BIENNIAL REPORT 2010-2011

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

Legislative Mandate:
“To provide for research on disease vectors associated with used and waste tires and the diseases they spread.”

Summary of Accomplishments by the Medical Entomology Program

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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 (Fig 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 arboviruses in the laboratory and natural infections of West Nile Virus (WNV) have also been documented in both species. Discarded tires also serve as breeding sites for many native mosquitoes most notably the northern house mosquito *Culex pipiens* (Fig 1), the vector for WNV and St. Louis encephalitis virus and the eastern treehole mosquito *Ochlerotatus (Aedes) triseriatus* the vector for LaCrosse encephalitis virus. 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 contribute to disease management efforts. The development and certification of biosafety level 2 arthropod-containment facilities at the Medical Entomology Program three years ago has provided a unique opportunity to study in great details how container-dwelling mosquitoes interact with arboviruses.

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

1. Vector-host and arbovirus interactions for West Nile virus in Illinois.

WNV was first detected in the United States in 1999, and has since spread across much of North America inflicting substantial morbidity and on humans and wildlife. A large outbreak of WNV in Illinois in 2002 followed by sustained endemic and epidemic transmission of the virus raised serious health concerns among medical entomologists, health care providers, and political leaders and in 2003, the Emergency Public Health Fund was established to enable researchers at the Illinois Natural History Survey address some of these concerns. The Medical Entomology Program at the Illinois Natural History
Survey has worked closely with Illinois Mosquito Abatement Districts to aid in arbovirus surveillance and related research in order to better understand the transmission dynamics of WNV and other related disease agents (St. Louis encephalitis virus).

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 monitors 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 2010 and 2011, The Medical Entomology tested 8,438 mosquito pools for West Nile virus RNA by polymerase chain reaction (TaqMan RT-PCR) and similar numbers of samples are anticipated during the 2012 season. Data generated from these efforts are generally disseminated to the public by the Medical Entomology program through peer reviewed journals and technical reports.

1b. Predicting the seasonal shift in mosquito populations preceding the onset of the West Nile virus in central Illinois.
The rise in infection rate in humans and vectors with West Nile virus is associated with a seasonal shift in the abundance of mosquitoes Culex restuans and C. pipiens populations in the Midwestern United States. Models to predict this seasonal shift in population based on daily temperature data have been run operationally since 2004 at the Midwestern Regional Climate Center located at the Illinois State Water Survey. In 2009, the models were modified to incorporate National Weather Service Model Output Statistics (MOS) 10-day temperature forecasts. This research evaluated the effectiveness of these models to predict the crossover date when the proportion of C. pipiens, the presumed epidemic vector of West Nile virus, becomes and remains equal and greater than that of C. restuans. Unique observations by the Illinois Natural History Survey of daily Culex egg-raft observations have been made since 1988, providing both the impetus for and the verification data for the predictive models. For 2002 – 2009, it was found that for 6 of the 8 years, at least one model was within 1 week of actual crossover date, and in all but one year, at least one of the models was within 2 weeks of the actual crossover date. However, the predictive models often converge on the crossover date with no lead time. Incorporation of MOS temperature forecasts for a 10-day period, while not improving the actual crossover date, dramatically improved the forecast lead time by 6-8 days. In 2009, the earliest crossover date for the 1988-2009 periods was observed but not predicted.


Pesticide contamination of environment is an emerging challenge for ecologists. The rising demand for food and renewed efforts to reduce the burden of vector-borne diseases has led to dramatic increase in pesticide use over the last few decades. In the United States alone, at least two billion kilograms of more than 80,000 registered pesticides are released into the environment each year. These pesticides enter the aquatic ecosystems including container habitats through direct application (e.g. mosquito control), leaching, surface runoff, and spray-drift from adjacent agricultural lands. Therefore, it is likely that mosquito larvae are frequently exposed to both lethal and sublethal concentrations of pesticides. One of our major goals is to investigate how exposure of mosquito larvae to sublethal concentrations of pesticides affects the fitness of the resulting adult mosquitoes and their ability to transmit arboviruses. Here, we provide evidence that sublethal concentrations of pesticides may indeed alter the dynamics of mosquito-borne diseases by altering one or more components of disease transmission.

2a. Larval environmental stress alters Aedes aegypti competence for Sindbis virus.

