Blackberry Debugging

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Background. *Culex pipiens* Linnaeus (Diptera: Culicidae), an important mosquito vector for West Nile virus in urban landscapes throughout the northeastern and midwestern United States, oviposits in a variety of natural and artificial containers such as small ponds, discarded tires, and storm water catch basins. These habitats are mainly fueled by plant-based detritus from the surrounding terrestrial vegetation. Detritus type and quantity determine the composition and abundance of microbial communities that form in container habitats as microbes break down terrestrial leaf litter. In turn, these bacteria and fungi provide a direct food source for mosquito larvae and influence oviposition behavior of gravid female mosquitoes through emission of oviposition attractants and stimulants. Thus, detritus from terrestrial plants and its associated microbes play a critical role in determining vector distribution, relative abundance, and life history traits that are important for vector-borne pathogen transmission including adult body size, longevity, biting rates, and vector competence. In this study, we test the hypotheses that leaf detritus of three native and three invasive shrubs asymmetrically affects oviposition site selection and adult emergence rates of *Cx. pipiens*.

Methods. Six focal plant species were selected among shrubs common within the geographic range of *Cx. pipiens*: *Lonicera maackii* (Dipsacales: Caprifoliaceae; Amur honeysuckle), *Elaeagnus umbellata* (Rosales: Elaeagnaceae; autumn olive), *Rosa multiflora* (Rosales: Rosaceae; multiflora rose), *Rubus allegheniensis* (Rosales: Rosaceae; blackberry), *Sambucus canadensis* (Dipsacales: Adoxaceae; elderberry), and *Amelanchier laevis* (Rosales: Rosaceae; serviceberry).

To test the hypothesis that leaf detritus species in the aquatic environment affects oviposition site selection of *Cx. pipiens*, six oviposition traps each containing 4 L of tap water and 80 g of fresh whole leaves of one of the six shrub species were placed 1 m apart from each other in partial shade at five sites located within a 5 km radius in a residential neighborhood. The 30 oviposition traps were monitored for egg rafts daily and the number of egg rafts collected in each substrate from June 24 to August 5, 2013 was recorded. A general linear mixed model (GLMM) with repeated measures was used to compare the abundance of egg rafts collected by leaf substrate, day, and their interaction throughout the study period.

To test the hypothesis that leaf detritus species affects adult emergence rates of *Cx. pipiens*, larvae were obtained by collecting egg rafts from five sites using grass infusion-baited oviposition traps. Egg rafts were individually hatched in petri dishes containing deionized water. To test for the effect of leaf substrate on intraspecific competition, 18 treatments were established with five replicates per treatment. Each treatment included one of three densities of first instar larvae of *Cx. pipiens* (10, 20, or 40 per container) and 360 mL infusion of one of the six leaf detritus species in 400 mL tri-pour beakers. Infusions were prepared by fermenting 80 g of fresh leaves of each plant species in 4 L of tap water for 7 days. The containers were monitored daily and pupae were removed from containers and housed individually in cotton-sealed plastic vials with deionized water. A GLMM with a factorial treatment structure was used to test the fixed effects of leaf species, competition, and their interaction on *Cx. pipiens* emergence rates.

Results. The number of egg rafts laid in oviposition traps containing the leaves of different native and invasive shrubs varied within and among leaf detritus species over the collection period, with significant

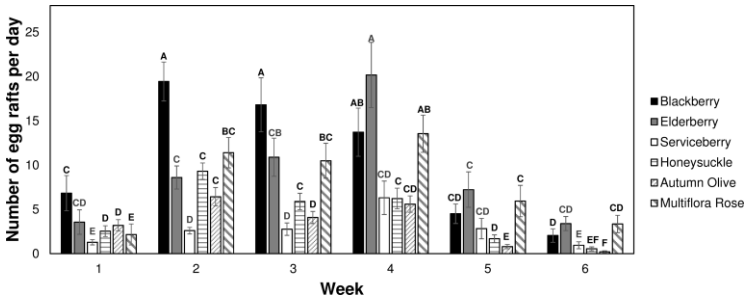

Figure 1. Mean (±1 standard error) for *Culex pipiens* egg rafts collected in oviposition traps per day from June 24 to August 5, 2013 (6 weeks) by leaf detritus treatment. Letters indicate significant pairwise differences at $\alpha = 0.05$.

effects of leaf species ($F = 7.25$; df = 5, 20; $P < 0.01$) and day ($F = 23.70$; df = 39, 912; $P < 0.01$) but not their interaction (Figure 1). Throughout the experiment, the greatest number of egg rafts were c
ollected in blackberry and elderberry leaf infusion, the lowest number of egg rafts per day were collected from water containing serviceberry, autumn olive, and honeysuckle leaves, and an intermediate number of egg rafts were collected from water containing multiflora rose.

