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Impact of Amur Honeysuckle (*Lonicera maackii*) Leachate on *Culex pipiens* Life History Attributes

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(*Lonicera maackii*) Leachate on *Culex
pipiens* Life History Attributes**



Honors Thesis

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Department: Biology

Advisors: Ryan McEwan, Ph.D. and M. Eric Benbow, Ph.D.

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Abstract

The purpose of this study was to look at the impact of a highly invasive plant, *Lonicera maackii*, on the primary vector of West Nile Virus in the Eastern United States, *Culex pipiens*. Three different assays using two different microcosms experiments were conducted to determine the rate of survivorship, pupation, emergence, and larval growth of mosquito larvae in native (*Platanus occidentalis* and *Acer saccharum*) and invasive (*L. maackii* leaf and flower) leachates. It was found that *L. maackii* has the potential to increase mosquito populations due to the fast decaying nature of its leaves and flowers which create an ideal leachate for mosquito larvae.



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Overview

1. Mosquito Biology

Mosquitoes are known to carry many pathogens that impact human health. One of these pathogens is West Nile Virus (WNV). In the summer of 2012, there was a large outbreak of WNV in the United States (Falco 2012). According to the Centers for Disease Control and Prevention (CDC), by December 2012, 286 people had died from WNV and 5,674 people were infected in 48 states (CDC 2012). In 2013, the numbers of cases of WNV decreased to 2,374 and deaths lowered to 114 (CDC 2014). However, WNV was still found in 48 states (CDC 2014). Figure 1 is from the United States Geologic Survey (USGS) and shows the location of cases of WNV in humans in 2013 (USGS 2014)

Mosquitoes can be found in wetlands, forests, parks, and even your own backyard (Cardo 2012; Chevillon 1995). One species of mosquito especially common in urban areas, and the primary vector of WNV and St. Louis Encephalitis in the Eastern United States is *Culex pipiens* (Crans 2012). *Culex pipiens* has been found to breed in storm drains, rain barrels, tires, and even polluted water (Crans 2012). Figure 2 shows the wide distribution of *C. pipiens* in North America based on suitable habitat (Ramanujan 2002).

Mosquitoes have a complex life cycle in which they spend part of their life cycle in water and part as flying aerial adults. Water is necessary for mosquitoes to breed. After a blood meal, female mosquitoes oviposit eggs on the surface of stagnant water (De Andrade 2010). Visual, chemical, and physical factors influence where these females will

oviposit (Bentley and Day 1989). Bacteria play an important role in *Culex pipiens* selection of an oviposition site (Rockett 1987). In aquatic systems, leaf leachate provides nutrients for bacterial growth in which mosquito larvae feed upon (Hall 1995; Giller & Malmqvist 1998; Gravgaard 2010; Merritt *et al.* 1992).

2. Amur honeysuckle (*Lonicera maackii*) as an environmental factor

One plant species that has been able to successfully invade areas where *C. pipiens* breeds and humans live is Amur honeysuckle (*Lonicera maackii*). Honeysuckle is an invasive species that was introduced to the United States in 1896 from Asia (Luken & Thieret 1996). Since then, it has successfully invaded 27 states (Luken and Thieret 1996). Figure 4 is a visual representation of the reported naturalized populations of honeysuckle in North America (USDA 2014). Amur honeysuckle has a long growing season, allelopathic chemicals, and other characteristics have allowed it to grow in a wide range of environments and out-compete native plant species (Gorchov and Trisel 2003; Hutchinson and Vankat 1997; McEwan *et al* 2009b).

There is little known about the effect of the chemicals found in honeysuckle leaves on aquatic organisms living in these infested riparian zones (McNeish *et al* 2012). Even less is known about the impact of these chemicals on organisms, like *C. pipiens*, that spend part of their life in the aquatic habitat and the other part of their lives in the terrestrial environment. As honeysuckle continues to spread, it is becoming a substantial source of nutrients for organisms living in aquatic habitats. Figure 5 illustrates how plant material is important in the overall dynamic of a riparian food web from Baxter *et al.*

(2005). The plant material provides sustenance for the bottom of the food web, which includes many macroinvertebrates (McNeish *et al.* 2012). Honeysuckle inputs organic material into the environment through its flowers, berries, and leaves. In the spring, honeysuckle inputs large amount of flowers into the environment. Most native ecosystems are not used to this large impulse of sugar rich organic material.

Honeysuckle leaves are thought to have allelopathic chemicals. These toxins are leached into the environment through a variety of ways. One such method is through rainfall. *L. maackii* has a dense canopy that intercepts a large amount of rainfall (McEwan *et al.* 2012). As rainwater passes through the leaves it can collect particles, which are washed directly off the leaves into the surrounding environment (McEwan *et al.* 2012). Additional, chemicals can be directly leached from the leaf surface as rainfall passes across it (McEwan *et al.* 2012). Some of this rain will get trapped on the leaves. Evaporation will occur and increase the concentration of the chemicals before finally making its way into the environment (McEwan *et al.* 2012).

Another way chemicals from honeysuckle enter the environment is through the natural senescing of honeysuckle leaves in late November early December. This large pulse of leaf material is much later than any other native plant (McEwan *et al.* 2009b). Honeysuckle leaves fall into many natural bodies of water and start to leach their chemicals.

Importance of the Study

If the chemicals found in honeysuckle leaves increase the survivorship, pupation rate, and growth of mosquito larvae, then honeysuckle could be associated with an increase in mosquito populations. This could have potentially hazardous repercussions on human health. Honeysuckle removal practices and programs would need to be instated, especially in urban areas, in order to protect human wellbeing.

On the other hand, the chemicals in honeysuckle could be found to reduce mosquito populations by creating less preferred breeding sites and decreasing survivorship and pupation rates. Honeysuckle could potentially be used for mosquito control. Using an unwanted invasive species to control an unwanted pest would be a possible forest management scenario. However, leaving honeysuckle unchecked and unmaintained in natural environments could potentially be detrimental to natural mosquito populations and the food web. The goal of this work was to investigate the role of honeysuckle invasion on mosquito populations, to differentiate between these two possibilities.

Scientific Introduction

Ecological systems are linked by material and energy that move across habitat boundaries (Polis, Anderson & Holt 1997). These cross-system subsidies are critical for ecosystem function, and can dictate food web structure (Baxter, Fausch & Saunders 2005). A quintessential example of this phenomenon is the linkage between aquatic

habitats and the surrounding terrestrial habitat (Bastviken *et al.* 2005; Giller & Malmqvist 1998). Indeed, vegetation inputs are crucial structuring elements in many aquatic systems and play a key role in supporting aquatic organisms (Baxter, Fausch & Saunders 2005; Gregory *et al.* 1991; McNeish *et al.* 2012). Feedbacks also exist between aquatic and terrestrial systems as aquatic organisms act as prey item and food resource, or through emergence as is the case with many aquatic insects (Baxter, Fausch & Saunders 2005; Polis, Anderson & Holt 1997).

Mosquitos are one of the most economically and culturally important insects on Earth. They transmit myriad diseases, are consistent pests, and inspire significant control efforts (Foote & Cook 1959, Merritt *et al.* 1992; Clements 2011). Mosquitos have an obligate aquatic life phase and, therefore, the ecology of water bodies is crucial to their survivorship (Gravgaard 2010; Merritt *et al.* 1992). One species of mosquito especially common in urban areas, and the primary vector of West Nile Virus and St. Louis Encephalitis in the Eastern United States is *Culex pipiens* (Crans 2012; Fonseca *et al.* 2004; Rudolf *et al.* 2013). *Culex pipiens* has been found to breed in stagnant pools of water in a range of natural and anthropogenic settings (Crans 2012). The biology of the small, stagnant, water bodies in which these organisms spend their aquatic life-phase is crucial to its population dynamics.

Lonicera maackii (Amur honeysuckle), is a non-native invasive plant that has successfully invaded areas where *C. pipiens* lives and breeds. Since its introduction from Asia ca. 1896, *L. maackii* has successfully invaded 27 states in eastern and central North America. *Lonicera maackii* has been able to successfully invade urban parks, riparian zones and also some less-disturbed, mature, second-growth forests (Luken 1988; Luken

& Thieret 1996; McNeish *et al.* 2012; Wilson *et al.* 2012). A suite of characteristics have been identified that give this species an advantage when it moves into habitats (Gorchov & Trisel 2003; Hutchinson & Vankat 1997; McEwan *et al.* 2009a, McEwan *et al.* 2009b). Many studies have found that *L. maackii* is capable of allelopathic suppression of native plants (Cipollini *et al.* 2008; Collier *et al.* 2002; Gorchov & Trisel 2003; Miller & Gorchov 2004) and evidence exists that suggests anti-insect herbivore properties (McEwan *et al.* 2009b; Lieurance and Cipollini 2013a; Lieurance and Cipollini 2013b).

We assessed the effects of *Lonicera maackii* invasion on *Culex pipiens* using a series of microcosm assays. We reported the results of (1) survivorship, (2) pupation, and (3) larval growth of *C. pipiens* in *L. maackii* flower and leaf leachates along with native leaf leachates. Since, *L. maackii* has been found to negatively influence terrestrial insects (Lieurance and Cipollini 2013a; McEwan *et al.* 2009) and has known allelopathic chemicals (Cipollini *et al.* 2008), we hypothesized that *L. maackii* would kill or greatly decrease the survivorship, pupation, emergence, and growth rates of *C. pipiens* larvae compared to native plants.

