

ABSTRACT

Mosquito-borne diseases impact health worldwide and control programs use ultra-low volume (ULV) aerosolized insecticide formulated products (FP) to control adult mosquitoes. The wind tunnel tested here mimics field ULV applications in a laboratory and uses fewer resources than a field trial. We evaluated droplet dispersal of ReMoa Tri® (FP) on field *Culex pipiens/quinqüefasciatus* using fluorescent dyes (Riboflavin and Tinopal®) and microscope with UV light filter. Mosquito mortality rates were monitored 2, 24, and 48 h post-treatment. Droplets deposited on mosquito body parts were counted post-treatment. No significant ($p > 0.05$) differences were observed in the mean number of droplets on different mosquito body parts. Mortality rate was significantly highest in the Tinopal® + Remoa Tri® treatment group. The findings from this study will be compared to a field trial. These results provide a basis for future research assessing droplet dispersal patterns in mosquitoes when exposed to FP in the wind tunnel and move this device closer to commercialization.

INTRODUCTION

- Mosquitoes are a global public health issue due to pathogens they transmit such as West Nile virus.
- Wind tunnels are used to apply and test efficacy of insecticides before widespread application in the environment.
- Mosquitoes are developing resistance to active ingredients (AI) used in FP.
- ReMoa Tri® is a new bacteria-based FP that contains three AI (abamectin, fenpropathrin, C8910 fatty acid blend) designed for use in resistant mosquitoes.
- Fluorescent dye (e.g., Tinopal®, Riboflavin) enables tracking of droplet dispersal patterns on mosquitoes under a microscope with ultraviolet (UV) light attachment.

MATERIALS AND METHODS

- *Culex pipiens/quinqüefasciatus* (F₀) from Pitt County, North Carolina.
- Female mosquitoes (4-5 d old) aspirated from cage and transferred to 6-in diameter wind tunnel cages (ca. 10-15 mosquitoes/cage; 4 replicate cages/group).
- Exposed (1.6 mL/min for 10 s) to ca. 3.3 µm aerosolized:
 - 1) Riboflavin alone
 - 2) Tinopal® alone
 - 3) Riboflavin + ReMoa Tri®
 - 4) Tinopal® + ReMoa Tri®
- Control groups exposed to air in wind tunnel.
- Mosquitoes transferred to separate 0.5 L cardboard cages post-exposure, 20% sucrose, housed in dark 28°C incubator.
- Mosquito mortality recorded at: 2 h, 24 h, 48 h post-exposure.
- Fluorescent droplets counted on mosquitoes post-exposure via Schott Intense Darkfield Ring Light Adaptor.
- Chi-square test ($p < 0.05$) to determine differences in mortality rates between groups; general linear model for differences in droplets between body parts (SAS Institute, Cary, NC).

RESULTS

Fig. 1. Mean numbers of droplets on mosquito body parts for all treatments 2 h post-exposure in wind tunnel.

