Volatile VUAA Based Excito-Repellents for Malaria Vector Mosquitoes

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Background

Insects vector several catastrophic human diseases and act as agricultural pests impacting global food security resulting in significant economic impacts. Malaria is transmitted to humans by adult female Anopheline mosquitoes during blood feeding (1). In 2020, there were 241 million clinical malaria infections and almost 627,000 deaths (2). We seek to use molecular approaches to develop next-generation excito-repellent strategies to reduce the ability of insects to attack agriculture and to transmit malaria and other diseases .

Introduction

Anopheles coluzzii mosquitoes utilize an intricate olfactory system to sense and discriminate a large spectrum of environmental and host cues to carry out blood feeding and other critical behaviors (Fig. 1) (2).

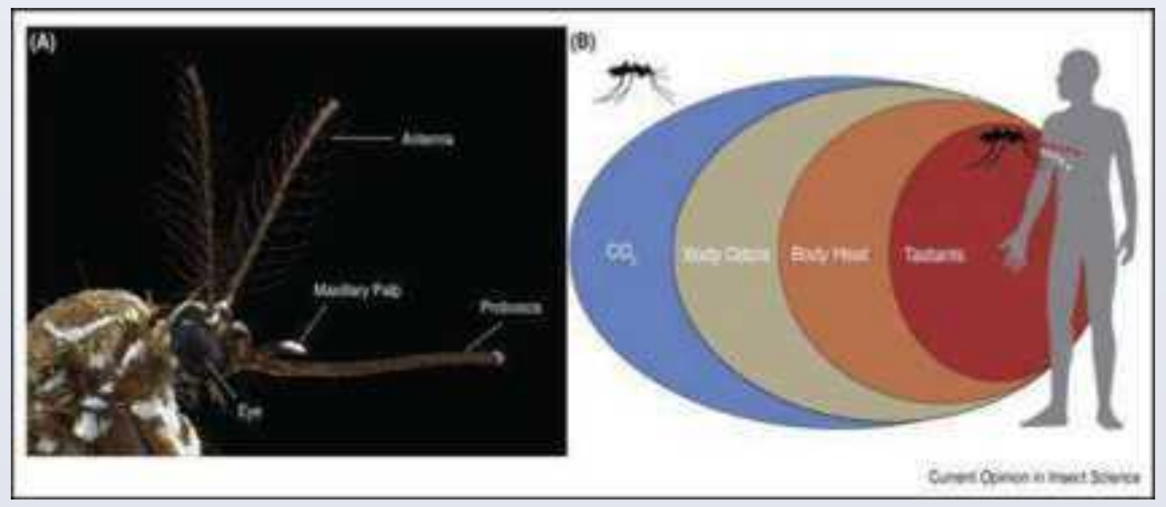

Figure 1. Olfaction Appendages and Odor Space (4)

The Zwiebel Lab focuses on the peripheral signal transduction pathways of the insect olfactory system that are modulated by several classes of chemosensory genes. Central to these pathways are several families of chemosensory receptors expressed on a variety of olfactory receptor neurons (ORNs) across the antennae and other olfactory appendances of adult and larval stage mosquitoes (3). Of these, a large superfamily of rapidly evolving odorant receptors (ORs) play critical roles. Insect ORs function as heteromeric complexes composed of highly divergent, odorant sensing "tuning" receptor and a highly conserved odorant co-receptor (Orco) (7). Utilizing a high throughput small molecule screening approach, Vanderbilt University Allosteric Agonist (VUAA)-class compounds were identified as novel Orco modulators that hyper-agonizes all Orco-containing olfactory neurons (Figure. 2) (4). VUAA-based actives are hypothesized to represent a novel class of broadly active insect excito-repellents against both agricultural pests and disease vectors (Figure 2).

