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Effects of Acorns on Populations of *Aedes* Mosquitoes

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The University of Southern Mississippi

Effects of acorns on populations of *Aedes* mosquitoes

by

Rachel E. Rogers

A Thesis
Submitted to the Honors College of
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Abstract

Aedes albopictus (Asian tiger mosquito) and *Aedes aegypti* (yellow fever mosquito) are two mosquito species that are non-native to North America and are of medical importance. Both species can be found in aquatic container habitats, such as discarded tires and tree holes. In container habitats, the influx of detritus from the outside environment is essential to mosquito production and can include a variety of plant and animal material. Seeds from oak trees (acorns) can seasonally contribute to the detritus of these container habitats. This study examined the effect of acorns from the southern live oak (*Quercus virginiana*) on the performance of *Ae. aegypti* and *Ae. albopictus*. Amounts of acorns were compared to oak leaves, which were based on tire samples from Mississippi. The mean, 50% of the mean, and 200% of the mean were used for each type alone, along with two asymmetrical mixtures of leaf and acorn. Tannins, secondary plant compounds that discourage herbivory, were measured across all treatment levels, and the levels were compared between leaf and acorn. Survival and λ' (an estimation of populations growth) were used to evaluate performance of *Ae. albopictus* and *Ae. aegypti*. A significant treatment effect was observed among tannin levels, with more detritus leading to higher tannins; there were no difference in tannins between detritus types. In addition, I found a significant treatment effect on survival of both mosquito species. Survival was the lowest in the mean acorn treatment regardless of species. Values for λ' between both species did not vary detritus types or amounts. Thus, although under certain circumstances acorns could limit mosquito performance, generally acorns and leaves represent similar resources for developing container *Aedes*.

Keywords: *Aedes aegypti*, *Aedes albopictus*, acorns, mosquito performance, tannins

Dedication

This thesis is dedicated to my grandmother, Louisa Elba Park. She encouraged my curiosity of nature and science when I was a child. Because of her, I am who I am today.

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I would also like to thank those who have helped me throughout the project. Dr. Kevin Kuehn identified the oomycete that was found growing in some containers during this project. Catherine Dean has been a big help throughout this entire process and has always cheered me on. Aaron Bermond checked my project for several days while I was away at a conference for which I am grateful. Zachary Taylor has been my rock throughout this. Also, the entire Yee Lab of Aquatic Insect Ecology has helped inspire me and helped me grow as a researcher. I would also like to thank all my professors who have taught me throughout my time at USM and the Honors College for making this thesis possible. I would not be where I am today without all of you. And thank you again to everyone who has encouraged me along this journey.

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List of Abbreviations

LTEC – Lake Thoreau Environmental Center

ANOVA – Analysis of Variance

Chapter I: Introduction

Insects in the Family Culicidae, commonly referred to as mosquitoes, are a group of true flies with over 3,000 species that occur on every continent except for Antarctica (Clements, 2000). Mosquitoes develop through a holometabolous, or complete, life cycle with an egg, four larval instars, pupa, and adult stages (Clements, 2000). In many mosquito species, the female requires a blood meal in order to produce eggs (Clements, 2000). Research conducted on various issues concerning mosquitoes is important because of their medical importance as vectors of important human and animal diseases. Certain species of mosquitoes can vector dangerous, if not deadly, diseases such as yellow fever, malaria, dengue, Zika, and chikungunya (Robert et al., 2016).

Many species of mosquitoes use small containers of water for larval development. Important human-made containers that can harbor mosquito larvae include cemetery vases and automobile tires (Yee, 2008). Cemeteries provide an important habitat for mosquito larvae because of the abundance of vases, where water and detritus can collect, along with flowers planted that provide the adults with nectar and plentiful resources that help the offspring survive and develop (Vezzani, 2007). Both cemeteries and tire dumps can be closely associated with human activity, and thus can provide female mosquitoes with a steady supply of blood meals. Two species that can utilize small human-made containers are *Aedes albopictus* and *Aedes aegypti* (Yee 2008). Both mosquitoes are of medical importance and are non-native to the United States.