Mosquito emergence rates varied across leaf detritus types and larval densities, with a significant interaction between leaf species and density ($F = 7.33$; df = 10, 72; $P < 0.01$; Figure 2). The lowest emergence rates were observed in blackberry and multiflora rose infusion; no adults emerged in the latter leaf detritus species at any larval density. The highest emergence rates were observed in honeysuckle and autumn olive infusions, although autumn olive-reared mosquitoes experienced a significant decline in emergence at the highest density while honeysuckle infusion mitigated the deleterious effects of intraspecific competition even at high larval densities. Among all other leaf species except for elderberry, higher larval densities yielded significantly lower emergence rates than lower larval densities.

Discussion. Using a combination of laboratory and field experiments, we identified two invasive shrubs that may promote growth and emergence of an important mosquito vector relative to native shrub species by improving the nutritional quality of the larval environment via leaf detritus inputs. *Culex pipiens* emergence rates were significantly higher in leaf infusions of honeysuckle and autumn olive compared to the other shrub species. We also noted that the deleterious effects of intraspecific larval competition were mitigated in honeysuckle treatments. These results complement a growing body of field studies that suggest landscaping with exotic – and potentially invasive – plants has the potential to influence local larval and adult mosquito abundance and distribution.

Our comparisons of native and invasive leaf detritus species on larval development and oviposition facilitated the discovery of a naturally-occurring ecological trap for *Cx. pipiens*. Ecological traps occur due to a mismatch between the attractiveness of a habitat and its quality for reproduction. The greatest number of egg rafts was collected in water containing leaves of blackberry, a native plant species found throughout the geographic range of *Cx. pipiens*. However, in laboratory assays, exceptionally low mosquito emergence rates were observed in blackberry infusions, with fewer than 20 percent of larvae surviving to eclosion even at the lowest larval density. Infusion of multiflora rose, an exotic shrub of limited importance in the Midwest but highly invasive in the northeastern United States, similarly

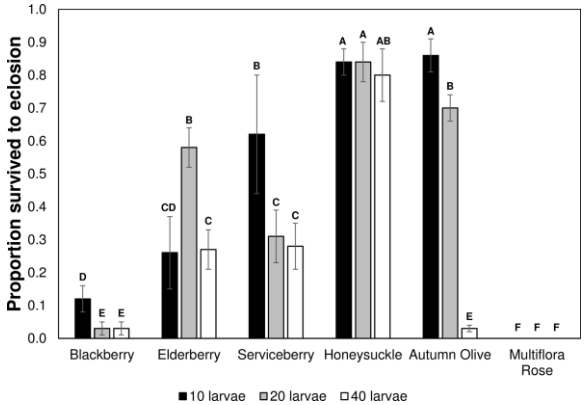

Figure 2. Mean (±1 standard error) for *Culex pipiens* male and female emergence rates across intraspecific competition by leaf detritus treatments. Letters indicate significant pairwise differences at $\alpha = 0.05$.

yielded lower emergence with no mosquitoes developing to eclosion across all density treatments. However, gravid females were better able to discriminate against this leaf detritus species and consequently water containing multiflora rose leaves collected fewer egg rafts than water containing blackberry leaves. Future research will determine whether exploitation of this ecological trap may yield a novel "attract-kill" approach (i.e., Blackberry Debugging) to control mosquito larvae in closed aquatic environments, such as rain barrels, buckets, and storm water catch basins.

In summary, we observed elevated emergence rates and more rapid development among *Cx. pipiens* mosquitoes reared in infusions of honeysuckle and autumn olive leaves, two exotic, invasive shrubs that occur throughout much of the northeastern and midwestern United States. In contrast, we discovered mosquito emergence was significantly reduced among mosquitoes reared in infusions of native blackberry and exotic multiflora rose leaves compared to those exposed to other leaf detritus species. Our results have applications in two areas. First, our findings that some exotic, invasive shrubs are favorable for mosquito production may be relevant to mosquito control and invasive plant management in the range of *Cx. pipiens*. Second, our discovery of a previously unknown ecological trap for an important vector of West Nile virus has the potential to lead to novel alternatives to conventional insecticides in mosquito control, exploiting the apparent "attract-kill" properties of this native plant species.

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