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# Oviposition preference and offspring performance in container breeding mosquitoes: evaluating the effects of organic compounds and laboratory colonisation

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## Abstract

1.  The preference-performance hypothesis predicts that organisms lacking parental care should oviposit in habitats that optimize offspring performance. We investigated preference-performance relationships for the Asian tiger mosquito (*Aedes albopictus* Skuse) and the southern house mosquito (*Culex quinquefasciatus* Say) (Diptera:Culicidae), two medically important container-breeding species, in response to an organic chemical blend mimicking decaying plant matter. Additionally, we evaluated the effects of long-term laboratory colonization of *Cx. quinquefasciatus* by using wild and laboratory strains.

2.  Oviposition bioassays were conducted by releasing gravid mosquitoes into field enclosures with automobile tires containing low and high concentrations of the chemical blend, and water controls. The offspring were then reared in water collected from the tires in which they were deposited.

3.  *Aedes albopictus* and wild *Cx. quinquefasciatus* laid more eggs in the chemical blend than water controls but did not differentiate between the low and high concentrations. Conversely, laboratory *Cx. quinquefasciatus* only preferred the high concentration to the low concentration. No statistical associations between oviposition preference and larval survival were found, as the chemical blend did not affect survivorship of either species.

4.  The oviposition preference for the chemical blend over water controls suggests that both species oviposit in the best available resource environment, but further studies are needed before conclusions regarding preference-performance relationships can be drawn.

5.    We found that long-term laboratory colonization affects oviposition behavior in *Cx. quinquefasciatus*, suggesting that behavioral studies on laboratory strains are not always applicable to wild populations.

## Keywords

Preference-performance hypothesis; optimal oviposition; laboratory colonization; *Aedes albopictus*; *Culex quinquefasciatus*

## Introduction

Oviposition decisions made by female insects are an important determining factor in the distributions of immature stages, especially in situations where the immature stages are limited to the habitat in which they hatch. Such is the case for mosquitoes (Diptera: Culicidae), which do not generally exhibit parental care, and their offspring, which develop in lentic bodies of water, are incapable of dispersing to alternative habitats in the larval and pupal stages (Clements, 2000). According to the preference-performance hypothesis (PPH), also known as optimal oviposition theory, organisms lacking parental care should place their eggs in the most suitable locations for their offspring, and selection should favor oviposition behavior that optimizes offspring performance (Jaenike, 1978). However, the few existing studies of preference-performance relationships for mosquitoes have offered varying levels of support for the PPH ranging from good (e.g., Kiflawi *et al.*, 2003; Eitam & Blaustein, 2004; Reiskind *et al.*, 2009) to poor (e.g., Roberts, 1996; Wong *et al.*, 2012), and preference-performance relationships may differ by species and for different types of variables. Understanding how certain factors influence both adult oviposition decisions and survival of immatures is integral to mosquito surveillance and control, as such knowledge allows for predictions for where mosquitoes are most likely to breed and serves to enhance management techniques. Independent assessments of oviposition behavior and larval distribution may be misleading, as has been illustrated by attempts to control the yellow fever mosquito (*Aedes aegypti* L.), a container-breeder, by targeting only the most productive container types. Although targeted control campaigns have proven successful in some countries, others have failed to reduce adult *Ae. aegypti* populations to intended levels (Tun-Lin *et al.*, 2009; Maciel-de-Freitas & Lourenço-de-Oliveira, 2011), as *Ae. aegypti* will oviposit in non-preferred container types (which are sometimes equally or more suitable for offspring performance) in the absence of preferred container types (Maciel-de-Freitas & Lourenço-de-Oliveira, 2011; Wong *et al.*, 2012). This example demonstrates that attempts to control populations of disease vectoring mosquitoes may merely cause a shift in oviposition behavior without drastically affecting population dynamics, and that such outcomes may be more foreseeable with a better understanding of mosquito preference-performance relationships.

A number of factors associated with an aquatic habitat can influence both adult oviposition behavior and offspring performance, including larval density (e.g., Edgerly *et al.*, 1998; Ellis, 2008; Yoshioka *et al.*, 2012) and the presence of natural enemies (e.g., Kiflawi *et al.*, 2003; Eitam & Blaustein, 2004; Wasserberg *et al.*, 2013). A key factor influencing performance of immature mosquitoes and oviposition behavior of adults is the type and

amount of organic detritus (e.g., senescent plant material, invertebrate carcasses) present in the aquatic environment (e.g, Daugherty *et al.*, 2000; Yee & Juliano, 2006; Reiskind *et al.*, 2009). Mosquito larvae feed primarily on aquatic microorganisms (e.g., bacteria, protists) that colonize detritus, as well as consuming tiny fragments of detritus itself (Merritt *et al.*, 1992). Mosquitoes tend to perform best on labile resources (e.g., grasses, insect carcasses) that support high microorganism productivity (Yee *et al.*, 2007; Murrell & Juliano, 2008; Costanzo *et al.*, 2011). However, detritus, or the bacteria that decompose it, release chemicals that can be detrimental to mosquitoes at high concentrations, and some species may be more susceptible to certain chemicals (e.g., plant secondary compounds) than others (David *et al.*, 2000; Murrell & Juliano, 2008). Detritus and its associated chemicals also influence oviposition behavior of adult mosquitoes (e.g., Ikeshoji, 1975; Isoe *et al.*, 1995; Allan *et al.*, 2005), suggesting that gravid females may use these chemical cues as indicators of habitat suitability for their offspring.

