

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/quinqüefasciatus* 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

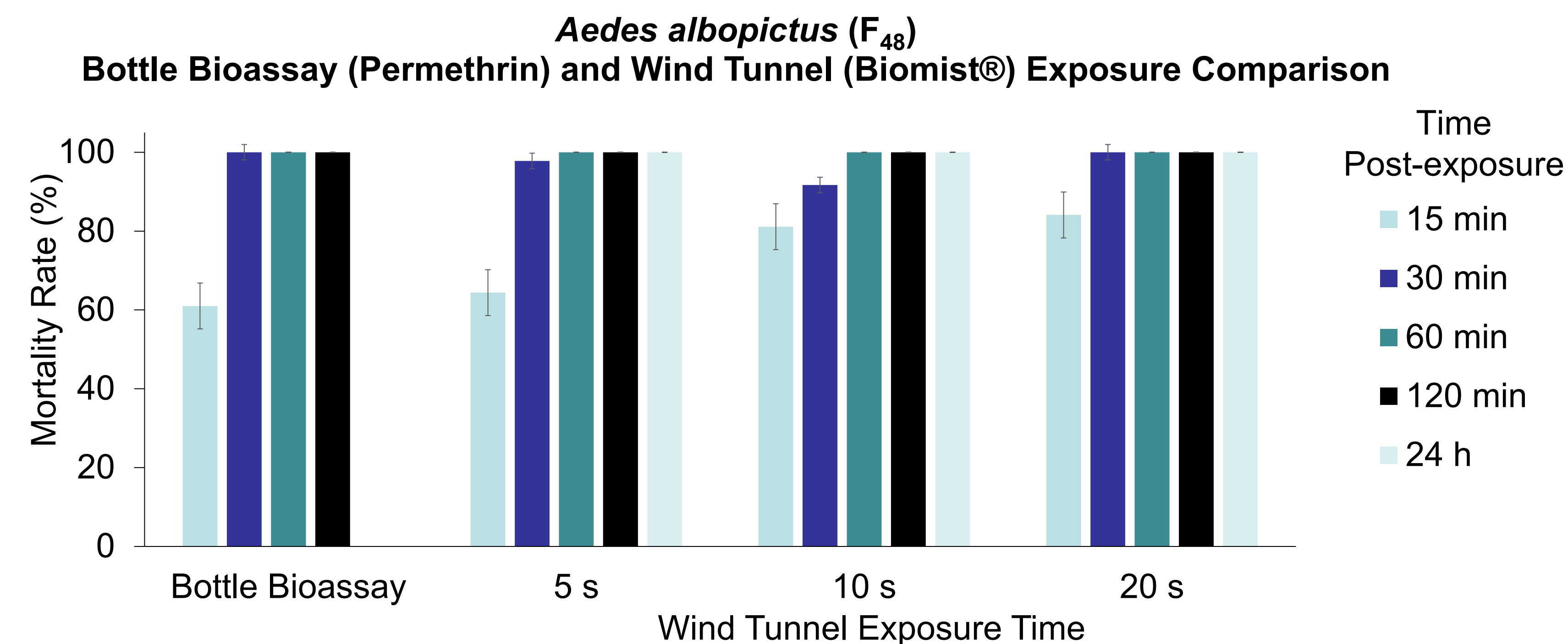
- Mosquitoes are a global public health issue due to the public health pathogens they transmit such as West Nile virus, dengue virus, and Zika virus.
- Insecticides help control mosquitoes, but mosquito control programs are facing issues with insecticide resistance.
- Mosquitoes can build resistance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

MATERIALS & METHODS

- Ae. albopictus* (F₄₈), *Ae. albopictus* (F₁) and *Cx. pipiens/quinqüefasciatus* (F₁) 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) customized for the wind tunnel and exposed to Biomist® via wind tunnel (1.6 mL/min for 5 s, 10 s, or 20 s) or 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.

