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Influence of Resource Levels, Organic Compounds and Laboratory Colonization On Interspecific Competition Between the Asian Tiger Mosquito *Aedes albopictus* (*Stegomyia albopicta*) and the Southern House Mosquito *Culex quinquefasciatus*

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Influence of resource levels, organic compounds, and laboratory colonization on interspecific competition between the Asian tiger mosquito (*Aedes albopictus*) and the southern house mosquito (*Culex quinquefasciatus*)

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Abstract

The mosquitoes *Aedes albopictus* (Skuse) and *Culex quinquefasciatus* (Say) (Diptera:Culicidae) are common inhabitants of tyres and other artificial containers, which constitute important peridomestic mosquito breeding habitats. We tested the hypotheses that interspecific resource competition between the larvae of these species is asymmetrical, that the concentration of chemicals associated with decomposing detritus affects their competitive outcome, and that wild and colonized strains of *Cx. quinquefasciatus* are affected differently by competition with *Ae. albopictus*. We conducted two laboratory competition experiments wherein we measured survivorship and estimated population growth (λ') of both species under multiple mixed-species densities. Under varying resource levels, competition was asymmetrical with *Ae. albopictus* causing competitive reductions or exclusions of *Cx. quinquefasciatus* under limited resources. In a second experiment, which used both wild and colonized strains of *Cx. quinquefasciatus*, organic chemical compounds associated with decomposing detritus did not affect the competitive outcome. The colonized strain of *Cx. quinquefasciatus* had greater survivorship, adult mass, and faster development times than the wild strain, but both strains were similarly affected by competition with *Ae. albopictus*. Competition between these species may have important consequences for vector population dynamics, especially in areas where tyres and artificial containers constitute the majority of mosquito breeding habitats.

Keywords

Aedes albopictus; *Culex quinquefasciatus*; competition; detritus; resources

Introduction

Abiotic (e.g., temperatures, chemical properties) and biotic (e.g., predation, competition) factors within an aquatic environment can affect an organism's survival and performance (Macan, 1961). Competition between species may occur in an environment where a shared

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resource exists in limited quantities (Tilman, 1982). When interspecific competition is asymmetrical, competitive exclusion (local extinction) or reduction of the weaker species are expected to occur (Lawton & Hassell, 1981; Connell, 1983; Lounibos, 2007). Competitive outcomes may be condition-specific, in which case the competitive advantage of a species is nullified or reversed under a different set of conditions (Dunson & Travis, 1991; Chesson, 2000). In larval mosquito communities, competition can affect larval survival and performance within an aquatic habitat and therefore influence which vector species will emerge as adults from that habitat (e.g., Smith *et al.*, 1995; Juliano, 1998; Costanzo *et al.*, 2011). Additionally, stress from competition in the larval stage can indirectly affect the susceptibility of adult mosquitoes to infection by diseases (Alto *et al.*, 2005; 2008a).

The Asian tiger mosquito (*Aedes albopictus*) is a worldwide invasive species that has become established on all continents except mainland Australia and Antarctica, primarily due to the international shipping of tyres and other artificial containers (Paupy *et al.*, 2009). This species invaded the eastern U.S. in the early1980s (Sprenger & Wuithiranyagool, 1986; Hawley, 1988) and has since become the most abundant species in tyres in the southeastern U.S. (Yee, 2008). *Aedes albopictus* is a competent vector of a number of arboviruses, including 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). The spread of *Ae. albopictus* has been associated with local extinctions or reductions of established species, most notably the yellow fever mosquito (*Aedes aegypti* L.) (O'Meara *et al.*, 1995; Braks *et al.*, 2003). Investigations of the mechanism of displacement revealed that *Ae. albopictus* is a superior resource competitor to *Ae. aegypti* (Juliano, 1998; Murrell & Juliano, 2008). Additionally, *Ae. albopictus* is a superior resource competitor to the eastern tree hole mosquito (*Ae. triseriatus* Say) (Yee *et al.*, 2007) and the northern house mosquito (*Culex pipiens* L.) (Carrieri *et al.*, 2003; Costanzo *et al.*, 2005b). The competitive superiority of *Ae. albopictus* over other species appears to be condition-specific. Nullifications or reductions of the competitive advantage of *Ae. albopictus* have been observed under dry conditions (Costanzo *et al.*, 2005a), in the presence of shared predators (Griswold & Lounibos, 2005b, 2006), and when more labile resources (i.e., grasses, invertebrate carcasses) are available (Yee *et al.*, 2007; Murrell & Juliano, 2008; Costanzo *et al.*, 2011).

The southern house mosquito (*Culex quinquefasciatus*) occurs worldwide in tropical and subtropical areas, and it is an established species in the southern U.S. (Vinogradova, 2000), where it is a vector of West Nile virus (Sardelis *et al.*, 2001; Goddard *et al.*, 2002; Molaei *et al.*, 2007) and St. Louis encephalitis (Hardy *et al.*, 1984; Savage *et al.*, 1993). *Culex quinquefasciatus* is predominantly an urban species (Subra, 1981; Lopes *et al.*, 2004), with its larvae found in a variety of anthropogenic habitats, including artificial containers, storm drains, drainage ditches, and septic tanks (Subra, 1981; Harbison *et al.*, 2009). *Culex quinquefasciatus* is one of the few pollution-tolerant mosquito species (Subra, 1981; Clements, 2000); larvae are usually found in water containing high concentrations of organic detritus, especially human and animal excreta (Barr, 1965; Subra, 1981). While *Cx. quinquefasciatus* is known to be common in these environments, there are no available

studies assessing the potential effects of organic conditions on the outcomes of interactions between *Cx. quinquefasciatus* and other species.

