

# Potential for sublethal insecticide exposure to impact vector competence of *Aedes albopictus* (Diptera: Culicidae) for dengue and Zika viruses

Stephanie L. Richards, Avian V. White, and Jo Anne G. Balanay

## Introduction

Dengue and Zika viruses (DENV and ZIKV, Family Flaviviridae, genus Flavivirus) are arboviruses that cause human epidemics. Due to lack of vaccines for many mosquito borne diseases, there is a need for mosquito control. In the United States and other regions, residual barrier insecticide sprays applied to foliage where female mosquitoes rest and/or sugar feed between blood meals are an important control method for anthropogenic day-active mosquitoes such as *Aedes albopictus* (vector of DENV and ZIKV). These mosquitoes are difficult to control using traditional sprays applied only at dusk or dawn when these mosquitoes are not active. In this exploratory study, we analyzed the extent to which ingestion of a sublethal dose of the active ingredient bifenthrin affected vector competence (i.e. infection, dissemination, and transmission) of *Ae. albopictus* for DENV and ZIKV.

## Objective

- To evaluate the effects of sublethal doses of insecticide exposure on vector competence of *Aedes albopictus* for DENV and ZIKV.

## Materials and Methods

- Louisiana *Aedes albopictus* F<sub>22</sub> colony
- Control group fed 20% sucrose solution; Insecticide group allowed to feed on 20% sucrose solution containing 0.128 µg/mL bifenthrin mixture for 4 hours
- Mosquitoes allowed to feed on infectious blood meals [Zika: Puerto Rican isolate (PRVABC59) 5.4 log<sub>10</sub> plaque-forming units (pfu) ZIKV/mL; Dengue: Southeast Asian DENV-2 isolate (16803) 5.4 log<sub>10</sub> pfu DENV/mL] for 45 minutes
- Fully engorged female mosquitoes transferred to 1L cages according to treatment
- Incubated at 28°C for duration of experiment
- After 7 and 14 day post infection, 5-10 live mosquitoes removed from each group and tested for ZIKV/DENV in bodies (infection), legs (dissemination) and saliva (transmission).
- Viral RNA isolated; qRT-PCR to quantify DENV/ZIKV



## Calculations

- Infection =  $\frac{\text{no. infected bodies}}{\text{no. of tested bodies}}$
- Dissemination =  $\frac{\text{no. of positive legs}}{\text{no. of infected bodies}}$
- Transmission =  $\frac{\text{no. of positive saliva}}{\text{no. of infected bodies}}$

## Results

**Table.1** The mean titers [log<sub>10</sub> plaque-forming unit equivalents (pfueq) DENV/mL or ZIKV/mL] ± standard error, infection rate, dissemination rate, and transmission rate for *Ae. albopictus* by pre-blood feeding meal, virus type, and incubation period.

Pre-blood feeding meal	Number tested	Number infected (%) <sup>1</sup>	Number disseminated (%) <sup>1</sup>	Number transmitted (%) <sup>1</sup>	Body Titer <sup>2</sup>	Leg titer <sup>2</sup>	Saliva titer <sup>2</sup>
7 days post-blood meal							
DENV							
Sugar	10	9 (90) <sup>a</sup>	3 (30) <sup>a</sup>	0	4.3±0.4 <sup>a</sup>	2.5±0.1 <sup>a</sup>	-
Sugar + Bifenthrin	10	8 (80) <sup>a</sup>	1 (10) <sup>a</sup>	0	4.3±0.3 <sup>a</sup>	2.5 <sup>a</sup>	-
ZIKV							
Sugar	10	10 (100) <sup>a</sup>	4 (40) <sup>b</sup>	1 (10)	5.1±0.3 <sup>a</sup>	0.1±0.0 <sup>a</sup>	1.4
Sugar + Bifenthrin	10	10 (100) <sup>a</sup>	10 (100) <sup>a</sup>	0	4.2±0.5 <sup>a</sup>	0.4±0.2 <sup>a</sup>	-
14 Days post -blood meal							
DENV							
Sugar	10	9 (90) <sup>a</sup>	2 (20) <sup>a</sup>	0	5.3±0.2 <sup>a</sup>	3.7±0.3 <sup>a</sup>	-
Sugar + Bifenthrin	10	4 (40) <sup>a</sup>	1 (10) <sup>a</sup>	0	3.5±1.1 <sup>b</sup>	2.3 <sup>a</sup>	-
ZIKV							
Sugar	10	10 (100) <sup>a</sup>	10 (100) <sup>a</sup>	0	4.9±0.4 <sup>a</sup>	2.1±0.6 <sup>a</sup>	-
Sugar + Bifenthrin	5	5 (100) <sup>a</sup>	5 (100) <sup>a</sup>	1 (20)	5.4±0.2 <sup>a</sup>	3.3±0.2 <sup>a</sup>	3.2

Analyses were conducted separately for each virus. <sup>1</sup>Same letter in the same column for each virus not significantly different between treatments by chi-square. <sup>2</sup>Same letter in the same column not significantly different between treatments by means comparison.

