



ILLINOIS NATURAL  
HISTORY SURVEY  
PRAIRIE RESEARCH INSTITUTE

2012–2014



**LEGISLATIVE MANDATE:**

*"To provide for  
research on disease  
vectors associated with  
used and waste tires  
and the disease  
they spread."*

# BIENNIAL REPORT

**Used Tire Management and Emergency Public Health Funds  
Report to Governor Pat Quinn and the Illinois General Assembly**

## SUMMARY OF ACOMPLISHMENTS BY THE MEDICAL ENTOMOLOGY PROGRAM

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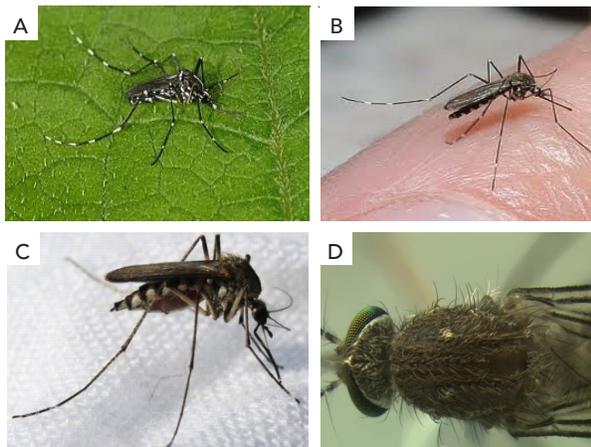


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## I. Medical Entomology Program.

The Medical Entomology Program at The Illinois Natural History Survey provides for research on vectors associated with used and waste tires and the diseases they spread. Waste tires are a serious public health issue because they are difficult to dispose and also provide ideal breeding sites for multiple indigenous and exotic mosquito species that vector arthropod-borne viruses (arboviruses). The Asian tiger mosquito *Aedes albopictus* and the Asian rock pool mosquito *Aedes japonicus* are two recent invaders that thrive well in waste tire environment (Figure 1). Both species are believed to have entered the United States through shipments of waste tires from Asia and have expanded their geographic range to become two of the most common nuisance mosquitoes. The two species are considered potential threats to human and wildlife health because they are competent vectors of several native and exotic arboviruses such as West Nile Virus (WNV), La Crosse Virus (LACV), Eastern Equine Encephalitis virus (EEEV), St. Louis Encephalitis Virus (SLEV), Dengue, and Rift Valley Fever. In addition, natural infections of West Nile Virus (WNV) have also been documented in both mosquito species. Discarded tires also serve as breeding sites for many native mosquito species most notably *Culex pipiens* and *Culex restuans* (Figure 1), the vectors for WNV and SLEV, and the eastern tree hole mosquito *Ochlerotatus (Aedes) triseriatus*, the vector for LACV.



**Figure 1.** Invasive and native mosquito species in Illinois. (A) *Aedes albopictus*, (B) *Aedes japonicus*, (C) *Culex pipiens pipiens*, and (D) *Culex restuans*.

Illinois passed the Waste Tire Act in 1989 to address issues associated with solid waste hazards. Funds associated with this legislation are used to provide a better understanding of the biology of container-dwelling arthropods, such as mosquitoes, and associated diseases which ultimately con-

tribute to disease management efforts. The development and certification of biosafety level 2 arthropod-containment facilities at the Medical Entomology Program five years ago have provided a unique opportunity to study in greater details how container-dwelling mosquitoes interact with arboviruses.