Artificial containers, especially tyres, constitute important peridomestic mosquito breeding habitats (Chambers *et al.*, 1986; Vezzani, 2007; Yee, 2008). Within their ranges, *Ae. albopictus* and *Cx. quinquefasciatus* are often the most abundant species of their respective genera found in these habitats (e.g., Chambers *et al.*, 1986; Sprenger & Wuithiranyagool, 1986; Lopes *et al.*, 2004). Despite this, virtually nothing is known about their interspecific interactions. In the southern U.S., *Cx. quinquefasciatus* has been found to be second in abundance to *Ae. albopictus* in tyres in both urban (Sprenger & Wuithiranyagool, 1986) and rural (Yee *et al.*, 2012) areas. Sprenger and Wuithiranyagool (1986) reported that *Ae. albopictus* and *Cx. quinquefasciatus* respectively comprised 53 and 22.7 % of larvae collected in a tyre survey in urban Harris County, TX, USA. Yee *et al.* (2012) found that *Ae. albopictus* and *Cx. quinquefasciatus* respectively comprised 73 and 13 % of larvae collected from tyres in rural Lamar and Perry Counties, MS, USA. In a tyre study in Brazil, larval *Cx. quinquefasciatus* was the predominate species in tyres in urban areas, but it became less abundant in rural areas, where *Ae. albopictus* predominated (Lopes *et al.*, 2004); the authors suggested that the observed pattern was due to competition, but this hypothesis has never been tested. The competitive superiority of *Ae. albopictus* to *Cx. pipiens* (Carrieri *et al.*, 2003; Costanzo *et al.*, 2005b), a species closely related to *Cx. quinquefasciatus* (Vinogradova, 2000), suggests that *Ae. albopictus* is likely superior to *Cx. quinquefasciatus*, but it cannot necessarily be assumed that ecological traits of *Cx. pipiens* apply to *Cx. quinquefasciatus*, as the ecologies of these two species have not been compared. If *Ae. albopictus* is indeed a superior resource competitor to *Cx. quinquefasciatus*, its competitive advantage may be condition-specific. Specifically, *Cx. quinquefasciatus* performs well in environments rich in organic matter due to its ability to tolerate pollution, but *Ae. albopictus* may be negatively affected by high concentrations of labile detritus (Murrell & Juliano, 2008). Therefore, excessive organic matter (or its associated chemicals) may serve to reduce or nullify the competitive advantage of *Ae. albopictus* by detrimentally affecting larval performance.

Our objectives were to test the hypotheses that 1) resource competition between *Ae. albopictus* and *Cx. quinquefasciatus* is asymmetrical, 2) the effects of interspecific competition on these species are condition-specific with regard to a ten-chemical blend (Du & Millar, 1999) associated with decomposing organic matter, and 3) wild and colonized strains of *Cx. quinquefasciatus* are affected differently by competition with *Ae. albopictus*. Laboratory experiments involving *Cx. quinquefasciatus* often use strains that have been selected for laboratory rearing (e.g.,Smith *et al.*, 1995; Agnew *et al.*, 2000; McCall & Eaton, 2001). Wild strains are not always dependable for generating enough larvae for experiments, as they are difficult to blood feed in captivity and are selective about oviposition substrates (personal observation; S. Allan, USDA/ARS, Gainesville, FL, personal communication). Despite the widespread use of laboratory strains in experiments, the effects of long-term laboratory colonization on *Cx. quinquefasciatus* ecological traits that may be affected by competition are largely unstudied.

We tested our first hypothesis in a laboratory experiment by evaluating the effects of resource levels and heterospecific larval density on the performance (i.e., survivorship, population growth) of both species. We predicted that *Ae. albopictus* would be less affected by high heterospecific density and limited resources than *Cx. quinquefasciatus*, due to the competitive superiority of *Ae. albopictus* to *Cx. pipiens* (Carrieri *et al.*, 2003; Costanzo *et al.*, 2005b; Costanzo *et al.*, 2011). We tested our second and third hypotheses in a similar laboratory experiment, except we manipulated chemical concentrations and not resource levels, and we used both wild and colonized strains of *Cx. quinquefasciatus*. We predicted that high chemical concentrations would reduce the competitive impacts of *Ae. albopictus* on *Cx. quinquefasciatus*, as *Ae. albopictus* appears to be less pollution tolerant than *Cx. quinquefasciatus*. We also predicted that the wild strain of *Cx. quinquefasciatus* would be less affected by interspecific competition than the colonized strain, as the colonized strain has not been reared with heterospecific larvae for hundreds of generations.

Materials & 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 on the University of Southern Mississippi (USM) campus in Hattiesburg, MS, USA (31°19.850’ N, 89°19.916’W). 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* that has been in colony since 1995 was provided by the USDA/ARS Center for Medical, Agricultural and Veterinary Entomology in Gainesville, FL, USA; a colony of this 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). Hereafter, *Cx. quinquefasciatus* from the Gainesville laboratory acclimated strain are referred to as ‘lab *Cx. quinquefasciatus*’, and larvae from the Hattiesburg strain are referred to as ‘F_2 *Cx. quinquefasciatus*’, as Hattiesburg *Cx. quinquefasciatus* larvae used in experiments were two generations removed from the field (F_2). Larvae of the two species were fed Purina® Puppy Chow® and brewers yeast (Acros Organics, Morris Plains, NJ, USA) on an eight-day schedule (see Gerberg *et al*., 1994) and reared to adults in environmental chambers (Percival Scientific, Inc., Perry, IA, USA) at 27 °C with a 14:10 hour day:night cycle. 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 lab *Cx. quinquefasciatus* (IACUC #A3851-01, 14 Aug 2009), and the arm of the experimenter was used to blood feed Hattiesburg-collected *Cx. quinquefasciatus*, as this colony would not feed on guinea pigs. Due to differing oviposition strategies of the two species, *Ae. albopictus* were provided black cups lined with wet paper towels for oviposition, and *Cx. quinquefasciatus* were provided black bowls containing larval rearing water as an oviposition stimulant. Eggs of both species were simultaneously hatched in a solution of 0.33 g Nutrient Broth (Difco™, BD, Sparks, MD, USA) per 750 mL reverse osmosis (RO) filtered water, and larvae were added to experiments within 24 h of hatching.

Resource Level Experiment

Experimental microcosms were 100 mL plastic beakers containing 99 mL of reverse osmosis (RO) water and 1 mL of microorganism inoculum (water collected from field tyres containing mosquito larvae and detritus in Hattiesburg, MS). Microcosms were housed in an environmental chamber (27 °C on a 14:10 hour day:night cycle) in plastic trays (0.50 × 0.35 m, 24 microcosms per tray). Microcosms were assigned to trays such that each factor level combination (see below) was equally represented in each tray. Microcosms were arranged randomly within trays, and tray positions were rotated within the environmental chamber every 24 hrs to control for effects of location within the incubator.