Methods

1. Collection of plant material

(a) *Lonicera maackii* Flower Collection

Naturally senesced *L. maackii* flowers were collected at five suburban forests near Dayton, Ohio on May 24, 2013. At each location two *L. maackii* shrubs approximately

2-3 meters tall were chosen haphazardly. A tarp was placed under each shrub, the shrub was shaken for approximately 1 minute, and the flowers that fell into the tarp were collected (Figure 7). The senesced flowers were transported to the lab in paper bags, and dried in a greenhouse for 72 hours and then stored in a dry, sealed cardboard box at room temperature. Flowers from each shrub ($n = 300$) were haphazardly chosen and divided into 6 samples of 50 flowers for calculation of ash free dry mass (AFDM) using standard procedures as follows: weighboats containing each of the 6 samples of 50 flowers were placed into a drying oven at 50 °C for 48 hours, then weighed. Then each boat was placed into an oven at 550°C to burn off any organic material and the boats were reweighed. The AFDM was calculated by subtracting the ash weight from the original weight of the flower. The Ash Free Dry Mass (AFDM) of the flowers across 5 locations was 0.4199 g for 50 flowers with an average, per flower, organic weight of 0.0084g.

(b) Leaf Collection

Sycamore (*Platanus occidentalis*) and sugar maple (*Acer saccharum*) leaves were collected on October 22, 2012 from Cox Arboretum MetroPark in Dayton, Ohio.

Lonicera maackii leaves were collected on December 15, 2012 from shrubs in a suburban setting in Spring Valley, Ohio. Leaves were collected directly off of several trees using latex gloves to avoid human oils being transferred to the leaves. The leaves chosen off the tree were the same color as the naturally senesced leaves on the ground surrounding the trees. The leaves were collected from multiple trees and bulked together into large bags for transport to the lab. The leaves were then placed on a tarp in a greenhouse to dry for

72 hours. The leaves were turned every 24 hours to aid in even drying and once drying was complete the leaves were stored in cardboard boxes, sealed, at room temperature.

2. Collection of *Culex pipiens* Eggs

Culex pipiens egg rafts used for all assays were collected in a suburban area of Spring Valley, OH. First, three 5 gallon plastic buckets were filled half way with tap water. A few handfuls of cat food was thrown into the water. The buckets were left to sitting in full sun to promote bacterial growth. The buckets were checked for egg rafts in the morning. Egg rafts were removed from the surface of the water using a paint brush. The eggs were placed into flat larval rearing trays. Eggs in Assay 1 and 2 were placed in treated tap water, and Assay 3 eggs were placed in pond water. The trays were placed into an incubator at 29°C. Within 24 hours, the eggs hatch into 1st instar larvae.

3. Leachates for Acute Toxicity and Larval Growth Tests

a. Assay 1 : effects on 2nd instar mosquito larvae in treated water

Culex pipiens eggs were collected July 17, 2013 and placed into trays with tap water treated with Crabworx Water Conditioner. This conditioner removes chlorine and heavy metals to make the water hospitable to mosquito larvae. The larval trays were

placed into an incubator at 29 °C. After the eggs hatched, the 1st instar larvae were fed crushed cat food for two days until they became 2nd instars.

Four leachates were made along with a tap water control that was treated with Crabworx Water Conditioner. Leachates were made from the leaves of *P. occidentalis*, and *A. saccharum* and both leaves and flowers from *L. maackii*. The leachates were started on July 17, 2013. For each leachate, 20 grams of dried leaves or flowers were placed in a 2 gallon plastic bucket then crushed for about a minute with a gloved hand until they were in fairly small pieces. One liter of treated water was poured over the leaves until submerged. An aeration system was added to the leachate which consisted of an air stone, clear tube, and Marina 50 air pump. Air was constantly pumped through the leachate and control to aerate the water to prevent the creation of an inhospitable environment for larvae. A lid was placed over each leachate to prevent the loss of water from evaporation. The leaves, flowers, and control water were left for three days at room temperature to leach. Then each leachate was strained through a 106 micrometer sieve into a 1 liter Nalgene bottle.

To account for evaporation, 100 mL of water treated with the Crabworx Water Conditioner were added to each leachate. Eight different leachates: 100% and 50% *L. maackii*, 100% and 50% *P. occidentalis*, 100% and 50% *A. saccharum*, 100% and 50% *L. maackii* flower and 1 control (tap water) were created for use in this assay. In each case, 50% concentrations were made by taking 250 mL of the main leachate and adding 250 mL of the treated tap water and then stirring until well mixed.

The toxicity assays were carried out by placing one 2nd instar *Culex pipiens* larva into a small cup with 20 mL of a leachate. Each of the 9 treatment had 20 replicates

(Figure 8). Replicates ($n = 180$) were mounted on boards and placed into the incubator at 29°C and covered with a clear acrylic glass to prevent evaporation. Every 12 hours the larvae were checked. The number dead, pupated, and emerged were recorded for 168 hours (7 days) ending on July 27, 2013. The dead larvae, pupae, and adults from throughout the trial were preserved in 90% ethanol. Cups with newly pupated larvae were covered with netting to prevent emerged adults from escaping into the incubator.

A parallel set of treatments were used to assess larval growth using the remaining leachate and larvae from the toxicity experiment. Initial size for the 2nd instar larvae was determined by measuring a random sampling of 20, 2nd instar larvae. Then, 20mL of each of the 9 treatments were placed into small cups along with five 2nd instar larvae. The cups were placed in the incubator at 29°C alongside the toxicity test. After 3 days, the larvae were removed from the leachate and preserved in 90% ethanol. The larvae bodies were measured from the top of the thorax to the beginning of the siphon to the nearest millimeter (Figure 9).

b. Assay2: effects on 1st instar mosquito larvae in treated water

Differences in larval development could lead to differences in response to the aquatic environment; therefore, we repeated the assay, using 1st (instead of 2nd) instar larvae along with some slight variations (explained below). The same 4 leachates as *Assay 1* were created for *Assay 2*. The leachates process began on September 3, 2013, with the straining of leachate through a 106 micrometer sieve three days later. One difference with *Assay 1* was the leachates created for this assay were placed back into the

original 2 gallon buckets with the aerators for an additional three days. This was done because no eggs were present in the collection buckets during the leachate process. *Culex pipiens* eggs were collected September 8, 2013 and placed in trays with tap water treated with Crabworx Water Conditioner. The larval trays were placed into an incubator at 29 °C until they hatched 24 hours later. On September 9, 2013 the leachates were placed into Nalgene bottles. 50% leachate concentrations were made following the procedures of *Assay 1*. Just as in *Assay 1*, 9 treatments were made: 8 leachates and one tap water control.

The toxicity trials were carried out by placing one, 1st instar *Culex pipiens* larva into a small cup with 20 mL of a leachate. Each of the 9 treatment had 20 replicates ($n = 180$) (Figure 8). The leachates were placed into the incubator at 29°C and covered with a clear acrylic glass. Every 12 hours the larvae were checked. The number dead, pupated, and emerged were recorded for 444 hours (19 days). Unlike *Assay 1* which was carried out for 168 hours, *Assay 2* was carried out until every larvae and adult died which took 20 days. During the experiment, the dead larvae, pupae, and adults were preserved in 90% ethanol. The trial was ended on September 29, 2013. Larval growth tests were run coinciding with the toxicity tests using 1st instar larvae instead of 2nd instar larvae as in *Assay 1*.

c. Assay 3: effects on 1st instar mosquito larvae in pond water

To test for potential effects of naturally occurring water, we replicated the assay using pond water instead of treated tap water. *Assay 3* was run at the same time as *Assay*

2. *Assay 3* was identical to *Assay 2* except *Assay 3* used pond water collected from Cox Arboretum as the control. Leachates were made using pond water and larvae were reared in the pond water. The trial lasted for 444 hours. Larval growth tests were also run as in *Assay 1 and 2*.

4. Data Analysis

Survivorship curves were created using the Kaplan-Meier method. This method calculates and portrays in the curves the portion of the population with a treatment still alive at a particular time (Graphpad 2014b). Curves were then compared using the log-ranked Mantel-Cox test. This is a non-parametric analysis which test the null hypothesis of no difference among the curves. All curves were first compared using this Mantel-Cox procedure then post-hoc comparisons were made between each curve and the control. To protect against a false positive result (type 1 error) the *P*-values were adjusted using a standard Bonferroni procedure where we set the *P*-value threshold at a value equal to the global value divided by the number of comparisons, which in this case yield a *P*-value threshold for significance of $P = 0.006$ (or $0.05/8$). Analysis of changes in larval growth, in each of the treatments was accomplished via a one-way ANOVA. Significant treatment effects were then tested via Dunnett's multiple comparisons test where each treatment was tested for difference against the control. All analyses were completed in Graphpad Prism version 6. (Graphpad 2014a)

Results

Assay 1: effects on 2nd instar mosquito larvae in treated water

The treatments had a significant overall effect on *C. pipiens* survivorship curves ($P < 0.001$) and the *L. maackii* leaf and flower treatments exhibited the highest survivorship after 180 hours (Figure 11). In fact, all *L. maackii* leachates had a survivorship over 60%. *Platanus occidentalis* had high survivorship until a step decline around 96 hours. The tap water control had an intermediate, final survivorship dipping just below 50%. All native leachates had lower final survivorships than the control, with 100% *A. saccharum* having the lowest survivorship. All larvae exposed to 100% *A. saccharum* died within 156 hours. The 50% concentration of the native leachates had higher final survivorships than their 100% native counterparts (Figure 11).