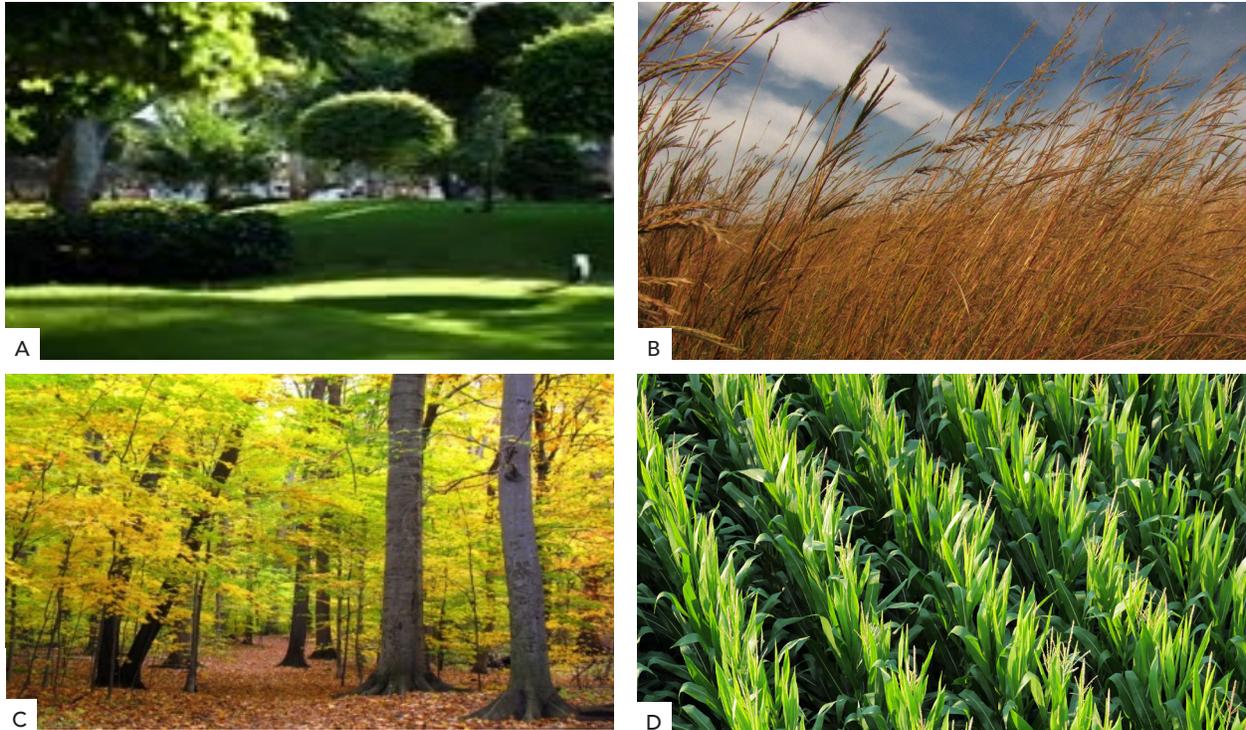
## II. Highlights of the Accomplishments and Achievements of the Medical Entomology Program.

### 1a. West Nile and St. Louis encephalitis virus surveillance in Illinois.

Since the arrival of West Nile virus (WNV) in Illinois, the Medical Entomology Program collaborates with Mosquito Abatement Districts (MADs) in Illinois to monitor the spatial and temporal variation in the risk of WNV and St. Louis encephalitis. The Medical Entomology Program tests mosquito pools from various MADs for WNV and St. Louis encephalitis. Multiple counties are involved in these efforts but much emphasis is focused on MADs in Cook County, an area with consistently high levels of WNV activity from year to year. During 2012 and 2014, The Medical Entomology tested 9,182 mosquito pools for WNV RNA by polymerase chain reaction (TaqMan RT-PCR). A similar number of mosquito pools are anticipated for the 2014–2015 seasons. Data generated from these efforts are generally disseminated to the public by the Medical Entomology Program through peer reviewed journals and technical reports.

### 1b. Land use patterns and the risk of WNV transmission in Illinois.

Since 2002 when large outbreaks of WNV occurred in Illinois, sustained endemic and epidemic transmission of this virus has continued to occur throughout the state. Urban residential areas are considered the main hotspots for WNV and major research efforts have been devoted towards the understanding of mechanisms underlying increased risk of WNV transmission in these areas. However, although many urban areas in Illinois are surrounded by farm lands and small-to-medium-sized woodlots, the potential role of these landscapes in WNV transmission are poorly understood. We conducted field studies to assess how four major land use categories in Illinois: row crops, prairies, forest fragments, and residential neighborhoods (Figure 2) influence mosquito species composition, abundance, and the risk of WNV transmission in central Illinois. Adult mosquitoes were collected every other week using gravid traps and Centers for Disease Control (CDC) and Prevention light



**Figure 2.** Typical land use categories in Illinois: (A) residential neighborhoods, (B) prairie, (C) woodlot, and (D) row crops.

traps. The mosquitoes were identified to species, counted and pools of the primary vectors of WNV *Culex pipiens* and *Cx. restuans* were tested for WNV-RNA by real-time polymerase chain reaction (PCR).

