

# EFFECTS OF VEGETATION AND COMPETITION ON THE DEVELOPMENT OF VECTOR SPECIES *AEDES ALBOPICTUS* AND *CULEX PIPIENS*

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*Abstract.* Mosquito-borne diseases are increasingly affecting public health with urban areas especially seeing changes in distribution and incidence of vector-borne pathogens. Anthropogenic influences are changing resource availability in aquatic juvenile habitats, influencing larval development and the adult mosquitoes emerging from urban aquatic breeding habitats, which can change the abundance and distribution of important mosquito vectors in urban areas. Leaf detritus and associated chemical makeup of aquatic larval habitats inform levels of competition among mosquito larvae and affect adult traits that can influence disease transmission. *Culex pipiens* and *Aedes albopictus* are both important disease vectors in urban areas that are known to co-occur in breeding habitats where resource conditions are mainly fueled by plant-based detritus from surrounding vegetation. This study investigated how differences in vegetation chemistry among plants commonly found in urban yards (*Agrostis spp.*, *Quercus rubra*) and vacant lots (*Ailanthus altissima*, *Catalpa speciosa*) influenced development, emergence and adult size of *Cx. pipiens* and *Ae. albopictus*. *Ailanthus* and *Agrostis* treatments had higher nitrogen content and both vector species developed more rapidly and achieved greater adult size when reared in these treatments. Although the numbers of *Cx pipiens* that pupated was not different across treatments, more *Ae albopictus* pupated in the lower nitrogen, *Catalpa* and *Quercus* treatment. There was no significant inter-specific competition detected, however *Ae albopictus* intra-specific competition was evident in the *Quercus* treatment only. Our findings demonstrate that the resource availability defined by the detritus inputs to container habitats can have an important influence on competition, development, adult size and vector fitness across urban environments.

## INTRODUCTION

Increases in cases of mosquito-borne diseases in urban populations have raised public health concerns across the globe in recent decades (Leisnham and Juliano 2012, Mari 2012, Tapia-Conyer et al. 2012, Weaver 2013, LaDeau et al. 2015, Robert et al. 2016). Factors such as vector abundance and species distribution are crucial for determining the distribution and incidence of mosquito-borne diseases. Understanding how environmental variables influence the abundance and distribution of mosquitoes is a key issue especially since this is an important predictor of the distribution and incidence of vector-borne pathogens (Juliano and Lounibos 2005, Freed and Cann 2013, Ferraguti et al. 2016). Although adult female mosquitoes are the direct vectors of disease, there is evidence that adult mosquito life history traits are influenced greatly by environmental conditions experienced during larval development (Juliano 2007, Noori et al. 2015). Further, the environment during the immature larval stages can influence traits that are relevant to disease transmission (Alto et al. 2008, Reiskind and Lounibos 2009, Westbrook et al. 2010).

Urbanization is often associated with abundant artificial container habitats in an environment with higher nutrient levels that are suitable for many mosquitoes (Costanzo et al. 2011, Li et al. 2014, Ferraguti et al. 2016). Two important mosquito species commonly found in cities across the eastern U.S. are *Culex pipiens* and *Aedes albopictus*. *Ae. albopictus*, the Asian tiger mosquito, has become an important vector in urban areas and is capable of transmitting several arboviruses, including dengue fever, chikungunya,

yellow fever virus, and West Nile virus (Sardelis et al. 2002, Turell et al. 2005, Lambrechts et al. 2010, Rochlin et al. 2013, Vega-Rua et al. 2014). *Cx. pipiens* is a competent and important vector of West Nile virus. *Ae. albopictus* and *Cx. pipiens* co-occur in a range of artificial, urban habitats and seem to coexist despite demonstrated larval competition for resources (Costanzo et al. 2005, Costanzo et al. 2011, LaDeau et al. 2013). The level of intra- and inter-specific competition for these species is resource and environment dependent (Juliano and Lounibos 2005). *Ae. albopictus* is a superior competitor when resources are limiting (Carrieri et al. 2003, Costanzo et al. 2005, Costanzo et al. 2011), but the species' competitive advantage is reduced when larval resources are high (Costanzo et al. 2005, Juliano 2010, Freed and Leisnham 2014). A proposed mechanism for this is that higher nitrogen inputs to juvenile habitats acts as a primary energy source for bacterial communities that are an integral part of larval mosquito diets (Sanford et al. 2005, Ponnusamy et al. 2010, Murrell et al. 2011, Yee et al. 2012, Noori et al. 2015).