Across the entire time course of Assay 1 (168 hrs.), the only larvae that pupated were those exposed to *L. maackii* plant material. There was 100% pupation in the larvae that survived through the trial for the 50% *L. maackii* flower treatment, while the higher concentration of flower leachate yielded 75% pupation (data not shown). Pupation rate in the lower concentration leachate from *L. maackii* leaves was 95% pupation, while that of the high concentration was 65%. Pupation for the control and all native leaf treatments was 0%.

Larvae exposed to *L. maackii* material exhibited more positive growth than other treatments (Figure 12). There was a highly significant treatment effect ($F = 19.7$, $P < 0.001$), and only larvae exposed to material from *L. maackii* exhibited growth trends that

were significantly different (all $P < 0.001$) than the control (which was near zero). The most significant, positive change in larval size was seen in the *L. maackii* flower leachates (Figure 12). *Acer saccharum* leachate resulted in the most negative larval growth. The tap water control, also had an overall decrease in body size. Native *P. occidentalis* had positive larval growth, but was not significantly different than the control.

Assay 2: effects on 1st instar mosquito larvae in treated water

Culex pipiens 1st instar larvae exhibited poorer survivorship in all treatments, in relation to the control (Figure 13). The treatments had a statistically significant effect on survivorship ($P < 0.0001$), and there was a statistically significant difference between each curve and the control (Table 1). Larvae exposed to *L. maackii* flower leachate was the only treatment to exhibit higher survivorship than the control, and that was only for one sampling that took place at 216 hours. After this point, there was a precipitous decline in survivorship. Observations at the time indicated that adult mosquitoes started dying off due to lack of food. This same decrease can be seen in the high concentration flower leachate. The tap water control had the highest overall survivorship throughout the 444 hour assay, with some larvae lasting until the very last day of the experiment. 50% *P. occidentalis* had a survivorship higher than all other native leachates while larvae exposed to the higher concentration *P. occidentalis* treatment exhibited relative poor survivorship and all larvae died within 72 hours. *Acer saccharum* 100% experienced a very similar die off, with all larvae perishing within 36 hours of exposure. The *L. maackii*

leaf leachates had an intermediate survivorship which was higher than the natives (besides 50% *P. occidentalis*) and below the control and *L. maackii* flower leachates.

The only larvae that pupated in Assay 2 (444 hours) were those exposed to *L. maackii* flower material (data not shown). The highest pupation rate of 95% was seen in the 50% *L. maackii* flower leachate while the 100% *L. maackii* had a pupation rate of 65%. No pupation occurred in all of the native leaf treatments, *L. maackii* leaf leachates, and the control.

Leachates had a highly significant overall effect on larval growth ($F = 107.4$, $P < 0.0001$) (Figure 14). *Culex pipiens* larvae exposed to *L. maackii* flower leachate in Assay 2 showed a highly significant ($P < 0.0001$) change in body length as compared to the control (Figure 14). Only larvae exposed to flowers from *L. maackii* and a low concentration of *P. occidentalis* exhibited growth trends that were significantly different (all $P < 0.01$) than the control. All treatments increased in body length over three days. *A. saccharum* grew the least of all of the leachates.

Assay 3: effects on 1st instar mosquito larvae in pond water

As was seen in Assay 2, there was a significant overall effect ($P < 0.0001$) of treatment on *C. pipiens* survival (Figure 15). No leachate treatment resulted in survivorship that was significantly higher than the control. 1st instar *C. pipiens* apparently had a very high survivorship in the *L. maackii* leachate until around 216 hours when mass adult death led to a steep decline. The 100% *L. maackii* flower leachate also numerically exceeded the control late in the assay; however, those larvae exhibited a crash at 216 hours that observationally seemed to be a result of bacterial growth in the experimental

units. Some larvae in the 50% *P. occidentalis* treatment survived until the last day of the experiment. *A. saccharum* leachates had the lowest survivorship; the high *A. saccharum* leachate crashed after 108 hours and the low concentration crashed around 132 hours.

Over the 444 hour time course of the experiment, the only larvae that pupated were those exposed to *L. maackii* flower leachate. The low concentration of *L. maackii* flower had the highest pupation rate of 95% while the 100% *L. maackii* had a pupation rate of 60%. These values were very similar to Assay 2.

In assay 3, leachate type had a significant overall effect ($F = 71.24$, $P < 0.0001$) on larval growth, and the only larvae that had a statistically discernable departure from the control were those treated with *L. maackii* materials (Figure 6). *Lonicera maackii* flower leachates had a significant positive impact on larval growth over 3 days as compared to the pond water control ($P < 0.001$). *Culex pipiens* exposed to *L. maackii* leaf leachates grew significantly more than the control, but nowhere near as much as the flower leachate. The control, native, and non-native leachates increased in body length over Assay 3.

Discussion

Invasive species often have unique traits, and also create dense stands, and therefore have the potential for significantly altering ecosystem structure and function (Arthur *et al.* 2012; Loomis & Cameron 2013). Invasive plants depositing significant quantities of leaf material with unique traits into ecosystems is known to drive shifts in ecosystem processes (Henkin *et al.* 2013) and impact aquatic food webs (Baxter, Fausch & Saunders 2005; Dudgeon 1999; McNeish *et al.* 2012). *Lonicera maackii* has

successfully invaded riparian forests (McNeish *et al.* 2012), fragmented forests (Luken & Goessling 1995), and secondary-growth forests all across eastern North America (Arthur *et al.* 2012). Once *L. maackii* establishes in an area, it forms a dense monoculture which decreases native plant species abundance and growth (Loomis & Cameron 2013). Organic material from *L. maackii* has the potential to disrupt and change native terrestrial and aquatic ecosystems.

All mosquitoes have a complex life cycle in which water plays an integral part. Water is necessary for mosquitoes to breed and for young to develop. In aquatic systems, leaf leachate provides nutrients for bacterial growth in which mosquito larvae feed upon (Hall 1995; Giller & Malmqvist 1998; Hazard *et al.* 1967; Gravgaard 2010; Merritt *et al.* 1992). Since bacteria are important for larval development, female *C. pipiens* are attracted to oviposition sites with high bacterial content (Reiskind *et al.* 2009; Rockett 1987; Suleman and Shirin 1981). The composition of the leaf litter, especially the leaf quantity and species, impacts mosquito growth (Carpenter 1982; Fish & Carpenter 1982). Mosquito larvae have been shown to have higher survivorship and larval growth in aquatic habitats containing rapidly decaying leaf species compared to slowly decaying leaf species (Fish & Carpenter 1982; Yee & Juliano 2006).

Lonicera maackii leaves have been shown to decay faster compared to native leaf litter (Arthur *et al.* 2012; Blair & Stowasswer 2009; McNeish *et al.* 2012). These fast decaying leaves have the potential to create an ideal habitat for mosquito larvae. Leaves are not the only organic material *L. maackii* inputs into the environment. In the spring, *L. maackii* inputs large amount of flowers into the environment. In most North American ecosystems this large impulse of sugar rich organic material is a unique phenomenon.

The primary food source of both male and female mosquitos is floral nectar (Vinogradova 2000). *Lonicera maackii* has the potential to provide an extremely large food source to adult *C. pipiens* in early spring when there was no such food source available before the *L. maackii* invasion. Not only are the flowers a food source to adults, but when senesced flowers fall into the aquatic environments they create a highly favorable leachate for larval growth. The results of this study show how favorable *L. maackii* flower leachates are to the growth and development of *C. pipiens* larvae. A similar study done by Chen *et al.* (2013) showed shrimp survivorship and growth increased when flowers from *Lonicera japonica*, a close cousin of *L. maackii*, were introduced into their environment. *Lonicera maackii* flowers are available at the right time to be utilized by *Culex pipiens* mosquitoes to potential increase in population early in the season. This may have hazardous repercussions on human health, if populations start high in the spring and get larger during prime mosquito time in summer.

Lonicera maackii has shown its ability to successfully invade areas in which humans and *C. pipiens* live and breed. This study showed *L. maackii* plant material has the potential to increase mosquito survivorship, pupation, and larval growth rates due to the fast decaying nature of *L. maackii*'s leaves and flowers. Future research is needed to test the results of this microcosm analysis in the field. Further advances could be made by creating leachates with a wide variety of leaf compositions to better understand what happens in natural conditions. Finally, it is possible that some co-evolutionary relationships have already begun forming between *L. maackii* and mosquitos and future work that tested *L. maackii* impacts on mosquito species that have never been exposed to this invasive species could be illuminating.

Tables

Assay 1	Chi Square	P value	Significant?	
ALL	72.90	<0.0001	yes	****
100% <i>L. maackii</i>	5.027	0.025	yes	*
50% <i>L. maackii</i>	2.436	0.1186	no	
100% <i>P. occidentalis</i>	2.274	0.1316	no	
50% <i>P. occidentalis</i>	0.2172	0.6412	no	
100% <i>A. saccharum</i>	10.01	0.0016	yes	**
50% <i>A. saccharum</i>	0.5001	0.4794	no	
100% <i>L. maackii</i> flower	1.817	0.1777	no	
50% <i>L. maackii</i> flower	4.736	0.0295	yes	*

Assay 2	Chi Square	P value	Significant?	
ALL	179.5	<0.0001	yes	****
100% <i>L. maackii</i>	32.52	<0.0001	yes	****
50% <i>L. maackii</i>	30.03	<0.0001	yes	****
100% <i>P. occidentalis</i>	37.17	<0.0001	yes	****
50% <i>P. occidentalis</i>	17.26	<0.0001	yes	****
100% <i>A. saccharum</i>	39.15	<0.0001	yes	****
50% <i>A. saccharum</i>	37.2	<0.0001	yes	****
100% <i>L. maackii</i> flower	20.84	<0.0001	yes	****
50% <i>L. maackii</i> flower	18.77	<0.0001	yes	****

Assay 3	Chi Square	P value	Significant?	
ALL	129.8	<0.0001	yes	****
100% <i>L. maackii</i>	18.19	<0.0001	yes	****
50% <i>L. maackii</i>	10.22	0.0014	yes	**
100% <i>P. occidentalis</i>	2.745	0.0975	no	
50% <i>P. occidentalis</i>	1.266	0.2605	no	
100% <i>A. saccharum</i>	35.61	<0.0001	yes	****
50% <i>A. saccharum</i>	24.75	<0.0001	yes	****
100% <i>L. maackii</i> flower	2.544	0.1107	no	
50% <i>L. maackii</i> flower	0.6658	0.4145	no	

Table 1. The survivorship curve analysis results using the Log-rank (Mantel-Cox) test for Assay 1-3. In each Assay, all survivorships curves of the 8 leachates were compared to each other (ALL) and to the control for significance.