Mosquito species diversity was significantly higher in forest habitats (wood lots) compared to residential, agricultural (row crops), and prairie land use categories. All the four landscape types were equally important habitats for WNV vectors *Culex pipiens* and *Cx. restuans*, contrary to previous findings that these species principally inhabit the residential areas. WNV-infected mosquito pools were observed in all land use types and the infection rates overlapped among land use categories. Thus, although our findings support the importance of residential habitats for WNV transmission to humans, they also establish that prairie, row crops, and wood lots are potentially important refuges for enzootic transmission. This is particularly important in urban ecosystems where these land use categories are small, interspersed fragments serving as potential refuge sites during periods of low rainfall.

## Reference

Garner, A. M., R. L. Lampman, and E. J. Muturi. 2014. Land use patterns and the risk of West Nile Virus transmission in central Illinois. *Vector Borne and Zoonotic Diseases*, 338–345.

## 2. Effect of container type on the relative abundance, body size, and vector competence of *Ochlerotatus triseriatus* for La Crosse virus.

The eastern tree hole mosquito *Oc. triseriatus* is the primary vector of LACV, the leading cause of mosquito-borne pediatric encephalitis in the United States and the second most reported mosquito-borne disease, behind WNV. This virus was historically confined to the Midwestern United States (which includes Illinois), but has recently emerged in the Appalachian region. *Oc. triseriatus* occurs throughout wooded regions east of the Rocky Mountains where it breeds in a variety of natural (e.g., tree holes) and artificial containers (e.g., waste tires, discarded plastic bins; Figure 3). However, it remains unclear how container type affects mosquito life history traits that are relevant to LACV transmission including body size, biting rates, longevity, and vector competence.

Accordingly, we conducted a series of field and laboratory studies at two woodlots (South Farms and Trelease Woods) in Urbana, IL to examine how container type influences vector abundance, body size, and vector competence for LACV. Mosquito pupae were collected from tree holes, plastic bins, and waste tires for 5 consecutive days each week (Monday–Friday) and eclosing adults were identified to species morphologically. Adult *Oc. japonicus* females were killed and their wings measured as an indicator of body

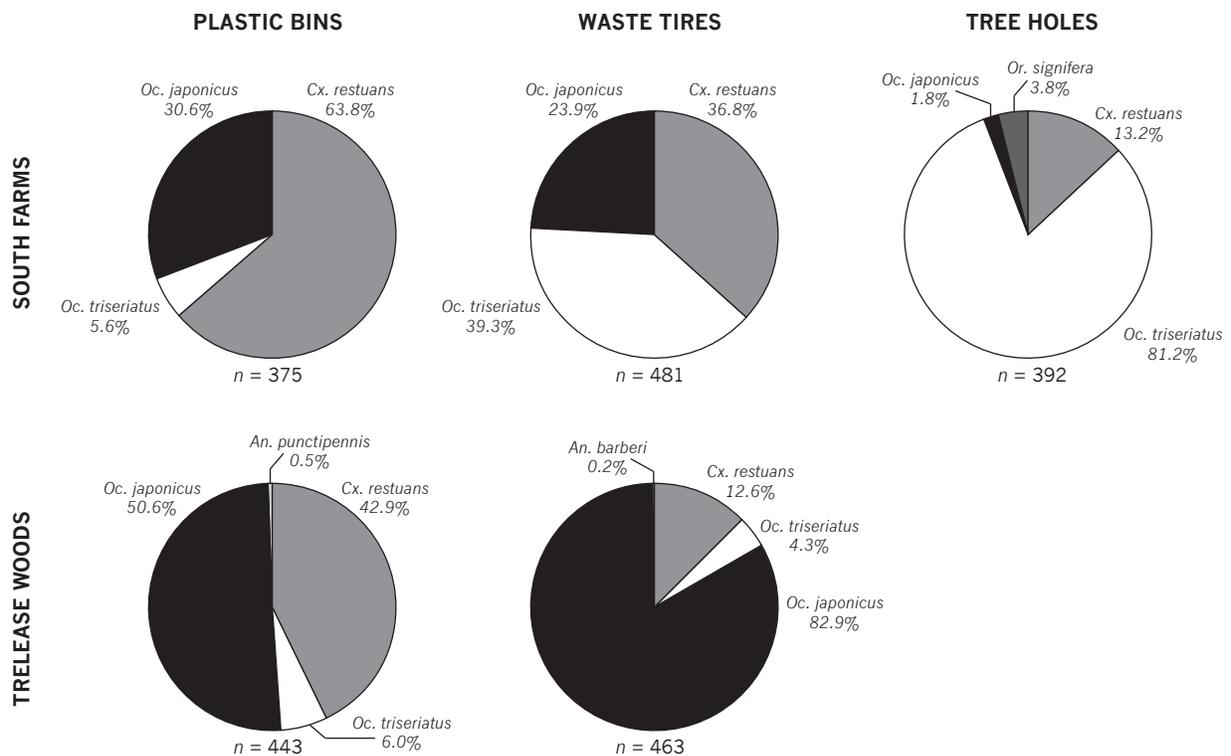
size. *Oc. triseriatus* adults were orally challenged with LACV and virus infection rates, dissemination rates, and titers were determined by reverse-transcriptase real-time PCR.

