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Insecticide Resistance Surveillance of a Zika Virus Vector, *Aedes albopictus*, in the State of Illinois

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Summary

The extent to which populations of two vector species in Illinois, the Asian tiger mosquito, *Aedes albopictus*, and the northern house mosquito, *Culex pipiens*, remain sensitive to two commonly-used insecticides (permethrin and malathion) was investigated using a series of CDC bottle assays. For *Ae. albopictus*, 6 colonies were established from collections in central and southern Illinois, while for *Cx. pipiens* eggs were collected in the North Shore of Cook County. The results suggest that the time until death occurred was longer in several of the more southern field populations for *Ae. albopictus* than that of a sensitive reference strain, for both malathion and permethrin at the recommended diagnostic dose. At a lower concentration of malathion, however, field populations appeared more sensitive than the reference strain. Further work will be required to confirm whether these conflicting results reflect environmental noise or a true genetic difference in the sensitivity status of these populations. The time until death occurred was also longer in field-collected *Cx. pipiens* than that of a sensitive reference strain when exposed to three different concentrations of permethrin.

The results of this initial insecticide resistance survey thus suggest that insecticide sensitivity varies among populations. Confirmation of and expansion on these results, through additional bioassays, inclusion of additional populations across Illinois, elucidation of the physiological mechanisms involved, and investigations performed over time will be required to develop a thorough understanding of the threat to public health associated with potentially increasing levels of resistance in IL mosquito populations.

Background

Zika virus (ZIKV) was initially isolated from a monkey in the Zika forest of Uganda in 1947 but remained relatively obscure for fifty years with only sporadic infections in humans being reported in Africa and Asia. This changed with a 2007 outbreak of ZIKV in Yap Island in the Federated States of Micronesia (Mlakar *et al.* 2016). From there, the virus continued to spread and caused epidemics in several Pacific Ocean islands in 2013-2014, and had reached 33 countries in Central and South America by 2015 (Mlakar *et al.*, 2016). In the US, 5,304 travel-associated and 226 locally acquired ZIKV infection cases were reported during the period between January 1, 2015 and November 22, 2017, and Illinois accounted for 110 travel-associated cases (CDC, 2017a). Most people infected with Zika virus are asymptomatic or they experience only mild symptoms (CDC, 2017b). However, the recent outbreaks in Central and South America suggest that ZIKV infection during pregnancy can result in microcephaly, other congenital anomalies, and miscarriages. Neurological disorders, such as Guillain-Barré syndrome, have also been associated with ZIKV infection (Mlakar *et al.*, 2016).

The appearance of locally-transmitted ZIKV in Florida, Texas, and U.S. Territories (Puerto Rico, American Samoa, and the U.S. Virgin Islands) has further intensified concerns about the occurrence and establishment of the two main vector mosquitoes, *Aedes aegypti* and *Ae. albopictus*. Currently, vector control is the most effective means of interrupting transmission of ZIKV (as well as other *Aedes*-borne viruses, such as dengue and chikungunya) since there are neither effective antiviral therapies nor vaccines available for it. Successful vector control relies on a thorough understanding of the behavior and ecology of the main vectors, their seasonal abundance, and their distribution patterns. Seasonal abundance depends on life history traits, such as the ability of a mosquito species to enter diapause. For instance, although none of the life stages of *Ae. aegypti* can survive the winter in Illinois, *Ae. albopictus* can survive freezing and overwinter in the egg stage (though likely only in the southern half of Illinois). As a result, populations can establish and become more widespread locally and appear earlier in the year than non-established invaders. This became clear during 2016 and 2017 surveys of the occurrence and abundance of *Aedes aegypti* and *Aedes albopictus* in 18 counties of Illinois (Kim *et al.*, unpublished data). It was found that multiple communities, for which occurrence records had previously not existed, were infested with *Ae. albopictus*, while *Ae. aegypti* was not found to be present. Considering that imported cases of Zika, dengue, and chikungunya viruses occur, along with the presence of a competent and increasingly abundant vector, *Ae. albopictus*, there is an urgency in preparing abatement and public health districts in Illinois for potential emerging arboviral outbreaks. Additionally, *Ae. albopictus* is a potential vector for a variety of vector-borne pathogens (e.g., La Crosse encephalitis virus) and a notorious nuisance biter. At high population densities, this species severely affects the quality and enjoyment of outdoor spaces in urban and suburban environments (Halasa *et al.*, 2014). Thus, even in the absence of localized arboviral outbreaks, a need for vector management methods targeting this species will be required.

