

Impact of Mosquito Age and Insecticide Exposure on Vector Competence of *Aedes albopictus* for Zika Virus

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Introduction

- Zika virus can cause birth defects, autoimmune, and other issues and is a serious public health concern.
- Primarily transmitted to humans by *Aedes aegypti* and *Aedes albopictus* mosquitoes; however, it can be transmitted in humans both sexually and congenitally.



Zika



- Zika virus in the United States: July 2016 in Florida.
- In 2017 there were 418 symptomatic Zika virus disease cases reported (CDC).
 - 409 of those cases were recorded from travelers returning from affected areas.
 - 4 cases were acquired through presumed local mosquito-borne transmission in Florida (N=2) and Texas (N=2).
 - 5 cases were acquired through sexual transmission.
- As of January 2018, there have been a total 223,477 confirmed Zika cases worldwide.
 - In August of 2016 there were only 107,888 total cases.

Vector Competence

- Vector competence is the ability of a mosquito to acquire, maintain, and transmit a pathogen.
 - Not all mosquito species can transmit a pathogen.
- Mosquito age is a biological factor that may influence vector competence.
- The immune response of mosquitoes to infection may weaken with age, thus influencing vector competence.



Bifenthrin

- Bifenthrin is a Type 1 pyrethroid that affects the central and peripheral nervous system of invertebrates, such as mosquitoes, by hindering the sodium channel gating, which leads to death.
- Insecticide exposure can cause two different effects, direct toxic effects which cause mortality or sublethal effects.
- Sublethal effects of insecticides can be behavioral (i.e. avoidance) or biological (i.e. changes in fecundity/fertility).

Insecticide Resistance

- Insecticide resistance refers to changes in an insect that increase its ability to withstand or overcome the effects of one or more insecticides.
- Since 2010, a total of 60 countries have reported mosquito resistance to at least one class of insecticide, with a total of 49 of those countries reporting resistance to two or more classes (WHO 2016).



Hypothesis

- Contact with sublethal doses of bifenthrin will enhance vector competence of *Aedes albopictus* for ZIKV and this relationship changes with age.



Study Design

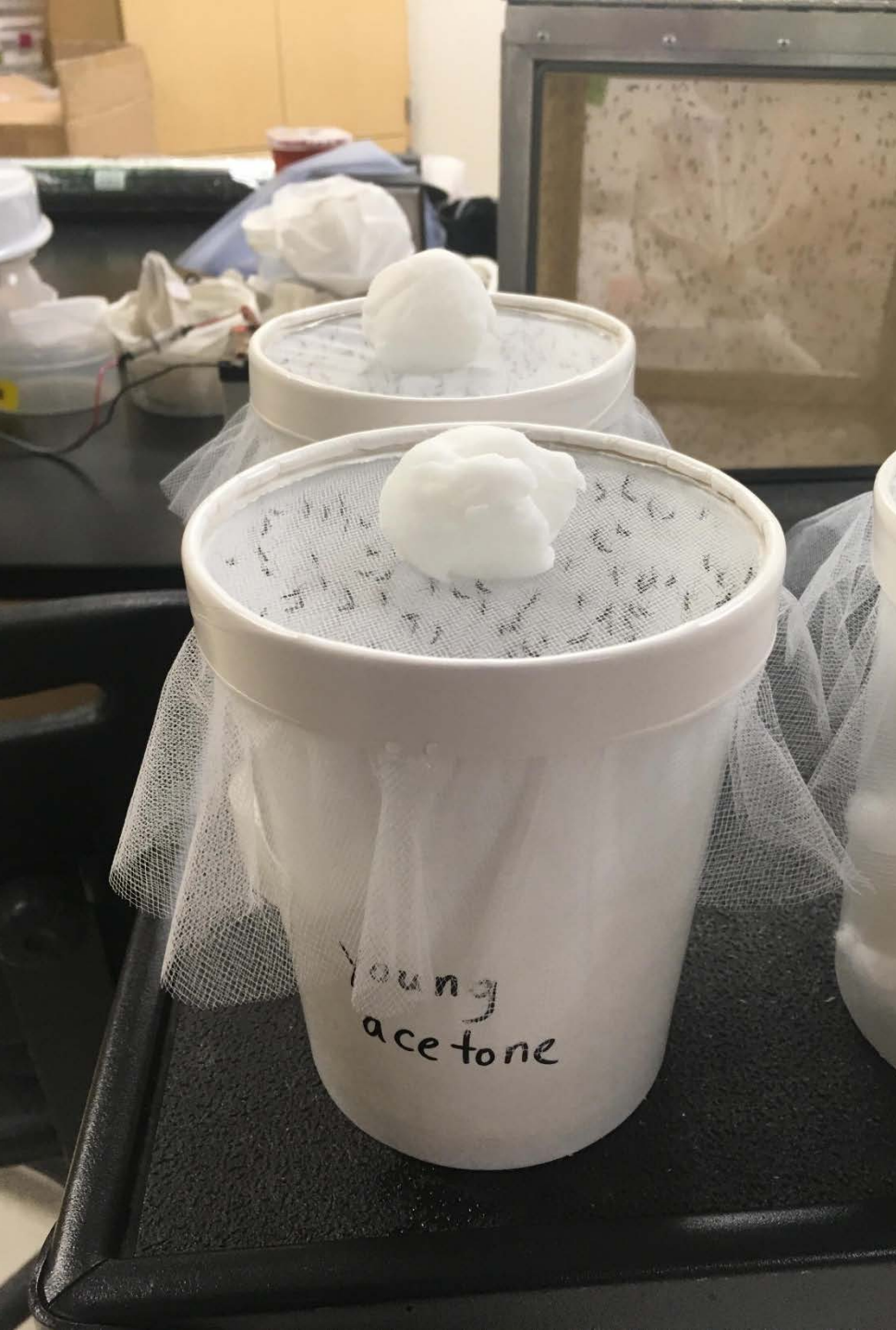
- *Aedes albopictus* (F₂₉)
 - Young, 6-7 days old
 - Old, 11-12 days old