A total of 2,154 mosquito pupae representing six mosquito species were collected from waste tires, plastic bins, and tree holes at the two study sites (Figure 4). *Oc. triseriatus*, *Oc. japonicus*, and *Cx. restuans* were the most abundant mosquito species and their relative abundance varied markedly by study site and container type (Figure 4). At the South Farm study site, *Oc. triseriatus* was the most dominant mosquito species in tree holes accounting for 81.2% of total collection while *Oc. japonicus* was the least abundant species accounting for 1.8% of total collection. Waste tires at this study site were also dominated by *Oc. triseriatus* (39.3%) followed by *Cx. restuans* (36.8%) and *Oc. japonicus* (23.9%). In contrast, plastic bins were dominated by *Cx. restuans* (63.8%) followed by *Oc. japonicus* (30.6%) and *Oc. triseriatus* (5.6%). At the Trelease Woods study site, *Oc. japonicus* was the dominant species in plastic bins and waste tires, with *Cx. restuans* and *Oc. triseriatus* ranking as the second and the third most abundant mosquito species, respectively. Adult females of *Oc. triseriatus* and *Oc. japonicus* from plastic bins were significantly larger than those from tree holes or waste tires (Figure 5). *Oc. japonicus* females from South Farms were also significantly larger

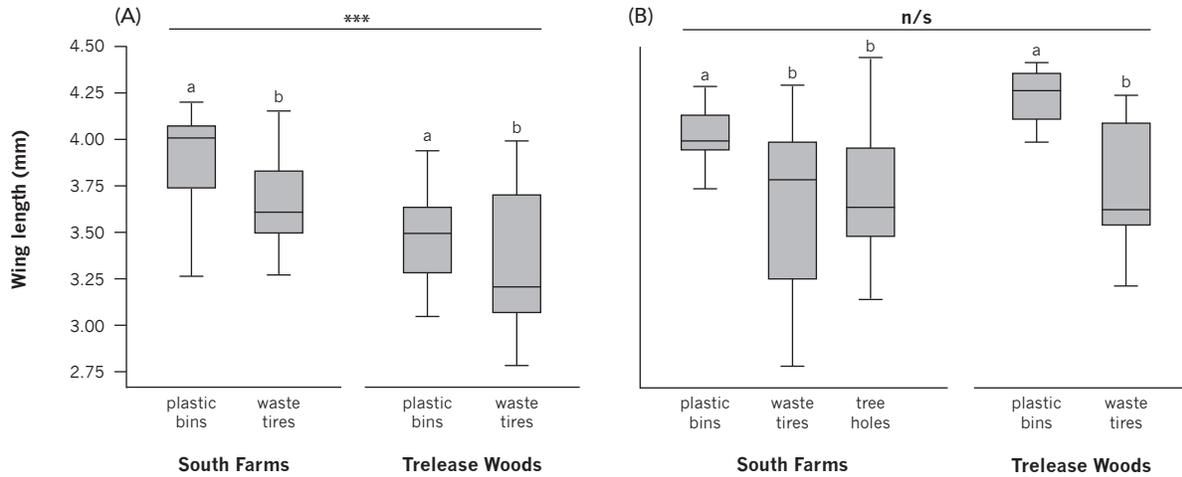


**Figure 3.** Natural and artificial container habitats for *Oc. triseriatus*. (A) Plastic bin, (B) tree hole, and (C) discarded tires.

than those from Trelease Woods (Figure 5). *Oc. triseriatus* females from plastic bins and waste tires had significantly higher LACV infection and dissemination rates than those from tree holes (Figure 6). In addition, *Oc. triseriatus* females from waste tires had significantly higher virus titers than those from tree holes. These findings suggest that container type likely contributes to the spatial and



**Figure 4.** Relative abundance of mosquito species collected from three container types at South Farms and Trelease Woods study sites from June 2<sup>nd</sup> to July 5<sup>th</sup>, 2013.