One of the factors hindering vector control efforts is insecticide resistance. Although the frequency and distribution of *Ae. albopictus* in southern Illinois has been investigated, the extent to which these populations are sensitive to commonly-used chemical insecticides is unknown. This prompted us to survey for insecticide resistance in *Ae. albopictus* populations in Illinois. Using CDC insecticide resistance “bottle assays” (CDC, 2017c), we screened female adult *Ae. albopictus* populations in southern and central Illinois for insecticide resistance to technical-grade pyrethroids (permethrin) and organophosphates (malathion). In addition, we performed initial resistance assays

on *Culex pipiens* (the major West Nile vector in Illinois), collected from areas in northern Illinois, for which control relies on a combination of larviciding and the application of chemical adulticides.

Methods

Study area

Guided by the survey results from 2016 and 2017, we selected communities in southern and central Illinois to collect *Ae. albopictus* eggs. The residential areas where we obtained a sufficient number of eggs to initiate experimental lab colonies were Champaign, Mt. Vernon, Paris, Fairfield, Albion, and Robinson. These communities are located along the I-57, IL-1 and IL-15 highways. The intent had been to include communities from northern Illinois as well, but these areas did not, at least this year, support sufficient *Ae. albopictus* populations. We did, however, establish a collaborative relationship with the Northshore Mosquito Abatement District (NSMAD) to test *Culex pipiens* from the district's survey areas for insecticide resistance.

Egg sampling

Eggs of *Aedes* mosquitoes were collected during the mosquito breeding season (July – October) in 2017. Using oviposition traps with germination paper lining and sugar maple infusion we operated the traps in the field for 12 consecutive nights to collect mosquito eggs. The eggs on the germination paper were stored in an incubator with temperature set at 13 °C until a sufficient number of eggs was collected to establish a colony for each location. The eggs were then hatched and reared following standard lab protocols. Adults were identified to species morphologically (Darsie Jr and Ward, 2005). The adult *Ae. albopictus* were provided with 10% sugar solution and blood-fed twice weekly using a Hemotek membrane feeding system (Hemotek Ltd., Blackburn, UK). Eggs were stored as above, until a sufficient number of F-1 eggs were obtained to perform CDC bioassays. As a sensitive reference strain, we used the ATM95 strain which was originally colonized from field collections in New Jersey in 1995 and whose insecticide sensitivity status has previously been characterized (Marcombe *et al.*, 2014).

For the bioassays on *Cx. pipiens*, egg rafts were collected by oviposition traps with grass infusion either on September 11, 2017 or on September 18, 2017. The GPS coordinates for the collection sites are listed in Table 1. The eggs were hatched and reared in an environmental chamber with its temperature set at 25 °C and a photoperiod set at 12-hour light and 12-hour dark. All adult mosquitoes were housed in 1-cubic-foot cages kept in an environmental chamber with the same temperature and photoperiod settings as the larval rearing conditions. The adult mosquitoes were given *ad libitum* access to a 10% honey solution before the bioassays, while no blood meal was provided. We also acquired eggs of a *Cx. pipiens* susceptible strain originally collected in Chicago from Dr. Janet McAllister at the CDC. The eggs of this strain were handled the same way as the eggs from NSMAD, and were used as a control in the CDC bottle assays.

Bioassay for insecticide resistance

We used the CDC bottle assay to investigate the susceptibility of reference and field populations of *Ae. albopictus* and *Cx. pipiens*. These bioassays were conducted for two insecticides commonly used to control adult mosquitoes: permethrin (pyrethroid) and malathion (organophosphate). To cover a wider range of insecticide concentrations than the discriminating doses established for a limited number of species by the CDC, we tested the effects of 7.5, 15 and 30 ppm permethrin, and 25, 50 and 100 ppm malathion (i.e., half and two times the recommended dose for *Aedes* spp.). Acetone was used to make working solutions with different concentrations of each insecticide.

Each replicate of the bioassay used 4 250-ml bottles with caps: a control bottle coated with pure acetone and 3 bottles coated separately with different concentrations of the insecticide. Between 10 and 20 female adult mosquitoes were placed in each bottle and monitored until all mosquitoes died or up to 2 hours. The number of dead mosquitoes in each bottle was recorded at a 10-minute interval.