Resources consisted of senescent live oak (*Quercus virginiana*) leaves (LO) and insect carcasses (IC) present in three different quantities at a constant 5:1 (LO:IC) ratio, as mosquitoes require less animal detritus than plant detritus to obtain similar growth rates, adult mass, survivorship, and population growth rates (Yee & Juliano, 2006). The three quantities of LO and IC (respectively) used were low (0.05 g, 0.01 g), medium (0.25 g, 0.05 g), and high (0.50 g, 0.10 g). These quantities fall within the range of what is found in mosquito-inhabited tyres in the field (Yee *et al.*, 2012) and were chosen based on the nutritional requirements of *Ae. triseriatus* (approximately 0.05 g of leaf material for one larva to complete development; Kaufman *et al.*, 2001), as data on leaf and insect detritus requirements for *Ae. albopictus* and *Cx. quinquefasciatus* were unavailable. Low resources were intended to induce competition at all larval densities (see below), medium resources were intended to induce competition at higher densities (> 10 larvae), and high resources were expected to allow coexistence at all densities. Leaves were collected from USM's Lake Thoreau Environmental Center, located approximately five miles west of the USM campus in Hattiesburg, MS. Insect carcasses consisted of fruit flies (*Drosophila melanogaster* Meigen; obtained from colonies within the Department of Biological Sciences, USM) and freeze-dried crickets (*Acheta domesticus* L.; Fluker Laboratories, Baton Rouge, LA, USA) present in a 4:1 (fly:cricket) ratio. Flies were freeze-killed and all detritus was oven dried for 48 h at 80°C to kill any pre-existing microorganisms prior to the start of the experiment. Water, inoculum, and detritus were added to beakers and stored in the incubators for three days prior to the introduction of mosquito larvae to allow time for microorganism population growth. Water levels in microcosms were refilled to 100 mL with RO water prior to the introduction of mosquito larvae and maintained at 100 mL thereafter. Mosquito larvae were rinsed with RO water to prevent additional microorganisms from being introduced to microcosms.

Aedes albopictus larvae were the progeny of field-collected specimens (F_1), and *Cx. quinquefasciatus* larvae were lab *Cx. quinquefasciatus* (we were unable to generate F_2 *Cx. quinquefasciatus* larvae for this experiment). Eight different density combinations of low (5) or high (10) numbers of mosquitoes (*Ae. albopictus* : *Cx. quinquefasciatus*) were used: 0:5, 0:10, 5:0, 10:0, 5:5, 5:10, 10:5, 10:10. These densities were chosen to avoid effects of crowding (i.e., spatial competition), as *Cx. quinquefasciatus* has a surface requirement of 1 cm^2 of water surface area per larva, *Ae. albopictus* requires 0.67 cm^2 per larva (Gerberg *et al.*, 1994), and our microcosms had a water surface area of approximately 24 cm^2 when filled to 100 mL. Each resource level (3) was replicated evenly across the eight density

combinations for a total of 24 resource × density combinations; each combination was replicated ten times for a total of 240 experimental units.

The experiment was ended 45 days after larvae were added (ample time for well-fed larvae to complete development at 27°C; Gerberg *et al.*, 1994). Mosquito larvae that did not pupate by day 45 were considered mortalities. Pupae were removed from microcosms each day and transferred to glass shell vials. Sex, species, date of pupation, and date of emergence were recorded for each newly eclosed adult, and adults were freeze killed and dried for 48 hrs at 50°C. After drying, mass was measured to the nearest 0.0001 mg using a XP2U ultra-microbalance (Mettler-Toledo Inc., Columbus, OH, USA). At the conclusion of the experiment, survivorship (the percentage of initial larvae surviving to adulthood), mean development time (number of days from hatching to pupation), mean adult dry mass, and a composite index of mosquito population performance were calculated for each species in each experimental unit. The performance index (λ') is an estimate of the finite rate of increase [$\lambda = \exp(r)$], where r is the per capita rate of population change (dN/N dt) (Smith & Smith, 2006). Values of λ' are commonly used to estimate the effects of competition on population performance for *Aedes* species (e.g., Juliano, 1998; Lounibos *et al.*, 2002; Yee *et al.*, 2007) and have also been used for *Culex* species (Costanzo *et al.*, 2011). A λ' value of 1 indicates a stable population, and values > 1 and < 1 indicate population growth and decline, respectively. A λ' value of 0 is assigned when no females in a cohort survive to reproductive age (Juliano, 1998). The estimated finite rate of increase is calculated as:

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

where *r'* is an estimate of *r* derived by Livdahl and Sugihara (1984), N_0 is the initial number of females in a cohort (assumed to be 50%), D is the time from eclosing to first oviposition (assumed to be 5 days for both species; Subra, 1981; Hawley, 1988), A_x is the number of females eclosing on day x, w_x is the mean mass of females eclosing on day x, and $f(w_x)$ is a function that estimates fecundity (i.e., number of eggs) from female mass based on regressions in the literature. For *Ae. albopictus* we used the relationship $f(w_x) = 19.5 + 152.7w_x$, which is a combination of regressions relating female mass (w) to wing length (l) [$l = 1.80 + 1.96w$; $r^2 = 0.805$, $P < 0.001$], and wing length to fecundity [$f(l) = -121.240 + 78.02l$; $r^2 = 0.713$, $P < 0.001$] (Lounibos *et al.*, 2002). Because relationships directly relating female mass to fecundity were not available for *Cx. quinquefasciatus*, a regression relating wing length to fecundity [$f(l) = -123.88 + 90.31l$; $r^2 = 0.05$, $P < 0.01$] (McCann *et al.*, 2009) was modified using regressions relating female wing length to female mass; these regressions, solved for wing length, were $l = [(w + 0.162)/0.021]^{1/3}$ ($r^2 = 0.75$, $P < 0.001$) for wild *Cx. quinquefasciatus*, and $l = [(w + 0.130)/0.018]^{1/3}$ ($r^2 = 0.92$, $P < 0.001$) for *Cx. quinquefasciatus* after two years of laboratory colonization (Nasci, 1990). The wing length regressions were substituted into the fecundity function to give the modified functions $f(w_x) = -123.88 + 90.31*[(w_x + 0.162)/0.021]^{1/3}$ and $f(w_x) = -123.88 + 90.31*[(w_x + 0.130)/0.018]^{1/3}$ relating mass to fecundity for wild and colonized *Cx. quinquefasciatus*,

respectively. Because the regressions of wing length with mass are significantly different between wild and colonized female *Cx. quinquefasciatus* (Nasci, 1990), we used the colonized function for lab *Cx. quinquefasciatus*. In the chemical experiment (see below), we used the wild function for F_2 *Cx. quinquefasciatus*. The wing length-fecundity relationship for *Cx. quinquefasciatus* has a low r^2 value because fecundity of this species is influenced by a significant interaction between wing length and age; thus, wing length considered independently of age explains less variation in fecundity (McCann *et al.*, 2009). We elected to use the wing length relationship despite the low r^2 value, as we were unable to know the age at which an individual female would oviposit, and the relationship between wing length and fecundity is still significant and positive (McCann *et al.*, 2009).