**Figure 5.** Wing lengths of (A) *Oc. triseriatus* and (B) *Oc. japonicus* adult females collected from three container types at South Farm and Trelease Woods study sites.

temporal dynamics of LACV transmission. These findings provide the impetus to develop vector control interventions that strategically target only those container types that are more likely to contribute to LACV transmission.

### Reference

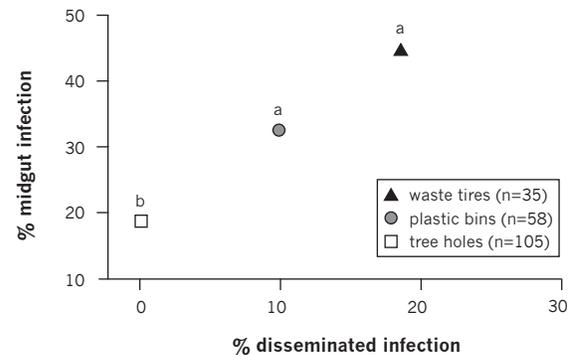
Bara, J. J., and E. J. Muturi. 2014. Container type influences the relative abundance, body size, and vector competence of *Ochlerotatus triseriatus* for La Crosse virus. *American Journal of Tropical Medicine and Hygiene* (under review).

### 3. Impact of honeysuckle on mosquito ecology

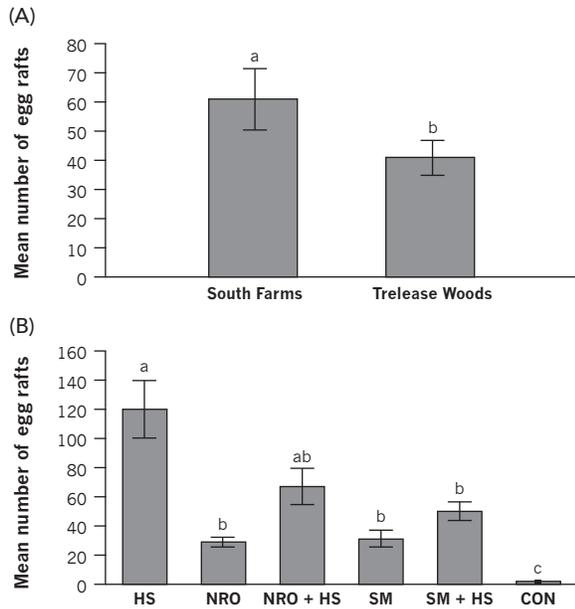
Alien invasive plants and animals (here after “invasive species”) are important agents of environmental change and pose a worldwide threat to native biodiversity, ecosystem functioning, economies, and human health. In the U.S. alone, invasive species are responsible for an annual economic loss of \$120 billion and a myriad of other ecological and societal damages that are difficult to quantify financially. Many ecosystems support multiple invasive species and it has become increasingly important to understand how a suite of invaders may interact with each other and with native communities. For example, invasive mosquitoes such as *Oc. japonicus* and *Oc. triseriatus* have invaded many ecosystems in the U.S. where they co-occur with native mosquito species such as *Oc. triseriatus*, *Cx. restuans*, and *Cx. pipiens*. Many of these ecosystems have also been invaded by one or more invasive plants. Honeysuckle is one of the most common invasive plants in rural and urban woodlots in eastern and central U.S. and its distribution range overlaps that of many native and invasive mosquito species. We therefore conducted field experiments at South

Farms and Trelease Woods study sites to determine how the interaction of alien invasive honeysuckle (*Lonicera maackii*) with two native tree species, sugar maple (*Acer saccharum*) and northern red oak (*Quercus rubra*) influences oviposition site selection by native *Culex restuans* and pupal production and adult body size of native *Oc. triseriatus* and alien invasive *Oc. japonicus*.

South Farm study site yielded significantly more *Cx. restuans* egg rafts and more and larger *Ochlerotatus* adults compared to Trelease Woods (Figure 7). Significantly more *Cx. restuans* egg rafts were deposited in honeysuckle infusions than in native tree species’ infusions. Mixing honeysuckle litter with native tree species’ litter had additive effects on *Cx. restuans* oviposition preference. There were no significant differences in *Ochlerotatus* pupal production among individual leaf species. However, mixing honeysuck-



**Figure 6.** LACV infections and dissemination rates among *Oc. triseriatus* adult females collected from waste tires, plastic bins, and tree holes.



