropics (Silberglied et al., 1979). In the United States, it is found in the southeast and has been recorded as a stray as far north as New England (Knerl & Bowers, 2013; Rawson, 1976). Like other members of the genus *Anartia*, it inhabits wet habitats, often along edges of bodies of water or disturbed areas (Rawson, 1976; Lederhouse et al., 1991). White Peacock butterflies are palatable to predators (Silberglied et al., 1979); however, palatability of larvae has not been tested. Larvae of White Peacock butterflies feed on a variety of plants in several families: Acanthaceae, Verbenaceae, Scrophulariaceae, Lamiaceae, and Plantaginaceae (Knerl & Bowers, 2013; Silberglied et al., 1979; Rawson, 1976). The most common host in North America is Water Hyssop (Plantaginaceae: *Bacopa monnieri*).

The White Peacock colonies used for this study were obtained from a laboratory population begun from individuals obtained from several Florida localities and from Butterfly Dan's, a butterfly farm based in Central Florida. Caterpillars were reared on two different plant species, the native host plant, *Bacopa monnieri* (hereafter *Bacopa*), and an introduced host plant, Narrowleaf

Plantain (*Plantago lanceolata*, family Plantaginaceae), which was recently reported as a larval host plant (Knerl & Bowers, 2013). In the laboratory, larvae were maintained in 16×10×6 cm plastic boxes in a growth chamber with 14 hours of light set to 25C° and 10 hours of dark set to 20C°. Adult butterflies were kept in insect tents (BugDorm) with the host plant available for oviposition and dental wicks placed in dilute honey solution (1:5, honey:water) for adult feeding. Once oviposition occurred, the host plants were removed from the insect tents and placed in the incubators and checked daily for neonate caterpillars. Neonate caterpillars were removed from the plant with paintbrushes and placed in rearing boxes with a damp paper towel and food.

Figure 1: *White Peacock adult (left) and 5th instar larva (right).*



Host Plants

Narrowleaf Plantain (Plantaginaceae: *Plantago lanceolata*) is a weedy herb that grows in rosettes commonly found in grasslands and disturbed areas (Hamre et al., 2010; Cavers et al., 1980). *Plantago lanceolata* was introduced to North America from Eurasia about 200 years ago (Cavers et al., 1980). It is known to contain iridoid glycosides, including catapol and aucubin (Bowers, 1991). It has become used

by several native North American butterfly species as a host plant, with some species sequestering the iridoids as a defense (Knerl & Bowers, 2013; Lampert et al., 2014).

Water hyssop (*Bacopa monnieri*) is a low growing succulent herb that is widespread in the tropics ranging from the Southeastern United States to India to Australia (Barrett & Strother, 1978; Aguiar & Borowski, 2013). It is described as a native North American species by the USDA database (<https://plants.usda.gov/core/profile?symbol=BAMO>). *Bacopa* prefers wetlands and damp habitats and is commonly found as a weed in rice paddies (Aguiar & Borowski, 2013; Barrett & Strother, 1978; Bhandari et al., 2007). *Bacopa* is used in traditional medicines including Ayurvedic medicine for memory enhancement and mental function (Bhandari et al., 2007; Aguiar & Borowski, 2013). Unlike *Plantago*, it does not contain iridoid glycosides or other chemicals that lepidopterans are known to sequester (Knerl & Bowers, 2013).

The host plants were grown in individual pots in greenhouses at the University of Colorado, Boulder. Potted plants were provided to adult White Peacocks for oviposition, but larvae were fed with harvested leaves of the host plants. Leaves were harvested from plants once a week and refrigerated.

Preliminary Experiments

To determine what behaviors White Peacock caterpillars exhibited when disturbed, six groups of 20 fourth and fifth instar caterpillars were selected from the colony. Each group was divided into 10 caterpillars reared on *Bacopa* and *Plantago*.

To prevent caterpillars from regurgitating during handling, they were removed on stems of the host plant and given one minute to acclimate to the petri dish for each repetition. Three preliminary experiments were performed. Trial A determined what defensive behaviors caterpillars displayed when pinched with soft forceps to stimulate a predator attack. Each caterpillar was pinched ten times and the behavioral response was recorded. The behaviors observed were thrashing, biting, regurgitation, escape attempts, and no reaction. In Trial B, how long to pinch the caterpillars to induce regurgitation was determined. The caterpillars were pinched and held in the soft forceps until regurgitation was induced. The duration of each pinch was measured using the stopwatch app on a smartphone. Behaviors leading up to regurgitation were recorded as well. The duration of a pinch to stimulate regurgitation was determined to be 10 seconds. Trial C explored how many times caterpillars can regurgitate until they stop. Most regurgitated five times before stopping. Based on these observations, three pinches was determined to be sufficient for future experiments.