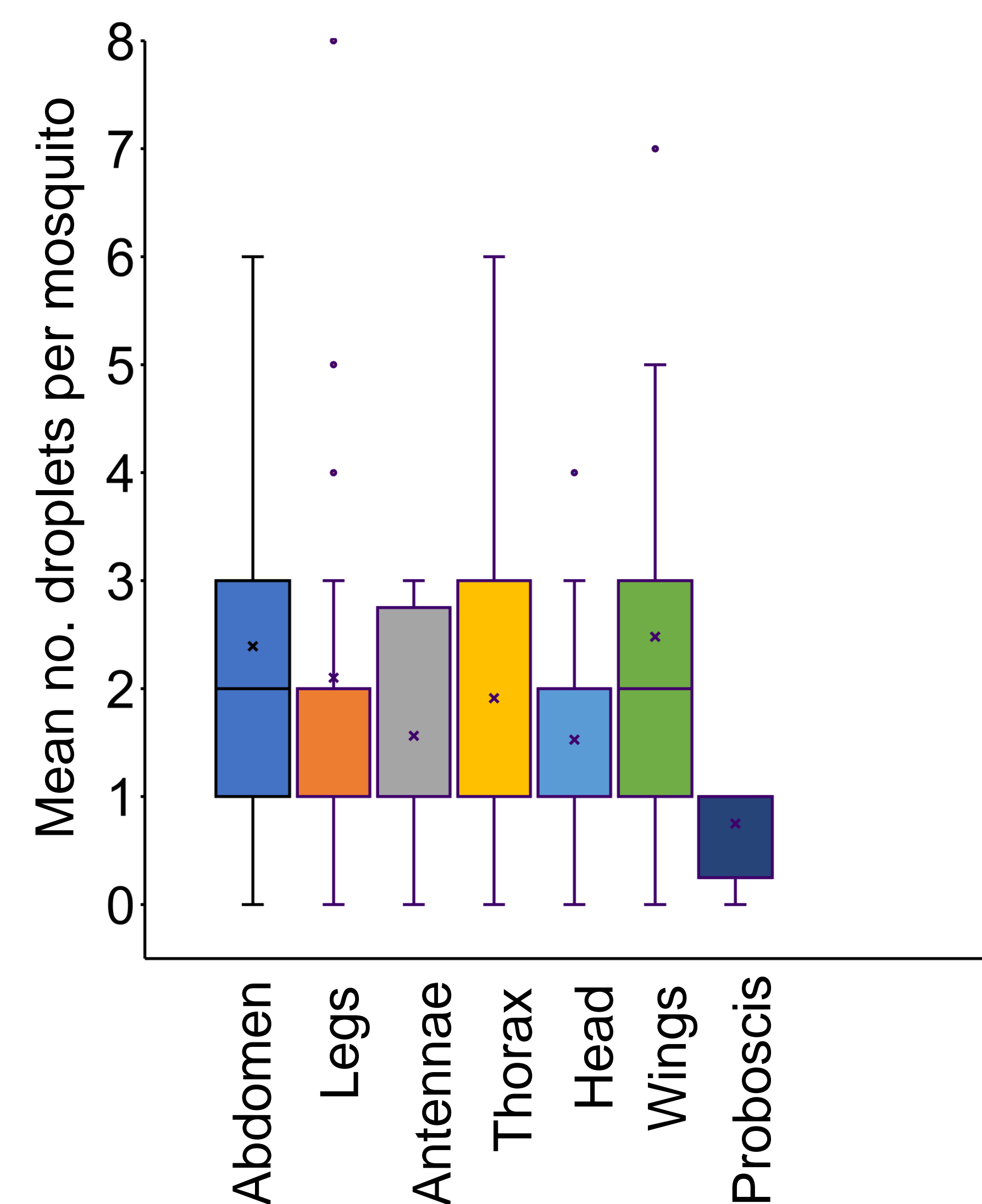
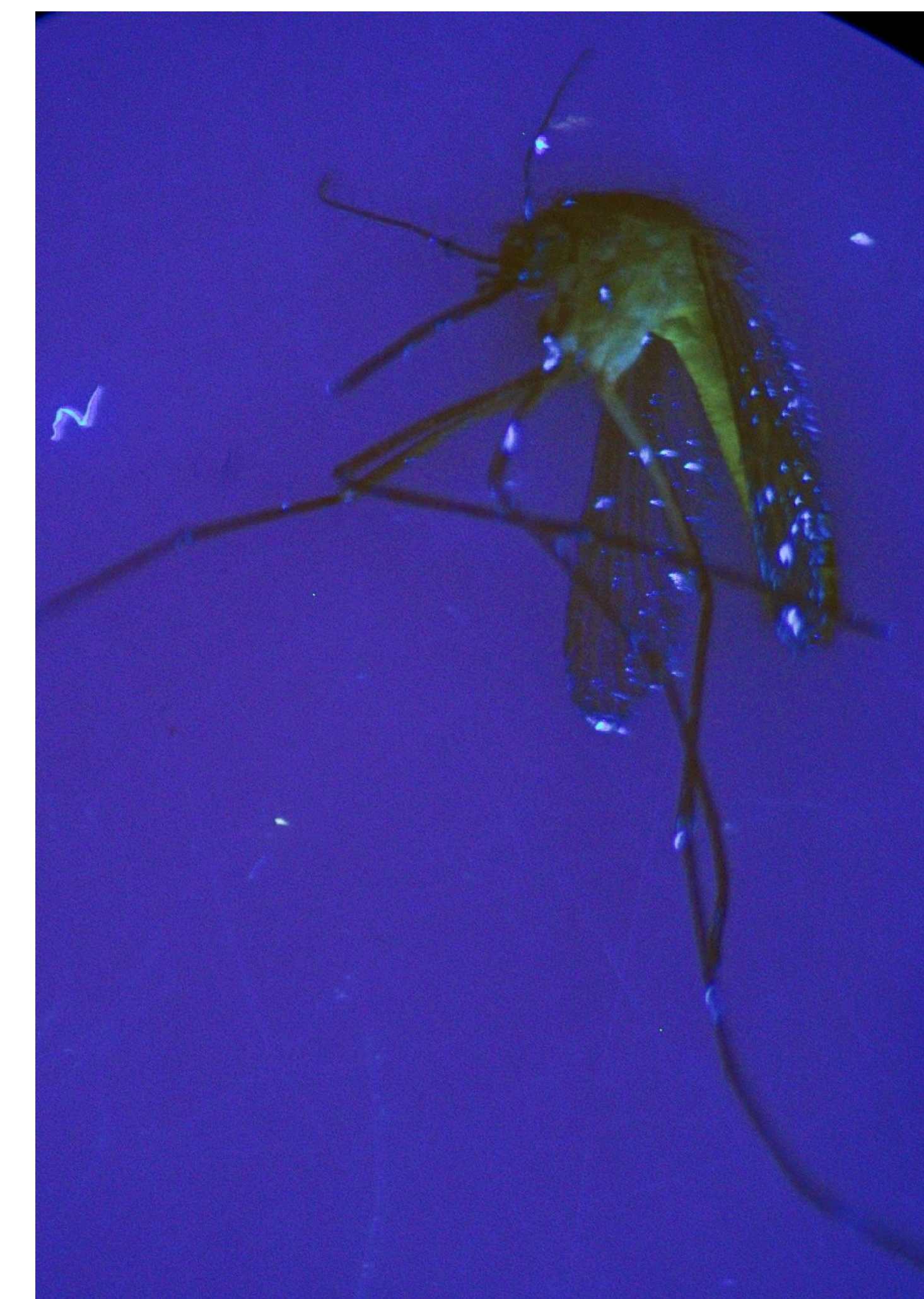