Chemical Experiment

A second experiment was conducted to determine the effects of chemicals associated with detrital decomposition on survivorship, development, and interspecific interactions of *Ae. albopictus* and *Cx. quinquefasciatus*. We used a blend of 10 chemical compounds (Table 1) associated with fermenting Bermuda grass infusions (Du & Millar, 1999). The blend was prepared by dissolving chemicals in diethyl ether to make stock solutions that produced the low and high concentration chemical blends (Table 1) when 100 μL of stock solution was added 100 mL of water (Du & Millar, 1999). Concentrations of compounds in the low concentration treatment reflect concentrations in headspace extracts above water containing 4.5 g/L Bermuda grass fermented with 0.27 g/L lactalbumin hydrolyzate and brewers yeast for nine days (Du & Millar, 1999). The low concentration was most effective for eliciting oviposition responses from gravid *Cx. quinquefasciatus*, whereas the blend at high concentration (100X the low concentration) was repellent to gravid *Cx. quinquefasciatus* (Du & Millar, 1999).

The same setup and procedures from the resource level experiment were used for this experiment, with the following changes: 1) in addition to 99 mL RO water and 1 mL of inoculum, each microcosm received 100 μL of chemical blend stock solution (low or high concentration; Table 1); the control was 100 μL of clean diethyl ether (Du & Millar, 1999), 2) a constant amount of detritus (the medium level used in the resource level experiment) was used across all treatments, as competitive asymmetry was strongest at this detritus level, and 3) both lab and F_2 *Cx. quinquefasciatus* larvae were used in this experiment. This last change was done to assess possible effects of lab acclimation on competitive outcomes, and to allow for comparable results between the two competition experiments, as only lab *Cx. quinquefasciatus* were used in the resource level experiment.

Each chemical concentration (3) was replicated evenly across each density combination (8) for a total of 24 chemical × density combinations; each combination was replicated ten times for a total of 240 experimental units. Within each chemical-density combination that contained *Cx. quinquefasciatus*, seven replicates contained F_2 *Cx. quinquefasciatus* larvae, and three replicates contained lab *Cx. quinquefasciatus* larvae (we were unable to generate enough lab larvae to use five cups per strain; no cups contained mixed strains).

Analyses

For both experiments, survivorship and λ' were analyzed for both species. Additionally, analyses of adult female mass are presented for both species in the resource level experiment (analyses of female mass in the chemical experiment are omitted for brevity, as the results were similar to those in medium resources in the resource level experiment). Each dataset was tested for normality and homogeneity of variances (SAS Institute, 2004). For the resource level experiment, *Cx. quinquefasciatus* mass was inverse transformed (1/x) and *Ae. albopictus* mass was square-root transformed (√x). For the chemical experiment, *Cx. quinquefasciatus* survivorship data were power transformed ($[x + 1]^2$). All other survivorship and λ' data sets did not meet parametric assumptions, and no transformation eliminated this problem. Kruskal-Wallis tests were used for these data sets. Because the Kruskal-Wallis test cannot directly test for an interaction, we tested for differences in survivorship and λ' among treatments levels (i.e., resource level or chemical concentration) within each density combination, and among density combinations within each treatment level. When multiple Kruskal-Wallis tests were used for the same dependent variable, the α level (set at 0.05) was adjusted using sequential Bonferroni correction (Rice, 1989) to reduce the likelihood of committing a Type I error. When Kruskal-Wallis tests indicated significant differences, Dunn's test for nonparametric multiple comparisons was used to reveal pairwise differences (Zar, 2010).

For female mass of both species in the resource level experiment, and *Cx. quinquefasciatus* survivorship in the chemical experiment, analysis of variance (ANOVA) was used to test for effects of treatment (i.e., resource level or chemical concentration), larval density combination, and a treatment × density interaction on dependent variables; for *Cx. quinquefasciatus* survivorship, strain was included as a block to account for differences between lab and F_2 strains.

To elucidate the effects of *Cx. quinquefasciatus* lab acclimation on competitive outcomes, ANOVA was used to test for effects of strain, density combination, and a strain x density interaction on *Cx. quinquefasciatus* survivorship, development time for each sex, and adult dry mass for each sex in the chemical experiment. Mass data for both sexes were log transformed (ln(x)), and development time data were power transformed ($x^{-2.8}$ for males; $x^{-2.3}$ for females) to meet parametric assumptions. When an ANOVA indicated significant factor effects or interactions, Tukey's Honestly Significant Difference (HSD) test was used to test for pairwise differences. All ANOVAs and Kruskal-Wallis tests were conducted using JMP® Version 8 (SAS Institute Inc., Cary, NC, 2010).

Results

Resource Level Experiment

Survival—Survivorship of *Cx. quinquefasciatus* was negatively affected by *Ae. albopictus* in limited (i.e., low and medium) resources. The low resource level was excluded from all analyses of *Cx. quinquefasciatus*, as no *Cx. quinquefasciatus* adults emerged from low resources except in the lowest density (A0:C5). *Culex quinquefasciatus* survivorship was significantly lower in medium resources in all density combinations where *Ae. albopictus*

was present (Table 2; Fig. 1A). Survivorship differed among larval density combinations within the medium resource level (Table 2), with significantly lower survivorship when *Ae. albopictus* density was high (Fig. 1A). Survivorship in high resources was not affected by *Ae. albopictus* density (Table 2).

Survivorship of *Ae. albopictus* was not affected by *Cx. quinquefasciatus* in high and medium resources, but differences were found within the low resource level (Table 2). In low resources, *Ae. albopictus* survivorship significantly declined when both con- and heterospecific density increased simultaneously, but not when *Cx. quinquefasciatus* density alone increased (Fig. 1B). When differences among resource levels occurred, fewer individuals survived in low versus medium and high resources (Fig. 1B).

Composite Index (λ')—Population growth of *Cx. quinquefasciatus* was negatively affected by *Ae. albopictus* under limited resources (Fig. 2A). Values for *Cx. quinquefasciatus* λ' were significantly lower in medium compared to high resources in all but one density combination (A5:C5) where *Ae. albopictus* was present (Table 2; Fig. 2A). Additionally, λ' was significantly lower in the medium resource level when *Ae. albopictus* density was high (Table 2; Fig. 2A). Mean values of λ' indicated positive population growth (i.e., $\lambda' > 1$) in all density combinations in high resources, and in medium resources in the absence of *Ae. albopictus*. Mean λ' values in medium resources with *Ae. albopictus* present indicated population decline (i.e., $\lambda' < 1$; Fig. 2A). All cohorts went extinct in low resources (i.e., $\lambda' = 0$), as no females emerged from that resource level.

