Influence of Leaf Detritus Type on Production and Longevity of Container-Breeding Mosquitoes

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ABSTRACT Freshwater ecosystems are positioned at low levels in the landscape and receive large inputs of diverse plant-based detritus, a major source of energy for consumers in aquatic ecosystems. We conducted field experiments in Urbana, IL to determine the independent and combined effects of leaves of common tree species including the northern red oak (Quercus rubra L.), sugar maple (Acer saccharum Marshall), and common hackberry (Celtis occidentalis L.) on the performance of container-dwelling mosquitoes, especially Culex restuans Theobald (Diptera: Culicidae). We tested the hypothesis that leaf species have asymmetric effects on adult mosquito production and longevity. Hackberry followed by combined leaf treatments and maple produced the greatest number of pupae, whereas oak leaves produced the fewest. Leaf treatments had no significant effects on adult female sizes but female longevity was significantly lower in oak leaf treatments compared with the other leaf treatments. These findings support the hypothesis that leaf species identity influences the performance of container-dwelling mosquitoes with potential consequences for the transmission of infectious diseases.

KEY WORDS Detritus, mosquitoes, longevity, bottom-up effects

A fundamental principle of food web theory is that populations of organisms often are regulated by a combination of bottom-up (e.g., resource availability) and top-down (e.g., predation) forces (Power 1992). Although the relative contribution of each has been the subject of much debate (Hastings et al. 1960, Murdoch 1966), it is becoming increasingly apparent that the contributions of such forces rely upon myriad factors including both within and between trophic level diversity, temporal and spatial heterogeneity, and fundamental biogeographic factors that regulate energy inputs at the ecosystem scale (Hunter and Price 1992). Because of such complexity, the contribution of bottom-up and top-down forces remains an invaluable framework for understanding the factors that determine the population dynamics of organisms.

The importance of top-down and bottom-up forces may become particularly pronounced in aquatic systems, where interactions between trophic levels may be dominated by one or a few species (Strong 1992). These habitats often are used by oviparous organisms with complex life cycles involving both aquatic and terrestrial life-stages, the reproductive success of which is partly dependent on the ability of adult females to discriminate among potential oviposition sites on the basis of expected hatching success and larval performance. The choice of oviposition sites is particularly important for organisms with complex life cycles because immature stages are unable to migrate to other habitats to escape harsh environmental conditions in their current habitats. Accordingly, theory predicts that gravid females will maximize their fitness by depositing their eggs in high quality habitats (Jainike 1978). This theory assumes that females can discriminate between suitable and unsuitable oviposition sites based on physical and chemical cues such as conspecific density, presence of competitors and natural enemies, temperature, age of the habitat, vegetation structure, degree of permanency, and the quality and quantity of food resources (Ressetarits and Wilbur 1989, Clements 1999, Reiskind and Wilson 2004). This hypothesis mostly has been tested for insect herbivores with some studies but not others, demonstrating a strong relationship between oviposition preference and offspring performance (reviewed by Mayhew 1997).

Fallen plant parts, especially senescent leaves, are the major resource base for many aquatic habitats occurring below forest canopy cover such as tree holes and artificial containers. These habitats are occupied mainly by detritivorous invertebrates that feed directly on decomposing leaf litter or the heterotrophic microbial communities supported by these detritus (Walker et al. 1991, Merritt et al. 1992). By breaking down the detritus through their metabolism, heterotrophic microbial communities release the nutrients trapped in the leaf matrix, thereby increasing availability of detritus-based nutrients to higher trophic levels (Kaufman and Walker 2006). The rate of plant
litter decomposition is influenced both by external (e.g., temperature and oxygen) and internal (e.g., leaf quality) factors (Webster and Benfield 1986). In general, however, leaf species that are rich in nitrogen-based secondary compounds decompose more rapidly than those with low levels of nitrogen and high levels of carbon-based secondary compounds (Peterson and Cummins 1974). Laboratory studies have found a strong positive correlation between the rate of leaf litter decomposition and performance of aquatic detritivores (Fish and Carpenter 1982, Murrell and Juliano 2008), stimulating the hypothesis that rapidly decomposing leaf litter is more immediately nutritious and more capable of promoting survival and growth of macroinvertebrate communities than slowly decomposing leaf litter.