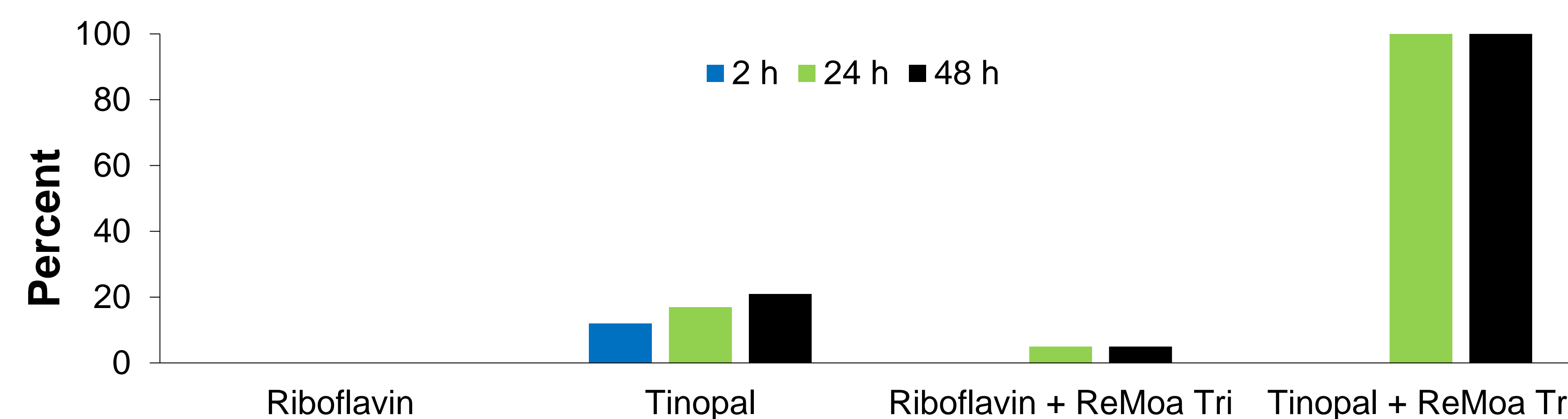