In this study, we investigated preference-performance relationships in response to chemicals associated with detrital decomposition for two medically important container-breeding mosquito species: the Asian tiger mosquito (*Aedes albopictus*) and the southern house mosquito (*Culex quinquefasciatus*). Within their ranges, *Ae. albopictus* and *Cx. quinquefasciatus* are often the most abundant members of their respective genera found in water-filled artificial containers, especially discarded automobile tires (Sprenger & Wuithiranyagool, 1986; Lopes *et al.*, 2004; Yee *et al.*, 2012; Yee *et al.*, 2015), which constitute important peridomestic mosquito breeding habitats (Chambers *et al.*, 1986; Yee, 2008). *Aedes albopictus* is a worldwide invasive (Paupy *et al.*, 2009) that was introduced to North America in the early 1980s (Sprenger & Wuithiranyagool, 1986). It is found predominantly in rural and suburban locations, and in vegetated urban areas (Hawley, 1988; Braks *et al.*, 2003; Lopes *et al.*, 2004). It vectors a number of important viruses, including Zika virus (Grard *et al.*, 2014), dengue virus (Hawley, 1988), chikungunya virus (Paupy *et al.*, 2009), and La Crosse virus (Grimstad *et al.*, 1989; Gerhardt *et al.*, 2001; Lambert *et al.*, 2010). *Culex quinquefasciatus* is established in the southern United States, having been introduced from Africa by ship sometime prior to the 19th century (Urbanelli *et al.*, 1985; Vinogradova, 2000). It is predominantly an urban species (Subra, 1981; Lopes *et al.*, 2004), and it vectors the viruses important for West Nile (Sardelis *et al.*, 2001; Molaei *et al.*, 2007) and St. Louis encephalitis (Hardy *et al.*, 1984; Savage *et al.*, 1993).

Although both species are common inhabitants of tires, they differ in their oviposition strategies and degree of habitat specialization. *Aedes albopictus* is a container specialist, primarily utilizing artificial (e.g., tires) and natural (e.g., tree holes) containers for larval development (Hawley, 1988). *Culex quinquefasciatus* is a habitat generalist and breeds in a variety of water types (e.g., ditches, storm drains, ponds, septic tanks) in addition to containers (Barr, 1965; Subra, 1981; Chambers *et al.*, 1986). *Aedes albopictus* females deposit desiccation-resistant eggs singly on container walls above the water line, and the eggs hatch when flooded (Hawley, 1988), whereas *Cx. quinquefasciatus* females lay their eggs on the water surface in floating clusters called rafts, and the eggs hatch in approximately 24 hrs (Subra, 1981). *Aedes albopictus* is capable of skip oviposition (i.e., distributing a single batch of eggs over multiple sites) (Clements, 1999), but *Cx. quinquefasciatus* is not known to exhibit this behavior. Both species show selectivity for

certain organic infusions (e.g., Allan *et al.*, 2005; Obenauer *et al.*, 2009), but *Cx. quinquefasciatus* seems to be more selective in its oviposition decisions than *Ae. albopictus* (Burkett-Cadena & Mullen, 2007, 2008; McPhatter & Debboun, 2009), and *Ae. albopictus* is apparently less influenced by olfactory cues (Trexler *et al.*, 1998). Despite the higher selectivity of *Cx. quinquefasciatus*, existing preference-performance studies for this species suggest that its oviposition decisions are suboptimal (Mian & Mulla, 1986; Roberts, 1996), whereas *Ae. albopictus* shows preference for conditions that support optimal offspring performance (Reiskind *et al.*, 2009; Yoshioka *et al.*, 2012). However, these studies evaluated preference and performance in response to different factors (salinity and presence of sewage effluent for *Cx. quinquefasciatus*; resource type and conspecific density for *Ae. albopictus*). No studies to date have tested preference-performance relationships for these species under the same types of variables.

Although both species are known to oviposit in water containing organic detritus (Allan & Kline, 1995; Allan *et al.*, 2005), *Cx. quinquefasciatus* is particularly attracted to malodorous infusions containing labile detritus (i.e., grass) or excreta (e.g., Isoe *et al.*, 1995; Mboera *et al.*, 1999), and its pollution-tolerant larvae are often found in water containing high levels of organic nutrients (Barr, 1965; Subra, 1981; Clements, 2000). *Aedes albopictus* appears less able to tolerate highly organic conditions (Murrell & Juliano, 2008; Allgood & Yee, 2014), and this species is not known to be associated with eutrophied water. Millar et al. (1992) identified skatole (3-methylindole), *p*-cresol (4-methylphenol), indole, phenol, and 4-ethylphenol as important chemical constituents of fermenting Bermuda grass (*Cynadon dactylon* L.) infusions, which have been found to be attractive to gravid *Cx. quinquefasciatus* in field surveys (e.g., Allan *et al.*, 2005; Burkett-Cadena & Mullen, 2007). A blend of these five chemicals elicited a greater oviposition response than clean water from *Cx. quinquefasciatus* in the lab (Millar *et al.*, 1992) and in the field (Beehler *et al.*, 1994). The five-chemical blend and its individual constituents do not affect oviposition responses of *Ae. albopictus* to the same degree as *Cx. quinquefasciatus* in the field (Allan & Kline, 1995). In a follow-up study to Millar et al. (1992), Du and Millar (1999) isolated 10 chemicals from the headspace odors above fermenting Bermuda grass; the 10 chemicals consisted of the five chemicals previously isolated by Millar et al. (1992), in addition to nonanal, 2-undecanone, 2-tridecanone, naphthalene, and dimethyl trisulfide. Nonanal and skatole were the most attractive to ovipositing *Cx. quinquefasciatus* when the chemicals were tested individually, but the blend of 10 chemicals elicited a significantly greater oviposition response than any of its individual constituents (Du & Millar, 1999). Although the 10-chemical blend has been used to test oviposition responses of *Cx. quinquefasciatus* under laboratory conditions, its effects on larval mosquito performance have not been investigated, nor have its effects on oviposition responses of *Cx. quinquefasciatus* or *Ae. albopictus* under field conditions.