Larval Defensive Behavior Experiment:

In order to investigate how host plant species might affect larval defensive behaviors and how this might change with instar, larvae were reared on either *Bacopa* or *Plantago*. First and second instar caterpillars were randomly chosen from the colony to be reared on either *Bacopa* or *Plantago*. Each host plant group (n=60) was randomly assigned to one of three groups that were reared to the third, fourth, or fifth instar (N = 20 in each group). Caterpillars were checked daily and

fed *ad libitum*. When larvae reached the appropriate stage, they were used for the behavioral tests. The experiments were conducted within three days of caterpillars reaching their respective group's target instar.

To investigate larval behavioral responses to simulated predator attack, the caterpillars were pinched with soft forceps for ten seconds which was repeated three times on each individual to simulate predator attacks (Bowers, 2003; Stamp, 1997). Once caterpillars reach the instar of their group, they were pinched with soft forceps for 10 seconds which was repeated three times with 30 seconds between each pinch. For the experiment, the caterpillars were not removed from the rearing cups to minimize stress and potential regurgitation caused by handling. The first behavior observed after the pinch was delivered was recorded as regurgitation, thrashing, biting, escape or no response. Each caterpillar was tested a single time.

The data were analyzed using chi square test using R (version R-3.6.1) to compare the effects of host plant species and larval instar on defense behaviors.

Regurgitation and Host Plant Effects on Larval Development

Because previous research had shown that regurgitation could negatively affect development and growth of other caterpillar species (Bowers, 2003; Higginson et al., 2010), and regurgitation was a common behavior observed in the previous set of experiments, we investigated the effects of host plant and regurgitation on larval development. Second instar caterpillars reared on either *Bacopa* (N=40) or *Plantago* (N=40) were randomly selected from groups of larvae that had been reared on either of these host plants. Each host plant group was split into a control (n=20)

and an experimental group (n=20). The caterpillars were reared in individual cups from first instar to pupation. The caterpillars were checked once a day from the first instar to the day of pupation. The date that caterpillars were placed in rearing cups was recorded. Once the caterpillars reached the third instar, the experimental group individuals were pinched with soft forceps for 10 seconds to simulate a predator attack once a day. For the control groups, the lid of the cup was simply removed. To prevent stress from handling, all treatments took place within the rearing cups. Caterpillars were only removed on plant stems or leaves when cleaning the cups due to frass build up. The date of pupation was recorded, and pupae were weighed on a digital scale to the nearest 0.1 mg. If a pupa was very fresh, it was measured the next day and that information noted on the data sheets. After weighing, pupae were placed in a rearing box for development to the adult stage.

The effects of regurgitation and host plant species on larval development time to pupation and pupal weight were analyzed with a two-way ANOVA using R (version R-3.6.1).

Iridoid Glycoside Content of Regurgitant:

Ten fifth instar caterpillars reared on *Plantago* were selected for collection of regurgitant for chemical analysis. Each caterpillar was placed in a petri dish on a piece of filter paper and then pinched for 10 seconds to induce regurgitation. The head of the caterpillar was tapped to prevent loss of regurgitation due to the caterpillar regurgitating on itself or reimbibing the regurgitant. If a caterpillar

defecated on the filter paper before regurgitating, the filter paper was removed and replaced with a new piece. If a caterpillar defecated on the paper while regurgitating on the filter paper, a different caterpillar was randomly selected to run the trial again to prevent any cross contamination. A ruler was placed next to each regurgitant sample and this was then photographed. The area of the regurgitant was then calculated using Image J (<https://imagej.nih.gov/ij/>). After photographing the sample, to extract the regurgitant sample and determine whether and how much iridoid glycosides were contained in the regurgitant, the filter paper was trimmed to include the portion that had regurgitant and placed in a test tube with 5mL of methanol for extraction of iridoid glycosides.