Figure 1. DENV 14 Day Post Infection

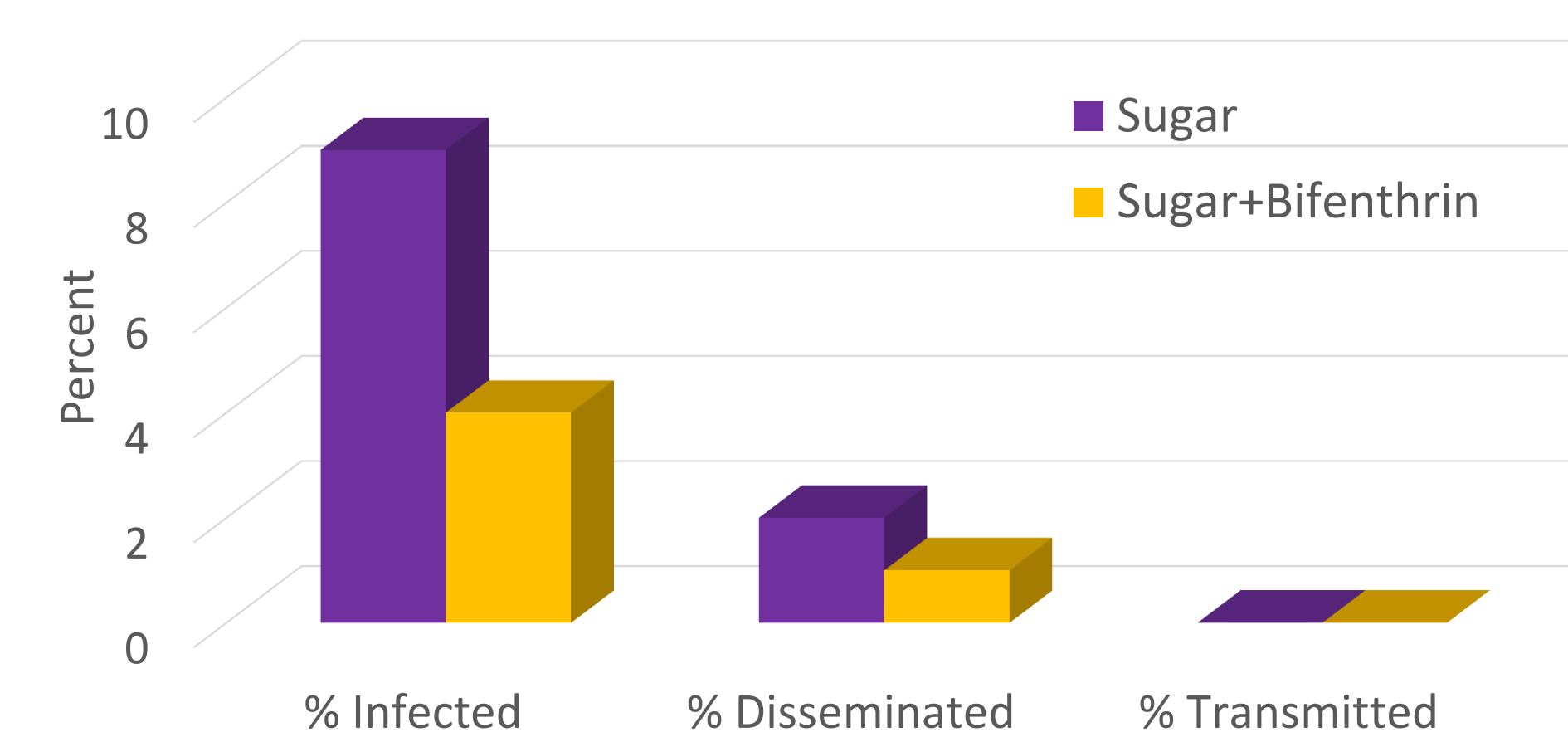
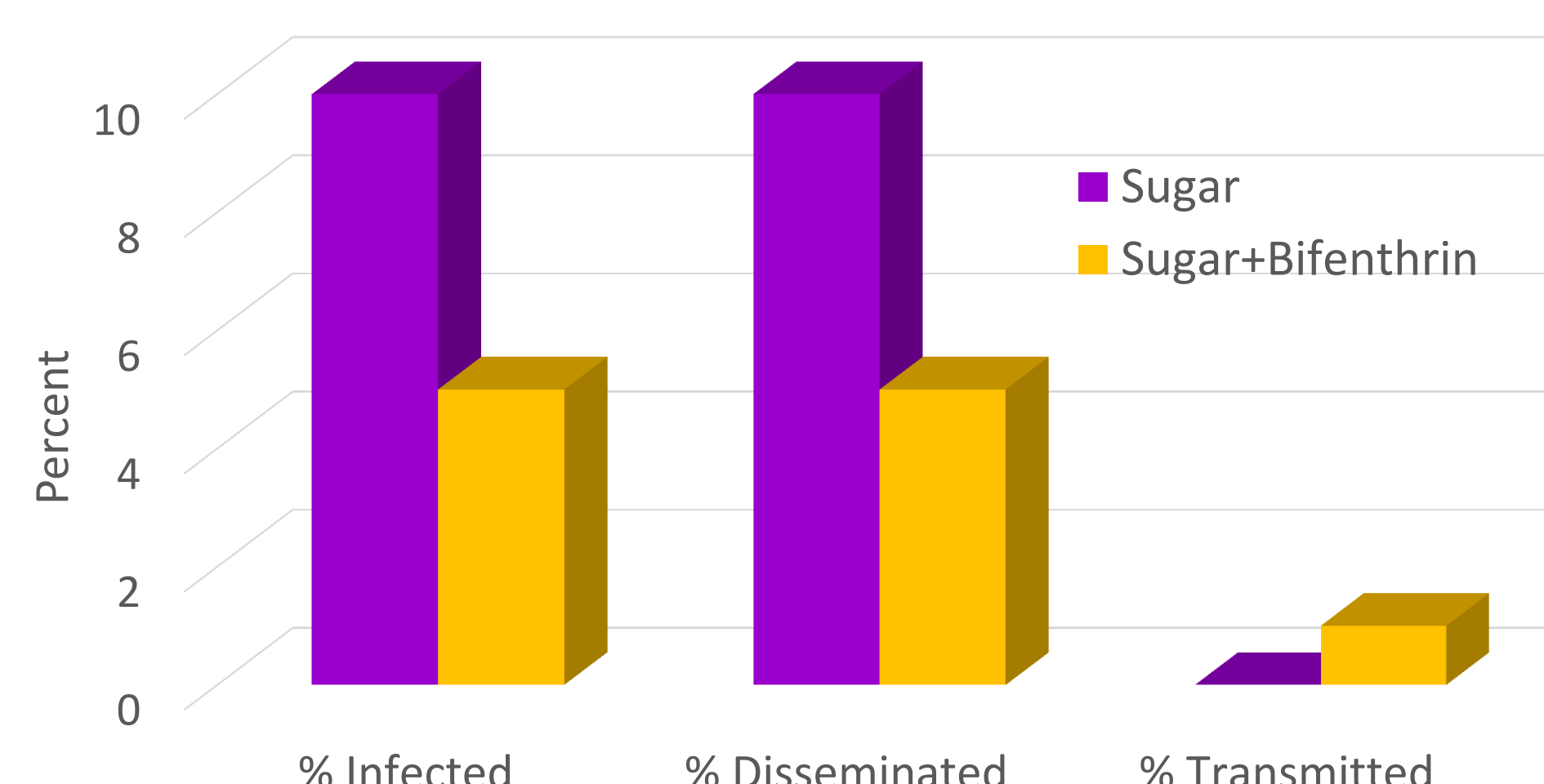


Figure 2. ZIKV 14 Day Post Infection



## Observations

- At 7 and 14 d IP, 100% infection of mosquitoes showed ZIKV infections
- 100% of Mosquitoes showed dissemination of ZIKV in bifenthrin group at 7 and 14 d IP
- At 7 and 14 d IP, sugar fed only group showed 40% and 100% ZIKV dissemination, respectively
- Two mosquitoes transmitted ZIKA (sugar group -7d IP, bifenthrin group -14 d IP)
- At 7d IP 80-90% DENV infection and 10 – 30% dissemination rates in both groups
- No DENV transmission observed

## Implications

There is potential for this population of *Aedes albopictus* to transmit Zika virus.

## Conclusions

- After 14 d IP, *Aedes albopictus* that ingested bifenthrin showed an increase in ZIKV transmission rate and a decrease in DENV infection rate
- Sublethal doses of insecticides may impact gut bacteria, mosquito immune response or affect other unknown factors that impact vector competence
- This knowledge is important to improve risk predictions of mosquito populations for virus transmission.
- \*Due to small sample size, more information is needed to elucidate the differences and further studies are planned on a larger scale.