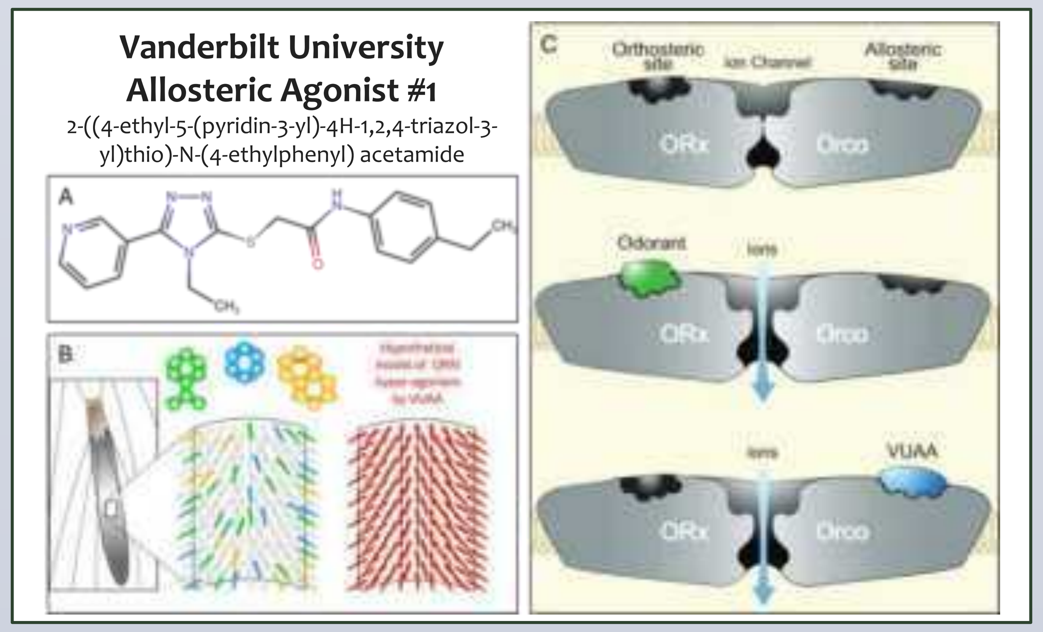

Figure 2. (A) Structure of VUAA1, (B) hypothetical model of VUAA hyper-agonism, (C) VUAA Postulated Mode of Action (6).

Our project's goal is to identify and characterize volatile VUAA-based actives (VUAIs) to act as efficient spatial insect repellents. We have used heating to reduce the VUAA structure into smaller, more volatile thermolysis substructure components that display Orco-specific activity as binary mixtures. We now use several behavioral assays to characterize the efficacy of those components as spatial insect repellents.

Glass Tube Assay

We have utilized a well-established high throughput repellent bioassay screen (9) to assess the efficacy of unitary or binary VUAIs on the behavior of adult *An. coluzzii* mosquitoes. Here, 10 female mosquitoes are exposed to the volatiles emitted from a segregated chemical (treatment) compound on one side and a control solution on the other of an enclosed glass tube (12.5cm in length) (Fig. 3). The simple goal in these assays is to manually observe the movement of mosquitoes in the tube to see which side they tend to move towards or away from.

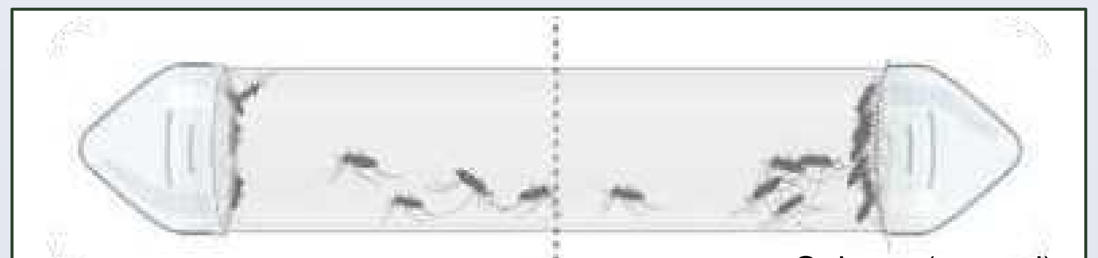

Figure 3. Glass Tube Apparatus Set-up.

On the treatment side of the tube, we will place several different analogs of VUAA (either alone or in binary combinations) to determine which is more efficient at repelling Anopheline mosquitoes. The solvent consists of 50% DMSO in acetone. The double-blinded studies are conducted for a total of 60-minutes, and at every 10-minute interval, the relative position of each mosquito is manually assessed. The treatment-induced knock-down of mosquitoes is also continually assessed during each 60-minute trial.