Assay 1	q	Mean Diff.	95% CI of diff.	Significant?	
100% <i>L. maackii</i>	4.989	-0.158	-0.2464 to -0.06957	Yes	***
50% <i>L. maackii</i>	4.8	-0.152	-0.2404 to -0.06357	Yes	***
100% <i>P. occidentalis</i>	2.147	-0.068	-0.1564 to 0.02043	No	ns
50% <i>P. occidentalis</i>	1.137	-0.036	-0.1244 to 0.05243	No	ns
100% <i>A. saccharum</i>	1.905	-0.064	-0.1578 to 0.02980	No	ns
50% <i>A. saccharum</i>	0.4421	0.014	-0.07443 to 0.1024	No	ns
100% <i>L. maackii</i> flower	7.768	-0.246	-0.3344 to -0.1576	Yes	****
50% <i>L. maackii</i> flower	7.957	-0.252	-0.3404 to -0.1636	Yes	****
Assay 2	q	Mean Diff.	95% CI of diff.	Significant?	
100% <i>L. maackii</i>	1.826	-0.037	-0.09310 to 0.01910	No	ns
50% <i>L. maackii</i>	2.346	-0.04533	-0.09882 to 0.008158	No	ns
100% <i>P. occidentalis</i>	1.826	-0.037	-0.09310 to 0.01910	No	ns
50% <i>P. occidentalis</i>	3.985	-0.077	-0.1305 to -0.02351	Yes	**
100% <i>A. saccharum</i>	1.147	0.02217	-0.03132 to 0.07566	No	ns
50% <i>A. saccharum</i>	0.2467	0.005	-0.05110 to 0.06110	No	ns
100% <i>L. maackii</i> flower	18.56	-0.3587	-0.4122 to -0.3052	Yes	****
50% <i>L. maackii</i> flower	16.66	-0.322	-0.3755 to -0.2685	Yes	****
Assay 3	q	Mean Diff.	95% CI of diff.	Significant?	
100% <i>L. maackii</i>	3.214	-0.07667	-0.1425 to -0.01088	Yes	*
50% <i>L. maackii</i>	3.174	-0.07083	-0.1324 to -0.009294	Yes	*
100% <i>P. occidentalis</i>	2.445	-0.05833	-0.1241 to 0.007455	No	ns
50% <i>P. occidentalis</i>	1.677	-0.04	-0.1058 to 0.02579	No	ns
100% <i>A. saccharum</i>	1.386	0.03467	-0.03433 to 0.1037	No	ns
50% <i>A. saccharum</i>	1.537	0.03667	-0.02912 to 0.1025	No	ns
100% <i>L. maackii</i> flower	13.55	-0.3233	-0.3891 to -0.2575	Yes	****
50% <i>L. maackii</i> flower	14.6	-0.3483	-0.4141 to -0.2825	Yes	****

Table 2. The larval growth analysis results using Dunnett's multiple comparison test for Assay 1-3. In each Assay, all of the 8 leachates were compared to the control for significance.

Figures

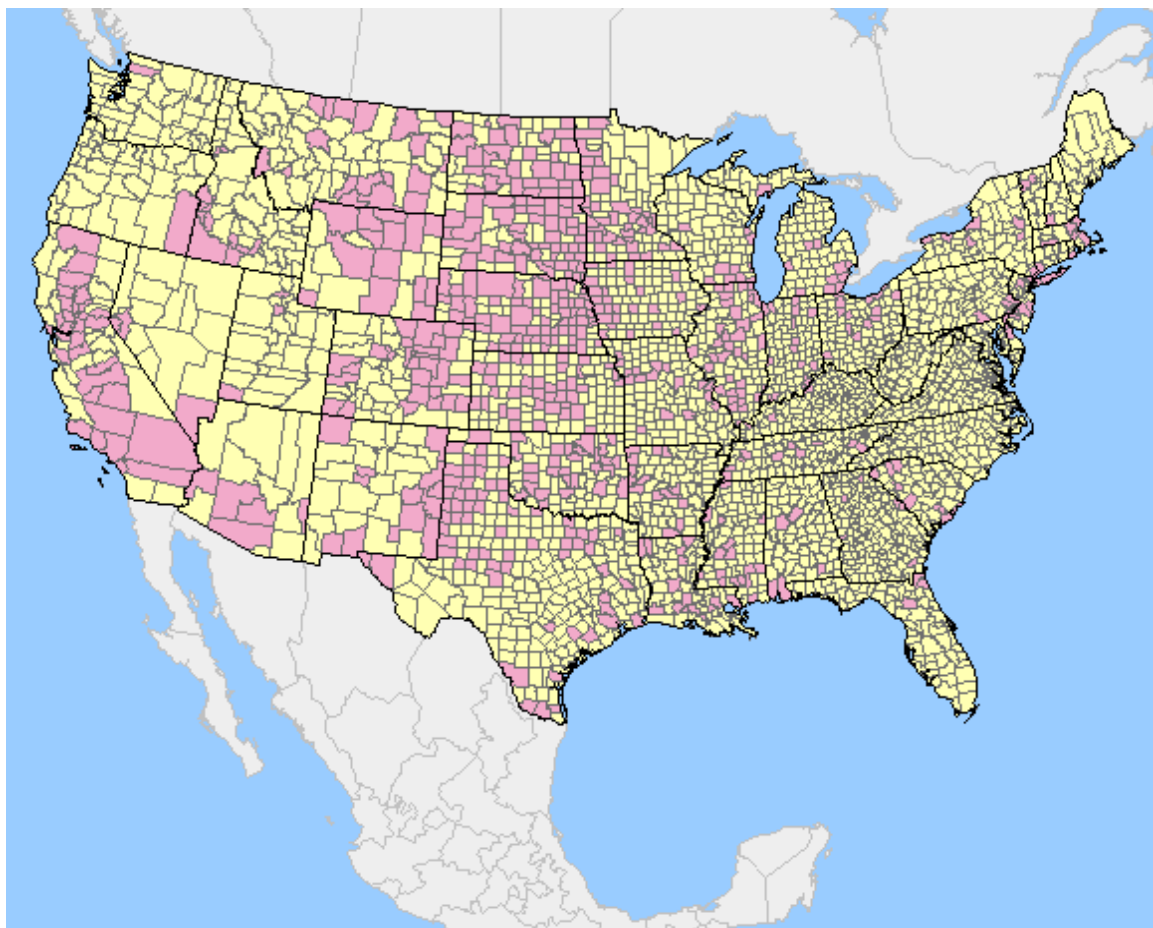


Figure 1. The pink counties in the United States have reported human cases of West Nile Virus in 2013. (USGS 2014)



Figure 2. The distribution of *Culex pipiens* in North America in 2002. The red area show the satellite image of the most favorable conditions for *C. pipiens*, the yellow outline is the known distribution of *C. pipiens* by other available data. (Ramanujan 2002)