In urban areas, larval mosquito habitats exist as small ponds, discarded tires, storm water catch basins, and other human made containers. The resource conditions in these habitats are mainly fueled by plant-based detritus from surrounding vegetation (Gardner et al. 2013). Thus, container habitat surrounded by managed lawns and yards receive different vegetation inputs from containers in abandoned lots, where vegetation is primarily opportunistic. Differences in the plant detritus in the aquatic larval environment can alter adult survival, development rate, as well as outcomes of intra- and inter-specific competition (Murrell and Juliano 2008, Juliano 2009, Costanzo et al. 2011, Gardner et al. 2015). Varying levels of nitrate from different plants was a predictor of larval *Culex* production in catch basins (Gardner et al. 2013).

This study aims to determine how leaf detritus and specifically, nitrogen concentrations influence intra- and interspecific competition between *Ae. albopictus* and *Cx. pipiens*. Higher levels of resources including nitrogen in aquatic breeding habitats have been seen to increase mosquito abundance (Sanford et al. 2005; Morrell et al. 2011). Larval competition for limited resources has been shown to prolong development, reduce survival, and foster production of small adults (Reiskind and Lounibos 2009). With higher levels of nitrogen *Aedes* and *Culex* have been seen to have a shortened development time, higher rates of survival, and larger body size (Xue et al. 2010; Winters and Yee 2012; Noori et al. 2015; Sanford et al. 2005). Larger mosquitoes produced from aquatic breeding habitats with more nitrogen available may be more fit as body size has been correlated with fecundity (Armbruster and Hutchinson 2002) and with the ability to obtain more blood meals (Xue et al. 2010). We reared *Ae. albopictus* and *Cx. pipiens* at two density treatments in aquatic conditions that reflect common vegetation in managed (*Agrostis spp.*, *Quercus rubra*) or unmanaged (*Ailanthus altissima*, *Catalpa speciosa*) urban habitats and that have known differences in nitrogen content.

We hypothesized that *Cx. pipiens* pupation, development rate, and adult size would all be greater in treatments where they were reared alone rather than in the presence of *Ae. albopictus*, but that this competitive effect would be decreased in treatments with the more nitrogen-rich plants. We hypothesized that *Ae. albopictus* performance would be less sensitive to the different plant treatments and expected to see the number of pupae, development rate, and wing size to be greater when *Ae. albopictus* were reared in the presence of *Cx. pipiens* because *Ae. albopictus* have been shown to be especially sensitive to intraspecific competition (Costanzo et al. 2011, Noden et al. 2016).

## MATERIALS & METHODS

*Ae. albopictus* and *Cx. pipiens* eggs were obtained from colonies reared in the Leisnham lab at the University of Maryland. We established seven treatment conditions with a volume of 100 mL, including a well-water control with no added nutrients. Four treatments had larvae reared in well water that was infused with different plant matter. Two of these treatments were made of plant infusion from plants