Results and Discussion

Aedes albopictus

Three replicate bottles for two concentrations of each insecticide were performed for each of six *Ae. albopictus* field populations and a sensitive colony (ATM95). The resulting percentages of mosquitoes that had died or were knocked down by 10-min increments are depicted (Fig. 1). A depiction isolating and more clearly highlighting the results of the sensitive strain is also provided (Fig. 2).

Based on parametric survival analyses, assuming log-normal distributions of error, there were statistically significant differences in survival times among populations, although these were not consistent among insecticides and the doses investigated. For malathion, at 50 ppm, all colonies except for Paris lived significantly longer than did ATM95. At 24 ppm, mosquitoes from Albion, Champaign, Fairfield, and Paris, but not Robinson and Mt Vernon, differed from the reference colony. Here, interestingly, the field populations all appeared more sensitive than the reference strain (Fig. 2). For permethrin, at the recommended dose of 15 ppm, only the Albion and Fairfield populations were significantly different from the reference strain, while at 7.5 ppm the differences between populations were more pronounced and all field populations lived longer than ATM95.

At the recommended diagnostic time of 30 minutes and the diagnostic doses (malathion at 50 ppm and permethrin at 15 ppm), none of the colonies has reached 100% mortality. For malathion, the populations from Albion and Mt. Vernon stand out as having a high survivorship at this time frame, while for permethrin Fairfield and Albion stand out. It is worth noting that at this time frame, one would wish to see 100% mortality in a sensitive strain. The fact that our reference strain (ATM95) falls short of this indicates that in the future, the diagnostic dose for *Ae. albopictus* may be higher than what we used here. Possibly, this could reflect that *Ae. albopictus* is in general a more resilient mosquito than *Ae. aegypti* (for which resistance assays will have been more commonly performed).

For future studies in Illinois, we plan to calibrate the diagnostic dose and time based on the ATM95 colony again.