Aedes albopictus, the Asian tiger mosquito, was originally from Asia and was probably introduced to the United States through shipments of tires (Hawley *et al.*, 1987). In areas where it is invasive, this species is more likely to be found in human-made

containers than natural containers such as tree holes (Sota and Mogi, 1992, Yee et al. 2015a). Their eggs are more desiccation resistant than the eggs of other *Aedes* species, which has aided their invasion into non-native habitats as they can survive some desiccation (Sota and Mogi, 1991). The larvae of this species are commonly found in tires throughout the United States (Yee, 2008). *Aedes albopictus* can be a superior competitor to both native and non-native species. It has been shown that under certain seasonal conditions, such as increased rainfall, it can outcompete other species such as *Aedes aegypti* and *Culex quinquefasciatus*, the southern house mosquito (Leisnham *et al.*, 2014).

Aedes aegypti, the yellow fever mosquito, was originally from Africa. It is found less commonly than *Aedes albopictus* throughout the United States. Its eggs are also desiccation resistant, which has helped it gain hold in new areas, and evidence suggests that its eggs are more desiccation resistant than *Aedes albopictus* (Sota and Mogi, 1992). However, larval survival is lower in *Aedes aegypti* than *Aedes albopictus* in some cases regardless of the detritus type used (Yee *et al.*, 2015b). Depending on weather or seasonal conditions, *Aedes aegypti* can be outcompeted by *Aedes albopictus* (Leisnham *et al.*, 2014).

Mosquito larvae are detritivores and feed upon the vast array of dead and decaying plant, animal, and sometimes even algal material in the water. The quality and quantity of detritus in microcosms can have significant effects on the larval populations that live there (Yee and Juliano, 2006). If mosquito larvae are grown in animal detritus, such as crickets or a mixture of plant and animal material, they experience higher growth and higher mass than larvae that only feed on less nutritious plant material (Yee *et al.*,

2015b). In containers, elements such as nitrogen or phosphorus can be limiting factors to larval growth (Yee and Juliano, 2006).

Tannins are water-soluble phenolic compounds that bind proteins that are produced by plant life to dissuade herbivory (Barbehenn and Constable, 2011). They exist in many different types of plants and in the various structures that plants possess (e.g., stems, leaves). Tannins can be either consumed directly by the insect or the plant material can break down in aquatic microcosms thus releasing tannins into the water. The toxicity of tannic acids in insects was first studied in Acridoidea (Bernays *et al.*, 1980). It was found that the introduction of tannic acid caused lesions in the midguts of graminivorous species of grasshoppers while no effect seemed to occur in phytophagous species (Bernays, *et al.*, 1980). Very little is known about the effects of tannins on the digestive tract, specifically of mosquito larvae. However, evidence has shown that tannins can have a negative impact on mosquito larvae. A study on *Aedes sierrensis* in tree holes illustrated that tannins could lengthen development times at high concentrations (Mercer and Anderson, 1994). Also, some *Aedes* species are more tolerant of tannins than others, with *Aedes albopictus* less susceptible to their effects than *Aedes aegypti* (Rey *et al.*, 1998). Thus, tannins must have some effect in disrupting their biological processes. However, tannin levels can decline as time passes (Yee and Juliano, 2006).

The southern live oak, or live oak (*Quercus virginiana*), is a common tree species in the south that produces tannins in their leaves. Live oak occurs commonly in Florida, areas in Texas, and along the Gulf Coast. Trees in the oak family, Fagaceae, produce seeds called acorns. In some years, seed production (i.e., mast) is high, perhaps due to weather patterns (Koenig *et al.*, 2016). In higher mast years, more acorn or leaf detritus

likely fall into containers, such as tires and cemetery vases containing mosquito larvae. Due to this influx of oak material, specifically acorns and leaves, the mosquito larval performance could be affected by factors such as the tannin composition or the nutrient content of the leaves and acorns.

Although several different types of detritus have been examined for their effect on container larvae, no other work has been done specifically focusing on acorns and their effects on mosquito performance. I hypothesized that leaves and acorns would have differing amounts of tannins, and that leaves and acorns would provide different resources for the mosquito larvae. In containers with higher tannin levels, it was predicted that there would be an overall lower performance in the mosquitoes. The larvae grown in the different resources would differ in performance (i.e., survival, λ').