typical to unmanaged urban areas. The first was made of leaves from *Ailanthus altissima* and the second was made of leaves from *Catalpa speciosa*. The other two plant treatments were made of plant infusion from plants typical to managed urban areas. The first was made from blades of common lawn grass (*Agrostis spp.*) and the second was made of leaves from a Northern red oak (*Quercus rubra*). Each plant infusion was made from 100 grams of fresh plant material consisting of the leaves and 1.75 L of well-water. With each experimental treatment having a volume of 100 mL, the amount of fresh plant material that would have produced the leachate for each container would have been around 5.7 g. This would equate to roughly 1 large *Ailanthus* leaf, 1 large *Catalpa* leaf, 8-10 average size *Quercus* leaves, and 20-30 blades of *Agrostis spp.* Initial well water had a nitrate concentration of 0.53 mg/L NO<sub>3</sub>-N. Two final treatments were well water with a potassium nitrate addition of 2 mg/L NO<sub>3</sub>-N (low) and a high nitrate treatment of 6mg/L NO<sub>3</sub>-N. Literature values demonstrate that nitrogen concentrations in field containers can range widely, from 0.0-18.0 mg/L NO<sub>3</sub>-N (Noori et al. 2015). A single replicate trial for each resource treatment included 3 containers: one with 20 *Ae. albopictus* first instar larvae, a second with 20 *Cx. pipiens* first instar larvae and a third with 10 *Ae. albopictus* and 10 *Cx. pipiens* first instar larvae. We aimed for densities approaching the observed 33 larvae per 100ml liquid from field studies in Baltimore, MD (Leisnham unpublished) but were limited to 20 total larvae per replicate due to productivity in the *Culex* colony. Each of these 3-container trials was replicated three times (12 experimental containers for control and plant-based treatments) or five times for the low and high nitrate controls (15 experimental containers per treatment). The full experiment included 75 containers, 750 *Cx. pipiens* and 750 *Ae. albopictus* larvae.

All replicate containers were placed in a programmable environmental chamber (Model LT36VLC9 Pervical Scientific, Inc., Perry, IA) that maintained 29 degrees Celsius for 16 hours of light and 24 degrees Celsius for 8 hours of dark and 80 percent relative humidity. Aquatic chemistry was analyzed for each of the plant infusions after one week to quantify NH<sub>4</sub>-N, NO<sub>3</sub>-N, and PO<sub>4</sub>-P. After treatments were established, the containers were checked and rearranged in the incubator daily. 10 mL of the appropriate treatment liquid was added every other day to maintain levels at 100 mL. Any pupae that developed were removed to single container for each treatment and held at the same environmental conditions until emergence. These containers were also checked daily for adults and any adults that emerged were removed and frozen at -20°C. The numbers of pupae and adults that emerged from each jar and treatment were recorded, as were the numbers of days until emergence and sex. Adult females were identified to species. A single wing was removed from each female and length was measured to the nearest 1/2 mm using a dissecting microscope. After 16 days, each container was checked and a final count of remaining larvae was recorded.

Data summaries and statistical analyses were computed using the R Statistical Software. Standard linear models were considered, with a significance threshold of alpha < 0.05.

## RESULTS

Of the 1500 mosquito larvae that were started in the experiment, 426 pupated (28.4%) and of those that pupated 391 (26.0%) emerged as adults. No larvae from the control, low nitrate, or high nitrate treatments reached pupation. There were 229 *Ae. albopictus* that emerged (50.2% female) and 162 *Cx. pipiens* (36.4 % female). After one week of incubation, the four plant infusions had wide-ranging nitrogen and phosphorus levels (Table 1). Ammonium (NH<sub>4</sub>-N) levels were an order of magnitude greater in the *Ailanthus* and *Agrostis* infusions than in *Quercus* or *Catalpa*. All four had nitrate (NO<sub>3</sub>-N) levels that were lower than our nitrogen added treatments, and were relatively similar to the well-water control. Phosphorus (PO<sub>4</sub>-P) levels were greatest in the *Agrostis* infusion, decreasing in the *Ailanthus*, *Catalpa*, and *Quercus* infusions, respectively.