We used a model system consisting of Sindbis virus (SINV) and the yellow fever mosquito Aedes aegypti to evaluate how stress at the larval stage alters adult mosquito performance and susceptibility to viral infection. Larvae were either reared under optimal conditions (control) or exposed to one of four types of stressors; suboptimal nutrients, starvation, elevated temperature, and a low dose of the insecticide malathion and adult females were fed SINV infectious blood meal. Differential expressions of stress, immune-specific and detoxification genes was measured in fourth instar larvae (HSP70, HSP83, cecropin, defensin, transferrin and CYP6Z6) and 3-day-old females (cecropin, defensin, transferrin) to identify plausible molecular mechanisms associated with mosquito response to stress. There were stress-specific variations in mosquito performance (survival, development time, female size), but all stressors had a consistent effect of significantly increasing susceptibility to viral infection and dissemination relative to the controls (Fig 2). Three genes were up-regulated in fourth instar larvae exposed to temperature stress (cecropin, defensin and CYP6Z6) compared to single genes in suboptimal nutrient (cecropin) and malathion (transferrin) stress treatments and down-regulation of all the six genes in starvation treatments. In adult samples, transferrin was up-regulated in all but starvation treatments while defensin was up-regulated in starvation and temperature stress treatments. These findings suggest that stress during larval
development may cause alterations in adult mosquito phenotype and immunity that can increase their susceptibility to pathogens.


2b. Can pesticides and larval competition alter susceptibility of Aedes mosquitoes (Diptera: Culicidae) to arbovirus infection?

Density-dependent processes such as larval competition may be important regulatory factors among some mosquito species. The application of pesticides used for control may alter these density-dependent interactions with consequences for the number of survivors and associated sublethal and chronic effects on these individuals. We examined how intraspecific competition among larvae and low concentrations of malathion alter Aedes aegypti L. and Aedes albopictus Skuse adult life history traits and competence for arboviruses using Sindbis virus as a model system. Larvae were reared at densities of 150 and 300 larvae per container and in the absence or presence of 0.04 parts per million of malathion, before surviving females were exposed to an infectious blood meal containing 10^5 plaque-forming units/ml Sindbis virus. For both species, competition and the presence of malathion reduced survival to adulthood. The presence of malathion eliminated the negative effects of competition that resulted in lengthened development time and smaller-sized adults. For Ae. aegypti, but not Ae. albopictus, high competition conditions and the presence of malathion independently led to an increase in virus dissemination from the midgut (Fig 3). Our results suggest that larval competition and chemical contaminants may influence disease transmission directly by altering adult mosquito fitness and indirectly by altering vector interactions with arboviruses.


2c. Larval environmental temperature and insecticide exposure alter Aedes aegypti competence for arboviruses.

Temperature is a key factor influencing mosquito growth and development and is also known to affect insecticide efficacy. We evaluated the effects of larval rearing temperature and exposure to insecticides on adult mosquito fitness and competence for
arboviral infection using Sindbis virus (SINV). We exposed newly hatched larvae of *Aedes aegypti* to an environmentally realistic level of insecticide malathion at 20°C and 30°C and allowed the resulting adults to feed on SINV-infected blood meal. Exposure to malathion significantly reduced survival to adulthood. Statistically significant interactions between temperature and malathion were observed for body size, estimated population growth, and SINV infection and dissemination. Malathion-exposed *Ae. aegypti* cohorts had significantly higher population growth at 20°C than at 30°C. Body size decreased with higher temperature and malathion-exposed females were larger than unexposed females at 20°C but not at 30°C. Viral infection and dissemination increased with larval rearing temperature and were higher in malathion-exposed than unexposed females at 30°C but not at 20°C (Fig 4). These results show that environmental factors, including those factors used in controlling mosquitoes, experienced by immature stages have latent effects that continue to adulthood and alter vector competence to arboviruses.


2d. Effect of temperature and insecticide stress on life-history traits of *Culex restuans* and *Aedes albopictus* (Diptera: Culicidae).

The chronic effects of exposure of *Culex restuans* (Theobald) and *Aedes albopictus* (Skuse) (Diptera: Culicidae) to low concentrations of malathion were examined by exposing larvae of the two species to four malathion doses at 20, 25, and 30 °C and maintaining the resulting adults at 25 °C. For both species, a significant
temperature by malathion interaction on survival was found. Greater temperatures at the highest malathion dosage had significantly lower survivorship than in other treatments, but this effect was not observed at 20 °C (Fig 5). For both species, temperature but not malathion had significant effects on female developmental time to adulthood and adult longevity. Temperature also affected adult female size for *Ae. albopictus* but not *Cx. restuans*. *Aedes albopictus* females developed faster as temperature increased, lived longer when larvae were maintained at 30 °C than at 20 °C, and were larger when larvae were maintained at 25 °C than at 20 or 30 °C. *Culex restuans* females developed faster at 25 and 30 °C than at 20 °C and lived longer at 25 °C than at 20 or 30 °C. The estimated finite rate of increase ($\lambda$') for *Cx. restuans* was significantly lower at 20 °C than at 25 and 30 °C, whereas that of *Ae. albopictus* was significantly influenced by an interaction between temperature and malathion with significantly lower $\lambda$' at 20 °C than at 25 and 30 °C for all malathion treatments except 0.014 mg/L. Understanding how pesticides interact with abiotic environmental conditions will contribute to management decisions about vector control practices.