Chapter II: Materials and Methods

Detritus

Acorns from the southern live oak (*Quercus virginiana*) from Lake Thoreau Environmental Center in Hattiesburg (LTEC), MS (31.3466° N, 89.4223° W) were collected and dried in the fall of 2016. The amount of acorn detritus used in each treatment was estimated using the mean dry mass of acorns and leaves collected from 498 tires in Mississippi from an earlier study (Yee et al., 2015a). As acorns were often fragmented within tires, the ratio of broken to unbroken acorns was held constant within treatment levels. I used three treatment levels of acorns: 200% of the mean from the earlier study (Yee et al. 2015a), the mean, and 50% of the mean, hereafter high (7.98 g), medium (1.98 g), and low (0.99 g), respectively. Acorn treatment levels were compared to the same levels of live oak leaves alone, and two combinations of leaf and acorn. The mixtures held total mass constant using the mean value (1.98 g) but varied the contribution of leaf + acorn (i.e., 1.48g + 0.50g, 0.50g + 1.48g). Leaves were collected senescent from *Quercus virginiana* located from the LTEC and were dried in the same manner as acorns. Leaves had the petioles remove and were fragmented into small pieces before being allocated to treatments. Detritus was added to 400 ml plastic tripour beakers to which I added 1 ml of water from field containers and 399 ml of deionized (DI) water. Beakers are hereafter referred to as microcosms. Inoculum added a microorganism community that was allowed to grow for 4 days before mosquito larvae were added. At that point, each microcosm received 20 *Aedes albopictus* or *Aedes aegypti* larvae. Eggs of F₂ generation were obtained from captive colonies, hatched, and the first instars were added to the microcosms. The microcosms were placed in an environmental incubator set

at 25 °C with a 12:12 day:night cycle and were checked daily for the presence of pupae. Water levels were maintained using new additions of DI water as needed.

Pupae were placed in individual 0.25 dram shell vials. After eclosing, adults were sexed and dried at 50 °C for > 48 hours before being weighed to the nearest 0.0001 mg using a XP2U ultra-microbalance (Mettler Toledo Inc., Columbus, Ohio). Development times from first instar hatching to eclosing as an adult was recorded for all individuals along with the numbers of larvae that survived to eclosing in each microcosm. Survival was compared across different detritus levels and between the two species using analysis of variance (ANOVA) with significant differences among means determined using mean separation with a Tukey adjustment. All analyses were performed in SAS (2004).

Tannins

Tannins were tested three times in each replicate once before larvae were added, 1 week post larval addition, and 3 weeks post larval addition. A tannin reagent and NaCO₃ were added to a water sample from the microcosm and compared to a color wheel (Hach, Loveland, CO). Tannin values were analyzed using ANOVA, with treatment level (8), time (3), species (2), as well as their interactions as independent variables.

Population Growth

Analysis of the estimate of population growth (λ') was performed on all replicates. This estimate is given by the equation:

$$\lambda' = \exp(r') = \exp\left(\frac{\ln\left[\frac{1}{N_0} \sum_x A_x f(w_x)\right]}{D + \left[\frac{\sum_x x A_x f(w_x)}{\sum_x A_x f(w_x)}\right]}\right),$$

where N_0 is the assumed number of females in a cohort (50%), and A_x represents the number of females eclosing on a given day. The dry weight of the females after they eclose is w_x and used in the function $f(w_x)$ that relates fecundity to female mass based on a regression. For *Aedes albopictus* the function used was $19.5+(152.7(w_x))$ (Lounibos et al., 2001), and in *Ae. aegypti* this function was $21.1+(42.9(w_x))$. The number of days it took after eclosion for a female to oviposit was assumed to be 14 days for *Aedes albopictus* (Grill and Juliano, 1996) and 12 days for *Aedes aegypti* (Lounibos et al., 2001). Values of $\lambda' > 1$ indicate that the population size is increasing, whereas a value of λ' of < 1 indicates a decreasing population size; λ' equal to 1 indicates a stable population. Values of λ' were compared across different detritus levels and between the two species, separately, using ANOVA, with significant differences among means determined using mean separation with a Tukey adjustment.