Contrary to our expectations, the number of pupae, development rate, and adult size were not significantly different between *Cx. pipiens* reared alone and those reared in the presence of *Ae. albopictus* in any of the plant infusions (e.g., Figure 1). Thus, we report on comparisons across the single-species density replicates for each of the performance metrics described below. There was no difference in the average numbers of *Cx pipiens* pupae (or adults emerged) across the four plant infusions (Figure 2), although significantly more larvae persisted after 16 days in the *Quercus* and *Catalpa* treatments ( $F=10.4$ ,  $df=4$ ,  $p<0.05$ ). While on average 0 ( $sd=0$ ) larvae persisted after 16 days in the *Agrostis* and *Ailanthus* treatments, an average of 4.83 ( $sd=1.33$ ) persisted in the *Quercus* and *Catalpa* treatments. *Cx. pipiens* developed faster by an average 3 ( $sd=1.62$ ) days ( $F=12.01$ ,  $df=3$ ,  $p<0.05$ ) (Figure 3), and female wing length was larger by an average of 0.36mm ( $df=41$ ,  $SE=0.067$ ,  $p<0.05$ ) when reared in the *Agrostis* and *Ailanthus* infusions compared to the *Catalpa* and *Quercus* treatments (Figure 4).

There was a significant differences in the average numbers of *Ae. albopictus* pupae across the four plant treatments ( $F=43$ ,  $df=6$ ,  $p<0.05$ ). Pupation was reduced in both the higher nitrogen treatments relative to *Quercus* and *Catalpa* that had an average of 17.66 ( $sd=2.65$ ) larvae, while an average of 13.33 ( $sd=4.93$ ) *Ae. albopictus* pupated from the *Agrostis* and an average of 9 ( $sd=3$ ) pupated from the *Ailanthus* treatments (Figure 4). However, *Ae. albopictus* developed faster by an average of 3.34 ( $df=69$ ,  $SE=0.420$ ,  $p<0.05$ ) days and achieved greater female wing length (0.3 mm,  $sd=.27$ ) in the *Agrostis* and *Ailanthus* treatments ( $F=18.8$ ,  $df=3$ ,  $p<0.05$ ) (Figure 5). Although intraspecific competition did not influence the number of pupae nor size of adult *Ae. albopictus*, *Ae. albopictus* reared alone developed more slowly but only in the *Quercus* infusion ( $F=?$ ,  $df=2$ ,  $p<0.05$ ). In the *Quercus* treatment, *Ae. albopictus* reared with *Cx. pipiens* emerged an average of 2.18 ( $df=30$ ,  $SE=0.377$ ,  $p<0.05$ ) days before those in the single-species treatment (Figure 6).

## DISCUSSION

Our results did not support the hypothesis that *Cx. pipiens* was an inferior competitor, as the number of pupae, development rate, and adult size were consistent across single and multi-species densities. This suggests that there were enough resources in all the plant-based treatments to sustain both species of larvae. This is consistent with the idea that the competitive advantage of *Ae. albopictus* is reduced with greater total resources available (Costanzo et al. 2011) and demonstrates that there are conditions that allow for co-existence of *Cx. pipiens* and *Ae. albopictus* (Carrieri et al. 2003, Costanzo et al. 2005, Juliano and Lounibos 2005, LaDeau et al. 2013). (Costanzo et al. 2011 should also be cited here).

*Culex pipiens* did have faster development, and larger wings when reared in plant infusions from plants with higher levels of nitrogen. The number of larvae did not change significantly between plant infusions, but pupae did develop faster with larger wings when in the *Agrostis* and *Ailanthus* infusions. This is consistent with other findings that demonstrate that with higher levels of nitrogen in larval breeding habitats *Culex* have been seen to have a shortened development time and larger body size (Winters and Yee 2012; Noori et al. 2015; Sanford et al. 2005). This is also consistent with the finding that *Cx. pipiens* have a greater tolerance for more eutrophic water (Costanzo et al. 2005).