**Figure 7.** Least-squares means ( $\pm$  SE) for effect of (A) study site and (B) leaf litter treatment on *Culex restuans* oviposition.

le with native tree species resulted in additive effects on *Ochlerotatus* pupal production. Honeysuckle litter resulted in significantly larger adult females of *Oc. triseriatus* (at both sites) and *Oc. japonicus* (at South Farm) relative to native tree species' litter. Mixing honeysuckle with native tree species resulted in additive effects on body size of *Oc. triseriatus* and *Oc. japonicus* females with the exception of honeysuckle and northern red oak litter mixture which had antagonistic effects on female body size of *Oc. triseriatus*. These findings suggest the potential for honeysuckle invasions to enhance production of native and exotic mosquito species and possibly the risk of arbovirus transmission.

## Reference

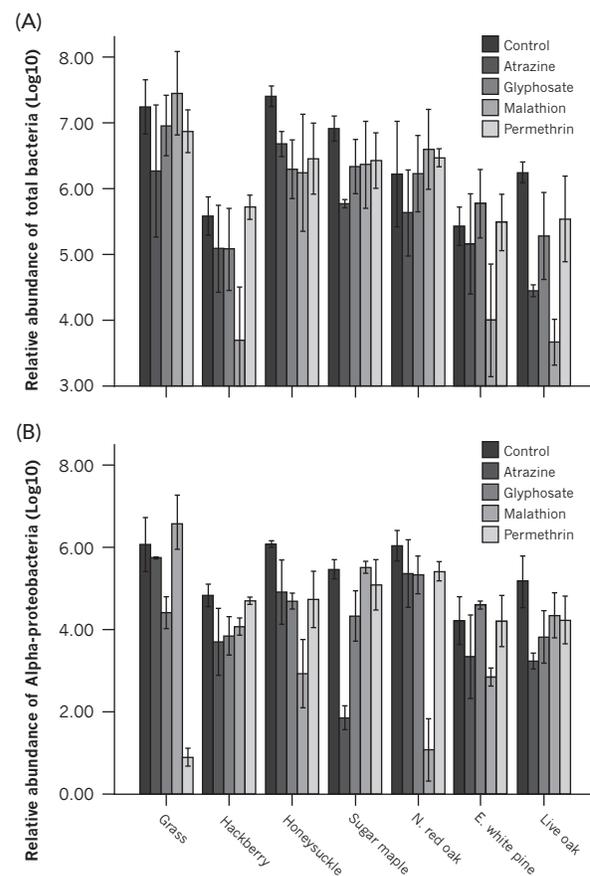
Muturi, E. J., A. M. Gardner, and J. J. Bara. 2014. Impact of invasive honeysuckle and native trees on ecology of native and invasive mosquitoes. *Bulletin of Entomological Research* (under review).

## 4. Impact of pesticide contaminants of bacterial communities in mosquito larval habitats

Bacterial communities in mosquito larval habitats serve many functions in mosquito life cycles. They produce chemical cues that act as oviposition attractants to gravid females and serve as the resource base for mosquito larvae. Some of the ingested bacterial species colonize the mosquito midgut and contribute to the digestion of sugar meals and blood meals as well as defense against

parasites and pathogens. The composition and structure of these communities may be altered by anthropogenic ecological disturbances. Environmental contamination with pesticides is one of the most common and widespread anthropogenic disturbances. Pesticides are deliberately introduced into the environment over large spatial scales in efforts to control agricultural and public health pests. These chemicals may unintentionally contaminate aquatic systems including those occupied by mosquito larvae through spray-drift, leaching, or surface runoffs and potentially alter the function and structure of microbial communities.

We used indoor aquatic microcosms to determine the abundances of major taxonomic groups of bacteria in leaf litters from seven plant species and their responses to low concentrations of four pesticides with different modes of action on the target organisms; permethrin, malathion, atrazine, and glyphosate. We tested the hypotheses that leaf species support different quantities of major taxonomic groups of bacteria and that exposure to pesticides at environmentally relevant concentrations alters bacterial abundance and community structure in mosquito larval



**Figure 8.** Mean number (with 95% CI) for (A) total bacteria and (B) *Alpha-proteobacteria* in different leaf and pesticide treatments.

habitats. We found support for both hypotheses suggesting that leaf litter identity and chemical contamination may alter the quality and quantity of mosquito food base (microbial communities) in larval habitats. The effect of pesticides on microbial communities varied significantly among leaf types (Figure 8), suggesting that the impact of pesticides on natural microbial communities may be highly complex and difficult to predict. Collectively, these findings demonstrate the potential for detritus composition within mosquito larval habitats and exposure to pesticides to influence the quality of mosquito larval habitats.