Mosquito larvae are among the most common aquatic macroinvertebrates that inhabit natural and artificial container habitats. Except for the predatory species, these larvae obtain food by browsing the microbial communities associated with detritus and container wall surfaces or by filter feeding on small detritus particles from the water column (Walker et al. 1991, Kaufman et al. 2008, Pelz-Stelinski et al. 2010). Results from laboratory studies have shown that mosquito performance is largely influenced by leaf species identity (Fish and Carpenter 1982, Yee and Juliano 2006, Reiskind et al. 2009); condition (fresh versus senescent, [Walker et al. 1991]); and ratio of leaf species combinations (Yee et al. 2007a, b; Reiskind et al. 2009). A laboratory study by Reiskind et al. (2009) did not find a direct relationship between the rate of leaf litter decomposition and mosquito population performance, suggesting that other factors may play a role in determining the contributions of specific leaf species to mosquito production. For example, there is evidence that certain leaf species contain toxic chemicals (e.g., tannins and lignins) that may be detrimental to the development and survival of macroinvertebrate communities (Rey et al. 1999, 2000; David et al. 2000). However, most of the studies that have investigated the effects of leaf litter inputs on mosquito production have been conducted under laboratory conditions and focused mainly on *Aedes* species and rarely on *Culex* species, despite their occurrence in container habitats and their important role in disease transmission. Most studies on this topic also use size as an indicator of fitness components, such as fecundity and longevity, but recent studies suggest that similar sized adults can differ in their fitness (Westbrook et al. 2010, Muturi et al. 2011a).

Our aim was to measure how different leaf species, and their combinations, influence the production and adult longevity of culicine mosquitoes. We tested the hypothesis that different leaf species, and combinations of leaf species, affect mosquito production and adult longevity under natural conditions. We focused on *Culex restuans* Theobald (Diptera: Culicidae), a vector of West Nile Virus that dominates early in the transmission season (Ebel et al. 2005, Kilpatrick et al. 2005, Lampman et al. 2006), and for which the impacts of leaf litter inputs have not been reported previously.

### Methods

#### Study Site.

The studies were conducted at the University of Illinois Trelease Woods Natural Area (40°09'N, 88°10'W) between 23 May and 7 July 2011. The study site is a 28.8-ha old growth deciduous upland forest located approximately 6 mi northeast of the University of Illinois at Urbana-Champaign campus. The site is primarily composed of mature oak, ash, hackberry, and maple species, a typical woodland community composition for the region. The site is characterized by a high closed canopy and moderately dense understory. On the south edge of the woods are two small man made seasonal ponds that serve as ideal habitats for aquatic invertebrates including mosquitoes. The site borders the Phillips Tract (a former farm currently used for large manipulative studies by University of Illinois researchers) to the west and agricultural land to the north, south, and east.

#### Leaf Litter Collection.

Experiments were conducted using three species of common North American trees that are abundant in the study region: northern red oak (*Quercus rubra* L.), sugar maple (*Acer saccharum* Marshall), and common hackberry (*Celtis occidentalis* L.). Freshly fallen leaves of each tree species were collected at multiple locations at the study site during the fall of 2010. Only whole leaves that had no direct contact with the soil were collected and used for the experiment. Leaves collected from multiple locations were pooled together and dried before assignment to experimental treatments.

#### Experimental Design.

The experiments were conducted in 17.9-liter plastic containers measuring 42.4 by 27.9 by 27.4 cm. Maple, oak, and hackberry leaf infusions were made by mixing 21 g of senescent whole leaves of each tree species with 3 liters of tap water. In addition, an infusion consisting of a mixture of all three leaf species was prepared by adding 7 g of each leaf species in 3 liters of tap water. Each treatment was replicated three times and random numbers were used to assign the location of each container. After 1 wk of fermentation under field conditions, the containers were monitored for the presence of mosquito pupae and other naturally-colonizing aquatic organisms 3 d per week for a 7-wk sampling period. On each sampling occasion, the contents of each container were emptied into larval pans and all pupae were removed before returning the contents to their respective containers. The pupae were transported to the laboratory in whirl-paks where they were counted and recorded. Approximately 30% of the pupae collected in each container during each sampling day were selected randomly and allowed to eclose. The adults were sorted by species (Darsie and Ward 1981) and sex and the females were housed in newspaper cages at 25°C. The adults were provided access to water (without sugar) and monitored throughout their life to determine their longevity. Upon death, the wings of the female adults were dissected and measured as an indicator of mosquito size.