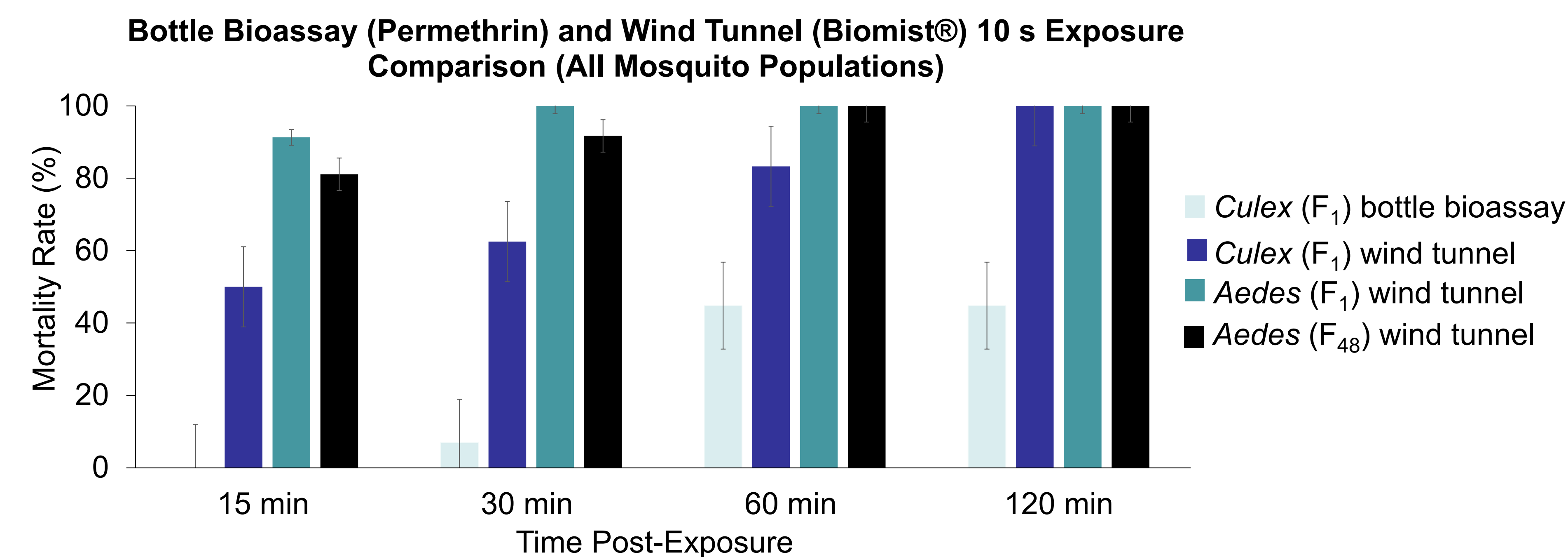
RESULTS

Figure 1. Method development experiment. Mortality rates after wind tunnel exposure to Biomist® and bottle bioassay exposure to permethrin for *Ae. albopictus* lab colony. No mortality observed in control groups.



- Mortality 15 min post-exposure marginally different than 30 min post-exposure (*P* = 0.054).
- Mortality significantly (*P* < 0.05) higher in exposed versus control groups, regardless of time.
- Bottle bioassay mortality comparable to wind tunnel.

Figure 2. Mortality after 10 s wind tunnel exposure to Biomist® for *Culex pipiens/quinqüefasciatus* (F₁), *Ae. albopictus* (F₁), *Ae. albopictus* (F₄₈). Bottle bioassay exposure to permethrin for *Culex pipiens/quinqüefasciatus* (F₁). No mortality observed in control groups.



- Mortality significantly (*P* < 0.05) higher in wind tunnel versus bottle bioassay exposed *Culex pipiens/quinqüefasciatus*.
- Culex pipiens/quinqüefasciatus* are susceptible to Biomist® but resistant to permethrin.
- Mortality significantly (*P* < 0.05) higher in *Ae. albopictus* versus *Cx. pipiens/quinqüefasciatus* 30- and 60-min post-exposure in wind tunnel.