This sample was extracted for two days and then the extract filtered to remove the filter paper. The extract was evaporated to dryness and an internal standard of phenyl- β D-glucopyranoside (PBG), at a concentration of 0.500 mg/ml was added to each sample. Samples were partitioned between water and ether. The ether portion, containing waste materials, was discarded and the water fraction evaporated. We then added 0.50 ml of methanol to each sample and a 200 μ l aliquot was removed, evaporated, and derivatized using Tri-Sil Z (Sigma-Aldrich Corporation), before being run on an Agilent 7890A gas chromatograph (GC; Agilent Technologies) equipped with a flame ionization detector (FID) and an Agilent DB-1 column. The GC was calibrated prior to running these samples with a standard containing purified PBG, catalpol and aucubin (for details see Bowers and Collinge

1992; Bowers et al. 1992; Fajer et al. 1992; and Gardner and Stermitz 1988). Data were processed with Agilent ChemStation software (version A.03.34).

Preliminary predation observations:

For the observational studies, mantids were chosen due to their use as a model predator (Yamawaki, 2017; Iwasaki, 1991) and availability. Mantids were collected from the CU-Boulder Campus and private property with permission. Mantids were reared in the same growth chamber as the caterpillars. Fourteen mantids were captured, but only nine were used in experimentation due to deaths (mortality=5). They were fed a variety of insects caught sweep netting on campus. Fourth and fifth instar White Peacock caterpillars were randomly selected from groups reared on either *Bacopa* or *Plantago*. The experiment was conducted in a clear 16×10×6 cm plastic rearing box. The mantids were starved for three days and then offered a caterpillar. The maximum time a caterpillar was left in with a mantis was 20 minutes. Mantids are sight predators and ambush if they see an insect move towards them (Rilling et al., 1959). If the mantis did not attack the offered caterpillar, the caterpillar was removed, and the mantis starved for another day and then offered another caterpillar. The trials were filmed and photographed using both a Canon Rebel T3i DSLR camera and a smartphone. If a mantis accepted a caterpillar, the behavior was noted.

CHAPTER III

RESULTS

Larval Defensive Behavior:

Overall, all caterpillars showed a number of different behavioral responses to the simulated predator attack (Table 1). Thrashing and escape attempts were the two most commonly observed responses. The least commonly observed defense behavior was biting. In all the demonstrated behaviors except escape attempts, caterpillar age had a significant effect on the frequency of behavior displayed (Table 2). However, the only behavior in which there was a significant effect of host plant on frequency was thrashing (Table 2); thrashing was more commonly observed on *Bacopa*. There was also an interaction of caterpillar age and host plant on the frequencies of regurgitation, thrashing, and no reaction behaviors, indicating that the frequencies of behaviors changed differently over the instars depending on the host plant.

Thrashing frequency was different between caterpillars reared on *Bacopa* and *Plantago* ($df=1$, $\chi^2=4.092$, $p=0.043$), but also among different instars ($df=2$, $\chi^2=13.44$, $p=0.001$). From Table 1, The *Bacopa* reared caterpillars had higher occurrences of thrashing. For caterpillars reared on *Plantago*, there was a significant difference in thrashing frequency among different instars ($df=2$, $\chi^2=14.938$, $p<0.001$). From Table 1, we can see that the number of occurrences of the thrashing behavior increased with each instar.

The probability of other behaviors such as biting and no response was most impacted by the caterpillar instar ($p < 0.001$). Older caterpillars were more likely to display these behaviors (see Table 1). For caterpillars reared on the same plant, the frequency of a caterpillar demonstrating other behaviors, was significantly different among instars (*Bacopa*: $p < 0.001$; *Plantago*: $p = 0.012$).

Regurgitation frequency was not affected by the host plant on which caterpillars fed ($df = 1$, $\chi^2 = 2.88$, $p = 0.089$). However, the frequency of regurgitation was affected by caterpillar age ($df = 2$, $\chi^2 = 12.16$, $p = 0.002$). Younger caterpillars are more likely to regurgitate than the older caterpillars (see Table 1). From Table 1, the instar that regurgitated the most was the third instar, especially those that were reared on *Plantago* ($df = 2$, $\chi^2 = 8.29$, $p < 0.001$). This runs counter to the hypothesis of older caterpillars regurgitating more than younger ones.

When behaviors were categorized as aggressive (thrashing, biting, and regurgitation) or evasive (escape attempts and no response) based on Greeney et al. (2012), there was no significant effect of either host plant ($p = 0.707$) or caterpillar age group ($p = 0.094$) (see Table 3). There was no difference between instars reared on the same host plant as well (*Bacopa*: $p = 0.07$; *Plantago*: $p = 0.435$).

Table 1: Number of observations of behaviors observed as the first response after stimulated attack (forceps pinch).