Fig. 2. Fluorescent droplets on mosquito under microscope with UV light post-exposure in wind tunnel.



- Droplets observed on mosquitoes exposed to aerosolized fluorescent dyes (Tinopal® and Riboflavin) in the wind tunnel.
- No significant differences ($p = 0.053$; $F = 2.12$; $df = 6$) in mean numbers of droplets between different body parts.

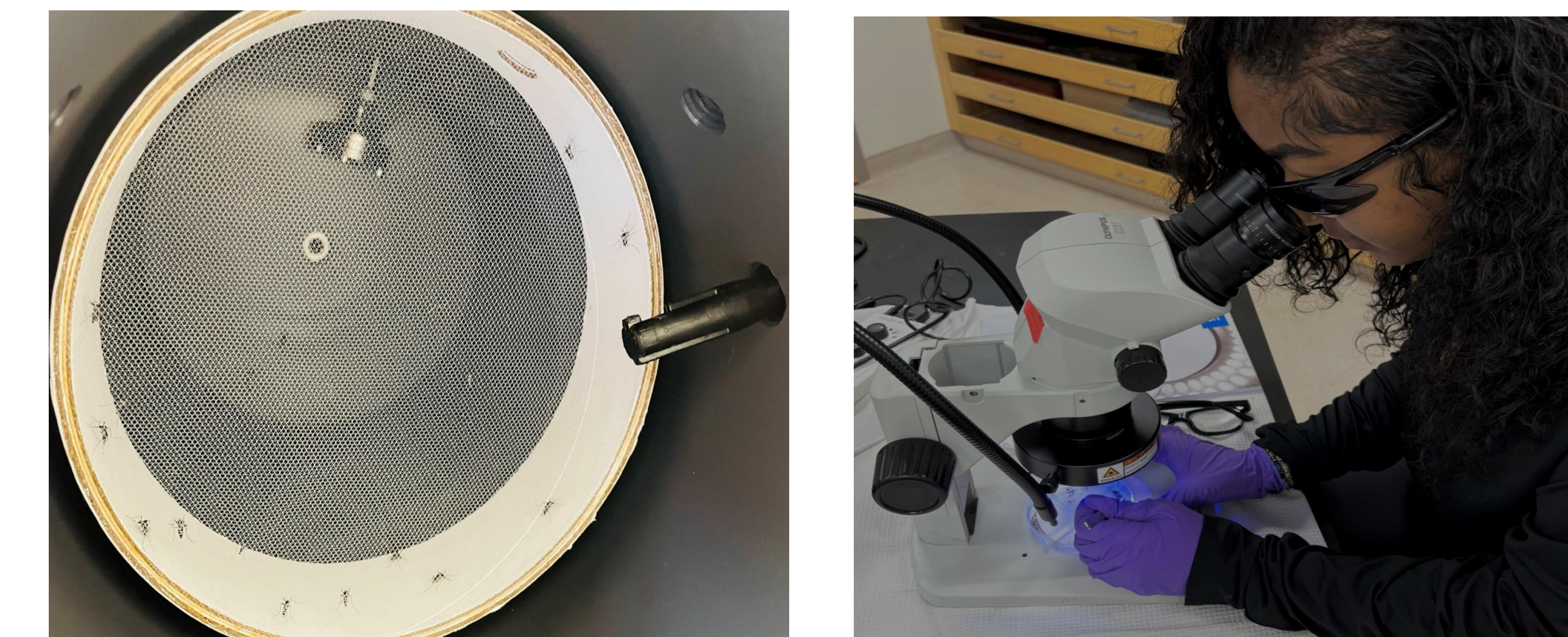
Fig. 3. *Cx. pipiens/quinqüefasciatus* mortality rates 2, 24, 48 h post-exposure.



- Mortality significantly ($p < 0.05$) highest in Tinopal® + ReMoa Tri® treatment at 24 and 48 h.
- Riboflavin did not cause mosquito mortality.

RESULTS (CONTINUED)

Fig. 4. Mosquitoes in wind tunnel and evaluation of fluorescent droplet dispersal.



DISCUSSION

- Both Tinopal® and Riboflavin fluorescent dyes can be aerosolized and visualized on mosquito body parts using the methods described here.
- Mosquitoes exposed to Tinopal® (dye) + ReMoa Tri® (FP) showed significantly higher mortality rates (100%) than other groups.
- No mortality was observed in the Riboflavin group, indicating this is a suitable dye to use in future droplet dispersal experiments.
- Future work will assess droplet dispersal patterns on mosquitoes exposed to water- and oil-based FP in the wind tunnel.

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- US Application 63/588137 was filed for the wind tunnel design on October 5th, 2023 by ECU Office of Licensing & Commercialization. Claims are directed towards a compact wind tunnel consisting of different mechanical and electrical elements for applying aerosolized solutions to determine arthropod resistance.

REFERENCES

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