Once every week, the physical and chemical characteristics of each container, including temperature,
dissolved oxygen, pH, salinity, conductivity, total dissolved solids (TDS), and tannins and lignins were recorded. Tannins and lignins were measured using a DR 890 m (Hach Company, Loveland, CO) with appropriate test kits. Temperature and dissolved oxygen were measured using EXSTIK DO600 (Extech Instruments Corporation, Waltham, MA); and pH, salinity, conductivity, and TDS were recorded using EXSTIK EC500 from the same company. TDS is the sum of all dissolved organic, inorganic, and suspended solids in water (Sattler et al. 2005) and may be a good indicator of the amount of food available in the habitat because aquatic invertebrates feed on these materials (Fish and Carpenter 1982, Leonard and Juliano 1995, Walker et al. 1997).

Data Analysis. Data analysis was conducted using SPSS version 19 (SPSS Inc., IBM) and SAS version 9 (SAS Institute 2002) statistical packages. Data were checked for normality and homogeneity of variances before conducting the statistical analyses. Mixed design repeated measures multivariate analysis of variance (MANOVA) was used to test for the effects of leaf treatments on the total number of pupae. One-way analysis of variance (ANOVA) was used to test the effect of leaf treatments on the size and longevity of adult females, whereas MANOVA was used to test leaf treatment effects on the physical and chemical characteristics of experimental mesocosms. When significant effects were obtained in ANOVA and MANOVA tests, pairwise differences between treatment means were compared using a Tukey’s posthoc test.

We used a time series autoregressive integrated moving average (ARIMA) model to test the relationship between the number of pupae collected in the containers and the measured physical and chemical characteristics. The data were plotted using sequence charts and assessed for stationarity and periodicity. Once the data were found to be both periodic and nonstationary, autocorrelation plots were used to assess the requirement for seasonal differencing. Based on autocorrelation charts, an ARIMA (0, 0, 0) (0, 1, 1) model was found to be the most appropriate, and then was used to assess the relationship between the number of pupae collected and dissolved oxygen, pH, salinity, temperature, conductivity, total dissolved solids, and tannins and lignins.

Results

Repeated measures MANOVA with a Greenhouse–Geisser correction revealed that the total number of pupae collected was significantly influenced by an interaction between leaf species and time (Pillai’s trace = 2.15; df = 18, 15; P = 0.048). Hackberry (65.0 ± 9.3, mean ± SE) treatments had the highest number of pupae followed by mixed leaf (39.3 ± 5.6) and maple treatments (26.2 ± 4.7), whereas oak (6.8 ± 1.5) treatments had the lowest number of pupae. The number of pupae in hackberry, maple, and mixed leaf treatments was highest during the second and third week of the sampling period and decreased rapidly thereafter (Fig. 1). This is in contrast with oak leaf treatments, where the number of pupae was highest during the first week of sampling and continued to decrease over time.

Leaf treatments had significant effects on the physical and chemical characteristics of the aquatic habitats (Pillai’s trace = 1.37; df = 21, 228; P < 0.001). Hackberry treatments were characterized by high values of pH, TDS, salinity, and conductivity compared with the other treatments (Table 1). They were also cooler compared with mixed leaf treatments and had higher levels of tannins than the oak treatments (Table
1). Oak treatments had lower values of pH, TDS, salinity, and conductivity than mixed leaf treatment but not maple treatments (Table 1). However, based on the ARIMA model, none of the physical and chemical characteristics of the containers were significant predictors of the number of pupae collected (Table 2).

In total, 3,700 pupae were allowed to eclose and the wing lengths and longevity were estimated in 1,640 adult females. These consisted of six mosquito species dominated by Cx. restuans (85.9%). Other species included Aedes japonicus Theobald (Diptera: Culicidae, 8.5%); Anopheles punctipennis Say (Diptera: Culicidae, 1.6%); Culex pipiens L. (Diptera: Culicidae 1.0%); Culex territans Walker (Diptera: Culicidae, 0.7%); Culex erraticus Dyar and Knab (Diptera: Culicidae, 0.1%); and indeterminate species of Culex (2.1%). Leaf treatments had no significant effects on the size of Cx. restuans (Fig. 2a and F = 1.05; df = 3, 44; P = 0.42) and Ae. japonicus (F = 0.188, df = 3, 44; P = 0.90) adult females (Fig. 2b). However, female longevity for both species was significantly lower in oak infusion than in the other treatments (Cx. restuans: F = 4.33, df = 3, 44; P = 0.04; Ae. japonicus: F = 4.91, df = 2, 44; P = 0.02; Fig. 2a, b).

The only other aquatic organisms that were observed in experimental mesocosms were Daphnia spp. (Cladocera: Daphniidae). No aquatic predators of mosquito eggs, larvae, or pupae were observed throughout the experiment.