<i>Bacopa</i>	Regurgitation	Thrashing	Escape Attempt	Other Response	<i>Total Caterpillars</i>
Third Instar	4	12	1	3	20
Fourth Instar	3	9	5	3	20
Fifth Instar	0	9	10	1	20
<i>Total</i>	7	30	16	7	60
<i>Plantago</i>					
Third Instar	9	4	2	5	20
Fourth Instar	0	10	7	3	20
Fifth Instar	0	12	6	2	20
<i>Total</i>	9	26	15	10	60

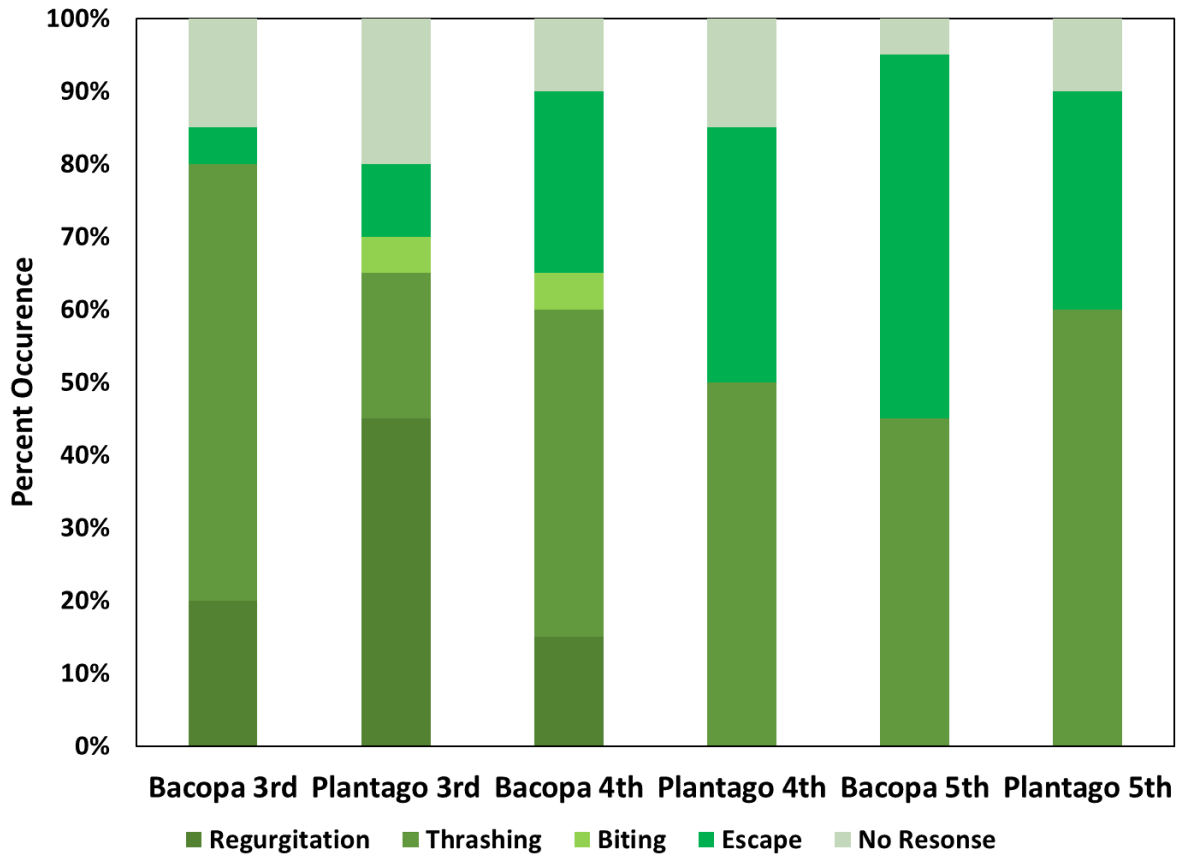
Table 2: Statistical analyses of defensive behavior displayed in response to stimulated attack. * indicates a significant effect. "Other responses" includes both biting and no response. Sample sizes of these two behaviors was too small to analyze separately, thus they were combined.

