Development of Novel Compact Wind Tunnel for Testing Efficacy of Insecticide Formulated Products in Mosquitoes

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ABSTRACT

Mosquitoes can develop resistance to insecticide active ingredients (AI) over time when exposed to sublethal doses. This is a public health risk as insecticides applied by mosquito control programs are one method for preventing mosquito-borne diseases. Mosquito exposure to insecticides during ultra-low volume (ULV) application occurs via direct liquid contact to formulated products (FP) while barrier applications expose mosquitoes to dried residual FP. We developed a method for exposing mosquitoes to FP using a compact wind tunnel apparatus. Initial wind tunnel testing was conducted on an *Aedes albopictus* lab colony, *Ae. albopictus* field population, and *Culex pipiens/quinquefasciatus* field population using a FP commonly used by mosquito control operators in North Carolina (Biomist® 3+15; AI permethrin). Future testing is planned for additional field populations and FPs.

INTRODUCTION

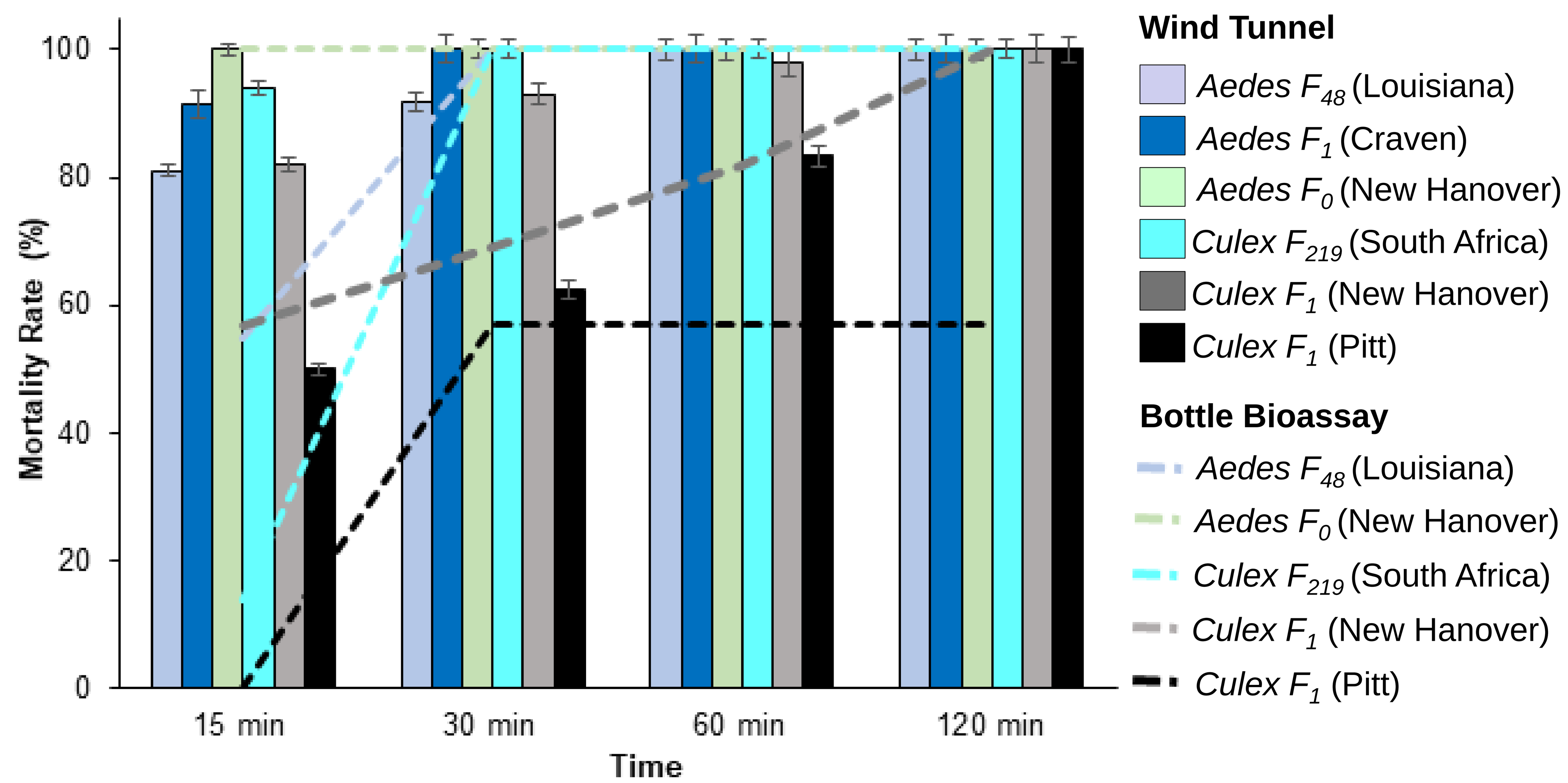
- Mosquitoes are a global public health issue due to the public health pathogens they transmit such as West Nile virus, dengue virus, and Zika virus.
- Insecticides help control mosquitoes, but mosquito control programs are facing issues with insecticide resistance.
- Mosquitoes can build resistance through "multigenerational" selection and other mechanisms.
- Biomist® (synthetic pyrethroid adulticide) is a FP that contains the AI permethrin.
- The wind tunnel exposes mosquitoes directly to aerosolized FP droplets.
- The Centers for Disease Control and Prevention (CDC) bottle bioassay exposes mosquitoes to AI residue but is not appropriate for FP exposure.