**2e. Interaction of a pesticide and larval competition on life history traits of *Culex pipiens***

Mosquito larval development occurs in aquatic habitats that are directly or indirectly exposed to chemical contaminants. Little is known about how interaction of these chemicals with other biotic and abiotic stressors impact mosquito populations. We used two levels of nutrient (low and high) and four larval densities (10, 20, 30, 40) to examine the effects of low concentrations of insecticide malathion, on *Culex pipiens* L. mosquitoes experiencing stress from larval competition. Addition of malathion at the high nutrient condition enhanced survival with increasing larval densities, but this effect was not observed at low nutrient condition (Fig 6). Males exposed to malathion were significantly larger than those from control treatments while the effect of malathion on size of females varied with larval density by malathion interaction on survival was found. Greater temperatures at the highest malathion dosage had significantly lower survivorship than in other treatments, but this effect was not observed at 20 °C (Fig 5). For both species, temperature but not malathion had significant effects on female developmental time to adulthood and adult longevity. Temperature also affected adult female size for *Ae. albopictus* but not *Cx. restuans*. *Aedes albopictus* females developed faster as temperature increased, lived longer when larvae were maintained at 30 °C than at 20 °C, and were larger when larvae were maintained at 25 °C than at 20 or 30 °C. *Culex restuans* females developed faster at 25 and 30 °C than at 20 °C and lived longer at 25 °C than at 20 or 30 °C. The estimated finite rate of increase ($\lambda$') for *Cx. restuans* was significantly lower at 20 °C than at 25 and 30 °C, whereas that of *Ae. albopictus* was significantly influenced by an interaction between temperature and malathion with significantly lower $\lambda$' at 20 °C than at 25 and 30 °C for all malathion treatments except 0.014 mg/L. Understanding how pesticides interact with abiotic environmental conditions will contribute to management decisions about vector control practices.


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![Fig 1: Effect of resource, larval density and malathion on survival (male+female, A) and male longevity (B) of *Culex pipiens*. Proportion survival data were arcsine square root transformed while longevity data was log transformed to meet the normality.](image-url)
density and the level of nutrients. Larval exposure to malathion and low nutrient resulted in significantly larger females with increases in larval densities compared with other treatments. The effect of malathion on male longevity varied with larval density and amount of nutrients (Fig 6). At higher densities, male longevity was consistently higher in low nutrient than in high nutrient conditions and addition of malathion in high nutrient treatment increased male longevity at the highest density. These effects are most likely attributable to release from competition among survivors after mortality from malathion and density-dependent effects. We conclude that biotic conditions of the larval habitat can alter the impact of low concentrations of chemical contaminants on mosquito populations in ways that may influence the pattern of disease transmission and the outcome of vector control efforts.


3. **Competition among container-dwelling mosquitoes.**

Intra- and interspecific competition is one of the major biotic processes that shape ecological communities. Both types of competition are particularly common in container habitats because they are often resource-deprived yet occupied by large numbers of diverse mosquito species. Our goal was to evaluate the outcome of competitive interactions between native and invasive mosquito species and how the conditions of the larval environment (e.g. resource type, quality and ratio) may influence these interactions. We also assessed how these interactions may influence the risk of arbovirus transmission.

3a. **Interspecific larval competition between invasive Aedes japonicus and native Aedes triseriatus (Diptera: Culicidae) and adult longevity.**

The Asian rock pool mosquito *Aedes japonicus* (Theobald) inhabits natural and artificial container habitats, some of which are occupied by the native treehole mosquito *Aedes triseriatus* (Say), a vector of LaCrosse encephalitis virus. A laboratory experiment was used to evaluate the effects of nutrient limitation and interspecific interactions between these species. The goal was to address two related hypotheses. First, interspecific interactions between these species show competitive asymmetry with the invasive mosquito *Ae. japonicus* being favored over *Ae. triseriatus*. Second, competitive stress at the larval stage alters adult longevity. There was minimal evidence for competitive asymmetry between these two species. Mosquito and population performance showed clear negative density-dependent effects with similar effects of intra- and interspecific interactions. Only *Ae. japonicus* development time showed competitive asymmetry over *Ae. triseriatus*, providing weak support for the first hypothesis. For both species, competition resulted in lower adult longevity compared with low competition, providing support for the second hypothesis. These results suggest both species are similarly affected by intra- and interspecific competition and underscore the importance of the effects of larval competition that continue into adulthood and alter parameters important to transmission of vector-borne diseases.
3b. The effects of resource type and ratio on competition with *Aedes albopictus* and *Culex pipiens* (Diptera:Culicidae).