Chapter III: Results

Tannins

An effect of time on tannin levels was found across all treatment levels ($F_{2, 48} = 9.05$, $P = 0.0005$). Tannins were lowest before larvae were added (week 0). During week 1, tannin levels had increased to their highest levels, and by week 3 they had leveled off into an intermediate level of tannins that was not significantly different to weeks 0 and 1 (Fig. 1).

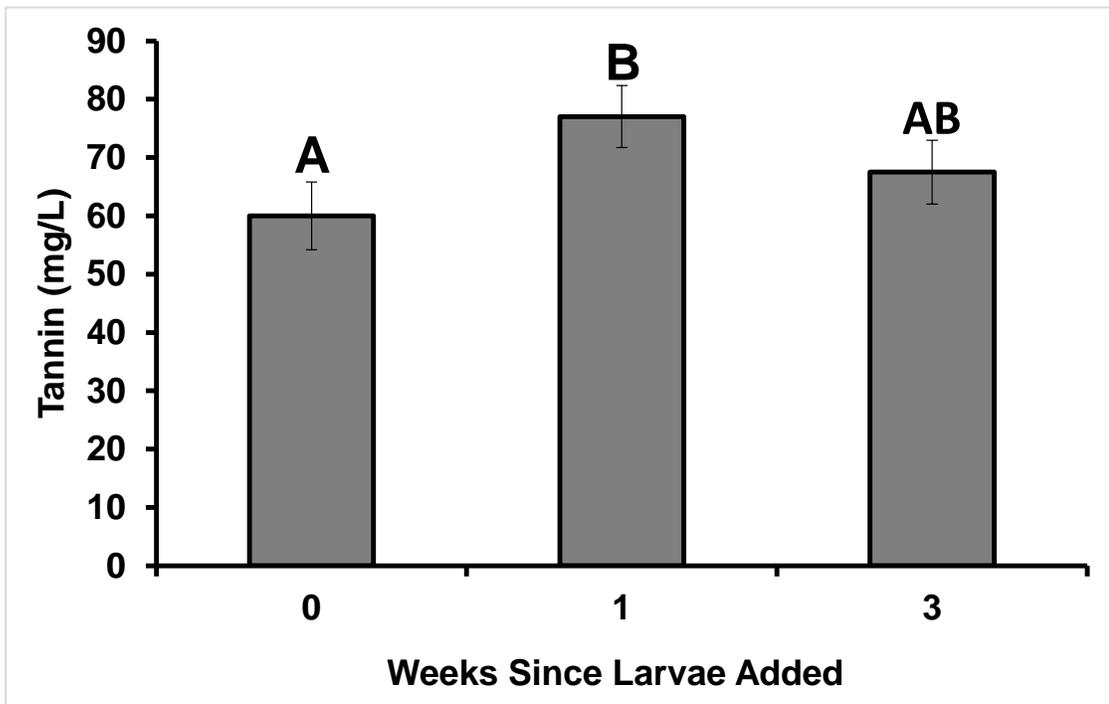


Figure 1. Concentrations of tannins (mg/L) across all treatments by weeks since larvae were added (0, 1, and 3 weeks).

A significant treatment effect was also detected ($F_{7, 48} = 80.29$, $P < 0.0001$). The highest amounts of leaf or acorn (7.94 g) had the highest amounts of tannins, with the lowest amount of leaf or acorn (0.98 g) having the lowest amount of tannins (Fig. 2). Other detritus amounts, including combinations of leaves and acorns, produced intermediate levels of tannins (Fig. 2).