In the present study, we evaluated oviposition preference and larval survival of *Ae. albopictus* and *Cx. quinquefasciatus* in response to two different concentrations of the ten-chemical blend under field conditions. Du and Millar (1999) tested several concentrations of the blend and found that the low concentration (Table 1) was most attractive to gravid *Cx. quinquefasciatus* under laboratory conditions, whereas the high concentration (100x the low concentration) was repellent; therefore, we were interested to see if concentrations known to produce positive and negative responses in gravid females would have corresponding effects

on offspring survival. We hypothesized that different concentrations of the chemical blend would affect oviposition responses of *Ae. albopictus* and *Cx. quinquefasciatus* under field conditions, and that there would be associations between the oviposition preferences of these species and the survival of their larvae in these chemicals. We predicted that, 1) *Cx. quinquefasciatus* would oviposit most often in water treated with the lower concentration chemical blend, as Du and Millar (1999) found this to be the most attractive concentration in laboratory oviposition bioassays; 2) *Ae. albopictus* would avoid the chemical blend and oviposit in clean water controls, as this species is not known to occur in polluted (i.e., highly organic) water; and 3) oviposition preference would correspond to larval survival for *Ae. albopictus*, but not for *Cx. quinquefasciatus*, as has been the trend in the few existing studies on these species. More specifically, we expected that *Ae. albopictus* survivorship would be negatively affected by the chemical blend, whereas *Cx. quinquefasciatus* would be unaffected, given that *Cx. quinquefasciatus* seems better able to tolerate highly organic conditions (Allgood & Yee, 2014).

Additionally, we investigated the effects of long-term laboratory colonization on *Cx. quinquefasciatus* oviposition behavior by testing predictions 1 and 3 on both field and laboratory strains of *Cx. quinquefasciatus*. Controlled oviposition bioassays involving this species often use laboratory strains to generate gravid females (e.g., Kramer & Mulla, 1979; Isoe *et al.*, 1995; Allan *et al.*, 2005), as wild strains are difficult to blood feed in captivity and may not feed in sufficient numbers to generate enough gravid females for experiments. However, the process of adapting mosquitoes to laboratory rearing over many generations may select for behaviors that differ from those of wild populations. Therefore, we hypothesized that data collected from wild and laboratory strains of *Cx. quinquefasciatus* would lead to different conclusions in our preference-performance study. Based on our experience rearing this species, we predicted that the lab strain would show less discrimination among oviposition substrates, as lab strains will readily oviposit in clean water, whereas wild strains will only oviposit in water containing organic substrates (D.W. Allgood, personal observation).

## Methods

### Mosquito Rearing

Colonies used to generate mosquitoes for experiments were established from *Ae. albopictus* and *Cx. quinquefasciatus* eggs and larvae collected from aquatic habitats in and around Hattiesburg, MS. Field-collected larvae were identified using keys by Darsie and Ward (2005) and reared to adults in the laboratory. A laboratory-acclimated strain of *Cx. quinquefasciatus* from Gainesville, FL that has been in colony since 1995 was provided by the USDA/ARS Center for Medical, Agricultural and Veterinary Entomology in Gainesville, FL. A colony of the Gainesville *Cx. quinquefasciatus* strain was established at USM in July 2010 and maintained using the methods described below; previous generations were maintained using the methods described in Allan *et al.* (2006). Larvae of the two species were reared to adults on Purina® Puppy Chow® and brewers yeast (Acros Organics, Morris Plains, NJ, USA). Adults were maintained in a colony room kept at approximately 27 °C on a 14:10 hour light:dark cycle with one hour of dawn and one hour of twilight and were

provided with a cotton pad soaked with 10 % sugar solution. Anesthetized guinea pigs were used to blood feed *Ae. albopictus* and the Gainesville laboratory strain of *Cx. quinquefasciatus* (IACUC #A3851-01, 14 Aug 2009), and the arm of the experimenter was used to blood feed the Hattiesburg field strain of *Cx. quinquefasciatus. Aedes albopictus* were provided black cups lined with paper towels and filled to 2.5 cm with reverse osmosis (RO) water for oviposition, and *Cx. quinquefasciatus* were provided black bowls filled to 2.5 cm with larval rearing water (Hattiesburg field strain) or fresh (tap or RO) water (Gainesville laboratory strain). Eggs were used to establish new colonies. Mosquito colonies were continually maintained and stocked using these methods.

Although oviposition preferences appear to be learned with each generation based on larval rearing conditions rather than inherited (McCall & Eaton, 2001), we attempted to standardize any possible differences in short-term conditioning by rearing Hattiesburg-collected *Ae. albopictus* and *Cx. quinquefasciatus* in the laboratory for two generations prior to oviposition bioassays. Additionally, eggs of the Gainesville laboratory strain of *Cx. quinquefasciatus* were collected in larval rearing water for two generations prior to oviposition bioassays for consistency with the Hattiesburg field strain (the latter would not oviposit in fresh water). Both the Hattiesburg field strain of *Cx. quinquefasciatus* (hereafter, wild *Cx. quinquefasciatus*) and the Gainesville laboratory strain (hereafter, lab *Cx. quinquefasciatus*) were used in experiments to test for effects of laboratory acclimation on oviposition response and preference-performance relationships.