The hypothesis that *Ae. albopictus* number of pupae, development rate, and adult size would be consistent across different plant infusion treatment was not supported by this experiment. We saw that the number of pupae were highest in the *Quercus* and *Catalpa* treatments that had the lowest levels of  $NH_4-N$ , which is consistent with the idea that *Ae. albopictus* are not as tolerant of eutrophic water (Costanzo et al. 2005). We also saw that development rate was faster and wing size was larger when larvae were reared in the *Agrostis* and *Ailanthus* treatments that had the greatest amounts of  $NH_4-N$ , which is more consistent with the findings that sufficient nutritional resources and higher levels of nitrogen allow for faster development of larger *Aedes* female mosquitoes (Xue et al. 2010; Winters and Yee 2012).

Our expectation to see the number of pupae, development rate, and wing size to be greater when *Ae. albopictus* were reared in the presence of *Cx. pipiens* rather than alone due to a sensitivity to intraspecific competition in higher larval densities (Alto et al. 2005; Noden et al. 2016) was only seen once. We saw that *Ae. albopictus* reared in the *Quercus* infusion with *Cx. pipiens* emerged two days faster than those reared solely with *Ae. albopictus* suggesting that the oak leaves did not leach enough resources to allow for full development when the density of *Ae. albopictus* was greater. This suggests that intraspecific competition was higher for *Ae. albopictus* reared alone in the *Quercus* infusion and some of this pressure was lifted when the density of *Ae. albopictus* was lessened in the presence of *Cx. pipiens*.

Overall, our results support previous findings that an increase in nitrogen allows for faster development and the production of larger female mosquitoes (Noori et al. 2015; Sanford et al. 2005; Winters and Yee 2012; Xue et al. 2010). Another study looking at plant detritus effects on mosquitoes determined that tannins from plants like oak could be toxic to mosquito larvae (Rey et al. 2000), but we saw significantly more *Ae. albopictus* pupating in the oak infusion. Our finding of only one instance of difference between single species treatments and treatments where the two species were reared together is consistent to what is seen with *Cx. pipiens* and *Ae. albopictus* coexisting in the field (Costanzo et al. 2005; Juliano and Lounibos 2005). There are conditions under which these two vector species are able to coexist, emerge faster, and emerge larger. Our experimental conditions were not unreasonably different from what could be found in the field. Although our NH<sub>4</sub>-N concentrations were higher than what has been observed in the field (Noori et al. 2015), our conditions could be seen if a relatively small amount of fresh leaves (i.e. 1 large *Ailanthus altissima* leaf, 1 large *Catalpa speciosa* leaf, 8-10 *Quercus rubra* leaves, and 20-30 *Agrostis spp.* blades) fell into a small mosquito breeding habitat with a volume near 100 mL and were allowed to decompose.

Future studies could repeat this or similar experiments with varying densities of larvae of *Cx. pipiens* and *Ae. albopictus* larvae to see how vegetation and larval density further affects competition and to find more conditions that allow for the production of these two vectors from larval breeding habitats that are commonly found in urban areas. Local abundance of vectors is one predictor of human illness in mosquito-vectored disease systems (Hayes and Gubler 2006; Halstead 2008). The coexistence of these vectors in urban areas with a greater human population could be medically important (Costanzo et al. 2005, Costanzo et al. 2011). This study contributes to the knowledge of ecological conditions that support larval production in an urban ecosystem, which could allow for more accurate identification of populations that are more at risk of disease. There is the potential for this information to be used for more targeted mosquito control efforts by identifying landscape features that can contribute to breeding sites for *Cx. pipiens* and *Ae. albopictus* that are especially likely to allow for the production of these two vectors of human disease.

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APPENDIX

TABLE 1. Chemical analysis of plant infusions after one week of incubation.