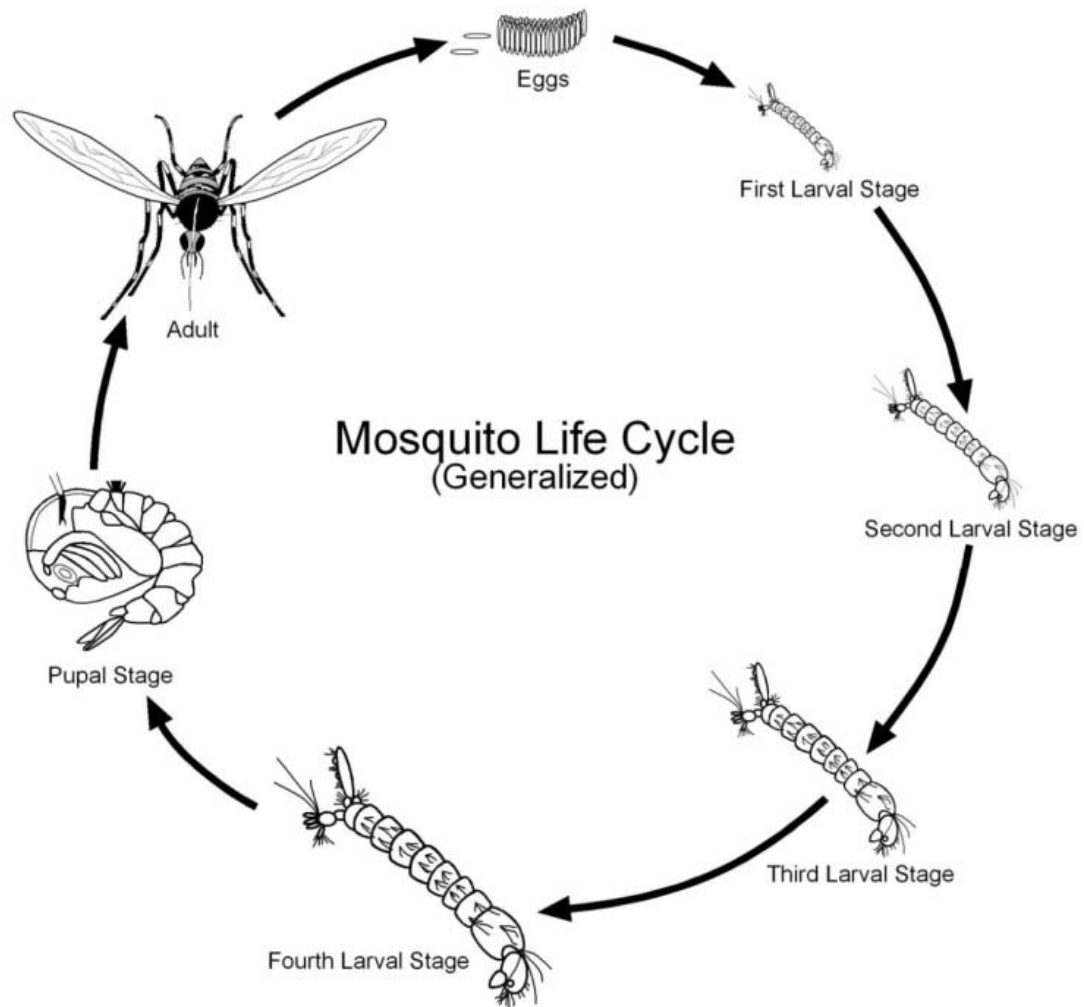


Figure 3. A generalized diagram showing the life cycle of a mosquito from egg to adult.

(Purdue Entomology *et al* 2011)

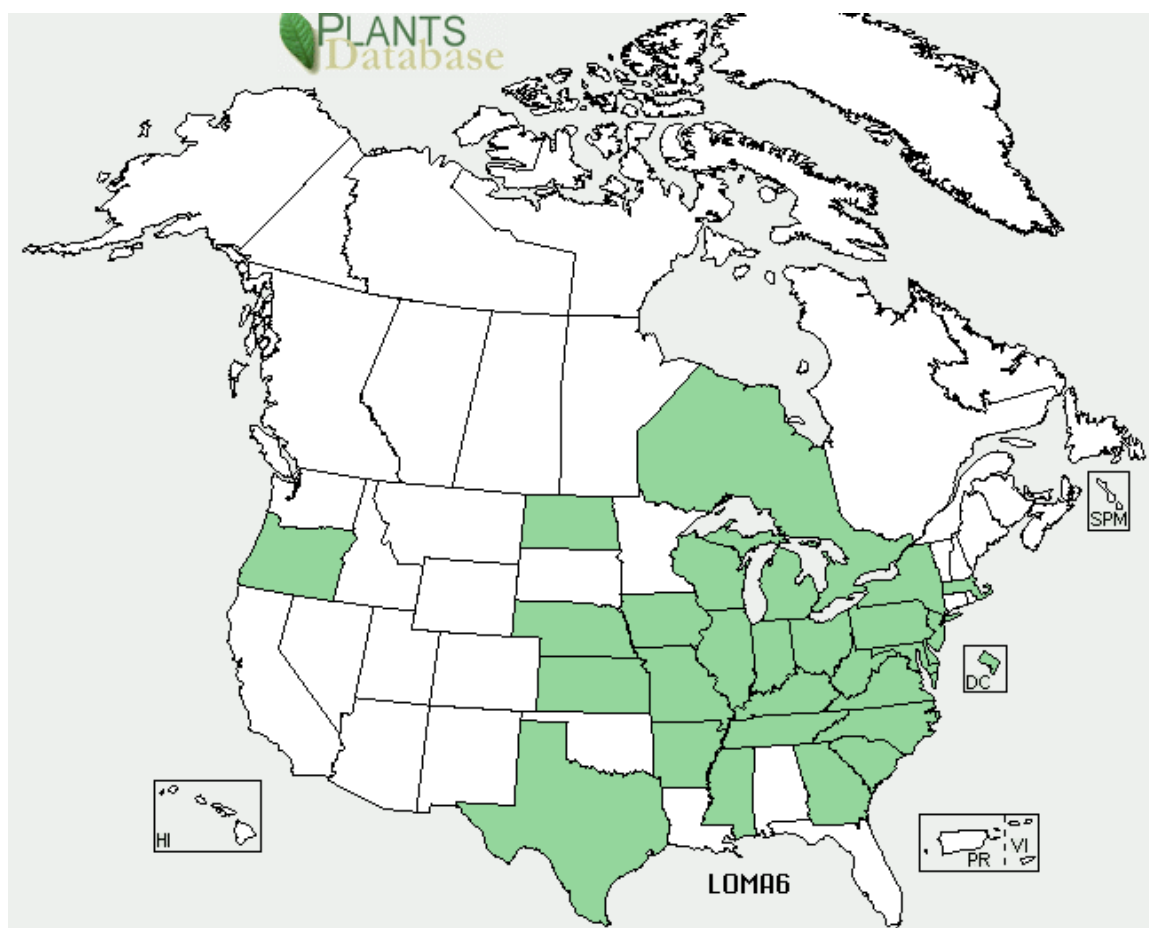


Figure 4. Distribution of *Lonicera maackii* in North America. The green areas show the location of reported naturalized populations of *L. maackii*. Not all populations have been reported. (USDA 2014)

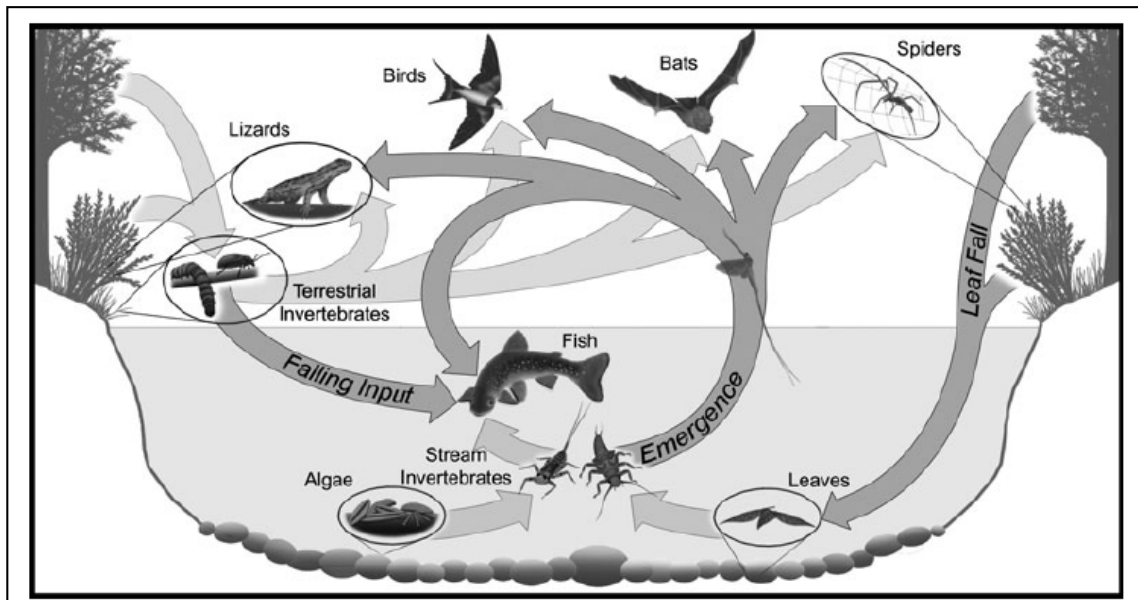


Figure 5. A generalized diagram showing the direct and indirect effects invertebrate prey and inputs of plant material have in stream and riparian food webs. (Baxter, Fausch & Saunders 2005)



Figure 6. The flower and fruit of *Lonicera maackii* taken by Dr. Ryan McEwan.



Figure 7. Picture of 5 gallon bucket in Spring Valley Ohio before cat food added (upper left corner). The middle image shows the bacterial growth on the surface of the water. The bottom left image shows a *Culex pipiens* egg raft.



Figure 8. Tarp placed under *L. maackii* in Spring Valley, Ohio.