MATERIALS & METHODS

- *Ae. albopictus* (F_{48}), *Ae. albopictus* (F_1), and *Cx. pipiens/quinquefasciatus* (F_1) propagated for use in experiments.
- Female mosquitoes (4-5 d old) aspirated from colony cage and transferred to 6-in diameter cages (*ca.* 10-15 mosquitoes/cage; 3 replicate cages/group) and exposed to Biomist®3+15 via wind tunnel (1.6 mL/min for 5 s, 10 s, or 20 s) or technical grade permethrin via CDC bottle bioassay.
- Control groups exposed to air for the same exposure times in wind tunnel and clean bottles for bioassays.
- Immediately after exposure, mosquitoes were chilled and transferred to separate 0.5 L cardboard cages.
- Mosquitoes provided 20% sucrose and housed in a 28°C incubator with 14 h light:10 h dark.
- After exposure, mosquito mortality monitored and recorded for all groups at these time intervals: 15, 30, 60, 90, 120 min and 24 h.
- Conducted *t*-test ($P < 0.05$) to determine significant differences between groups.

RESULTS

Figure 1. Mortality over time after 10 s of exposure to aerosolized Biomist®3+15 in the wind tunnel compared to constant bottle bioassay exposure to permethrin residue. No mortality was observed in control groups.



- Mortality was significantly higher in *Ae. albopictus* compared to *Cx. pipiens/quinquefasciatus* populations tested here at 15 min (P=0.0002), 30 min (P=0.0001), and 60 min (P=0.0007) post wind tunnel exposure.
- Mortality was significantly higher in *Ae. albopictus* compared to *Cx. pipiens/quinquefasciatus* populations tested here at all time points during the bottle bioassay (15 min: P<0.0001; 30 min: P<0.0001; 60 min: P<0.0001; 120 min: P<0.0001).
- At the 15 min time point, significant differences were observed in mortality rates in *Ae. albopictus* populations between wind tunnel and bottle bioassay exposures (P<0.005).
- *Cx. pipiens/quinquefasciatus* showed significant differences in mortality between wind tunnel and bottle bioassay exposures at all time points (15 min: P<0.0001; 30 min: P=0.010; 60 min: P<0.0001; 120 min: P<0.0001).
- Mortality rates were generally higher in mosquito populations tested in the wind tunnel versus those tested using bottle bioassays.

Figure 2. General schematic of wind tunnel prototype (not to scale)