<i>Regurgitation</i>	<i>Df</i>	<i>X²</i>	<i>P-Value</i>
Host Plant	1	2.88	0.089
Caterpillar Age	2	12.16	0.002*
Age of Caterpillars Raised on <i>Bacopa</i>	2	0.47619	0.788
Age of Caterpillars Raised on <i>Plantago</i>	2	17.374	1.69x10 ⁻⁴ *
<i>Thrashing</i>			
Host Plant	1	4.0926	0.043*
Caterpillar Age	2	13.44	0.001*
Age of Caterpillars Raised on <i>Bacopa</i>	2	1.538	0.5616
Age of Caterpillars Raised on <i>Plantago</i>	2	14.938	5.704x10 ⁻⁴ *
<i>Escape Attempts</i>			
Host Plant	1	0.13714	0.711
Caterpillar Age	2	2.5371	0.281
Age of Caterpillars Raised on <i>Bacopa</i>	2	1.7647	0.413
Age of Caterpillars Raised on <i>Plantago</i>	2	2.9167	0.233
<i>Other Responses</i>			
Host Plant	1	0.223	0.637
Caterpillar Age	2	25.492	2.915x10 ⁻⁶ *
Age of Caterpillars Raised on <i>Bacopa</i>	2	17.5	1.585x10 ⁻⁴ *
Age of Caterpillars Raised on <i>Plantago</i>	2	8.88	0.0118*

Table 3: *Statistical analyses of aggressive versus evasive behaviors displayed in response to stimulated attack.*

<i>Treatment</i>	<i>Df</i>	<i>X²</i>	<i>P-Value</i>
Host Plant	1	0.14101	0.707
Caterpillar Age	2	4.724	0.094
Age of Caterpillars Raised on <i>Bacopa</i>	2	5.311	0.070
Age of Caterpillars Raised on <i>Plantago</i>	2	1.667	0.435

Figure 2: *Percent occurrence of defensive behaviors in response to stimulated attack by host plant and caterpillar age group.*



Larval Development

Results showed that both host plant and whether caterpillars regurgitated significantly affected pupal weight (Figure 3). However, there was no interaction between the host plant and treatment ($df=1$, $p=0.136$). Overall, the pupae that had been reared as caterpillars on *Plantago* were heavier than those on *Bacopa* (Figure 3, Table 4; $df=1$, $p=0.002$). In the caterpillars reared on *Bacopa*, the control larvae were heavier than those who had been induced to regurgitate (see Table 4; $df=1$, $p=0.008$). The larvae reared on *Plantago* showed a similar pattern, as pupal weight was higher in the control group compared to that of larvae in the group that had been induced to regurgitate (see Table 4: $df=1$, $p=0.008$).

The time that it took the caterpillars to reach the pupal stage was significantly different between host plants ($df=1$, $p<0.001$). The *Bacopa* groups took 18 days on average to reach the pupal stage, compared to the *Plantago* group which took 26 days on average. Results from a previous study showed that White Peacock caterpillars reared on *Plantago* developed more slowly than those reared on *Bacopa* (Knerl & Bowers, 2013). However, there was no difference in development time between the control and experimental groups reared on the same plant ($df=1$, $p=0.965$). This indicates that the host plant is potentially more important in development duration of caterpillars than if a caterpillar regularly regurgitates.

Table 4: *Analysis of variance of pupal weights and time to pupation.*

Pupal Weight	d	Sum of Squares	Mean of Squares	P-Value
Host Plant	1	0.3356	0.3356	0.002 *
Treatment	1	0.2453	0.2453	0.008 *
Host plant x Treatment	1	0.0740	0.0740	0.136

Time to Pupation				
Host Plant	1	2.9012	2.9012	2.0x10 ⁻⁶ *
Treatment	1	0.000	0.000	0.965
Host plant x Treatment	1	0.000	0.000	0.965

Figure 3: Pupal weight of larvae reared on *Plantago* and *Bacopa* that were exposed or not exposed to simulated predation. Means and +/- standard error are shown.