### Chemical Blend

The blend of 10 chemical compounds (Du & Millar, 1999) was prepared by dissolving chemicals in diethyl ether to make stock solutions that produced either a low or high concentration of the chemical blend (Table 1) when added to water in a 1:1000 (stock solution:water) ratio (Du & Millar, 1999). Amounts of compounds in the low treatment reflect concentrations in headspace extracts above infusions containing 4.5 g/L Bermuda grass fermented with 0.27 g/L lactalbumin hydrolyzate and brewers yeast for nine days (Du & Millar, 1999).

### Oviposition Experiment

Female *Ae. albopictus* and *Cx. quinquefasciatus* were blood fed 2–8 d and 5–12 d, respectively, after reaching adulthood. Mosquitoes were removed from colony cages via aspiration after blood feeding and knocked out with $CO_2$, and blood-engorged females were separated from other mosquitoes and transferred to separate colony cages. Blood-fed females of *Ae. albopictus* and *Cx. quinquefasciatus* were held for 3 and 7 d, respectively, after which time they were presumed gravid. At this time, gravid females were knocked out with $CO_2$ and counted, transferred to 40 mL vials stopped with cotton, and introduced to field bioassay cages within 2 h.

Field bioassays were conducted beneath a wood-framed structure with a shade-cloth ceiling (~ 3 m high) and a concrete floor (hereafter, pad) at the USM Science Park in Hattiesburg, MS (31°21′11.9″N, 89°21′35.0″W). The area immediately surrounding the pad was a grassy lawn, leaving no vegetative canopy above the pad. Experimental tires (passenger car

or light truck tires with radial construction and a wheel diameter of 16 in) were placed 24 h prior to the introduction of gravid females. Before each run of the experiment, tire interiors were treated with 10% bleach solution, scrubbed with a scour pad, thoroughly rinsed with tap water, and allowed to dry for 24 h before water and chemicals were added. Chemical concentrations and mosquito species were randomly assigned for each tire and tire pair, respectively, for each run of the experiment. Each tire received 3.5 L RO water and 3.5 mL of appropriately concentrated stock solution; control tires received 3.5 mL of pure diethyl ether. The inner surfaces of tires receiving *Ae. albopictus* eggs were lined with brown paper towels, as *Aedes* mosquitoes oviposit on container walls just above the water surface (Hawley, 1988), whereas *Culex* mosquitoes oviposit directly on the water surface (Subra, 1981). Each experimental unit consisted of a pair of tires containing differing chemical concentrations (control and low concentration, control and high concentration, or low and high concentration) covered with a cage made from plastic PVC piping ($1.5 \times 0.8 \times 0.8$ m), mosquito netting, and clear plastic covering on top to prevent inputs of organic detritus, rainwater, intrusion by other animals, and escape of adult female mosquitoes. Forty-eight tires were used, yielding 24 experimental units for each run of the experiment. The arrangement of treatments within each cage was randomized. Because three different species/strains (*Ae. albopictus*, wild *Cx. quinquefasciatus*, lab *Cx. quinquefasciatus*; hereafter, strains) were used across three different pairwise chemical concentration pairings (hereafter, combinations), we were unable to divide experimental units evenly among strains within a single run of the experiment while maintaining a balanced design with respect to combinations. Therefore, we divided the 24 experimental units among the three strains in a 6-6-12 arrangement and ran the experiment three times, with each strain receiving 12 experimental units in one run of the experiment, and six units in the other two runs. Each combination was replicated two times per strain when six experimental units were used, and four times per strain when 12 units were used. This produced eight replicates per combination per strain after three runs of the experiment.

Ten gravid female *Ae. albopictus* or 20 gravid *Cx. quinquefasciatus* were released into each cage at the center of the west facing side of the cage. We used a higher number of *Cx. quinquefasciatus* in order to increase the number of observations per replicate, as a single egg raft (attributable to one female) was considered one observation. Individual eggs were considered independent observations for *Ae. albopictus*, as this species lays eggs singly and exhibits skip oviposition (unlike *Cx. quinquefasciatus*). Only one strain was released into each cage. *Aedes albopictus* was released into cages at 0800 h, and *Cx. quinquefasciatus* was released into cages at 1700 h on the same day. Mosquitoes were released at different times so as to allow both species time to acclimate to their surroundings prior to their peak oviposition times (afternoon for *Ae. albopictus* (Trexler *et al.*, 1997), dusk for *Cx. quinquefasciatus* (Beehler *et al.*, 1993; Mboera *et al.*, 2000)). Eggs were collected at 0800 h the next morning. For *Cx. quinquefasciatus*, we quantified the number of egg rafts laid in each tire, whereas for *Ae. albopictus* we quantified individual eggs laid in each tire.

### Survival Experiment

After eggs were collected from all tires, hand pumps were used to collect a 500 mL aliquot of water from each tire; pumps were moved around the circumference of the tire while

removing water to mix the water and obtain a representative water sample. Water samples were stored in airtight bottles at room temperature until needed.

Eggs and tire water were taken back to the lab, at which time *Ae. albopictus* eggs were counted. Egg rafts of *Cx. quinquefasciatus* were transferred to plastic cups containing RO water, and newly hatched larvae were added to cups containing tire water the next day; *Ae. albopictus* eggs were stored in an incubator (Percival Scientific, Inc., Perry, IA, USA) at 24 °C and ~85 % relative humidity for four d after oviposition to allow ample time for egg counting and embryogenesis, and the eggs were then placed in a solution of 0.33 g Nutrient Broth (Difco™, BD, Sparks, MD, USA) per 750 mL deionized water for hatching.