**Table 1.** Mean (± SE) of the physical and chemical characteristics of experimental containers in response to leaf treatments during the 7-wk study period

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hackberry</th>
<th>Maple</th>
<th>Oak</th>
<th>Mixed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>20.46 ± 0.12a</td>
<td>20.53 ± 0.10ab</td>
<td>20.60 ± 0.08ab</td>
<td>21.07 ± 0.14b</td>
</tr>
<tr>
<td>pH</td>
<td>8.15 ± 0.04a</td>
<td>7.60 ± 0.07bc</td>
<td>7.56 ± 0.03b</td>
<td>7.79 ± 0.02c</td>
</tr>
<tr>
<td>TDS (mg/liter)</td>
<td>400.83 ± 21.50a</td>
<td>173.17 ± 3.18bc</td>
<td>154.00 ± 7.13b</td>
<td>210.08 ± 3.09c</td>
</tr>
<tr>
<td>Salinity (ppm)</td>
<td>461.50 ± 23.57a</td>
<td>206.25 ± 4.95bc</td>
<td>190.75 ± 8.63b</td>
<td>243.35 ± 2.46c</td>
</tr>
<tr>
<td>Conductivity (µS)</td>
<td>720.42 ± 38.90a</td>
<td>324.09 ± 8.02bc</td>
<td>283.92 ± 13.56b</td>
<td>384.93 ± 5.22c</td>
</tr>
<tr>
<td>DO (mg/liter)</td>
<td>2.04 ± 0.10a</td>
<td>3.87 ± 1.68a</td>
<td>1.60 ± 0.10a</td>
<td>2.13 ± 0.04a</td>
</tr>
<tr>
<td>Tannins (mg/liter)</td>
<td>26.19 ± 3.21a</td>
<td>28.70 ± 5.34a</td>
<td>9.28 ± 2.63b</td>
<td>17.24 ± 0.74ab</td>
</tr>
</tbody>
</table>

Different lowercase letters indicate significant differences between means.

Discussion

Our results are consistent with a primarily bottom-up driven assembly of mosquito communities, as there was significant variation in pupal abundance between leaf treatments, whereas no aquatic predators were observed during the 7-wk sampling period. Hackberry treatments produced the greatest number of pupae, whereas oak treatments produced the fewest. Because of the difficulties in distinguishing between previously enumerated egg rafts from newly deposited ones, we did not quantify the number of eggs deposited in our experimental containers. Thus, it is uncertain whether the observed treatment differences in pupal production were because of variations in oviposition preference, or because of asymmetric egg hatching success and larval survivorship among leaf treatments, or both. On many sampling occasions, however, we observed large numbers of egg rafts (and larvae) in hackberry treatments and low numbers in oak leaf treatments suggesting that gravid females discriminated the containers for oviposition based on the leaf treatments. These findings are consistent with previous laboratory findings that different leaf species have asymmetric effects on mosquito performance (Fish and Carpenter 1982, Murrell and Juliano 2008, Reiskind et al. 2009). Interestingly, none of the measured physical and chemical characteristics were significant predictors of pupal abundance, suggesting that the observed heterogeneity in production may be affected either by many variables each contributing small effects or by other variables that were not measured in this study.

Oak leaves may generate poor food resources for mosquito larvae in part because their decomposition and associated microbial growth are slow (Murrell and Juliano 2008). Further, oak leaves are known to contain high levels of tannins (Walker et al. 1997, Murrell and Juliano 2008). Further, oak leaves are known to contain high levels of tannins (Walker et al. 1997, Murrell and Juliano 2008). Interestingly, the oak leaf treatment in our study generated lower levels of tannins in the aquatic environment than maple or hackberry. Tannin levels were determined from water samples collected from the leaf treatments, and because of the slow decomposition rate for the oak leaves, it is possible that the insoluble component of tannins and lignins in this leaf species were trapped in the leaf matrix. Poor production from the oak leaf treatment, despite the low levels of tannins and lignins and the lack of a significant relationship between tannins and the number of pupae collected, indicates that tannins and lignins had negligible effects on mosquito production in this

**Table 2.** Regression estimates from ARIMA model showing the relationship between the number of pupae collected and the physical and chemical attributes of the experimental containers