Exposure to Insecticide

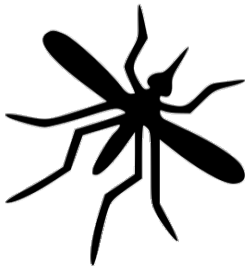
- Treatment bottles coated with 1mL of bifenthrin stock (0.128 $\mu\text{g}/\text{mL}$).
- Control bottles coated with 1mL of acetone.
- Coated the day before the bioassay and allowed to dry overnight.
- Female mosquitoes exposed for 30 minutes to bottles coated with either bifenthrin or acetone.
- Approximately 100 mosquitoes/bottle





Mosquito Infection

- One day after bifenthrin/acetone exposure, mosquitoes allowed to blood feed on ZIKV-infected blood meal.
- Fully engorged mosquitoes were transferred to separate 1 L cardboard cages with mesh screening and maintained in incubators for 7 days at 28°C and provided 20% sucrose *ad libitum*.

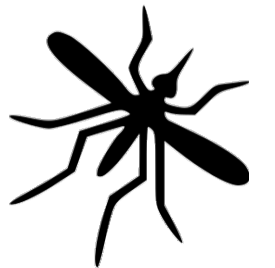


Mosquito Processing

- Mosquitoes surviving the 7-day incubation period removed from their cages.
- Bodies and legs processed separately (qRT-PCR).
- Virus found in the body but not the legs represented a non-disseminated infection limited to the midgut.
- Virus found in both the body and legs was considered a disseminated infection.

Data Analysis

- Chi-Square analysis of the infection and dissemination rates and ANOVA of ZIKV titers compared between insecticide and control groups between ages.



Results

Group	Sample Size (Day 0)	Sample Size (Day 7)	Percent Mortality
Old Acetone	29	16	45
Old Bifenthrin	63	14	78
Young Acetone	36	24	33
Young Bifenthrin	50	28	44

- 10-20% blood feeding rate.
- Significant Differences:
 - Age at Bifenthrin exposure (p -value 0.0017)
 - Difference between Old Bifenthrin and Old Acetone significant (p -value 0.0002)

Results

- The results show that there is a midgut barrier preventing dissemination into the legs for the groups Old and Young Acetone, and Young Bifenthrin.
- Old Bifenthrin group showed a high dissemination rate in the legs.

Table 1.

<i>Description</i>	<i>No. Tested</i>	<i>No. ZIKV</i>		<i>ZIKV Body Titer</i>	<i>ZIKV Leg Titer</i>
		<i>Body</i>	<i>Leg</i>		
		<i>Infection (%)</i>	<i>Infection (%)</i>		
Old Acetone	16	15(94)	3(19)	3.8±0.3	0.9±0.3
Old Bifenthrin	16	16(100)	14(88)	4.9±0.3	2.6±0.4
Young Acetone	25	25(100)	1(4)	4.7±0.1	0.1±0.1
Young Bifenthrin	30	29(97)	5(1)	4.3±0.2	1.6±0.3

The mean titers (\log_{10} PFUeq ZIKV/ mL) \pm standard error and rates of infection (% with ZIKV positive bodies) for

Aedes albopictus fed ZIKV-infected blood meals ($5.5 \log_{10}$ PFUeq blood meal titer) and held at 28°C for 7d.

Conclusion

- Preliminary testing has shown that for this population of mosquitoes age at time of insecticide exposure is a significant factor in the mortality rate of the mosquito.
- For this population of mosquitoes, bifenthrin does seem to significantly impact the ability for Zika virus to exit the midgut and disseminate through the mosquito at the old age point.

Discussion

- Impacts of insecticides should be considered in risk assessments of potential vector populations.
- We expect these relationships to change under different biological and environmental conditions, and with different mosquito populations and virus strains.

Acknowledgments

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QUESTIONS?