Semi-Field Greenhouse Experiments

We have taken advantage of the SC2 BSCI greenhouse facility (Figure 4a) to conduct larger-scale, over-night semi-field bioassays. Here, 100 female *An. coluzzii* mosquitoes are released each evening into a 20x20 sq. ft. greenhouse, which also holds a Biogents mosquito trap (Figure 4b) fitted with an attractive bait consisting of a malodorous human sock and a carbon dioxide diffuser. Treatment or solvent controls are released from a passive sponge diffuser placed into the trap. The next morning, the number of mosquitoes in the trap are counted. The goal of this study is to assess whether test compounds that were initially identified in tube assays maintain efficacy as spatial repellents in a larger setting over a longer period of time.

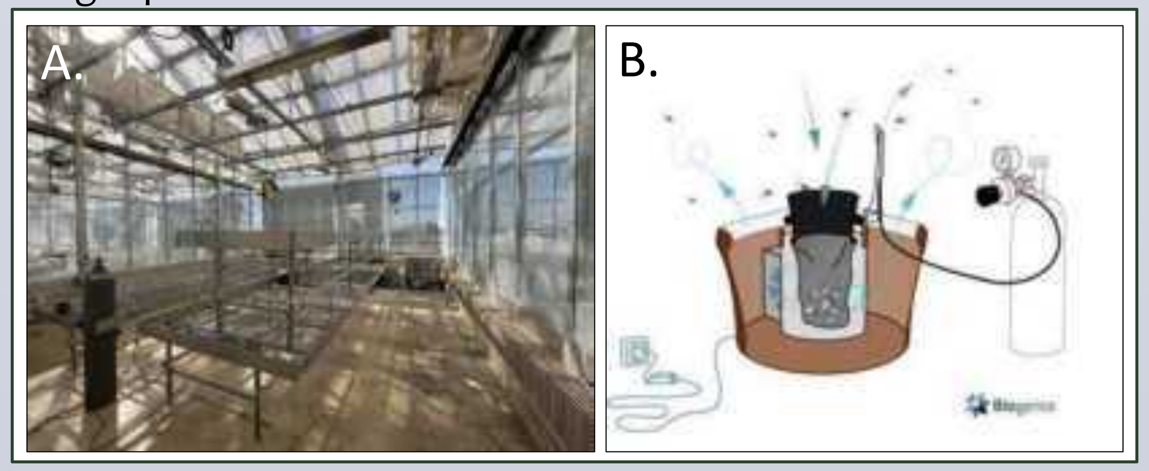

Figure 4. (A) SC2 Greenhouse (B) Biogents® mosquito trap.