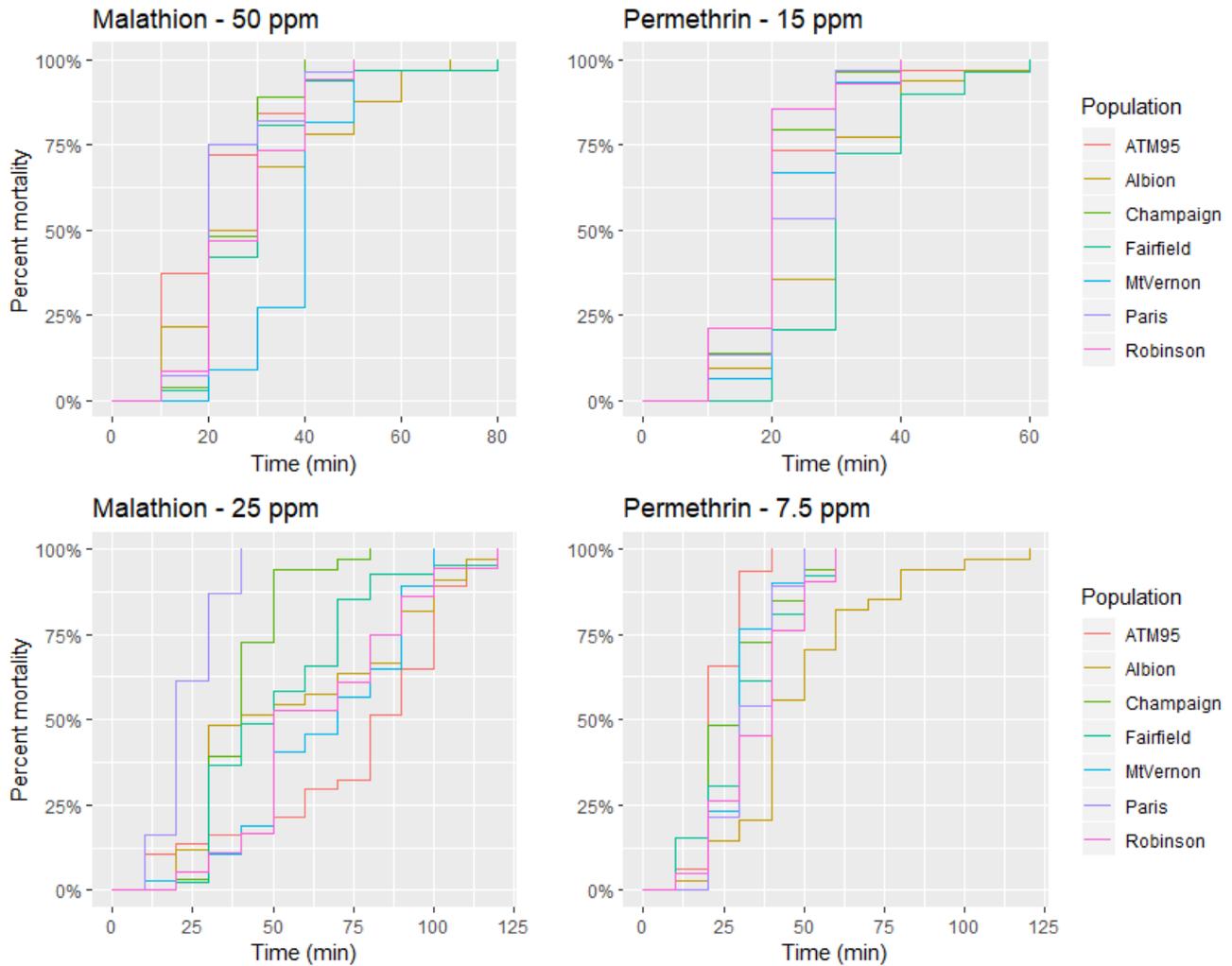


Fig 1: Percentage mortality by time in minutes following introduction into insecticide-coated bottles. Different colors indicate mosquitoes from different populations, with ATM95 being the sensitive reference strain.

Overall, these results for *Ae. albopictus* suggest that certain populations in Illinois are more susceptible to insecticides than others. In particular, Fairfield, Albion and Mt. Vernon – all among the more southern populations we sampled – appear potentially resistant. Given the inconsistency among results (e.g., the outcomes for malathion at the lower concentration) and the incomplete mortality of the sensitive reference strain at the recommended diagnostic time, care should be taken with the interpretation and further work investigating this in more detail is recommended.