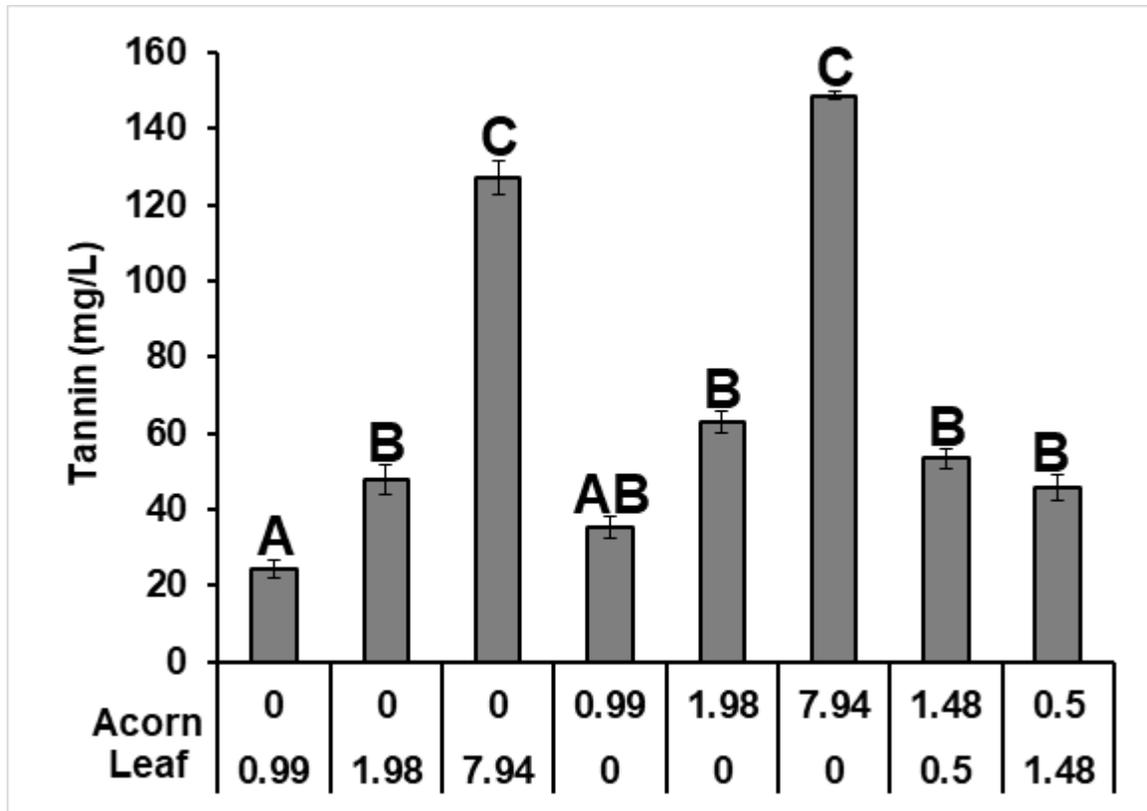


Figure 2. Concentrations (mg/L) of tannins across all treatment levels.

Survival

No differences in survival existed between species ($F_{1, 48} = 0.92$, $P = 0.3430$). However, significant differences in survival existed among detritus treatment levels ($F_{7, 48} = 5.00$, $P < 0.001$). The treatments with the mean amount of acorns (1.98 g) had the lowest survival of all treatments, but this was only significantly different from the mean amount (1.98 g) of leaves and the two mixtures (1.48 g + 0.50 g, 0.50 g + 1.48 g), which generally had the highest survival (Fig. 3).