One hundred mL of water from each tire was added to a 100 mL plastic cup. For consistency, only larvae from eggs deposited in the preferred tire from each replicate were used, as some tires received 100 percent of the eggs deposited. Within 24 h of hatching, 10 larvae were introduced to both cups corresponding to the respective tire pairing from which the larvae originated, and the cups were placed in an incubator set to 27 °C on a 14:10 h day:night cycle (approximate photoperiod [www.fcc.gov] and mean temperature [www.weather.com] for June-August in Hattiesburg, MS). Larvae were fed ground Purina® Puppy Chow® and brewers yeast (Acros Organics, Morris Plains, NJ, USA) on an eight-day schedule using amounts of food per larva given in Gerberg *et al.* (1994). Water levels within cups were maintained at 100 mL on a daily basis using RO water. Pupae were removed each day, transferred to glass shell vials, and stored in an incubator with the same settings described for larval rearing. The experiment ended 45 d after larvae were introduced to cups, and any larvae that had not pupated were considered mortalities. Individuals surviving to adulthood where quantified for each cup. Three runs of the oviposition bioassay and subsequent survival experiment were conducted in May-August 2011; gravid females were released into enclosures on May 31, June 14, and June 29.

### Analyses

To compare oviposition responses of each strain among chemical concentrations, treatment pairings for each species were analyzed separately using analysis of variance (ANOVA) in JMP® Version 8 (SAS Institute Inc., Cary, NC, 2010) to test for effects of chemical concentration on number of eggs (*Ae. albopictus*) or egg rafts (*Cx. quinquefasciatus*) allocated to each treatment. To assess the effects of chemical concentration on larval survival, we used ANOVA in SAS/STAT 9.1 (SAS Institute Inc, 2004) to test for effects of chemical concentration on survivorship (i.e., the number of larvae surviving to adulthood) of each strain. For all oviposition and survivorship analyses, blocks for experimental run and pair (i.e., replicate, nested within run) were included to account for variation due to time and paired samples, respectively. No transformations were used for the oviposition data, or for *Cx. quinquefasciatus* (wild and lab) survivorship data, as raw data met assumptions of normality and homogeneity of variances. *Aedes albopictus* survivorship data was arcsine square root transformed (arcsin(sqrt(x))) to meet assumptions.

To examine associations of oviposition response with larval survival, we calculated an oviposition preference index ($O$) and a larval survival index ($S$) for each experimental unit.

The term *preference* is used to refer to oviposition response, although responses may be involuntary (i.e., due to stimulatory effects of the chemicals). Preference was calculated as,

$$O = \frac{O_H - O_L}{O_H + O_L}$$

where $O_H$ is the number of eggs or egg rafts deposited in the preferred tire (i.e., the tire that received more eggs/rafts), and $O_L$ is the number eggs or egg rafts deposited in the non-preferred tire. Values of $O$ can range from 0 to 1, with 0 indicating no preference between tires, and 1 indicating complete preference for one tire over the other. An index measuring relative survival in the preferred oviposition site was calculated as,

$$S = \frac{S_{OH} - S_{OL}}{S_{OH} + S_{OL}}$$

where $S_{OH}$ is the number of larvae that survived to adulthood in water from the preferred oviposition tire, and $S_{OL}$ is the number of larvae that survived in water from the non-preferred tire. Values of $S$ can range from –1 to 1, with positive values indicating that the preferred habitat had higher survivorship, negative values indicating that the non-preferred habitat had higher survivorship, and 0 values indicating that both habitats had equal survivorship. $S$ does not measure the overall suitability of a habitat; rather, it is a measurement of the degree to which one habitat is more suitable relative to the other.

Separate statistical analyses were conducted for each strain. Values of $O$ and $S$ were analyzed using analysis of covariance (ANCOVA) in JMP® Version 8 (SAS Institute Inc., Cary, NC, 2010), with chemical concentration pairing as a factor, $O$ as a covariate, and $S$ as the response variable. Data were pooled across runs, as preliminary ANCOVA indicated that slopes and intercepts of the regression lines for each run did not differ for any strain.

## Results

### Oviposition Experiment

Oviposition responses of both *Ae. albopictus* and wild *Cx. quinquefasciatus* significantly differed between at least one chemical concentration and water controls, and no effects of run or cage were found for any strain (Table 2). *Aedes albopictus* laid significantly more eggs in the high concentration than in the control, but oviposition responses did not differ significantly in other concentration pairings (Table 3). Wild *Cx. quinquefasciatus* deposited significantly higher numbers of egg rafts in both chemical concentrations than in water controls, but the number of egg rafts did not differ between low and high concentrations (Table 3). In contrast, the lab strain deposited a significantly higher number of egg rafts in the high concentration than in the low concentration, but differences between either chemical concentration and water controls were not significant (Table 3).

## Survival Experiment

Analyses of survivorship revealed random effects of run for *Ae. albopictus*, and random effects of pair (i.e., replicate nested within run) for lab and wild *Cx. quinquefasciatus*, but chemical concentration did not affect survivorship for any strains (Table 4). For all strains, no significant relationship was found between oviposition preference and relative survivability of the larval habitat (Table 5).