Plant Infusion (100g/1.75L)	Ailanthus	Catalpa	Grass/Lawn	Oak
Nitrate-N (mg/L)	<0.02	0.05	<0.02	0.06
Ammonium-N (mg/L)	141	0.82	128	0.77
Phosphate-P (mg/L)	41.9	30.4	101	12.5

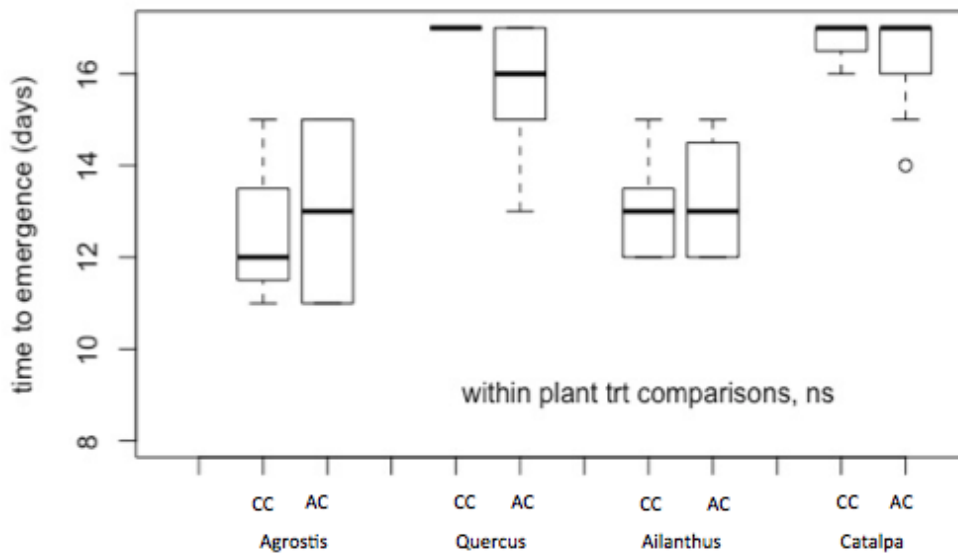
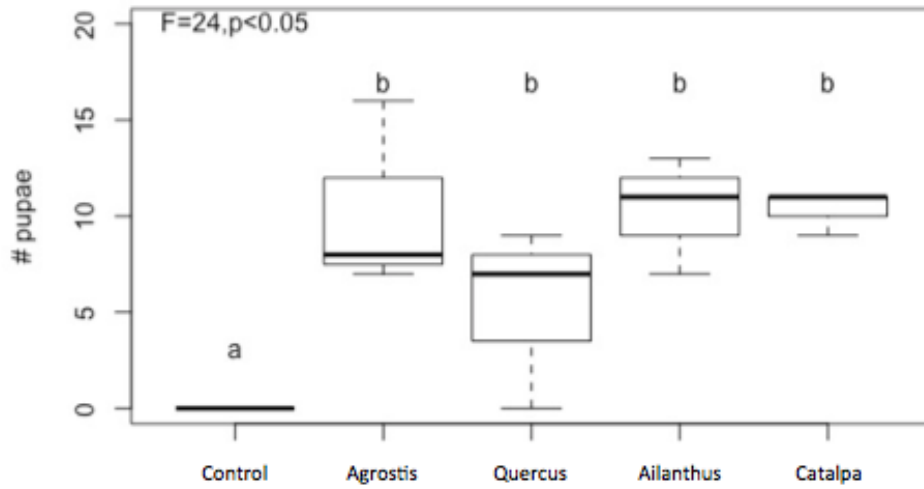
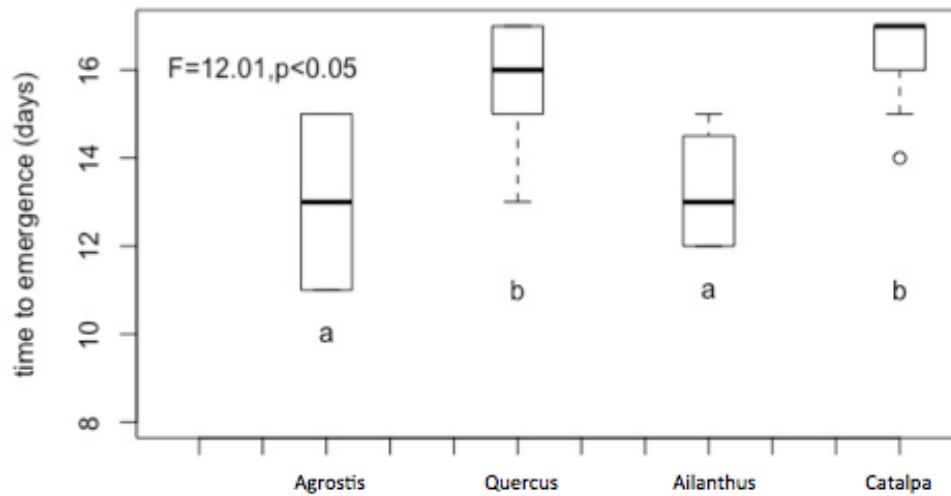