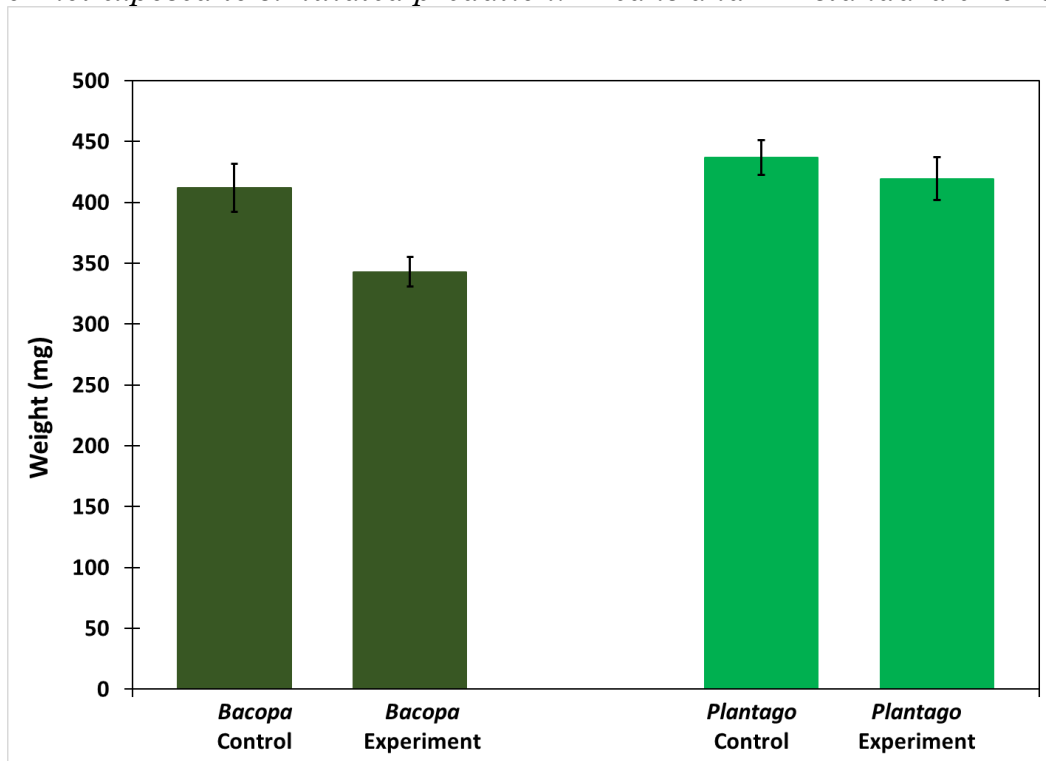
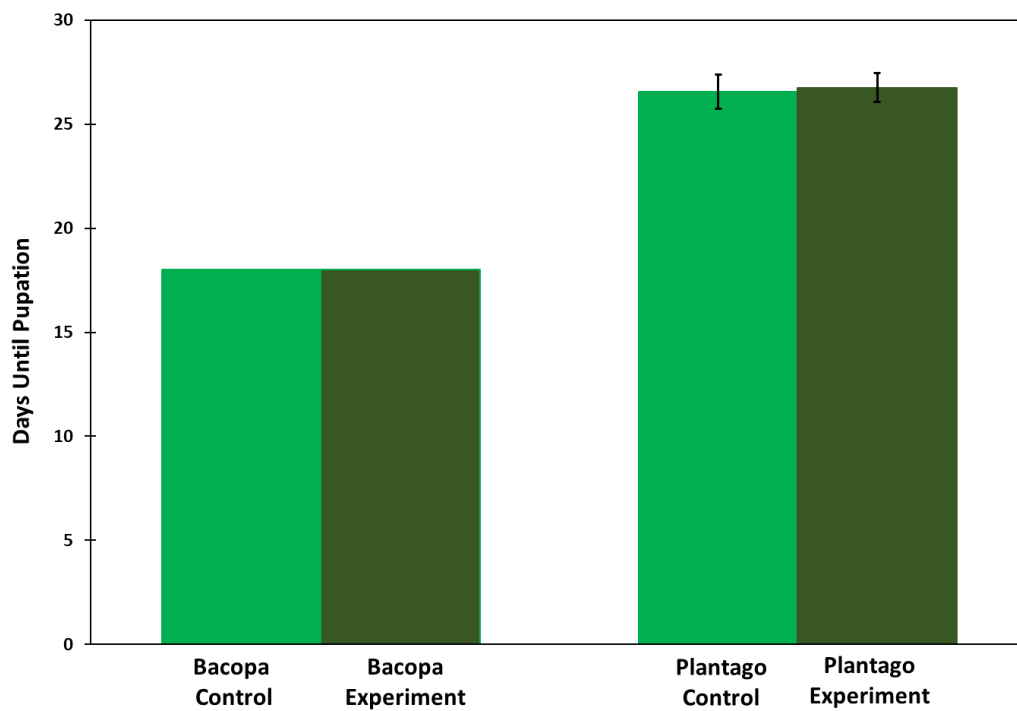


Figure 4: *Time to pupation of caterpillars reared on Plantago and Bacopa and that were exposed or not to simulated predation. Means and +/- standard errors are shown. All larvae in both treatments that were reared on Bacopa pupated on the same day, thus there are no error bars.*