Results

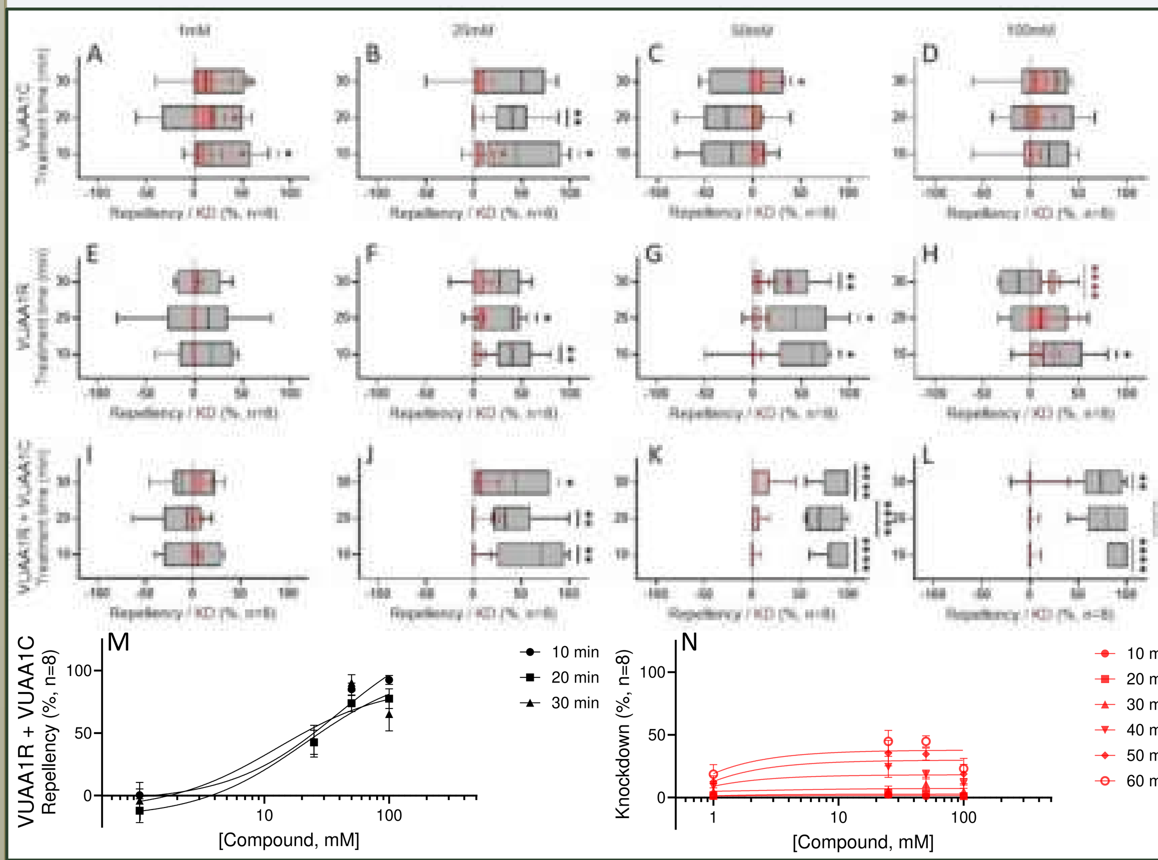

Figure 5. A-L: Repellency (black) and knockdown (red) bioassays for wild type female mosquitoes. M-N: Concentration response curves for VUAA1R + VUAA1C.

Tube assay repellency data was collected for An. coluzzii adult females across a 1-100mM concentration range of VUAA1R, VUAA1C, and a 1:1 binary combination of VUAA1R+VUAA1C. In this studies, increasing the concentration of VUAA1R from 50 mM to 100 mM resulted in greater knockdown that may indicate a toxic treatment effect (Fig. 5 G and H). On the other hand, knockdown was not observed when the concentration of VUAA1C increased (Fig. 5 C and D). Indeed, the binary combination of VUAA1R+VUAA1C resulted in significantly stronger repellency across all time intervals together with minimal knockdown effect observed at 25 mM and 100 mM at early time points (Fig. 5 J-N), which is an indicator of a potentially efficacious spatial repellent that does not harm insects.