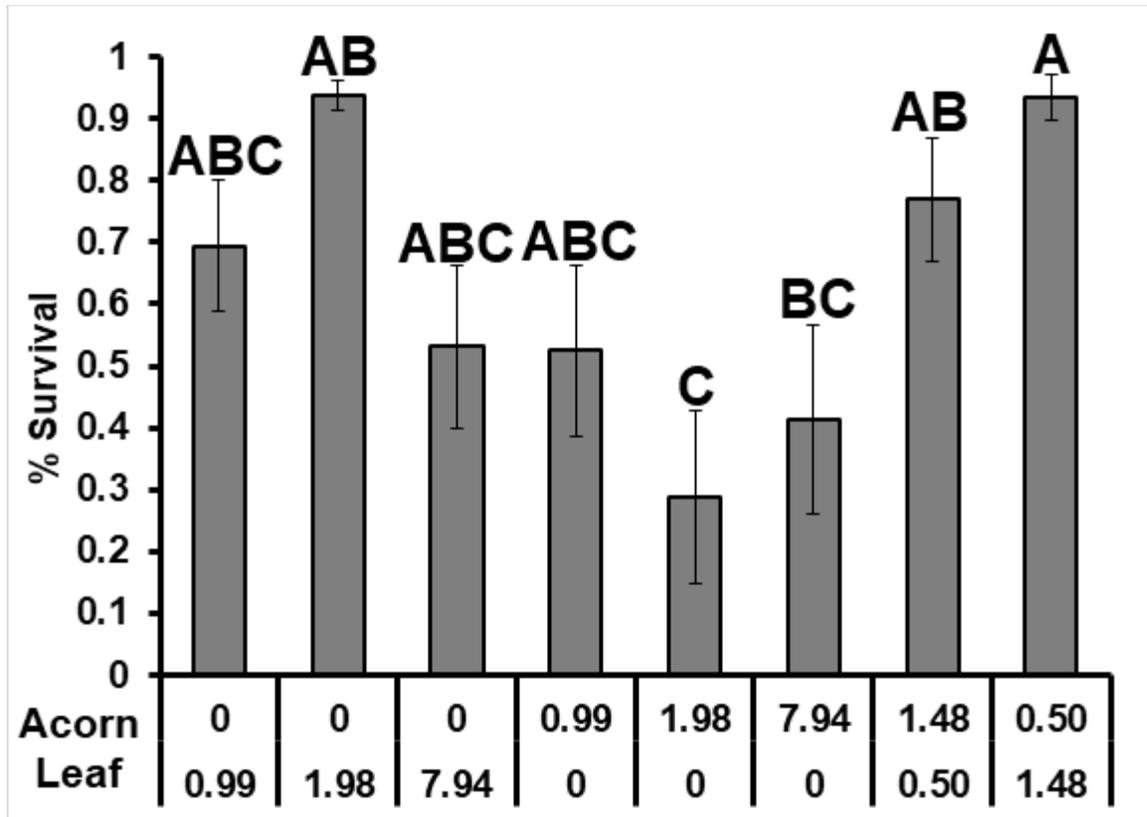


Figure 3. Mean \pm SE of percent survival of mosquitoes across acorn or leaf treatment levels. Means that share a letter are not significantly different.

Population Growth

The λ' values across all detritus treatment levels and between the two species were all >1 , suggesting positive population growth. For *Aedes albopictus*, λ' showed no differences across detritus environments ($F_{6, 18} = 2.31$, $P = 0.0792$, Fig. 4). No *Aedes albopictus* females survived in the mean acorn treatment (1.98 g) and is zero for this treatment level.

For *Aedes aegypti*, there were significant differences among detritus treatment levels ($F_{7, 19} = 2.82$, $P = 0.0340$). However, mean separation failed to detect differences among specific means although differences between the 7.94 g leaf treatment and 1.98 g leaf treatment did approach significance (Fig. 5).

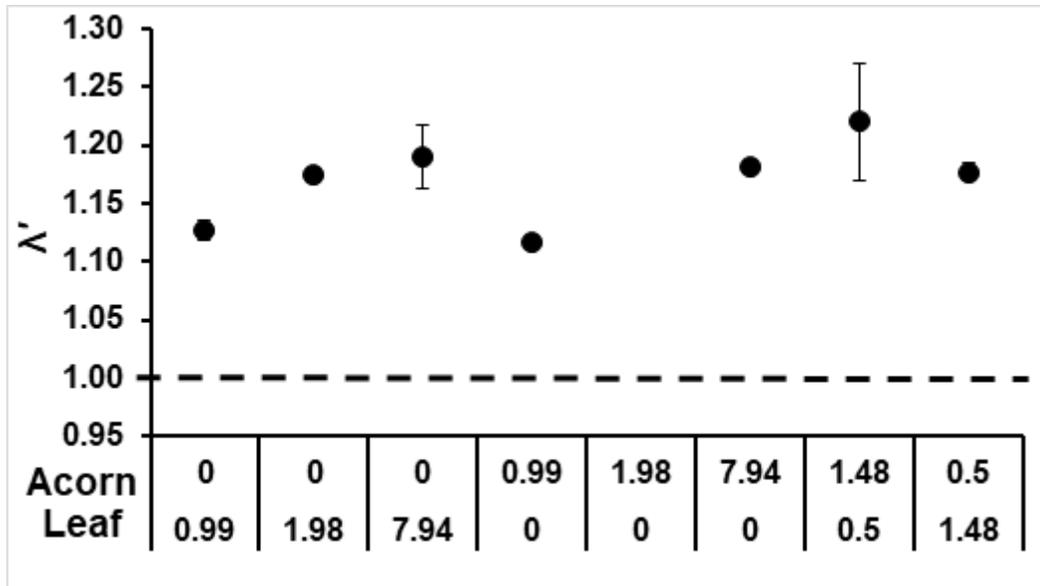


Figure 4. Mean \pm SE λ' for *Aedes albopictus* across all detritus type treatment levels. The dashed line at 1.00 indicates no population growth.