## Discussion

Results of the oviposition experiment supported our hypothesis that the chemical blend would affect oviposition responses of both species under field conditions, but our predictions that *Ae. albopictus* would avoid the chemical blend and that *Cx. quinquefasciatus* would show the strongest preference for the low concentration were not supported. Wild *Cx. quinquefasciatus* laid more eggs in both concentrations than in water controls (Table 3), and *Aedes albopictus* laid significantly more eggs in the high concentration compared to water controls (Table 3). *Aedes albopictus* did not discriminate between water controls and low concentration, or between low and high concentration, suggesting that lower magnitudes of difference between concentrations of these chemicals do not lead to differential oviposition responses. *Aedes albopictus* may be less selective in response to oviposition cues because it is able to "hedge" offspring success by distributing a single batch of eggs over multiple sites with different conditions. Conversely, *Cx. quinquefasciatus* may be more selective because it deposits its entire batch of eggs in one location and therefore faces greater selective pressure to oviposit in a suitable habitat for its offspring.

Comparisons of lab and wild strains of *Cx. quinquefasciatus* supported our hypothesis that oviposition preferences would be inconsistent between strains. The lab strain did not show significantly higher preference for the chemical blend over fresh water, while the wild strain preferred to oviposit in water containing the chemical blend (Table 3). This demonstrates that the use of laboratory acclimated mosquito strains in oviposition bioassays may lead to different conclusions than would be drawn from wild strains. Therefore, when it is impractical to use wild mosquitoes in controlled oviposition bioassays, it is advisable to corroborate the results with field surveys (e.g., Beehler *et al.*, 1994; Allan *et al.*, 2005) before assuming that the observed effects (or lack thereof) are applicable to wild populations.

Our hypothesis that oviposition preferences would correspond to larval survival in the chemical blend was not supported, as we found no association between oviposition preference and larval survival for any strain. Previous studies have shown that high concentrations of labile detritus can be detrimental to *Ae. albopictus* larvae (Murrell & Juliano, 2008; Allgood & Yee, 2014), suggesting that toxicity of detrital chemicals plays a role in larval performance for this species. We predicted that the chemical blend would detrimentally affect *Ae. albopictus* survivorship, but the chemical concentrations we used were insufficient to affect larval survival (Table 4). Our concentrations were based on the amount of each chemical present in headspace extracts above water containing fermenting Bermuda grass (Du & Millar, 1999) and may not have reflected the concentrations present in the water itself. Therefore, the chemical blend may affect preference-performance

relationships at higher concentrations than those used in our study. Additionally, there may have been some chemical evaporation from the tires during the field portion of our study, in which case the chemicals would have been less than their intended concentrations when brought back to the lab for the survival experiment. However, we also found no effects of the chemical blend on survival when we tested the same concentrations on our study species entirely under laboratory conditions (Allgood & Yee, 2014). It is also possible that the detrimental effects of copious detritus on *Ae. albopictus* noted in previous studies were attributable to non-chemical factors (e.g., scum formation or alterations to the microbial food environment) or chemicals not present in the blend.

Even though our chemical concentrations did not affect larval survival, not all treatments received equal oviposition responses. If oviposition preferences of these species reflect larval habitat quality, then the preference of both species for the chemical blend may reflect perceived resource availability rather than toxicity. Tires in our study were not supplemented with resources, but the presence of these chemicals in the wild is associated with the presence of decomposing organic matter harboring microorganisms, the primary food source of larval mosquitoes (Merritt *et al.*, 1992). Mosquito density in tires is positively associated with resource abundance (Kling *et al.*, 2007), which is consistent with our finding that both species generally preferred to oviposit in higher chemical concentrations. The performance (i.e., survivorship, development time, adult mass) of both species is negatively affected by intra- and interspecific competition when resources are limited (Agnew *et al.*, 2000; Costanzo *et al.*, 2005; Allgood & Yee, 2014), and these negative effects can be reduced or nullified by higher resource levels, or by types of resources that promote higher bacterial productivity (e.g., grasses, insect carcasses) (Carrieri *et al.*, 2003; Murrell & Juliano, 2008; Costanzo *et al.*, 2011). *Culex* spp. tend to be more detrimentally affected by limited resources than *Ae. albopictus* (Costanzo *et al.*, 2005; Costanzo *et al.*, 2011; Allgood & Yee, 2014), which may partially explain why *Cx. quinquefasciatus* exhibited a higher selectivity for chemical concentrations indicative of greater bacterial productivity. Future studies involving simultaneous comparisons of oviposition preference and offspring performance in differing resource environments rather than synthetic chemicals would likely produce more informative results.

Although direct comparisons of mosquito preference and performance are limited, oviposition studies using differing resource environments are consistent with the results of our oviposition experiment. *Culex quinquefasciatus* prefers secondary sewage effluent over distilled water (Mian & Mulla, 1986), likely due to increased bacterial productivity, and *Culiseta longiareolata* (Macquart) (which also deposits egg rafts) oviposition is positively associated with resource levels (Blaustein & Kotler, 1993). *Aedes albopictus* also shows some preference for resource types and amounts that favor offspring success (Reiskind *et al.*, 2009; Wasserberg *et al.*, 2013), but its preferences for optimal resource conditions appear to interact with conspecific egg density. Yoshioka *et al.* (2012) found that oviposition was driven by an interaction between resource level and conspecific egg density, with oviposition more strongly affected by egg density (larval survival was more strongly affected by resource level); females with large egg batches oviposited in containers with higher egg densities, and females with small egg batches preferred low densities. Wasserberg *et al.* (2013) observed that *Ae. albopictus* oviposition had a positive association with resource