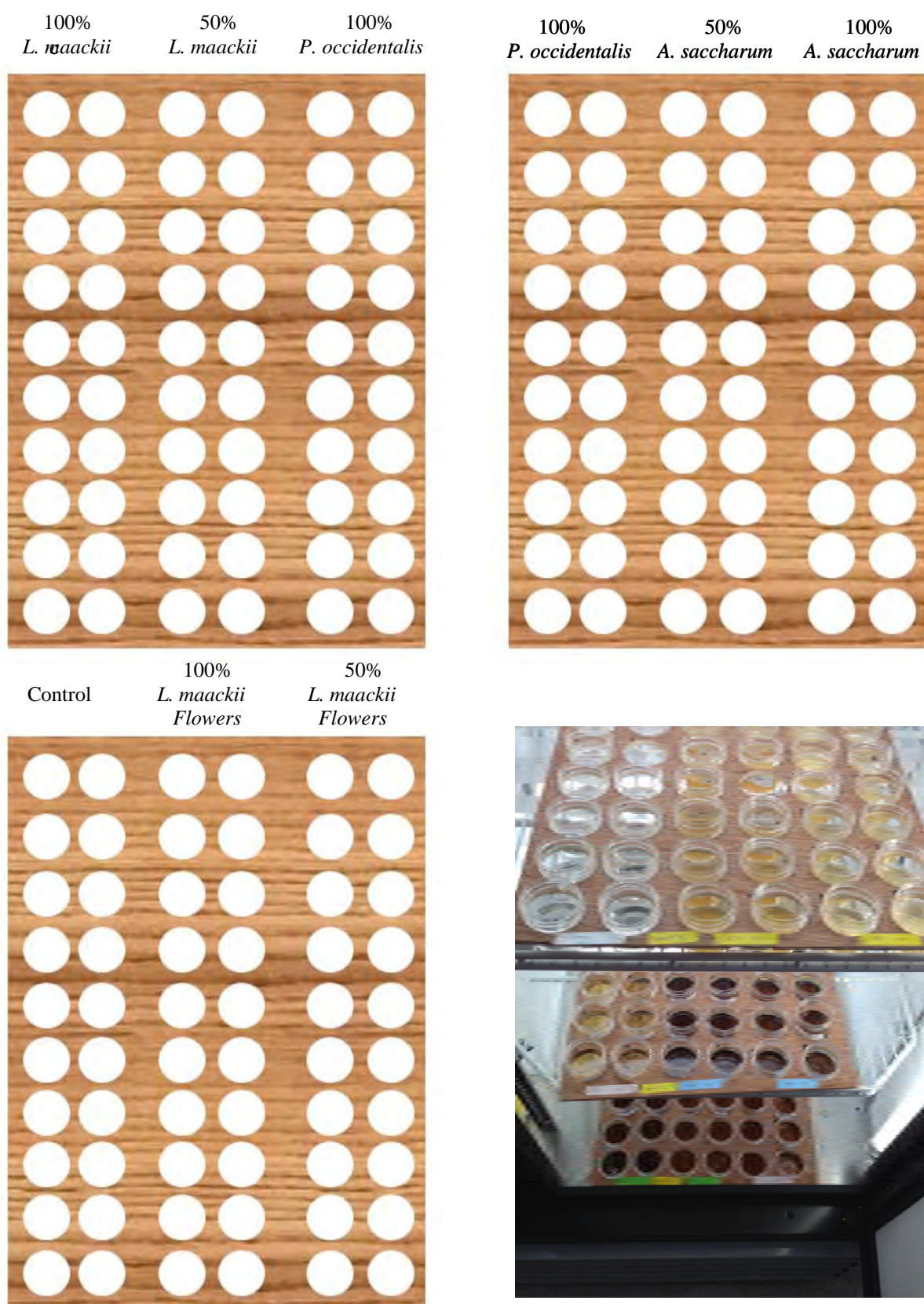


Figure 9. The generalized set up of the toxicity tests for Assays 1-3.

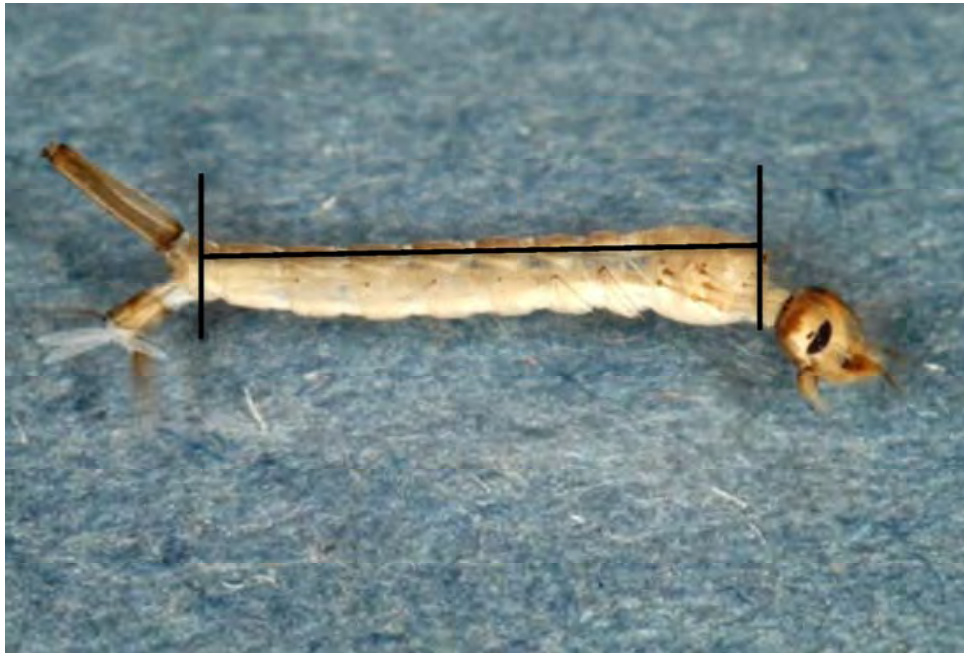


Figure 10. The top picture is the setup of Assay 1 larval growth, the bottom represents the measurements taken of the larvae from the top of the thorax to the beginning of the air tube.

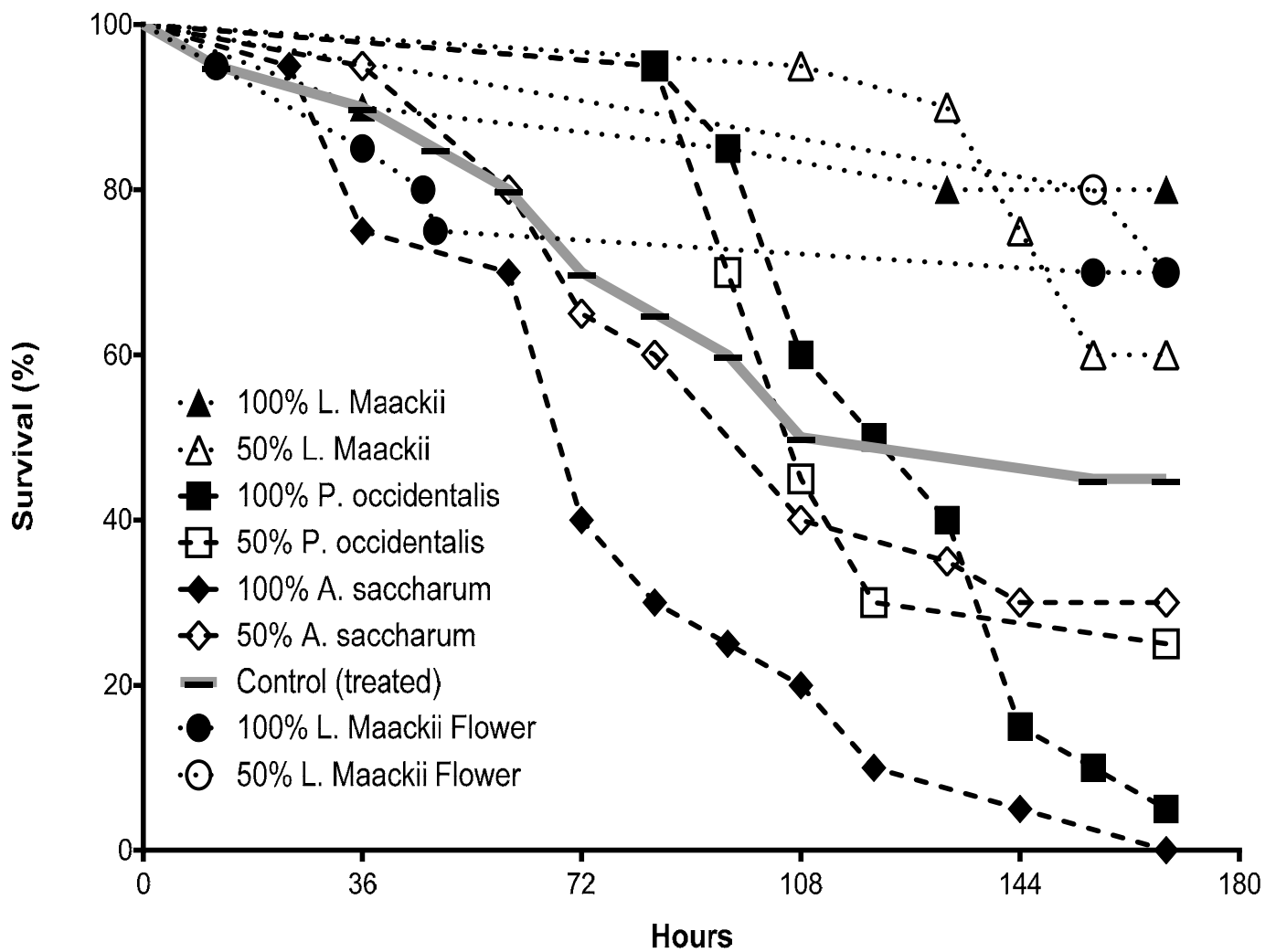


Figure 11. Percent survivorship of 2nd instar *Culex pipiens* larvae in Assay 1 in various leachates and a treated water control over 168 hours at 29 °C.