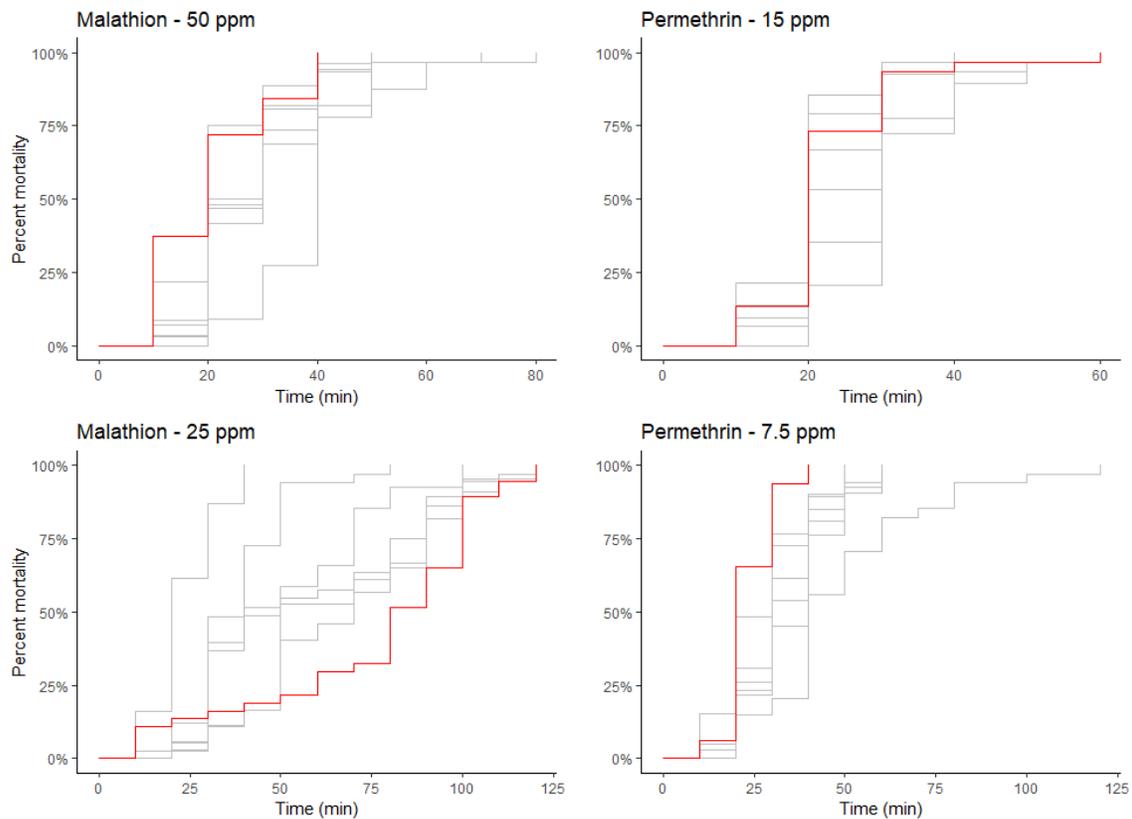


Fig. 2: Percentage mortality by time in minutes following introduction into insecticide-coated bottles. Results are as in Fig. 1, with red lines here representing the reference strain (ATM95) and all other field populations presented in grey.

Culex pipiens

For *Cx. pipiens* we performed bottle assays using three concentrations (7.5, 15, and 30 ppm) of permethrin. For the field populations, we used 4 replicate bottles for the lowest concentration, 3 for the middle concentration, and 7 for the highest concentration. Due to a low number of females available from the reference strain, we were only able to perform a single replicate per concentration for these mosquitoes.

Here, we detected a delay in mortality of *Cx. pipiens* from NSMAD compared to that of the susceptible strain obtained from the CDC (Fig. 3). The time to death was significantly longer in the field populations than in the reference strain for all three concentrations. At the recommended diagnostic dose and times (following the CDC recommendation for *Aedes* spp., as one for *Culex* spp. is not available), mortality among the field populations was only 50%, which would suggest there is some resistance present in these populations.

These findings should again be interpreted with care. For instance, the reference strain did not experience 100% mortality at 15 ppm. At the higher dose of 30 ppm, the reference strain reached 100% mortality within the diagnostic time frame, while the field populations still had a proportion of mosquitoes surviving. Another reason for caution is that due to the low number of individuals from the reference strain used in the bottle assays, we were only able to test one replicate bottle with 20 females of this strain for each concentration. Even so, the apparent decreased sensitivity of the field

populations, for which we had sufficient replication, suggests these findings should be followed up on with additional tests performed over subsequent summers and with *Culex pipiens* populations from a wider geographic area, while work on the molecular and physiological basis of this (incipient) resistance in Illinois *Culex* populations also appears to be warranted.

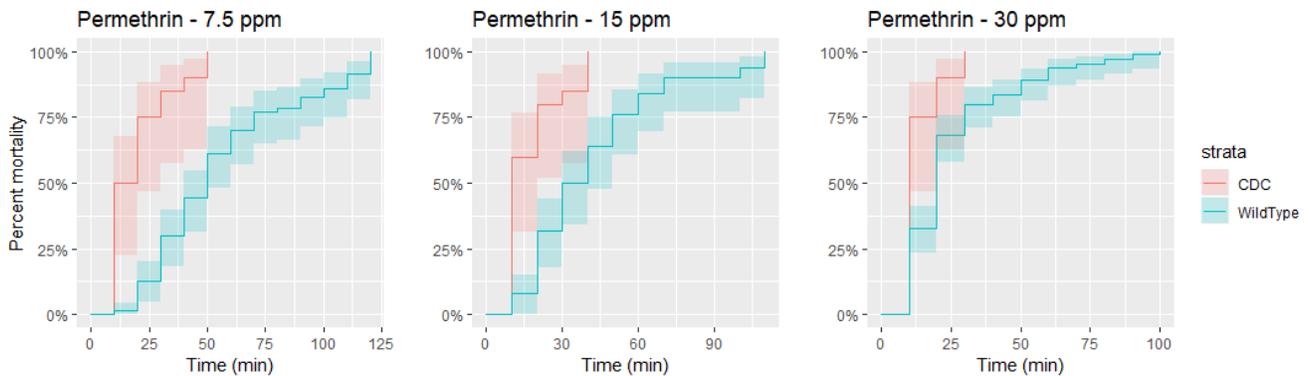


Fig. 3: Percentage mortality by time in minutes following introduction into insecticide-coated bottles for *Culex pipiens* females. Lines show the average survival outcomes over all replicate bottles and shaded areas the associated confidence interval.

Acknowledgements

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