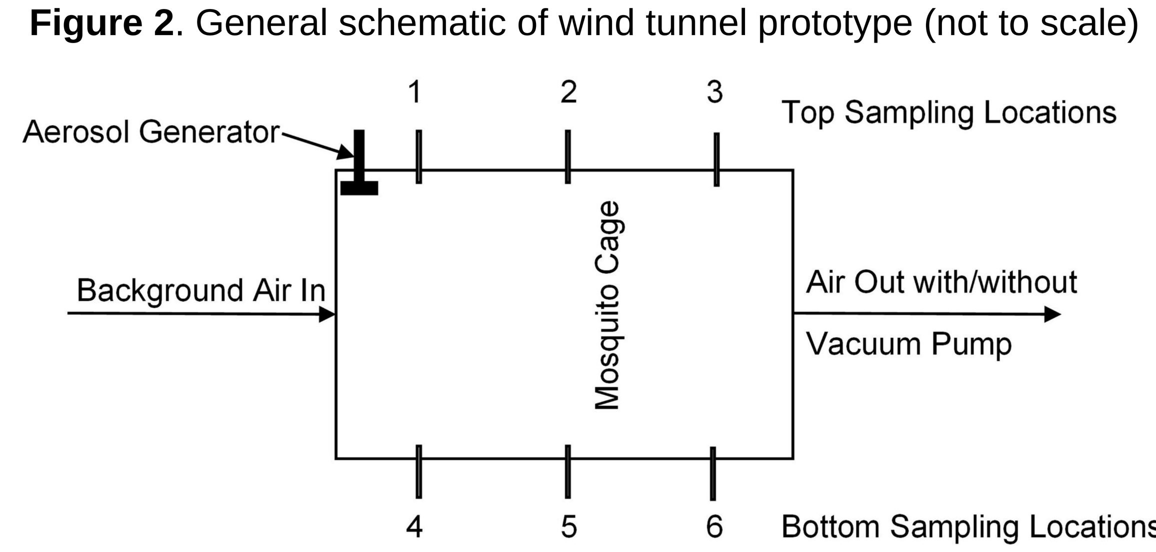


Figure 3. *Aedes albopictus* (left) and *Culex pipiens/quinquefasciatus* (right)

RESULTS (continued)

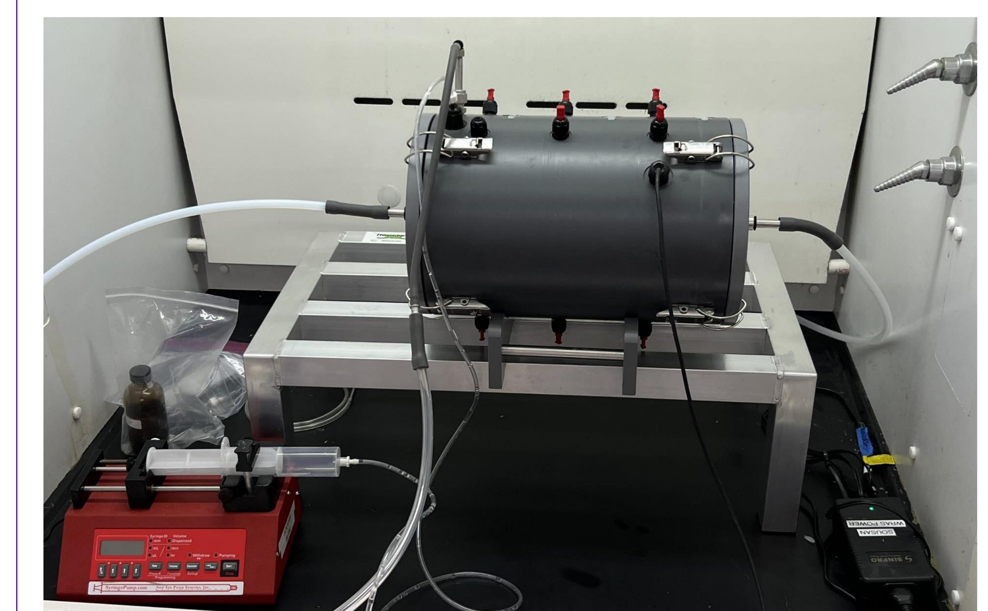


Figure 4. Wind tunnel prototype within chemical hood

Figure 5. Placing mesh cage into wind tunnel

Figure 6. Mosquitoes in wind tunnel

Figure 7. Mosquitoes in bottle bioassay

DISCUSSION

- The wind tunnel was developed as a mitigation tool that can be used to apply insecticide FP.
- A subset of field mosquitoes (or other arthropods) can be used as a proxy to determine if insecticides would be effective in killing mosquitoes in the environment.
- This protects public health, reduces the spraying of ineffective pesticides into the environment, and helps increase efficacy of mosquito control.
- The wind tunnel could allow for a regional approach to FP testing in which smaller programs could transfer mosquitoes to universities or larger programs for testing.
- Results from these experiments will be used to further develop the wind tunnel prototype.
- Dr. Richards and Dr. Sousan have a pending patent and their innovation aims to solve the problems and costs associated with current testing approaches. US Application 63/588137 was filed for the wind tunnel design by the ECU Office of Licensing & Commercialization.
- Manuscript is "in press" at Pest Management Science:

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