A comparison of the egg laying behavior of two mosquito vectors of West Nile Virus in aquatic habitats enriched with turfgrass and cattail clippings.

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Mosquitoes complete the first part of their life cycle (larval and pupal stages) in aquatic environments, where the larvae of many species feed on small suspended particles, including bacteria and other microorganisms. Adult female mosquitoes looking for a suitable place to lay their eggs often rely on chemical compounds released by the microbial decomposition of organic detritus to identify aquatic habitats that are likely to contain sufficient food for the development and survival of their offspring. The diversity and abundance of these microorganisms, and therefore the nature of the chemical signal released from an aquatic environment, is greatly affected by the amount and type of organic material added. Plant detritus (example: fallen leaves, grass clippings, etc.) is often the main source of organic enrichment in the types of aquatic habitats typically used by mosquitoes.

The two mosquito species most important to the transmission of West Nile Virus (WNV) in the northeastern and much of the central U.S. (including Illinois) are *Culex pipiens* and *Cx. restuans*. Females of these species deposit their eggs in a variety of aquatic habitats as a single mass ("egg raft") often consisting of more than 100 eggs. Some of the most productive sources of these mosquitoes in urban environments are man-made structures used to manage stormwater runoff. While aboveground stormwater management structures (examples: ditches and dry detention basins) are typically landscaped with turfgrass or other managed vegetation, they often become colonized by cattails and other invasive, aquatic weeds. Managers of these structures will often mow them periodically throughout the season to control undesirable plant growth and maintain stormwater management functions.

Our previous research had found that the abundance of mosquito larvae in stormwater ditches and detention basins greatly increases following mowing practices that deposit plant material in the aquatic environment. It was also found that the source of the plant material influences the mosquito species composition in these structures after mowing - with larvae of *Cx. pipiens* most abundant in recently-mowed sites where turfgrass was the dominant vegetation, and *Cx. restuans* more abundant species in recently-mowed sites colonized by cattails. The purpose of this experiment was to test whether differences in the egg laying behavior of these two mosquito species could be a reason for this previously observed relationship between plant type and the species composition of mosquito larvae in recently-mowed stormwater management structures. Specifically we wanted to compare whether *Cx. pipiens* and *Cx. restuans* have differences in their relative preference for depositing their egg rafts in aquatic habitats enriched with cattail and turfgrass clippings.

Methods. Artificial aquatic habitats (i.e. "ovitraps") containing various amounts of cattail or turfgrass clippings were used to evaluate how the type and amount of decaying plant material affects the egg laying preferences of *Cx. pipiens* and *Cx. restuans*. Ovitraps were constructed from 5 gallon plastic pails containing 8 liters of water and a submerged packet of plant material. A total of 7 treatments were compared: ovitraps enriched with packets containing 6, 12 or 36

grams of turfgrass clippings, and ovitraps enriched with packets containing 6, 12, 36 or 72 grams of cattail clippings. Seven ovitraps (one for each treatment) were placed at each of five locations in Urbana, IL, including 2 municipal parks (AMBUCS Park and Crystal Lake Park), and 3 sites on the University of Illinois Campus (U of I Arboretum, U of I Plantation and a wooded area adjacent to the Ashton Woods Family and Graduate housing building). Mosquito egg rafts were collected from ovitraps and counted every day for 4 weeks (24 June – 21 July, 2014). Since the egg rafts of these two mosquito species appear very similar, we hatched each egg raft individually in the laboratory so that we could identity eggs rafts to species using the physical characteristics of the newly-hatched larvae.

Results. We found that the egg laying response of these two mosquito species to turfgrass and cattail clippings was consistent with the species distribution of larvae previously observed in recently mowed stormwater ditches and detention basins. The largest number of *Cx. restuans* egg rafts was collected in ovitraps that contained the greatest amount of cattail clippings (72 g per ovitrap). The largest number of *Cx. pipiens* egg rafts was collected in ovitraps that contained the greatest amount of turfgrass clippings (36 g per ovitrap).

Significance. Our study confirmed that the type and amount of plant material introduced into aquatic habitats can affect their potential to be colonized by these two mosquito vectors of WNV. Female *Cx. pipiens* mosquitoes are more likely to deposit their eggs in aquatic habitats that receive large amounts of clippings from managed turfgrass, while female *Cx. restuans* mosquitoes prefer aquatic habitats collecting large amounts of cattail clippings. These results indicate that plant management practices within or adjacent to aquatic habitats may have implications for WNV transmission risk. Both of these mosquito species differ in their seasonal patterns of activity and their ability to support virus transmission. In Illinois, *Cx. restuans* is most abundant in the spring and early summer months. The abundance of *Cx. pipiens* tends to peak later in the summer when WNV transmission activity (and human exposure to WNV) is greatest, and laboratory studies have suggested that *Cx. pipiens* is a more efficient vector of WNV than *Cx. restuans*. Our results suggest that mowing of stormwater management structures landscaped with turfgrass during the summer months could increase their likelihood to support the production of *Cx. pipiens* during the period of peak WNV transmission. Conversely, mowing of cattails in stormwater management structures in the spring or early summer season can increase the risk that they will be colonized by *Cx. restuans*, potentially enhancing the early season transmission of WNV.

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