Aedes albopictus performed best in medium resources; effects of density varied within each resource level, but negative effects of high density were found only in low resources. Values of λ' differed among resource levels at four larval density combinations (Table 2), with significantly greater values generally occurring in high and medium resources than in low resources (Fig. 2B). Differences among density combinations were found in medium and low resources (Table 2), but significant pairwise differences were slight in the medium resource level and were attributable to conspecific density rather than *Cx. quinquefasciatus* in the low resource level (Fig. 2B). Mean λ' values indicated population growth in all density combinations in medium resources (Fig. 2B). Higher resources exerted sufficient stress such that the population index indicated declining growth except when conspecific density was high and *Cx. quinquefasciatus* was present (Fig. 2B). Population decline also was indicated in low resources, with the exception of two density combinations at low conspecific density (A5:C0, A5:C10; Fig. 2B).

Mass—Adult female mass of both species was negatively affected by decreased resource levels, and by the presence of heterospecific larvae. ANOVA indicated *Cx. quinquefasciatus* mass was significantly affected by resource level ($F_{1, 73} = 64.7833$; $P < 0.0001$), density ($F_{5, 73} = 9.4135$; $P < 0.0001$**)**, and their interaction ($F_{5, 73} = 3.0627$; $P = 0.0145$**)**. In general, females were significantly heavier in high resources than in medium resources (Fig. 3A). In medium resources, mass was negatively affected by both increased con- and heterospecific density (Fig. 3A).

For *Ae. albopictus*, ANOVA indicated significant effects of resource level ($F_{2, 118}$ = 433.1607; P <0.0001), density ($F_{5, 118}$ = 25.7078; P < 0.0001), and their interaction ($F_{10, 118}$ = 4.0766; P < 0.0001). In general, females became smaller as resource levels decreased (Fig. 3B). In high and medium resources, mass was generally lower in the presence of *Cx. quinquefasciatus* (Fig. 3B). Significant decreases in mass within low resources reflected simultaneous increases in both con- and heterospecific density (Fig. 3B).

Chemical Experiment

Survival—Survivorship of *Ae. albopictus* was unaffected by the chemical blend; survivorship of *Cx. quinquefasciatus* differed between the high and low chemical concentrations in one density combination (A10:C10), but there were no density combinations where *Cx. quinquefasciatus* survivorship in the chemical blend differed significantly from the control (Fig. 4A). Effects of density combinations were similar to those in the resource experiment. For *Cx. quinquefasciatus*, ANOVA indicated effects of chemical concentration ($F_{2, 157}$ = 3.2013; P = 0.0434), density ($F_{5, 157}$ = 35.9128; P < 0.0001), their interaction ($F_{10, 157}$ = 2.2420; P = 0.0180), and *Cx. quinquefasciatus* strain ($F_{1, 157}$ = 9.6278; P = 0.0023). Survivorship in all chemical concentrations significantly declined when *Ae. albopictus* density increased from absent to high at high conspecific density (Fig. 4A). *Aedes albopictus* survivorship did not differ among chemical concentrations at any density, and did not differ among density combinations at any chemical concentration (Table 3; Fig. 4B).

Composite Index (λ')—*Culex quinquefasciatus* population growth was not affected by the chemical blend, and trends for density were similar but less pronounced than in the resource experiment. Values of λ' differed among density combinations within all chemical concentrations, but did not differ among chemical concentrations within any density combination (Table 3). In all chemical concentrations at high conspecific density, λ' was significantly lower when *Ae. albopictus* density increased from absent to high (Fig. 5A). At low conspecific density, λ' decreased significantly with *Ae. albopictus* density only in the control (Fig. 5A). Mean values of λ' indicated positive population growth in all but the highest density combination (A10:C10; Fig. 5A).

For *Ae. albopictus*, there were no differences in λ' among chemical concentrations within any density, or among density combinations within any chemical concentration (Table 3). Mean values of λ' indicated positive population growth in every treatment combination except for one density (A5:C5) in high concentration (Fig. 5B).

Culex quinquefasciatus *Laboratory Acclimation*—Analysis of variance indicated a significant effect of strain for *Cx. quinquefasciatus* survivorship, and development time and mass of both sexes (Table 4). Specifically, lab *Cx. quinquefasciatus* had higher survivorship, faster development times for both sexes, and larger adults of both sexes than F_2 *Cx. quinquefasciatus* (Table 4). There was no significant density by strain interaction for any of these dependent variables (Table 4),

Discussion

The results of the resource level experiment supported our prediction that *Ae. albopictus* is a superior resource competitor to *Cx. quinquefasciatus*. Competitive asymmetry was produced when resources were limited (i.e., medium or low). *Culex quinquefasciatus* in medium resources experienced population decline in the presence of *Ae. albopictus* (Fig. 2A), but *Ae. albopictus* in medium resources maintained positive population growth within all density combinations (Fig. 2B). Moreover, *Cx. quinquefasciatus* went extinct in low resources after one generation, as no females emerged from that resource level; *Ae. albopictus* in low resources experienced population decline in most density combinations, but it maintained population growth at one mixed-species density (A5:C10; Fig. 2B). Therefore, *Ae. albopictus* appears to be capable of competitively reducing or excluding *Cx. quinquefasciatus* in the limited resource levels tested. Our λ' values for *Cx. quinquefasciatus* may have been less precise than those for *Ae. albopictus*, due the more complex relationship between fecundity and female body size in *Cx. quinquefasciatus* (McCann *et al.*, 2009); however, our hypothesis for asymmetrical competition was also supported by the survivorship data (Fig. 1), which contained the same trends present in the population growth estimates (Fig. 2).

The observed competitive asymmetry is possibly due to the differing foraging strategies of the two species and the decay rates of the detritus used. Mosquitoes perform better in rapidly decaying detritus (e.g., grass, insect carcasses) that supports high microorganism productivity (Dieng *et al.*, 2002; Murrell & Juliano, 2008), but species differ in their ability to exploit slowly decaying detritus (e.g., oak leaves). *Aedes albopictus* can exploit both resource types (Yee *et al.*, 2007), and it appears to better able to exploit slowly decaying resources (e.g., oak and elm leaves) than competitors (e.g., *Ae. aegypti*, *Ae. triseriatus*, and *Cx. pipiens*) (Barrera, 1996; Yee *et al.*, 2007; Murrell & Juliano, 2008; Costanzo *et al.*, 2011). This is possibly due to the superior ability of *Ae. albopictus* to harvest resources and efficiently convert them to biomass (Carrieri *et al.*, 2003; Yee *et al.*, 2004a). Additionally, *Ae. albopictus* allocates more time to browsing detrital surfaces for microorganisms than some competitors (Yee *et al.*, 2004a, 2004b), which may serve as an advantage when microorganism productivity is low. Further studies are needed to determine how foraging behavior, efficiency of resource assimilation, and overall competitive outcomes between *Ae. albopictus* and *Cx. quinquefasciatus* compare in different resource environments.