FIGURE 1. Average time to adult emergence for *Cx. pipiens* including those reared in the presence of solely *Cx. pipiens* and those reared in the presence of *Ae. albopictus*. This figure shows a boxplot with distributions of data and the dark line representing the median. Letters represent pairwise comparisons done with a Bonferroni correction. CC refers to replicate containers with solely *Cx. pipiens* and AC refers to replicate containers with *Cx. pipiens* and *Ae. albopictus*.

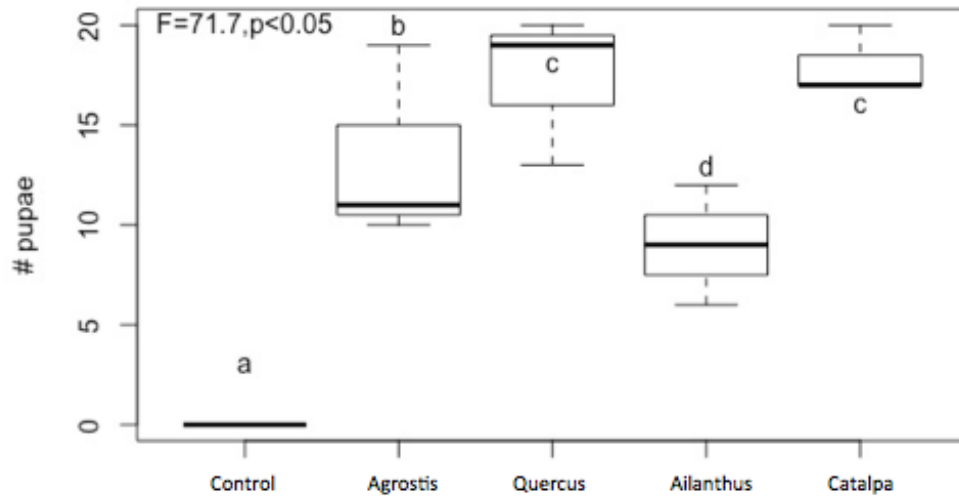




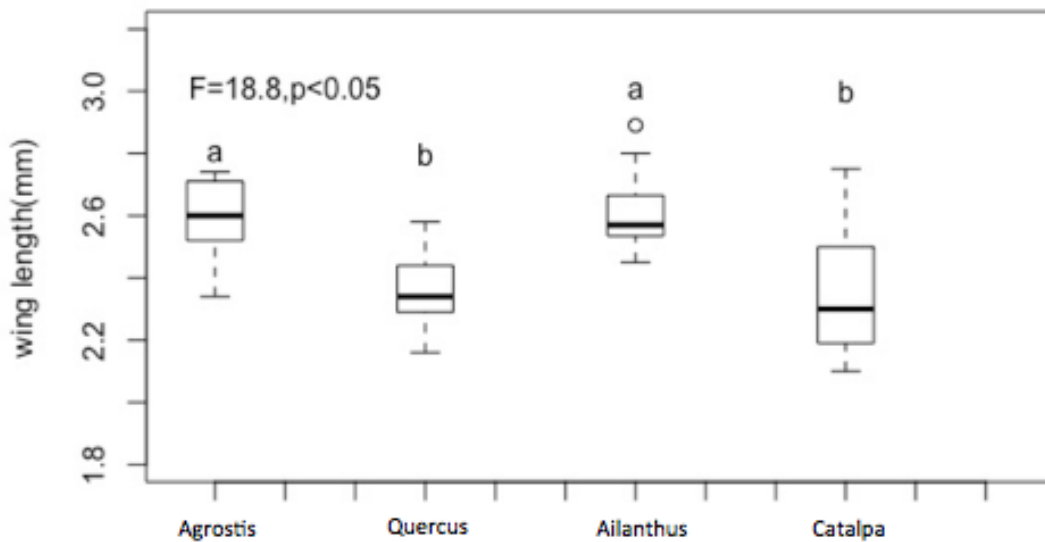
**FIGURE 2.** Average number of pupae developed from different treatments for *Cx. pipiens* in single species treatments. This figure shows a boxplot with distributions of data and the dark line representing the median. Letters represent pairwise comparisons done with a Bonferroni correction.



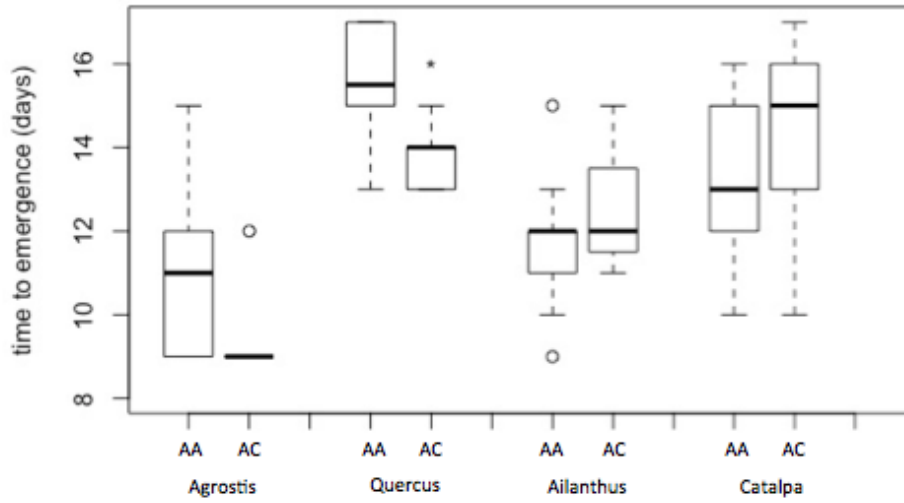
**FIGURE 3.** Average time to adult emergence for *Cx. pipiens* single species treatments. This figure shows a boxplot with distributions of data and the dark line representing the median. Letters represent pairwise comparisons done with a Bonferroni correction.



**FIGURE 4.** Average number of pupae developed from different treatments for *Ae. albopictus* in single species treatments. This figure shows a boxplot with distributions of data and the dark line representing the median. Letters represent pairwise comparisons done with a Bonferroni correction.



**FIGURE 5.** Average wing length across treatments for adult female *Ae. albopictus* in single species treatments. This figure shows a boxplot with distributions of data and the dark line representing the median. Letters represent pairwise comparisons done with a Bonferroni correction.



**FIGURE 6.** Average time to adult emergence for *Ae. albopictus* including those reared in the presence of solely *Ae. albopictus* and those reared in the presence of *Cx. pipiens*. This figure shows a boxplot with distributions of data and the dark line representing the median. Letters represent pairwise comparisons done with a Bonferroni correction. AA refers to replicate containers with solely *Ae. albopictus* and AC refers to replicate containers with *Ae. albopictus* and *Cx. pipiens*.