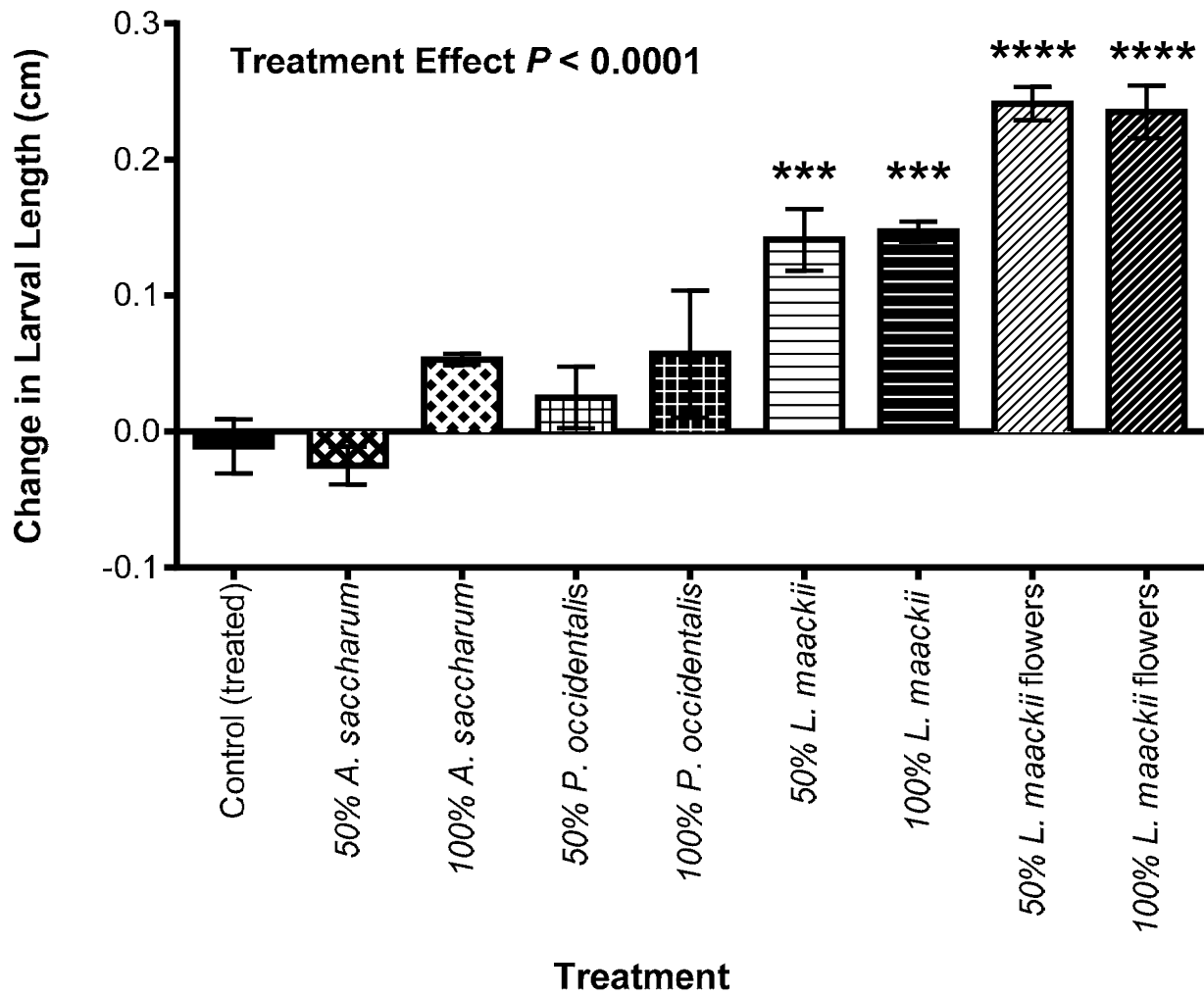


Figure 12. The average change in body length of 2nd instar *Culex pipiens* larvae in Assay 1 after three days of exposure to various leachates and a tap water control.

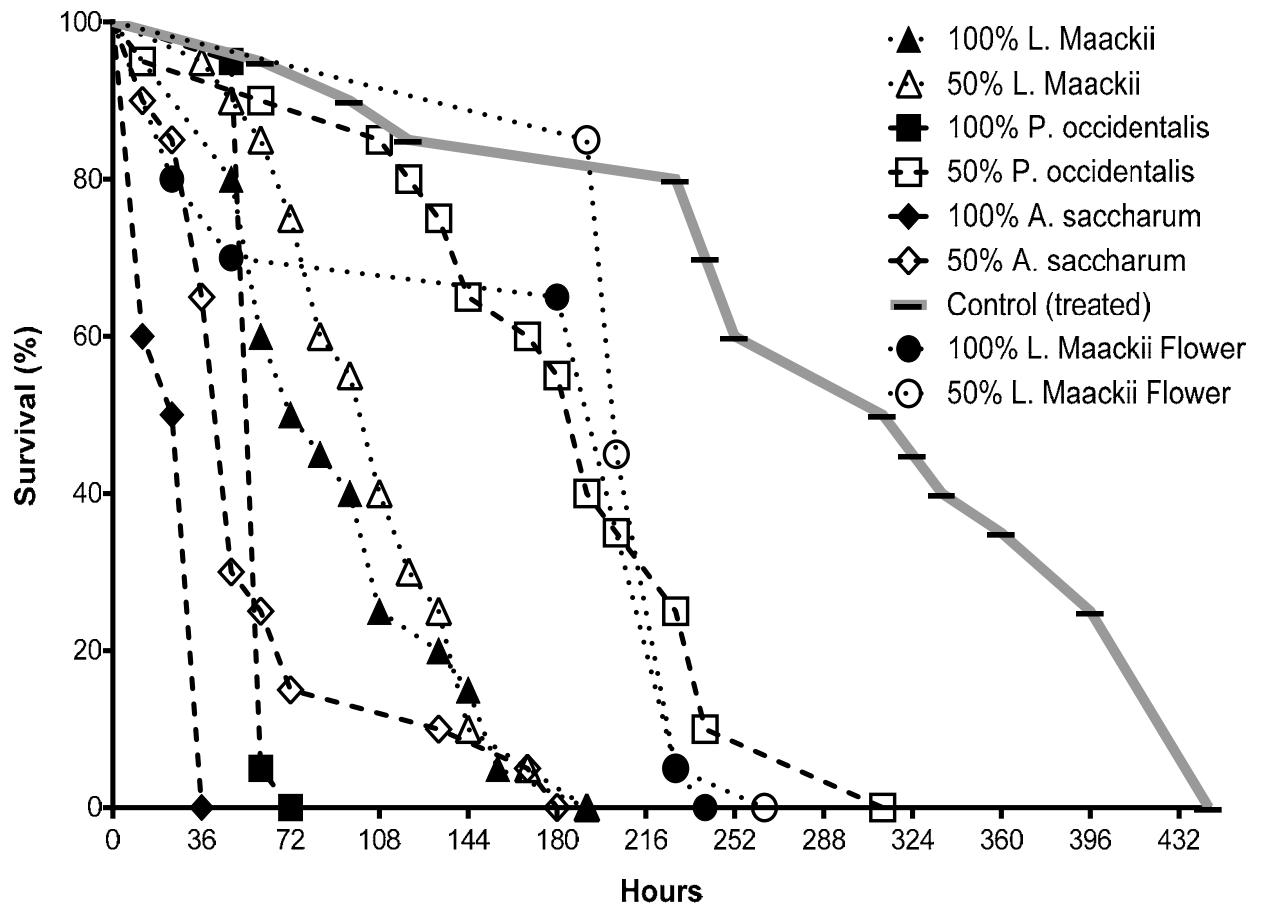


Figure 13. Percent survivorship of 1st instar *Culex pipiens* larvae in Assay 2 in various leachates and a treated water control over 444 hours at 29 °C.