Aedes albopictus survivorship and population growth were generally unaffected by *Cx. quinquefasciatus*, but competition from *Cx. quinquefasciatus* had clear effects on *Ae. albopictus* adult female mass. In medium resources, and in high resources at high intraspecific density, *Ae. albopictus* adults were smaller when *Cx. quinquefasciatus* was present (Fig. 3B). The effects of *Cx. quinquefasciatus* on *Ae. albopictus* adult mass may have important implications for disease transmission patterns, as smaller females stressed by competition are more prone to arbovirus infection (Alto *et al.*, 2005; 2008b). Thus, competition between these species appears to be highly asymmetrical in favor of *Ae. albopictus*, but subtle effects of *Cx. quinquefasciatus* on *Ae. albopictus* may still have consequences for disease dynamics.

For *Ae. albopictus*, survivorship and population performance appeared to have opposite associations with increasing density in low and high resources. When grown at low conspecific density, survivorship in low and high resources was intermediate and similar regardless of *Cx. quinquefasciatus* density; this diverged at high conspecific density, with survivorship increasing in high resources and decreasing low resources as *Cx. quinquefasciatus* density increased (Fig. 1B). This trend was also observed for population growth, where negative population growth ($\lambda' < 1$) was observed in high resources except when conspecific density was high and *Cx. quinquefasciatus* was present (Fig. 2B). In contrast, *Cx. quinquefasciatus* attained positive population growth in all high resource treatments regardless of density (Fig. 2A). The observed pattern may have been due to the increased amount of insect detritus in high resources, which putrefies the water and may be toxic to *Ae. albopictus* larvae in high amounts (Murrell & Juliano, 2008); *Cx. quinquefasciatus* is less likely to be affected by this, as it is highly tolerant to organic pollution (Subra, 1981). High con- and heterospecific densities may serve to facilitate *Ae. albopictus* performance in high concentrations of labile detritus (e.g., grasses, invertebrate carcasses) via increased control of microbial communities (Kaufman *et al.*, 1999). Past experiments have demonstrated that these detritus types can reduce or nullify (but not reverse) the competitive advantage of *Ae. albopictus* (Yee *et al.*, 2007; Murrell & Juliano, 2008; Costanzo *et al.*, 2011), but these experiments used grass and insect concentrations ≤ 1.5 and 0.5 g/L, respectively. Further studies are needed to determine how higher concentrations of labile detritus affect interspecific interactions, and to elucidate the relationship between *Cx. quinquefasciatus* density and *Ae. albopictus* performance under highly organic conditions.

The chemical experiment did not support our hypothesis that chemicals associated with decomposition would affect interspecific competition between *Ae. albopictus* and *Cx. quinquefasciatus*, as there were no cases in which survivorship or λ' for either species differed between the control and either concentration of the chemical blend. This suggests that these chemicals either were not responsible for the negative effect of high detritus on *Ae. albopictus* in the resource level experiment, or that the concentrations used in the chemical experiment were insufficient to affect the performance of either species. The concentrations of the chemicals present in the blend are based on the amounts present in headspace extracts above water containing decomposing grass (Du & Millar, 1999), and therefore may not reflect the amounts present in the water itself. Further studies of the chemicals released into the water column by detrital decomposition and their concentrations at various detritus levels are needed to assess what effects, if any, these chemicals have on mosquito survival and interspecific interactions.

Our hypothesis that wild and laboratory strains of *Cx. quinquefasciatus* would be differently affected by interspecific competition was not supported. Although there were effects of lab acclimation on *Cx. quinquefasciatus* life history traits, these effects did not interact with larval density, indicating *Ae. albopictus* competition has the same negative effect on F_2 and lab *Cx. quinquefasciatus*. Therefore, results of the resource level experiment, which used only lab *Cx. quinquefasciatus*, should be applicable to F_2 *Cx. quinquefasciatus*. We do note that λ' values for F_2 would likely have been lower overall, as F_2 *Cx. quinquefasciatus* had

lower survivorship and mass, and longer development times. It is possible, albeit unlikely, that the use of different blood meal sources (guinea pig for lab *Cx. quinquefasciatus*, human for F_2 *Cx. quinquefasciatus*) contributed to the differences in life history traits. We know of no studies that investigate the effects of blood meal source on offspring life history traits for *Cx. quinquefasciatus*, but a study of *Ae. aegypti* and four *Anopheles* species found that larvae generated from guinea pig blood and human blood did not differ in survivorship (with the exception of one *Anopheles* species) or development time (Phasomkusolsil *et al.*, 2013). Additionally, parental rearing conditions (including nutritional stress) do not affect survivorship, development time, or body size of offspring in *Anopheles stephensi* Liston (Grech *et al.*, 2007). Our results suggest that laboratory strains of *Cx. quinquefasciatus* are suitable for larval competition experiments, but further studies are needed to determine how behavior, life history, and fitness of this and other species are affected by long-term colonization.

This is the first study to investigate larval interactions between *Ae. albopictus* and *Cx. quinquefasciatus*. We demonstrated that *Ae. albopictus* is a superior resource competitor and appears to be capable of competitively reducing or excluding *Cx. quinquefasciatus* from an individual container after one generation under limited resources. Because the competitive advantage of *Ae. albopictus* over other mosquito species is often condition-specific (e.g., Barrera, 1996; Costanzo *et al.*, 2005a; Griswold & Lounibos, 2005a), more studies are needed to understand the effects of extraneous factors (e.g., predation, weather patterns, resource types) on competition between *Ae. albopictus* and *Cx. quinquefasciatus*. Additionally, *Cx. quinquefasciatus* lays its eggs on the water surface, and the eggs hatch after one day (Subra, 1981), whereas *Ae. albopictus* lays the majority of its eggs on container walls above the water surface, and the eggs hatch when flooded (Hawley, 1988). Therefore, egg hatching times of these species are not necessarily synchronous and may vary due to rainfall patterns, meaning that interspecific competition between *Aedes* and *Culex* in the field is likely to occur between different larval instars. Future work could test the effects of non-synchronous egg hatching on competitive outcomes between these species. Although it is unlikely that *Ae. albopictus* will displace *Cx. quinquefasciatus* on a regional scale, as *Cx. quinquefasciatus* also utilizes non-container habitats (Subra, 1981), interspecific competition between these species clearly has the potential to affect vector population dynamics, especially when containers represent the majority of available mosquito breeding habitats.