## Reference

Muturi, E.J., B. O. Orindi, and C. H. Kim. 2013. Effect of leaf type and pesticide exposure on abundance of bacterial taxa in mosquito larval habitats. *PLoS One*. e71812.

## 5. Effect of temperature and malathion on mosquito life history traits.

Malathion is one of the most commonly used insecticides and a leading source of water pollution around the world. We evaluated how larval rearing temperature influences the impact of malathion on the fitness of the yellow fever mosquito *Aedes aegypti*. Larvae were exposed to water control, and low (0.03 mg/L) or high (0.05 mg/L) malathion dose at 20°C, 25°C, and 30°C. Emergence rate, time to emergence, female fecundity, and expression of genes encoding two antimicrobial peptides (defensin, cecropin) and an iron-binding protein (transferrin) were quantified. High malathion dose at 25°C and 30°C resulted in significantly lower emergence rates compared to control and low malathion dose, but this effect was not observed at 20°C. Female time to emergence was inversely proportional to temperature and was significantly shorter in high malathion dose than in control and low malathion dose at 25°C and 30°C, but not at 20°C. Regardless of temperature treatment, females from high malathion dose were significantly larger and laid more eggs than their counterparts in control and low malathion dose. Relative to the controls, two immune genes were significantly over-expressed in adult females from malathion-exposed treatments at 20°C (defensin and cecropin) and 25°C (defensin and transferrin), and one gene (defensin) was significantly under-expressed at 30°C. These findings suggest that larval rearing temperature can modify the effect of malathion on fitness traits in mosquitoes.

## Reference

Muturi, E. J. 2013. Larval rearing temperature influences the effect of malathion on *Aedes aegypti* life history traits and immune responses. *Chemosphere*, 92:1111–1116.

## 6. Herbicides affect mosquito life history traits.

Approximately 180–185 million pounds of glyphosate and 73–78 million pounds of atrazine are used each year, ranking them as the first and the second most commonly used pesticides in the world. These herbicides are among the most frequently detected pesticide contaminants in aquatic ecosystems worldwide. Because mosquito larvae develop in a variety of aquatic habitats within and around agricultural lands, they are likely to be exposed to these chemicals on frequent basis. We investigated how an environmentally relevant concentration of the two herbicides, affects mosquito life history traits. One hundred and fifty (150) first instars of either *Aedes (Stegomyia) aegypti* (L.) or *Aedes (Stegomyia) albopictus* (Skuse) were reared in 1.6 L of live oak leaf (*Quercus virginiana*) infusion in the presence (5mg/L) or absence (0 mg/L) of one of two herbicides. The containers were monitored daily to determine the emergence rates, sex ratio, male and female emergence times, and female body size. For *Ae. aegypti*, atrazine exposure significantly increased emergence rate, the proportion of females emerging, and time to emergence compared to the control or glyphosate treatment. For both mosquito species, a sex ratio distortion with male bias was observed in control and glyphosate treatments, but this effect was eliminated in atrazine treatments. For *Ae. aegypti*, atrazine treatment resulted in significantly higher emergence rates and longer emergence time relative to the control or glyphosate treatments, but had no significant effect on female body size. For *Ae. albopictus*, atrazine treatment resulted in significantly longer emergence time and larger females relative to control or glyphosate treatments. In addition, atrazine treatment had significantly higher emergence rates compared to glyphosate, but not the control treatment. These results demonstrate the potential for atrazine, a widely used herbicide, to influence epidemiologically relevant life history traits of mosquitoes.

## Reference

Bara, J. J., A. Montgomery, and E. J. Muturi. 2014. Sublethal effects of atrazine and glyphosate on life history traits of *Aedes aegypti* and *Aedes albopictus* (Diptera:Culicidae). *Parasitology Research*, DOI10.1007/s00436-014-3949-y.