The introduction of *Aedes albopictus* (Skuse) in the United States has been associated with declines in abundance of resident mosquito species, presumably because of resource competition, as larvae of *Ae. albopictus* have been illustrated as superior competitors under certain resource conditions. We evaluated the hypothesis that varying the type and ratio of two food resources (Foxtail grass: American elm) alters the competitive outcome of *Ae. albopictus* and *Culex pipiens* (L.). We measured survivorship, development time, size, and adult longevity, and estimated the population growth index ($\lambda'$) of populations raised both alone and in equal number with the interspecific competitor, across five ratios of the two food resources. Competition was asymmetric with *Ae. albopictus*, the superior competitor across all resource treatments; however, the competitive advantage *Ae. albopictus* had over *Cx. pipiens* was reduced as grass became the predominant resource. With elm as the predominant resource, the population growth index ($\lambda'$) for both *Ae. albopictus* and *Cx. pipiens* was lower in intraspecific and interspecific competition treatments, respectively. The treatments also impacted adult life history, as life spans of both *Ae. albopictus* and *Cx. pipiens* varied when they emerged from larval conditions with different resource and competition treatments. We discuss the possible differences in the two species' efficiencies in exploiting the two resource types. Despite some resource conditions alleviating the competitive effects of *Ae. albopictus* on *Cx. pipiens*, competition remained asymmetric; thus, additional mechanisms are likely operating under field conditions when the two species coexist.


3c. Trait-mediated effects of predation across life-history stages in container mosquitoes.

This study examined whether the predatory midge *Corethrella appendiculata* Grabham imposes a fitness cost in a native mosquito, *Ochlerotatus triseriatus* Say, and an invasive mosquito, *Aedes albopictus* Skuse. We tested the hypothesis that decreased activity of immature prey in the presence of predator cues is associated with life history costs through all life cycle stages. In experiment 1,
individual larvae of *O. triseriatus* or *A. albopictus* were raised in the presence or absence of predation cues at two resource levels. Prey were video recorded to detect behavioural responses and to measure development time, size at emergence, and adult longevity. In experiment 2, prey populations were reared in similar environments and the frequency of predator cue additions was varied. Only *O. triseriatus* reduced its activity in the presence of predation cues (Fig 7). Predation cues were associated with longer immature development times and shorter adult life spans in *O. triseriatus*, whereas in *A. albopictus*, the cues were associated with a larger size of emerging adults. We conclude that behavioral modifications during the larval stage can affect mosquitoes through multiple stages of their complex life cycle. The species-specific behavioral differences are probably attributable to the longer evolutionary history *O. triseriatus* has with predators, relative to the invasive *A. albopictus*.


3d. Relationship between leaf litter identity, expression of Cytochrome P450 genes and life history traits of *Aedes aegypti* and *A. albopictus*.

The role of toxic component of leaf litter in mediating the outcome of mosquito species interactions is not well documented. To examine the effect of leaf litter toxins on mosquito performance and interspecific interactions, we reared monospecific and heterospecific cultures of *Aedes aegypti* L. and *A. albopictus* Skuse larvae in microcosms with one of five leaf species and measured the expression of five cytochrome P450 genes and life history traits of the two mosquito species. For both mosquito species, survival to adulthood was significantly higher in black alder, black walnut, and cypress infusion compared to sugar maple and eastern white pine infusion. In pine but not in other leaf treatments, the presence of *A. albopictus* had significant positive effects on *A. aegypti* wing length and development time to adulthood. *Aedes albopictus* from heterospecific cultures were larger than those from monospecific cultures and were smaller and took longer to develop in pine and sugar maple infusions than in the other infusions. Up regulation of CYP6Z6 and CYP9M9 in *A. aegypti* and *A. albopictus* respectively appeared to be closely associated with the deleterious effects of sugar maple infusion on mosquito performance as was the down regulation of CYP6N12 (in *A. aegypti*) and lack of induction of CYP6Z6 and CYP9M9 (in *A. aegypti* and *A. albopictus* respectively) in pine infusion. Results suggest that metabolic capabilities that enable the two species to tolerate natural xenobiotics are associated with a fitness cost.

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.

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

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


Poster and paper presentations (Illinois Mosquito Vector Control Association, American Mosquito Control Association, Entomological Society of America, American Society of Tropical Medicine and Hygiene).

Article contributions to Illinois Natural History Survey Reports.


Professional reviews provided to peer-reviewed journals.