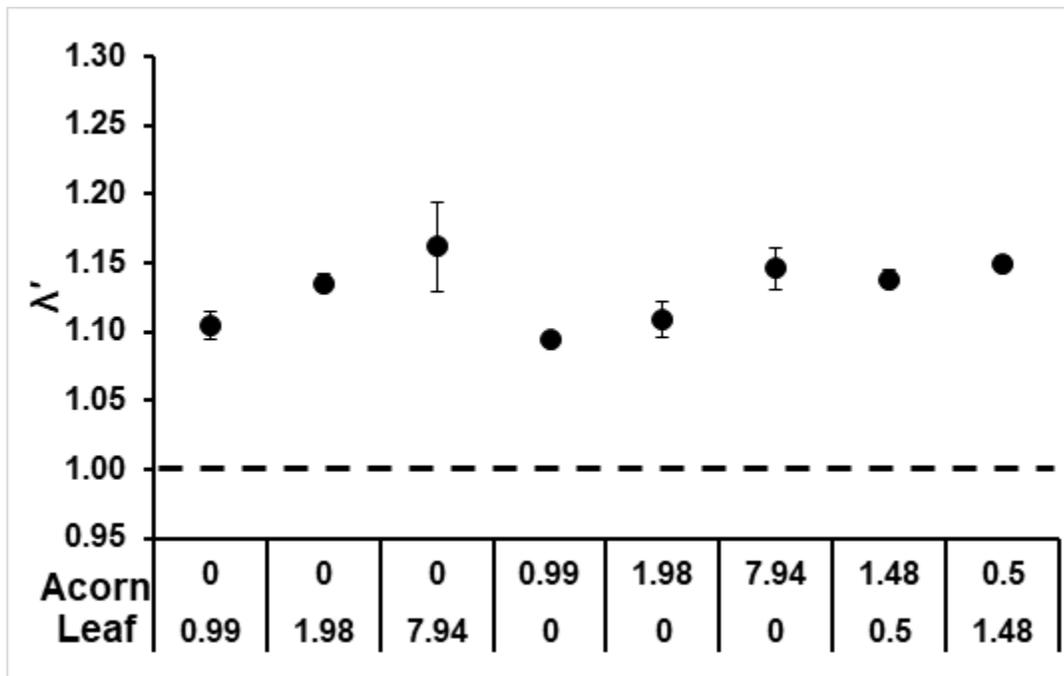


Figure 5. Mean \pm SE λ' for *Aedes aegypti* across all detritus treatment levels. The dashed line at 1.00 indicates no population growth.

Chapter IV: Discussion

Unexpectedly, there were no significant differences in tannin levels between oak leaves and acorns. Thus, my hypothesis that leaves and acorns would have different tannin levels was unsupported. As predicted, the highest tannin levels were found in the highest amounts of both detritus types, but these values did not seem to negatively affect mosquito survival. In other studies of detritus, such as grass, pine needles, or animal material, tannins have been found to be significantly lower than in systems with oak detritus (Murrell and Juliano, 2008). However, it appears that leaves and acorns did represent different resources for larvae. The mean acorn treatment level (1.98 g) had the lowest survival compared to the same amount of leaves, suggesting something else besides tannins was affecting larvae. This effect also seemed to be specific, as no *Aedes albopictus* females survived in this treatment level. Because of the similar tannin levels across all treatment levels, this suggests that another factor, like nutrient levels, could inhibit the survival of larvae in the acorn only treatments. Treatments containing leaves and acorns had a higher survival rate than treatments only containing acorns. This could suggest that oak leaves contain nutrients that are lacking in acorns. No significant differences in survival were detected between *Aedes albopictus* and *Aedes aegypti* across detritus treatment levels. This comes into conflict with how *Aedes albopictus* has been previously shown to be less susceptible to tannins than *Aedes aegypti* (Rey et al., 1998), as here their survival was nearly identical across microcosms.