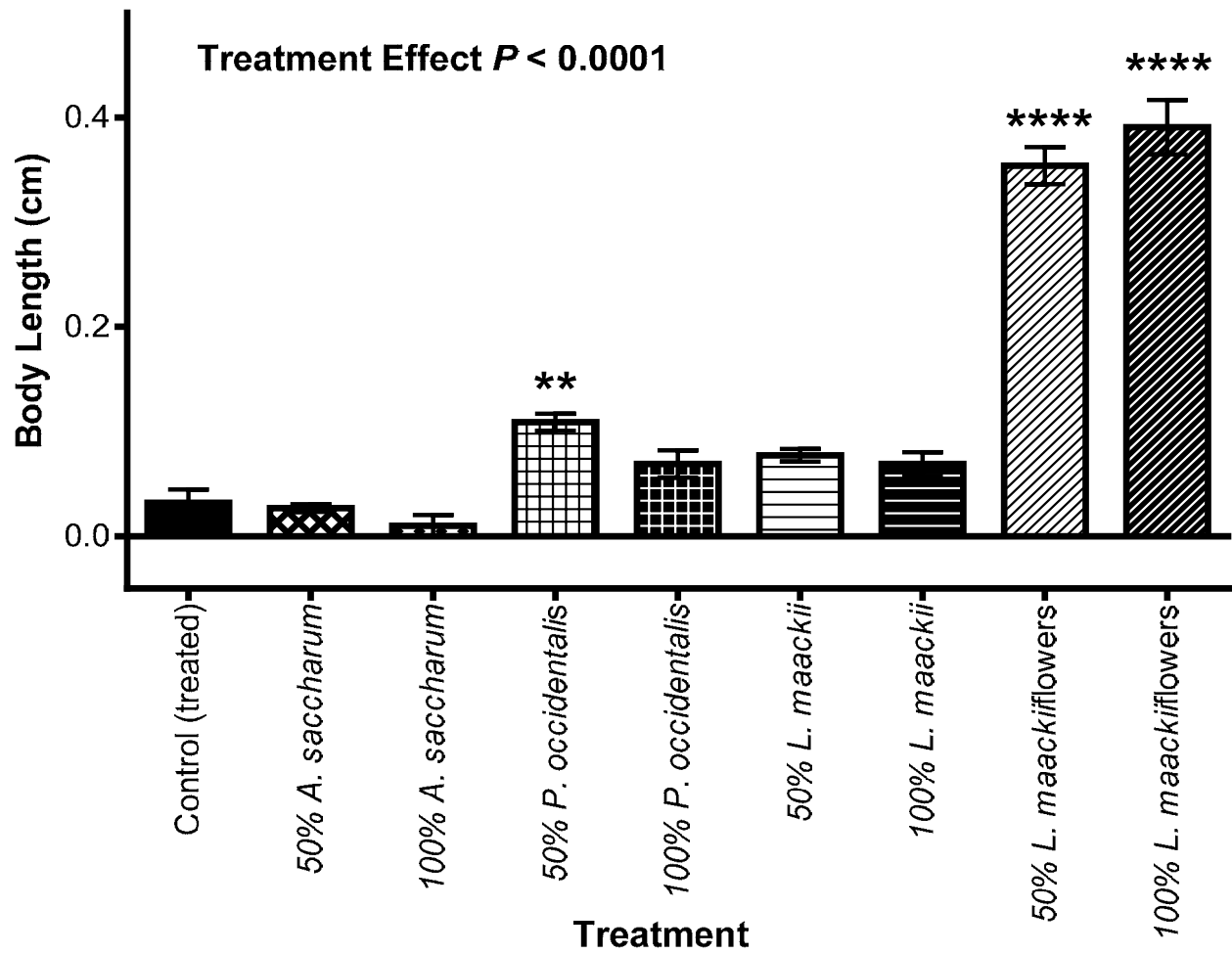


Figure 14. The average change in body length of 1st instar *Culex pipiens* larvae in Assay 2 after three days of exposure to various leachates and a tap water control.

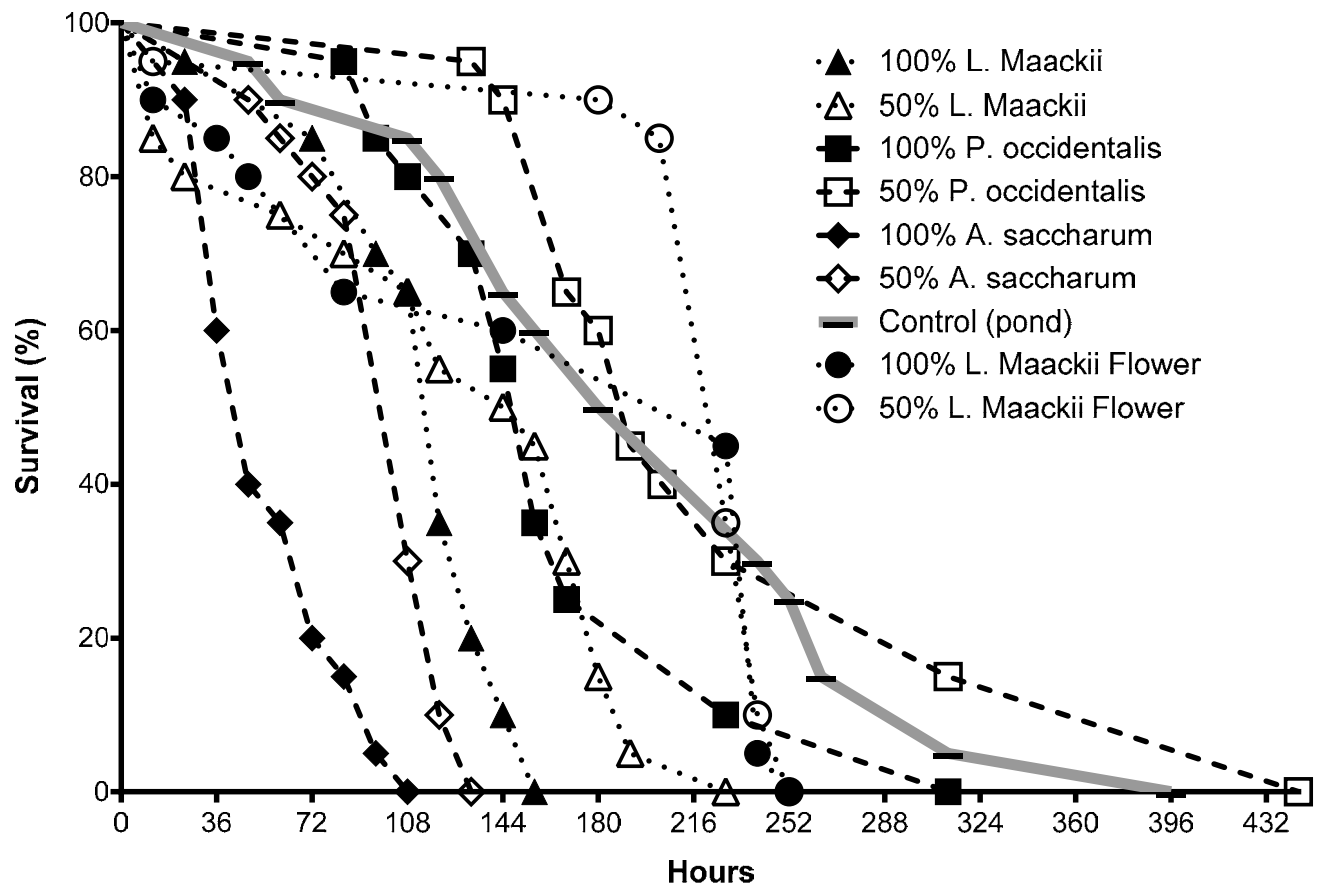


Figure 15. Percent survivorship of 1st instar *Culex pipiens* larvae in Assay 3 in various leachates and a treated water control over 444 hours at 29 °C.

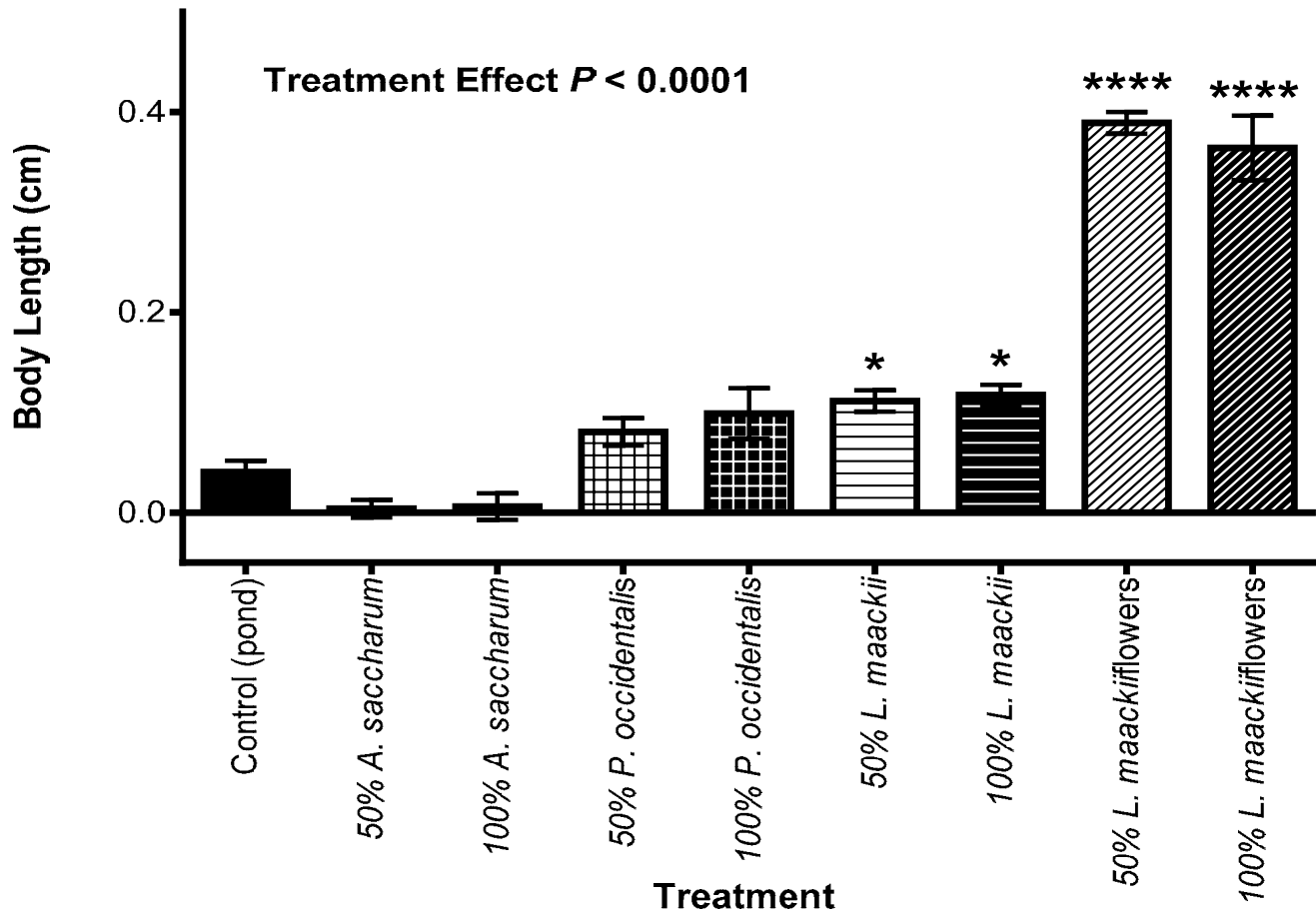


Figure 16. The average change in body length of 1st instar *Culex pipiens* larvae in Assay 3 after three days of exposure to various leachates and a pond water control.

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