Figure 3. Larvae and pupae of mosquitoes used in experiments



Figure 4. *Aedes albopictus*



Figure 5. *Culex pipiens/quinqüefasciatus*



RESULTS, continued



Figure 6. Checking mosquito mortality rates post-exposure



Figure 7. Mosquitoes in cardboard cages post-exposure



Figure 8. Mosquitoes in wind tunnel

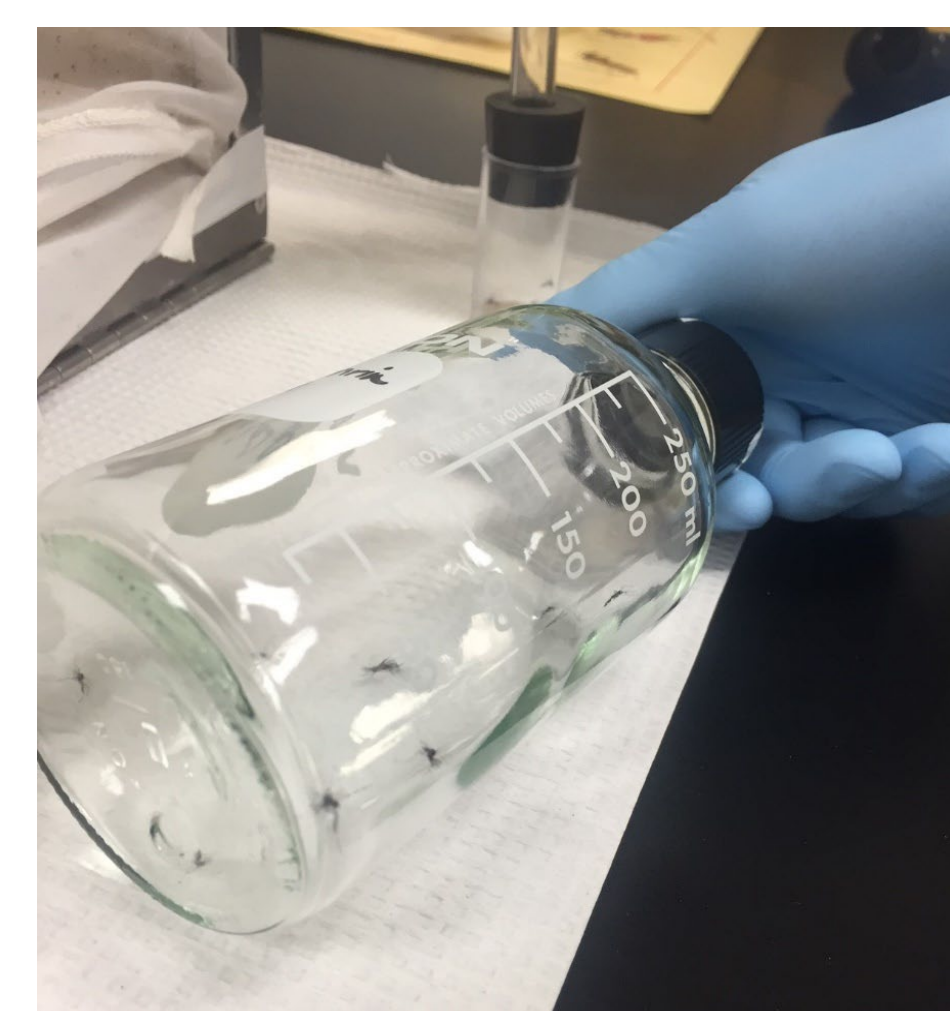


Figure 9. Mosquitoes in bottle bioassay

DISCUSSION

- Mortality significantly higher in Biomist®-exposed (wind tunnel) and permethrin-exposed (CDC bottle bioassay) groups compared to control groups.
- Findings here indicate the wind tunnel is an effective device for exposing mosquitoes to FP and provides an additional tool for assessing insecticide resistance.
- Results from these preliminary experiments will be used to further develop the wind tunnel prototype. This compact device could be used by a variety of mosquito control programs as a tool for assessing FP exposure in mosquito populations.

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