## 7. Impact of oxidases in the tolerance of *Aedes* mosquitoes to leaf infusions.

*Aedes (Stegomyia) aegypti* (L.) and *Aedes (Stegomyia) albopictus* (Skuse) larvae rely on oxidases to reduce toxicity of water soluble toxins from some senescent tree leaf infusions. The mortality of third instar *Ae. aegypti* larvae in live oak and pin oak leaf infusions increased significantly in the presence of piperonyl butoxide (PBO), a broad inhibitor

of cytochrome P450s (CYPs). In contrast, PBO treatment did not increase mortality in water controls or infusions of northern red oak or sugar maple leaf infusions for *Ae. aegypti* larvae. A similar pattern was observed for *Ae. albopictus* larvae, that is, an increase in mortality when CYPs were inhibited in live oak leaf infusions and no increase in sugar maple leaf infusions or water controls. However, the fresh live oak leaf infusion (5 d old) was the most toxic infusion to *Ae. aegypti*, but appeared less toxic to *Ae. albopictus* than the older infusions. A direct comparison of survival between the two *Aedes* species revealed *Ae. aegypti* exhibited a greater mortality than *Ae. albopictus* in PBO-treated live oak leaf infusions. These findings suggest that toxic components of some leaf litter in larval habitats may impose cryptic energy costs (detoxification).

### Reference

Lampman, R. L., C. H. Kim, and E. J. Muturi. 2014. The importance of oxidases in the tolerance of deciduous leaf infusions by *Aedes (Stegomyia) aegypti* and *Aedes (Stegomyia) albopictus* (Diptera: Culicidae). *Journal of Medical Entomology*, 51:68–75.

## 8. Immune responses of *Aedes aegypti* to larval density and Sindbis virus

Stressful environmental conditions during mosquito larval development may enhance susceptibility of adult mosquitoes to viral pathogens. Although anti-viral defense system in mosquitoes remains uncertain, stress-related enhancement of mosquito susceptibility to viral pathogens may be due to alteration of signaling pathways such as the Toll and immune deficiency (IMD) pathways. To test the influence of larval density and Sindbis virus (SINV) infection on mosquito Toll/Imd pathways, 100 or 200 *Aedes aegypti* larvae were reared in 2L of live oak (*Quercus virginiana*) leaf infusion and the adults were fed on SINV-infected (treatments) or non-infected (controls) bovine blood. SINV infection status and expression of genes encoding three antimicrobial peptides (cecropin, defensin, dipterin), an iron-binding protein (transferrin), and four regulators of Toll/Imd pathways (caspar, cactus, Rel1A, Rel2) were quantified by RT-qPCR at 7 and 14 days post blood meals. Irrespective of larval density, females incubated for 14 days after an infectious blood meal had significantly higher SINV titers compared to females from low density treatments that were incubated for 7 days. For both larval densities and time intervals, there was significant down-regulation of the Toll/Imd regulator genes in SINV-infected mosquitoes compared to controls. At day 7 post-infection, there was significant down-regulation of cecropin, defensin, dipterin, and transferrin in SINV-infected mosquitoes at low larval density, but this effect was only observed for dipterin at high larval density. These genes remained suppressed

on day 14, except cecropin which was significantly up-regulated at both larval densities, and transferrin which was similar to controls at low larval density. We conclude that SINV infection suppresses Toll/Imd pathways, but high larval density enables SINV to attain maximum titers in *Ae. aegypti* much earlier compared to low density treatments despite the up-regulation of cecropin.

### Reference

Kim, C. H., and E. J. Muturi. 2013. Effect of larval density and Sindbis virus infection on immune responses in *Aedes aegypti*. *Journal of Insect Physiology*, 59:604–610.

## III. Outreach

Medical Entomology Display panels at Illinois Natural History Survey, University of Illinois.

Guest lecturers for Department of Entomology, University of Illinois, Integrated Pest Management and biology of disease vectors.

Guest lecturers for Department of Pathobiology, University of Illinois, Ecotoxicology.

Invited Seminars (University of Illinois, Illinois State University).

Poster and paper presentations (Illinois Mosquito Vector Control Association, American Mosquito Control Association, Entomological Society of America, Society for Vector Ecology).

Article contributions to Illinois Natural History Survey Reports.

Professional reviews provided to peer-reviewed journals.

Contacts with Illinois Mosquito Abatement District Directors, Assistant Directors, Biologists, Illinois Department of Public Health, and Champaign-Urbana Public Health District personnel regarding mosquito surveillance efforts.

Cover Photo © Joseph L. Spencer, INHS  
Eastern Inland Floodwater Mosquito (*Aedes vexans*)