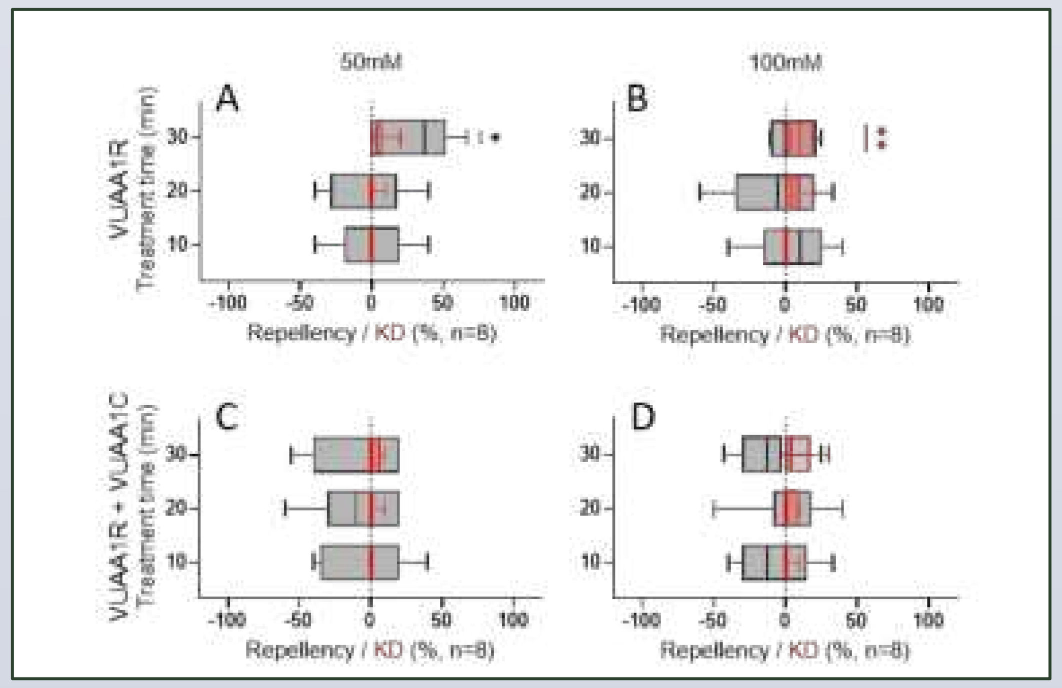

Figure 6. A-D: Repellency (black) and knockdown (red) bioassays for Orco knockout female mosquitoes.

In important controls that demonstrate VUAI's Orco-centric mode of action, VUAIs combinations that were active against wild-type *An. coluzzii* mosquitoes were tested against Orco knockout mosquitoes which carry the loss-of-function mutations (7). In Orco mutant mosquitoes, the excito-repellent activity of VUAA1R and the binary combination of VUAA1R + VUAA1C were largely absent (Fig. 6. A-D). This is consistent with the hypothesis that VUAIs specifically targets the Orco co-receptor.

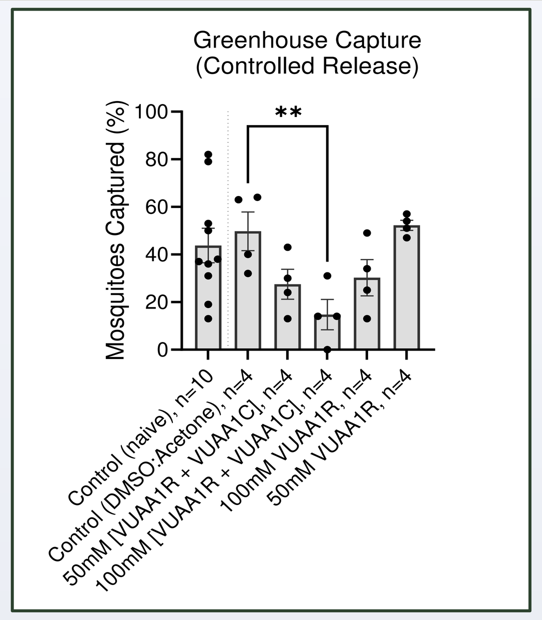

Figure 7. VUAI-based repellents are effective in semi-field (greenhouse) baited trap bioassays.

The greenhouse studies utilized two concentrations of the binary compounds (1R+1C) and VUAA1R alone. When measured against the solvent (DMSO/Acetone) control, 100 mM of the binary mixture elicited a significant difference in capture percentage (Fig. 7). This highlights that a larger concentration of test compound is needed in these larger scale, longer duration bioassays for there to be significant repellency. Also, in these more realistic bioassays, the VUAA1R components did not exhibit any significant difference in capture percentage. This preliminary data suggests that higher concentrations of binary compound formulated in control-release devices can act as long-lasting spatial repellents against mosquitoes.

Discussion/Future Directions

VUAI-based excito-repellents represent a paradigm shifting approach for the design and development of broadly active, next-generation of insect repellents. This class of actives are expected to provide near universal protection against insects that act as disease vectors, as well as those that act as economically important agricultural pests and nuisance insects. Importantly, in contrast to insecticides which, because of their inherent lethality, drive the rapid emergence of a variety of resistance mechanisms, insect repellents are expected to remain viable tools for insect control for extended periods.

Volatility remains a key issue that needs to be further resolved in order to spur the utility of VUAI based spatial repellents. Also, testing of VUAI analogs will be conducted in the glass tube and greenhouse assays to identify whether there are other compounds that may exhibit strong repellency effects.

In addition, other pest/vector insects such as aphids, flour beetles, thrips and Aedine mosquitoes will be introduced into these bioassay studies to test the range of VUAI-repellency.

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Other contributions: L. Martinez led the experimental team as bench mentor, contributed to experimental and poster design, and generated all data figures. Dr. L. J. Zwiebel acted as overall project PI in the design and implementation of all experiments/poster design.

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