Iridoid Glycoside Analysis

Low levels of iridoid glycosides were detected in regurgitant through gas chromatography. The total levels of iridoid glycosides ranged from $1.84 \times 10^{-4} \text{mg/cm}^2$ to $3.913 \times 10^{-2} \text{mg/cm}^2$ with an average of $7.79 \times 10^{-3} \text{mg/cm}^2$ (standard error: $4.09 \times 10^{-3} \text{mg/cm}^2$). Aucubin levels ranged from $1.88 \times 10^{-4} \text{mg/cm}^2$ to $3.19 \times 10^{-2} \text{cm/mg}$ with an average level of $6.19 \times 10^{-3} \text{mg/cm}$ (standard error: $3.33 \times 10^{-2} \text{mg/cm}^2$). The catalpol levels ranged from undetectable to $7.25 \times 10^{-3} \text{mg/cm}^2$ with an average level of $1.60 \times 10^{-3} \text{mg/cm}^2$ (standard error: $7.70 \times 10^{-4} \text{mg/cm}^2$).

Predation Observation Study

When attacked by a mantis, caterpillars often did not have the opportunity to display defensive behaviors. If they were able to regurgitate, the regurgitant often missed the target predator and instead landed on the caterpillar itself or the experimental set up. In one case, a White Peacock caterpillar did regurgitate on the mantis which had captured it. The regurgitant also got on the caterpillar. The mantis appeared to ingest some of the regurgitant when feeding on the caterpillar. The regurgitant was observed to travel through the esophagus into the gut of the mantis before coming back up to be expelled through the mouth several times which appeared to be an unsuccessful attempt remove the regurgitant from the mantid's body. This mantis did not finish eating the caterpillar after the attempt to expel the regurgitant and was found dead the next day. The mantis was examined under a microscope and photographed. Although there did not appear to be any regurgitant on the mouth parts, there was dried regurgitant on its head near the clypeus and on

the forelegs (see Figures 6-7). The true cause of the death of this individual mantis is unclear. Overall, the predation experiments were inconclusive and need to be repeated.

Figure 5: *Mantis Eating White Peacock Caterpillar.*



Figure 6: *Dried regurgitant on deceased mantis' head.*



Figure 7: *Dried Regurgitant on deceased mantis.*



CHAPTER IV

DISCUSSION

The results of these experiments showed that the age of the caterpillar was generally more important in determining the defensive behaviors shown, rather than the host plant on which the caterpillars had fed (although this was not the case for thrashing in which host plant did affect the frequency of this behavior). Although caterpillars frequently regurgitated, this was not their primary defense behavior; instead they more commonly thrashed or attempted to escape. However, there was no difference between aggressive and evasive defense strategies. Grouping behaviors may not be the most valuable approach in this species.

Host plant did, however, influence larval development time. Caterpillars which were reared on *Plantago* grew more slowly than those on *Bacopa*. Despite the time to pupation being longer on *Plantago*, the weight of pupae reared on *Plantago* was higher than that of reared on *Bacopa*. Regurgitation also affected larval development. Caterpillars which regurgitated regularly had lower pupal weights than the control group. However, there was no interaction between host plant and regurgitation frequency found indicating there is no impact of host plant on the behavior. The regurgitant collected from caterpillars reared on *Plantago* did contain low levels of iridoid glycosides.

Of the defenses displayed, regurgitation appears to incur tradeoffs from both the frequency of occurrence and impacts on development. The tradeoffs associated

with regurgitation may come from the gut contents being lost; these include plant matter that is disgorged (Rhainds et al., 2011) and thus not available for nutritional needs. Furthermore, feeding on *Plantago* may require more energy for the caterpillars to detoxify the effects of iridoid glycosides (Knerl & Bower, 2013). However, the caterpillars were observed to reingest regurgitant which may lessen the loss of nutrients.

Although low levels of iridoid glycosides were found in the regurgitant, the effect of the chemicals on the efficacy of regurgitant as a defense was not explored. Sequestered chemicals found in regurgitant have been shown to not influence the regurgitant's potency to predators (Desurmont et al., 2017). The surfactant properties of regurgitant is what appears to be the main source of efficacy as a defense (Desurmont et al., 2017; Rostas & Blassman, 2009). Thrashing and escape attempts do not involve potential plant matter loss which may be the reason for their high occurrence, unlike regurgitation.