level and a negative association with conspecific egg density, and that this species still utilized low quality containers (e.g., low resources, high predation risk), albeit to a lesser degree than higher quality containers. This suggests that *Ae. albopictus* oviposition patterns follow an ideal-free distribution (i.e., the population is distributed such that resource availability with respect to density is balanced in all habitats) (Fretwell & Lucas, 1969). As a container specialist, *Ae. albopictus* likely faces greater selective pressure to optimize offspring performance in small bodies of water by distributing eggs in this manner. This is consistent with our finding that *Ae. albopictus* oviposited in both the control and high concentration tires despite exhibiting a preference for the high concentration. In contrast, *Cx. quinquefasciatus* strongly preferred any concentration of the chemical blend over the control. Ovipositing *Cx. quinquefasciatus* females tend to aggregate at oviposition sites, as conspecific egg rafts contain an aggregation pheromone that acts additively or synergistically with other attractive or stimulatory oviposition cues (Mordue *et al.*, 1992; Blackwell *et al.*, 1993; Mboera *et al.*, 1999). Therefore, this species does not appear to distribute eggs in an ideal-free manner. *Culex quinquefasciatus* is a habitat generalist that also utilizes larger bodies of water (e.g., ditches, septic tanks) (Barr, 1965; Subra, 1981; Chambers *et al.*, 1986). Given that *Cx. quinquefasciatus* larvae compete intraspecifically for space (Smith *et al.*, 1995) as well as resources, surface area and water volume likely interact with resource availability to affect preference-performance relationships. A statewide tire survey in Mississippi revealed that larval *Cx. quinquefasciatus* were positively associated with water volume and microorganism abundance (Yee *et al.*, 2015), and laboratory oviposition experiments show that it prefers to oviposit in the largest available container (Daniels *et al.*, 2015). Therefore, *Cx. quinquefasciatus* preference-performance relationships may be optimal for large, nutrient-rich bodies of water, but not for small containers. Although this species is cosmopolitan in tires, its occurrence in containers may be incidental in the context of the larger population. Tire surveys have revealed similarities in abundances of different life stages for *Aedes* spp., but not *Culex* spp., across environmental factors in tires (Yee *et al.*, 2010; Yee *et al.*, 2015). The presence of early instars is an indirect indicator of oviposition preference, whereas the presence of late instars indicates habitat suitability; therefore, these surveys support the notion that oviposition strategies of container-dwelling *Aedes* spp. are consistent with offspring survival, while *Culex* spp. show a disconnect between oviposition preference and offspring survival in tires. This was not reflected in our survival experiment, as we did not investigate the effects of container size and the resulting larval densities from the numbers of eggs deposited in each tire. Because relationships between oviposition preferences and offspring performance may be density dependent (Ellis, 2008), future work could examine the effects of conspecific egg and larval densities, and how they interact with other habitat variables to influence mosquito preference-performance relationships.

Although our direct quantitative comparison of oviposition preference and larval performance yielded unclear results, our comparison of oviposition behaviors of wild and laboratory strains of *Cx. quinquefasciatus* offer some support for the PPH. The lab strain of *Cx. quinquefasciatus* is selected for laying eggs in clean water, which has apparently diluted over time its selectiveness for organic substrates. In the wild, offspring deposited in clean water would likely starve to death due to lack of nourishment (i.e., detritus and

microorganisms). However, laboratory-reared offspring have an equal chance of survival regardless of oviposition substrate, as all eggs are transferred into larval rearing trays with ample nutrients. Thus, oviposition preference for organic substrates over clean water likely optimizes offspring performance with regard to resource availability in the wild, but not in the laboratory. The differences in oviposition preference between the wild and laboratory strains in our study suggest that optimal oviposition behavior with regard to cues indicating resource availability is selected for in wild *Cx. quinquefasciatus* populations. Furthermore, the laboratory strain indicates that this species is able to adapt its oviposition behavior to facilitate offspring production under novel conditions, as decreased selectivity may be required to produce offspring when only one oviposition substrate (e.g., clean water) is provided.

In summary, this work demonstrates that the blend of 10 chemicals identified by Du and Millar (1999) is effective at eliciting increased oviposition responses from *Ae. albopictus* and *Cx. quinquefasciatus* in tires under field conditions, and that oviposition behaviors may differ between wild and laboratory mosquito strains. We did not find a clear relationship between oviposition preferences and larval survival with regard to the chemical blend, as the blend affected oviposition responses but not larval survival. Our results suggest that both species oviposit in optimal resource environments for their offspring, but further work testing oviposition and offspring survival in detrital environments (as opposed to synthetic chemicals) is needed to test this hypothesis. Future studies are also needed for these and other mosquito taxa to determine how resource conditions interact with other variables such as density, predation risk, and abiotic factors (e.g., temperature, water volume, chemical environment) to affect preference-performance relationships and mosquito distribution.

## Acknowledgments

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**Table 1**

Concentration of each chemical present in low and high concentration treatments.

| Chemical | Low | High |
|---|---|---|
| Dimethyl trisulfide | 576 ng/L | 57.6 µg/L |
| Phenol | 29 ng/L | 2.9 µg/L |
| p-Cresol | 980 ng/L | 98.0 µg/L |
| Nonanal | 39 ng/L | 3.9 µg/L |
| 4-Ethylphenol | 5 ng/L | 0.5 µg/L |
| Naphthalene | 25 ng/L | 2.5 µg/L |
| Indole | 52 ng/L | 5.2 µg/L |
| 2-Undecanone | 22 ng/L | 2.2 µg/L |
| 3-Methylindole | 804 ng/L | 80.4 µg/L |
| 2-Tridecanone | 15 ng/L | 1.5 µg/L |

Concentrations are based on those used by Du and Millar (1999).