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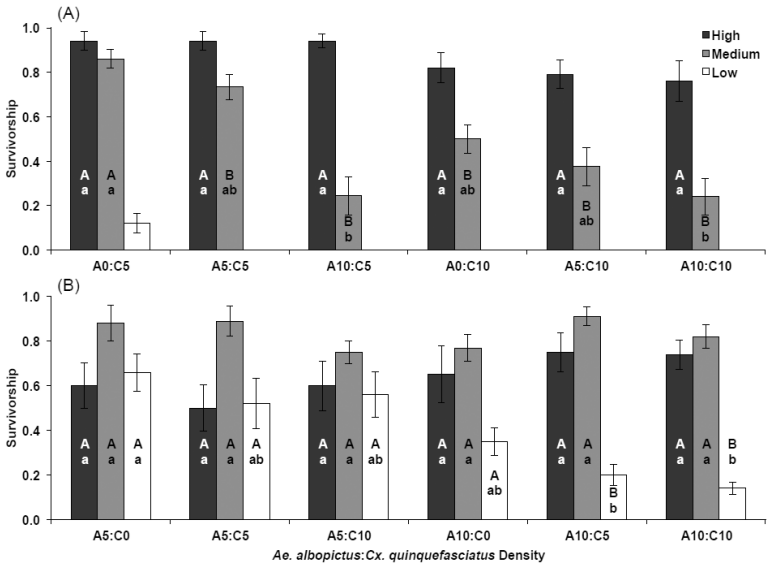

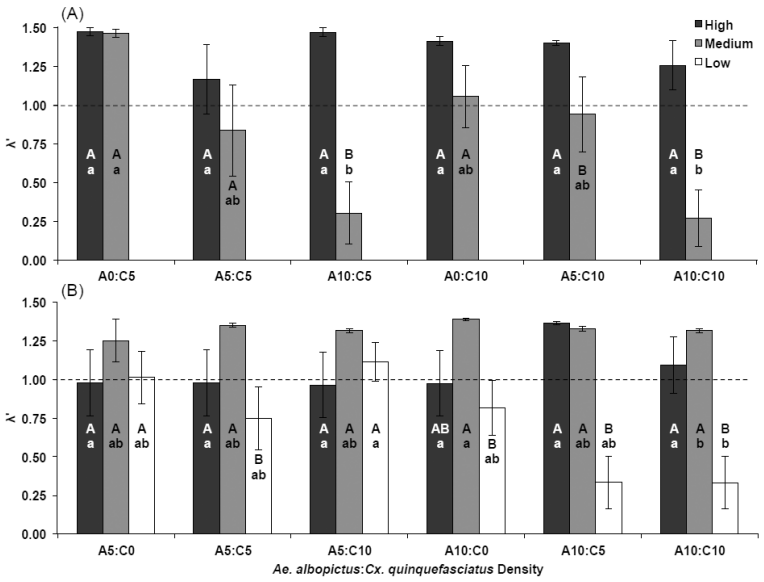

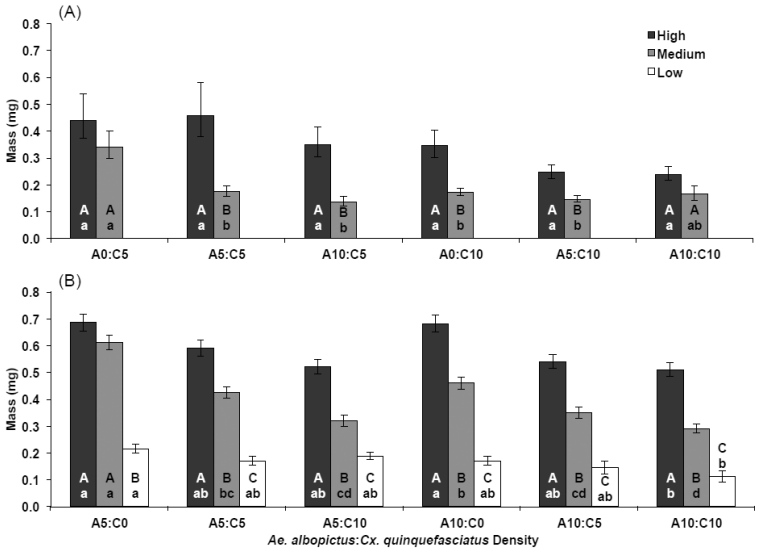

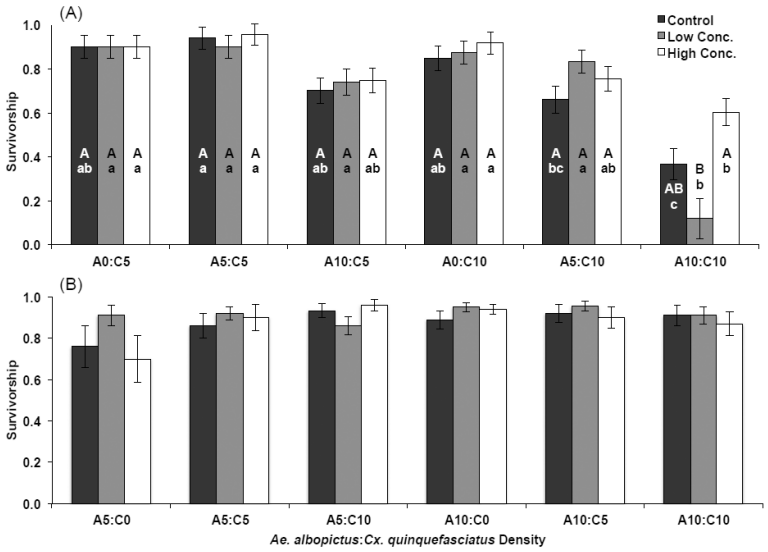

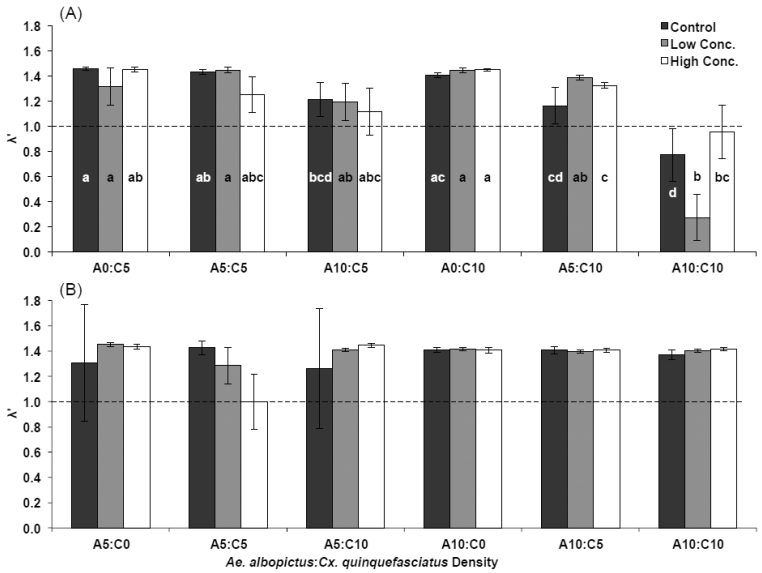

Table 1

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

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

Concentrations are based on Du and Millar (1999).