Despite the slower time to pupation of caterpillars reared on *Plantago*, the resulting pupae were heavier than those of caterpillars reared on *Bacopa* even for those that were in the regurgitant treatment. This could indicate that *Plantago* has a higher nutritional value than *Bacopa* (Knerl & Bowers, 2013). In research by Coley et al. (2006), the nutrient levels of the host plant not only affected caterpillar growth but also defenses in a variety of Lepidoptera and plant species surveyed in Panama. Generalist caterpillars which fed on lower quality hosts grew more slowly and demonstrated more defenses (Coley et al., 2006).

Furthermore, the defenses of the plants may also play an important role in insect defensive behavior, beyond chemical sequestration. In oleander aphids which feed on milkweeds, the aphids that feed on milkweed species with less physical defenses, such as trichomes, display more frequent defensive behavior against parasitoids than those on more heavily protected milkweeds (Colvin & Yeargan, 2013). The physical defenses of the plants to discourage herbivory worked in the favor of the aphids since more trichomes and tougher leaves can make aphids potentially harder for parasitoids to detect (Colvin & Yeargan, 2013). Similar effects may also occur in this study system, as *Plantago* and *Bacopa* are very different plants in terms of morphology including the presence of trichomes and leaf toughness; *Plantago* having tougher leaves and more trichomes (Barrett & Strother, 1978; Cavers & Crompton, 1980).

Host plants can also affect other behaviors including foraging and diapause (Friberg & Wiklund, 2010; Muller & Muller, 2017). In Friberg & Wiklund (2010), generalist butterflies that were studied used two host plants, one that occurred at lower elevations in meadows and one that occurred in higher elevation forests. It was shown, that regardless of temperature and photoperiod, the caterpillars reared on the forest host plant would enter diapause instead of directly developing like those on the meadow host plant (Friberg & Wiklund, 2010). In chrysomelid beetles that feed on cabbage and watercress, it was shown that their foraging and reproductive behavior were different on the different plants (Muller & Muller, 2017). Beetles which fed on cabbage were more active in foraging which resulted in

higher reproductive output because actively foraging beetles were more likely to encounter potential mates (Muller & Muller, 2017). In these two cases, it was the identity of the plant that triggered the changes in behavior.

Introduced host plants can have a detrimental effect on Lepidoptera (Yoon & Read, 2016). For some butterflies such as checkerspots (*Euphydryas sp.*), *Plantago* is considered an inferior host (Bowers et al., 1992). In other cases, *Plantago* has been shown to increase the performance of larvae (Brown et al., 2017; Reudler et al., 2011). Knerl & Bowers (2013) found mixed results when the White Peacock fed on *Plantago* regarding fitness. This study indicates that White Peacocks that feed on *Plantago* may have higher fitness than those that feed on *Bacopa* however have a trade off with longer development. Despite the tradeoff with development could make them more susceptible to predators, these caterpillars may be better defended due to the sequestration of iridoid glycosides (Knerl & Bowers, 2013) which are also found in the regurgitant, as shown in this thesis.

The predator observation study had several challenges, including time limitations and insect behavior and should be redone for more robust results due to the interesting preliminary observations. It may be better to use a non-ambush predator since, when the offered prey did not move, the mantids would not notice it and time of the trial would elapse before any interaction. Ants have been used as a predator to evaluate defenses of caterpillars and may be a better choice since they actively search for prey (Smedley et al., 1992). Further study can also quantify if there are any differences in palatability in caterpillars reared on *Plantago* and

Bacopa since this experiment focused on whether White Peacock caterpillars were palatable in general. The observations during the study suggest that regurgitation may not be the best defense since caterpillars accepted by the mantids either did not have the chance to regurgitate or regurgitated on themselves and/or the rearing box.

Further exploration into the behavior of White Peacock caterpillars outside of defense on the novel host plant may reveal more differences between *Plantago* and *Bacopa* reared caterpillars. Further research is needed to determine how the defensive behaviors are coupled since they often did not occur alone. It was observed that the White Peacock caterpillars often thrashed in order to target where the stimulus was occurring before demonstrating a different behavior. The order of behavior displayed may shed more light on the defensive strategies of this species.

The White Peacock is not well studied despite being a widespread and common butterfly in the Southeastern United States, Central America, and the Caribbean (Knerl & Bowers, 2013; Rawson, 1976). As a study system, the White Peacock presents an opportunity to explore many questions about host plant and caterpillar interactions due to the recent shift to *Plantago*. This study establishes a baseline for defense behavior in this species which is useful for future research on this species.

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