For λ' values, I found them to be increasing across all detritus environments, although detritus levels affected some life history aspects (e.g., 1.98 acorn level had zero survival for *Ae. albopictus*). With all populations increasing, the performance of both *Aedes*

albopictus and *Aedes aegypti* do not seem to be negatively affected by tannin levels or resource amounts. The values for detritus I chose were reflective of a study that measured acorns in tires from across the state of Mississippi (Yee et al., 2015a). The tires sampled all had some larvae (most contained *Aedes albopictus*), suggesting that detritus levels were either high enough or tannin levels low enough to have positive effects on mosquito growth. Thus, in the future, lower amounts of detritus (producing lower nutrients) or higher amounts of detritus (producing higher tannins) could be used to test for their effect on mosquito populations.

In container habitats, detrital inputs can come from a variety of sources, including leaves, seeds, flowers, grasses, and animal material. Entering tire systems, twigs, leaves, seeds, and fine detritus could all be found (Kling *et al.*, 2007). Other plant material such as oak flowers have been shown to increase the size of the female in *Aedes triseriatus* (Lounibos *et al.*, 1993). Animal material, such as dead insects, generally produces greater performance than plant material (Murrell and Juliano, 2008, Yee *et al.*, 2015b). Tannins in oak leaves, specifically *Quercus virginiana*, when compared to other detritus, such as pine needles, grass, and animal material, have been found to contain more tannins overall (Murrell and Juliano, 2008). Tannins of all types of detritus seem to decline as the oak leaves and acorns did here (Yee and Juliano, 2006). Thus, as I found that tannins were similar between oak leaves and acorns, tannin levels in *Quercus virginiana* acorns can be assumed to be greater than the tannin levels in pine, grass, and animal detritus.

In addition to the potential effects of tannins on mosquito growth, I did observe that a fungus-like growth occurred in all acorn only treatments, and that was seemingly more abundant in treatments with the highest acorns (7.94 g). This was identified to be an

oomycete, specifically in the Order Saprolegniales (K. Kuehn, personal communication). Some species in Order Saprolegniales are known pathogens on mosquito larvae, such as *Leptolegnia chapmanii*, which can be lethal to mosquito larvae (Pelizza *et al.*, 2008). Another oomycete, *Lagenidium giganteum*, has been shown to have a larvicidal effect on *Aedes aegypti* larvae (Maldonado-Blanco *et al.*, 2011). This oomycete could have affected the survival of the larvae in the acorn-containing microcosms; however, further examinations of the effect of this oomycete, its specific identity, and its affinity for acorns in nature needs to be conducted.

I found that acorns, at least at certain concentrations, can lower the survival of mosquito larvae in a given microcosm. This is unlikely due to tannins, as oak leaves had similar amounts of tannins, and the decrease in survival was not observed in leaves. Habitats, such as tire dumps or cemeteries where *Aedes albopictus* or *Aedes aegypti* occur, are often surrounded by live oaks (D. Yee, personal communication). A higher mast year for southern live oaks could contribute greatly to the acorn detritus amounts in human-made microcosms that harbor mosquito larvae. Thus, in these years, acorns would have a greater chance of affecting mosquito performances. This relationship between the detritus as a resource and its possible toxicity via tannins or the growth of an oomycete is important in understanding the ecology of *Aedes albopictus* and *Aedes aegypti*.

For future directions, as both species in this experiment were non-native to North America, we might not expect them to respond to the presence of North American acorn species. A good candidate for future testing would be *Aedes triseriatus*, the eastern tree hole mosquito. Because *Aedes triseriatus* are often found in tree holes (Wilton, 1968), it is possible that their larvae may have historically come into contact with *Quercus*

virginiana leaves and acorns more often than *Aedes albopictus* and *Aedes aegypti*. Also, to attempt to explain the lower survival in acorn compared to leaves, the nutrient composition of live oak leaves and acorns could be measured. This could be via measuring the carbon and nitrogen ratios of mosquitoes grown in every treatment and comparing them. Whatever the cause, acorns can have a negative impact on the performance of *Aedes albopictus* and *Aedes aegypti*.

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