Table 2

Kruskal-Wallis test results on *Ae. albopictus* and *Cx. quinquefasciatus* survivorship and estimated population growth (λ') differences among resource levels within each density combination and among density combinations within each resource level.

	Survivorship			λ'		
	χ^2	d.f.	*P*	χ^2	d.f.	*P*
Cx. quinquefasciatus						
Resource (A0:C5)	2.4429	1	0.1181	0.0079	1	0.9292
Resource (A5:C5)	7.2068	1	**0.0073**	1.5267	1	0.2166
Resource (A10:C5)	13.5034	1	**0.0002**	12.1784	1	**0.0005**
Resource (A0:C10)	7.5476	1	**0.0060**	4.5106	1	0.0337
Resource (A5:C10)	8.1856	1	**0.0042**	7.8799	1	**0.0050**
Resource (A10:C10)	9.2208	1	**0.0024**	8.6133	1	**0.0033**
Density (Low)	not tested			not tested		
Density (Medium)	31.6395	5	**<0.0001**	22.9391	5	**0.0003**
Density (High)	12.1891	5	0.0323	6.8047	5	0.2356
Ae. albopictus						
Resource (A5:C0)	6.3633	2	0.0415	6.7773	2	0.0338
Resource (A5:C5)	8.2059	2	0.0165	8.7838	2	**0.0124**
Resource (A5:C10)	1.5046	2	0.4713	7.0001	2	0.0302
Resource (A10:C0)	9.0634	2	0.0108	12.5513	2	**0.0019**
Resource (A10:C5)	17.9120	2	**0.0001**	20.3121	2	**<0.0001**
Resource (A10:C10)	20.0099	2	**<0.0001**	15.2431	2	**0.0005**
Density (Low)	24.3455	5	**0.0002**	22.9629	5	**0.0003**
Density (Medium)	9.8768	5	0.0788	20.6825	5	**0.0009**
Density (High)	4.7697	5	0.4446	0.6136	5	0.9874

Significance at sequential Bonferroni adjusted significance levels is shown in bold.

Table 3

Kruskal-Wallis test results on *Ae. albopictus* and *Cx. quinquefasciatus* survivorship and estimated population growth (λ') differences among chemical concentrations within each density combination and among density combinations within each chemical concentration.

	Survivorship			λ'		
	X^2	d.f.	*P*	X^2	d.f.	*P*
Ae. albopictus						
Chemical (A5:C0)	1.7767	2	0.4113	0.4314	2	0.8060
Chemical (A5:C5)	0.7181	2	0.6983	1.6046	2	0.4483
Chemical (A5:C10)	3.7928	2	0.1501	3.2089	2	0.2010
Chemical (A10:C0)	1.0690	2	0.5860	1.3239	2	0.5159
Chemical (A10:C5)	0.3114	2	0.8558	0.2359	2	0.8888
Chemical (A10:C10)	0.2867	2	0.8664	6.6759	2	0.0355
Density (Control)	3.2581	5	0.6603	11.1504	5	0.0485
Density (Low)	3.6811	5	0.5962	7.0237	5	0.2189
Density (High)	3.9901	5	0.5508	6.6650	5	0.2468
Cx. quinquefasciatus						
Chemical (A0:C5)				0.0335	2	0.9834
Chemical (A5:C5)				2.9961	2	0.2236
Chemical (A10:C5)				0.6838	2	0.7104
Chemical (A0:C10)				2.8824	2	0.2366
Chemical (A5:C10)				7.2218	2	0.0270
Chemical (A10:C10)				6.3589	2	0.0416
Density (Control)				35.7225	5	**<0.0001**
Density (Low)				30.4275	5	**<0.0001**
Density (High)				19.3746	5	**0.0016**

Significance at sequential Bonferroni adjusted significance levels is shown in bold. *Culex quinquefasciatus* survivorship was analyzed using ANOVA and is omitted from the table.

Table 4

Results of two-way ANOVA (density combination and strain) on transformed values, and back-transformed least squared means (± standard error) for *Cx. quinquefasciatus* survivorship, development time (days), and mass (mg) for males (m) and females (f).

	ANOVA			Mean ± SE			
Effect	**d.f.**	***F***	***P***	**Wild (F_2)**		**Lab**	
Survivorship							
Density	5, 164	21.9399	**<0.0001**				
					+0.0164		+0.0242
Strain	1, 164	10.5274	**0.0014**	0.7279		0.8244	
					−0.0165		−0.0246
Density × strain	5, 164	1.6030	0.1620				
Development Time (m)							
Density	5, 151	9.6309	**<0.0001**				
					+0.0610		+0.0810
Strain	1, 151	12.9564	**0.0004**	6.5250		6.1532	
					−0.0589		−0.0772
Density × strain	5, 151	1.6069	0.1615				
Development time (f)							
Density	5, 143	16.6996	**<0.0001**				
					+0.0888		+0.0910
Strain	1, 143	36.6042	**<0.0001**	7.4008		6.6210	
					−0.0855		−0.0870
Density × strain	5, 143	1.1386	0.3428				
Mass (m)							
Density	5, 149	49.0756	**<0.0001**				
					+0.0034		+0.0063
Strain	1, 149	10.4604	**0.0015**	0.2170		0.2392	
					−0.0033		−0.0061
Density × strain	5, 149	1.6425	0.1522				
Mass (f)							
Density	5, 134	43.4058	**<0.0001**	0.2903	+0.0061	0.3254	+0.0102
Strain	1, 134	9.3531	**0.0027**		−0.0060		−0.0099
Density × strain	5, 134	1.1086	0.3585				

Significant effects are shown in bold.