**Table 2**

Results of ANOVA within each chemical concentration combination on number of *Ae. albopictus* eggs and *Cx. quinquefasciatus* (wild and lab) egg rafts deposited in each chemical concentration. Significant effects are shown in bold.

| Effect Tests | Control vs. Low | | | Control vs. High | | | Low vs. High | | |
|---|---|---|---|---|---|---|---|---|---|
| | df | F | P | df | F | P | df | F | P |
| *Ae. albopictus* | | | | | | | | | |
| Run | 2,5 | 2.8109 | 0.1520 | 2,5 | 1.9162 | 0.2411 | 2,5 | 0.5975 | 0.5852 |
| Pair | 5,7 | 1.2083 | 0.3947 | 5,7 | 2.8972 | 0.0990 | 5,7 | 0.3603 | 0.8606 |
| Concentration | 1,7 | 0.4119 | 0.5415 | 1,7 | 16.6590 | **0.0047** | 1,7 | 0.2264 | 0.6487 |
| *Cx. quinquefasciatus* (wild) | | | | | | | | | |
| Run | 2,5 | 0.4325 | 0.6711 | 2,5 | 1.8243 | 0.2541 | 2,5 | 3.3235 | 0.1207 |
| Pair | 5,7 | 1.1850 | 0.4035 | 5,7 | 0.1121 | 0.9858 | 5,7 | 0.2324 | 0.9363 |
| Concentration | 1,7 | 7.2000 | **0.0314** | 1,7 | 6.8182 | **0.0349** | 1,7 | 0.1094 | 0.7505 |
| *Cx. quinquefasciatus* (lab) | | | | | | | | | |
| Run | 2,5 | 1.0067 | 0.4292 | 2,5 | 0.2531 | 0.7858 | 2,5 | 0.5898 | 0.5889 |
| Pair | 5,7 | 0.2562 | 0.9235 | 5,7 | 0.0986 | 0.9893 | 5,7 | 0.1855 | 0.9590 |
| Concentration | 1,7 | 0.8826 | 0.3788 | 1,7 | 2.5409 | 0.1550 | 1,7 | 9.1755 | **0.0191** |

[*]
For all analyses, run and pair are included as random effects; pair is nested within run.

**Table 3**

Least-squared mean (± 1 SE) number of *Ae. albopictus* eggs or *Cx. quinquefasciatus* egg rafts deposited in each chemical concentration within each chemical concentration combination. Bold pairs are significantly different (determined by ANOVA).

|  | *Ae. albopictus* | *Cx. quinquefasciatus* (wild) | *Cx. quinquefasciatus* (lab) |
|---|---|---|---|
|  | Mean ± SE | Mean ± SE | Mean ± SE |
| Control | 108.4 ± 17.1 | **1.7 ± 0.8** | 6.0 ± 1.8 |
| Low | 123.5 ± 17.1 | **4.7 ± 0.8** | 8.4 ± 1.8 |
| Control | **114.3 ± 17.3** | 2.1 ± 1.0 | 3.5 ± 2.3 |
| High | **211.7 ± 17.3** | 5.8 ± 1.0 | 8.6 ± 2.3 |
| Low | 209.4 ± 50.6 | 3.7 ± 1.1 | **3.5 ± 1.6** |
| High | 176.2 ± 50.6 | 4.2 ± 1.1 | **10.1 ± 1.6** |

**Table 4**

Results of ANOVA on survivorship in each chemical concentration for each mosquito strain. Significant effects are shown in bold.

| | df | F | P |
|---|---|---|---|
| *Ae. albopictus* | | | |
| Run | 2,17 | 40.84 | **<0.0001** |
| Pair | 17,18 | 0.87 | 0.6068 |
| Concentration | 2,18 | 0.25 | 0.7792 |
| *Cx. quinquefasciatus* (wild) | | | |
| Run | 2,16 | 0.14 | 0.8683 |
| Pair | 16,17 | 3.39 | **0.0085** |
| Concentration | 2,17 | 0.26 | 0.7762 |
| *Cx. quinquefasciatus* (lab) | | | |
| Run | 2,18 | 0.09 | 0.9158 |
| Pair | 18,19 | 3.06 | **0.0099** |
| Concentration | 2,19 | 2.11 | 0.1483 |

*
For all analyses, run and pair are included as random effects; pair is nested within run.

**Table 5**

Results of ANCOVA on relative habitat suitability for each mosquito strain with oviposition preference as a covariate and chemical concentration combination as factor.

|  | df | F | P |
|---|---|---|---|
| *Ae. albopictus* | | | |
| Preference | 1,13 | 0.6561 | 0.4325 |
| Combination | 2,13 | 0.2684 | 0.7687 |
| Preference x combination | 2,13 | 3.0894 | 0.0799 |
| *Cx. quinquefasciatus* (wild) | | | |
| Preference | 1,12 | 1.4690 | 0.2488 |
| Combination | 2,12 | 0.1514 | 0.8611 |
| Preference x combination | 2,12 | 0.3153 | 0.7354 |
| *Cx. quinquefasciatus* (lab) | | | |
| Preference | 1,15 | 0.2839 | 0.6020 |
| Combination | 2,15 | 0.0605 | 0.9415 |
| Preference x combination | 2,15 | 0.1961 | 0.8240 |