METHODS · VOLATILE INSECTICIDES · ANOPHELES & AEDES

Standard methods for exposing mosquitoes to volatile insecticides: the Two Tube Volatile Assay (TTVA) and the Peet-Grady chamber

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1 Context and objectives

Volatiles are arriving

Spatial emanators releasing volatile pyrethroids such as transfluthrin are entering vector control, and volatile actives are being explored against *Aedes* vectors of dengue, chikungunya and Zika. There have already been trials of volatiles against Aedes.

The gap

WHO tube and CDC bottle tests measure contact exposure. There is no standard way to ask whether a population is still susceptible when the route of entry of the insecticide is as a vapour.

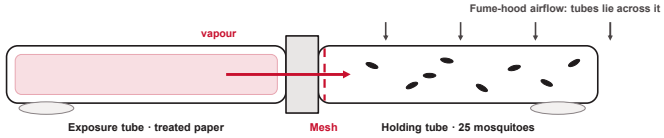
Our objective

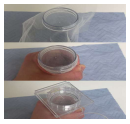
Standard, transferable methods for exposing mosquitoes to volatiles and recording multiple endpoints: the benchtop TTVA and a Peet-Grady chamber protocol, shown here in *An. gambiae* and *Ae. aegypti*.

2 Two standard methods for volatile exposure

A Two Tube Volatile Assay (TTVA)

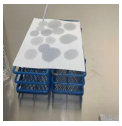
Benchtop · intrinsic activity and susceptibility · vapour-only exposure





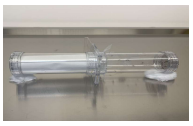
1 · Holding tube

Untreated netting is pulled taut across the tube end and clamped by the screw-on door. 25 mosquitoes are aspirated in through the side hole, sealed with parafilm.



2 · Treated paper

The dose is applied in 1 ml acetone aliquots, spread evenly. A 1 ml acetone rinse of the dilution bottle follows, then the paper dries for 30–60 s.



3 · Exposure

Paper goes into the exposure tube; the holding tube is attached, door closed. Tubes lie level across the airflow; opening the door starts the assay.

Exposure	1 h; knockdown scored at 5, 10, 15 and 60 min
Endpoints	Knockdown and 24 h mortality
Replication	Biological = testing day; technical = dose, testing day, tube
Analysis	Pooled % per dose, 95% CI by bootstrap over testing days

B Peet-Grady chamber

Detailed characterisation · caged or free-flight exposure



Treated papers in the chamber.



Cages positioned in the chamber.



Camera setup for cage filming.



Chamber and fan.

Caged

Dual cages hold two strains side by side; 1 h or 18 h overnight exposure.

Caged + blood-feeding

Blood-feeding offered to caged mosquitoes to score blood feeding inhibition (BFI), can be done in parallel.

Free flight

Mosquitoes released into the chamber to expose them in a larger arena, closer to real life exposure and behaviour.

Dual cages: several tests in one exposure



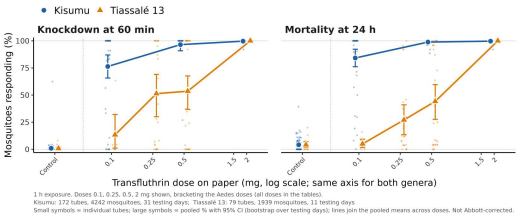
Each dual cage holds two strains, species or replicates side by side, so they are exposed together under the same conditions. More cages can be added in principle; our standard setup of 4 dual cages in each of 2 chambers gives **16 tests** in one exposure.

Exposure	1 h (knockdown every 5 min) or 18 h overnight; 24 h holding
Delivery	Transfluthrin on filter paper suspended at the chamber centre; heated pads available for other volatile actives
Air & climate	Medium fan speed, set from wind-speed analysis; 27 °C, 70% RH
Endpoints	Knockdown time course, KT50, 24 h mortality, blood-feeding; video tracking of position and movement
Controls	0 mg on the same day; Abbott's correction only against same-day controls

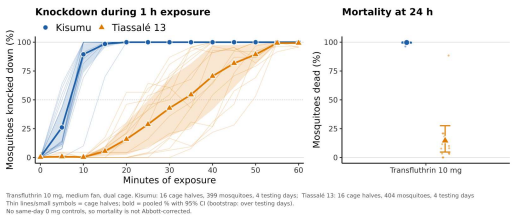
3 Example data: what the methods can show

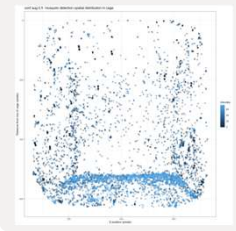
***An. gambiae*: Kisumu (susceptible) vs Tiassalé 13 (resistant), transfluthrin**

A · TTVA



B · Peet-Grady chamber, 10 mg

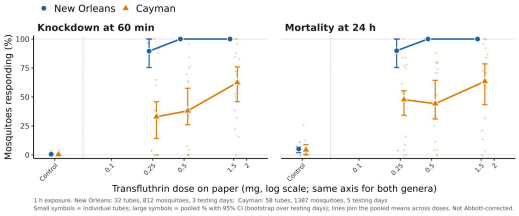




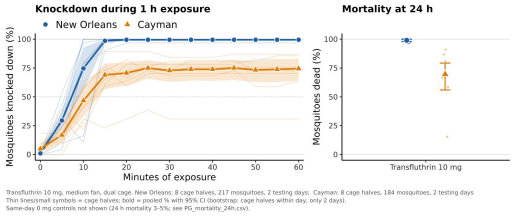
Detected Susceptible Mosquito Positions over 1h from Video tracking

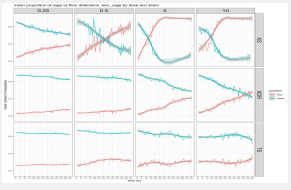
***Ae. aegypti*: New Orleans (susceptible) vs Cayman (resistant), transfluthrin**

A · TTVA



B · Peet-Grady chamber, 10 mg





Counts of mosquitoes in upper vs floor of cage

Across methods. Both methods give consistent knockdown and mortality results that separate susceptible and pyrethroid-resistant strains: the TTVA captures a dose-dependent response suited to measuring intrinsic activity, and the Peet-Grady chamber adds a detailed picture of the response over time. The resistant strains respond differently: Tiassalé 13 is knocked down more slowly and then recovers, whereas a fraction of Cayman is not knocked down at all. Questions like this, comparing populations or genera, are what the methods can now be applied to.

4 Using the methods

Susceptibility testing. Labs already running WHO tube tests can use the TTVA to test susceptibility to volatiles such as transfluthrin, in *Anopheles* and *Aedes*, with the same equipment.

Characterising responses. Knockdown time course, 24 h mortality, blood-feeding and video tracking pick up strains that are knocked down but survive and characterise the sublethal and behavioural response.

Screening actives. Standard exposure conditions, several exposure formats and multiple endpoints let candidate volatiles be tested and compared across strains directly.

Next. Optimising and validating both methods, expanding the endpoints, and applying them to real-world questions such as comparing populations and genera.



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TTVA

Peet-Grady

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