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimates</th>
<th>Std error</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMA*</td>
<td>-0.45</td>
<td>0.11</td>
<td>-4.16</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Temperature</td>
<td>1.05</td>
<td>1.18</td>
<td>0.90</td>
<td>0.37</td>
</tr>
<tr>
<td>pH</td>
<td>-1.19</td>
<td>2.66</td>
<td>-0.49</td>
<td>0.66</td>
</tr>
<tr>
<td>TDS (mg/liter)</td>
<td>-0.03</td>
<td>0.19</td>
<td>-0.17</td>
<td>0.86</td>
</tr>
<tr>
<td>Salinity (ppm)</td>
<td>-0.23</td>
<td>0.19</td>
<td>-1.23</td>
<td>0.22</td>
</tr>
<tr>
<td>Conductivity (µS)</td>
<td>0.20</td>
<td>0.22</td>
<td>0.57</td>
<td>0.38</td>
</tr>
<tr>
<td>DO (mg/liter)</td>
<td>-4.50</td>
<td>5.69</td>
<td>-0.55</td>
<td>0.58</td>
</tr>
<tr>
<td>Tannins and lignins</td>
<td>0.86</td>
<td>0.17</td>
<td>3.40</td>
<td>0.08</td>
</tr>
</tbody>
</table>

* SMA: seasonal-moving average component.
We did not estimate the rate of microbial growth, but water chemistry analysis suggests that hackberry followed by mixed leaf species treatments had the highest values of TDS, indicating more food resources to support mosquito growth.

The size of adult females of *Cx. restuans* and *Ae. japonicus* was not influenced by leaf treatments but their longevity was significantly lower from oak leaf treatments compared with other treatments. These findings are consistent with previous reports that size is not a good indicator of fitness (Muturi and Alto 2011, Muturi et al. 2011b). Reduced female longevity for pupae that emerged from the oak leaf infusion illustrates the carryover effects of larval environment on subsequent adult populations and is yet another indication that oak leaves are poor food resources for mosquito larvae, perhaps because of their slow decomposition and microbial growth rate (Murrell and Juliano 2008), chronic toxic effects, or both (Feeny 1970). In nature, adult mosquitoes procure sugar meals from surrounding plants to replenish their reserves from the larval environment. To eliminate the confounding effects of adult nutrition on longevity, emergent adults were fed a diet of water with no sugar. Thus, we cannot answer the question as to whether adults from resource-poor larval habitats, such as those from oak leaf infusion, can compensate for reduced longevity through sugar feeding. Studies are under way to test this hypothesis.

Treatments with mixed leaf species had higher number of pupae and greater adult female longevity than those from oak leaf treatments, suggesting that a mixture of leaf species may modulate the negative effects of any one leaf species that has deleterious effects on invertebrate consumers. The values of pH, TDS, and salinity were significantly higher in mixed leaf treatments than in oak leaf treatments, suggesting that alterations of water chemistry may be one of the mechanisms by which mixed leaf species may modulate the negative effects of a given leaf species. Previous studies indicate that multiple leaf species can provide a superior diet for invertebrate consumers compared with single leaf species diet by accelerating microbial growth, supporting diverse microbial communities, or both (Reiskind et al. 2009). However, our data suggest that this is only true for some leaf species but not others. Further, maximum production was achieved during the second and third week of sampling (except in oak leaves) and declined gradually thereafter. This was expected because *Cx. restuans* is a seasonal mosquito that peaks in abundance early in spring and declines thereafter. Moreover, the nutri-

![Fig. 2. Effect of leaf species on the size and longevity of A) Cx. restuans and B) A. japonicus females. Different lowercase and uppercase letters indicate significant differences between means. n values are represented in brackets.](image-url)
tional quality of the containers likely decreased over time because no food supplements were added. An accumulation of top-down effects (e.g., consumption by predators) could also play a role in temporal decline in pupal densities, but in our study the containers were not colonized by aquatic predators during the study period.

Our study supported the hypothesis that different leaf species in the aquatic environment influence mosquito production and longevity of the subsequent adult stage. Different leaf species had significant effects on pupal production and adult longevity but had no effect on adult sizes. Longevity may be a particularly important factor in influencing the transmission dynamics of infectious diseases because of the lengthy extrinsic incubation period of many mosquito-borne infectious agents. Environmental factors that extend the longevity of adult female mosquitoes may both increase their probability of exposure to an infectious agent and the probability of surviving a sufficient period postinfection to contribute to transmission. Thus, our findings demonstrate the importance of leaf species identity in determining the performance of invertebrate consumers in container habitats, both during and after the aquatic life-stage, with consequences that may cascade through ecosystems and ultimately influence human health. We advocate for further studies on the influence of bottom-up forces, particularly leaf litter inputs in small aquatic communities where many mosquito species thrive, on the chain of interactions that govern the reproductive success of mosquito populations and their potential to transmit pathogens.

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