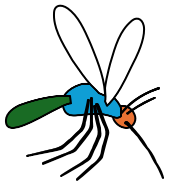
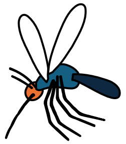
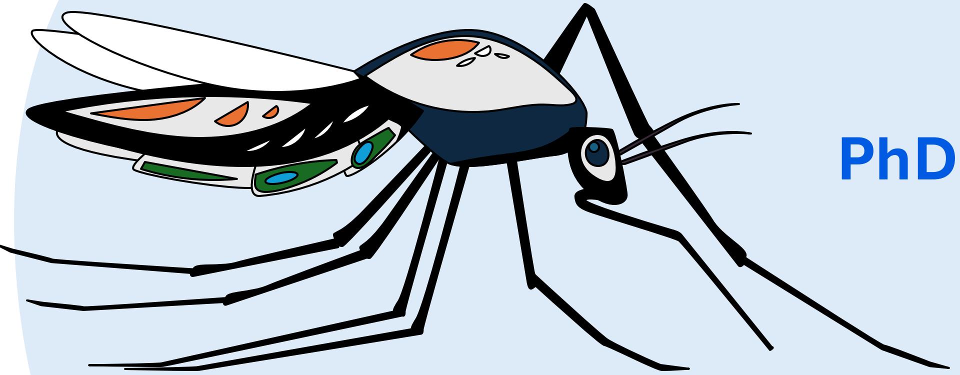
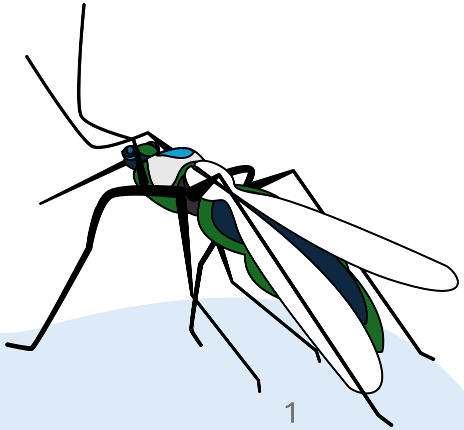


Assessing Behavioral Resistance to Targeted Insecticide Spraying in Field Populations of *Aedes aegypti* across Yucatan, Mexico





David Jimenez-Vallejo
PhD Candidate – Prokopec Lab
PBEE Seminar Series
October 10th, 2025



Developed in the **1940s**, **DDT spraying** was greatly used to **combat** malaria, typhus, and other **insect-borne disease**

Wikimedia Commons

Anopheles, CDC

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Wikimedia Commons

Time, June 30th, 1947

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Wikimedia Commons

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In 1947, 2 Sanitarians from the United States Public Health Service investigating *Anopheline* behavior wrote:

Anopheles, CDC

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Wikimedia Commons

Time, June 30th, 1947

In 1947, 2 Sanitarians from the United States Public Health Service investigating *Anopheline* behavior wrote:

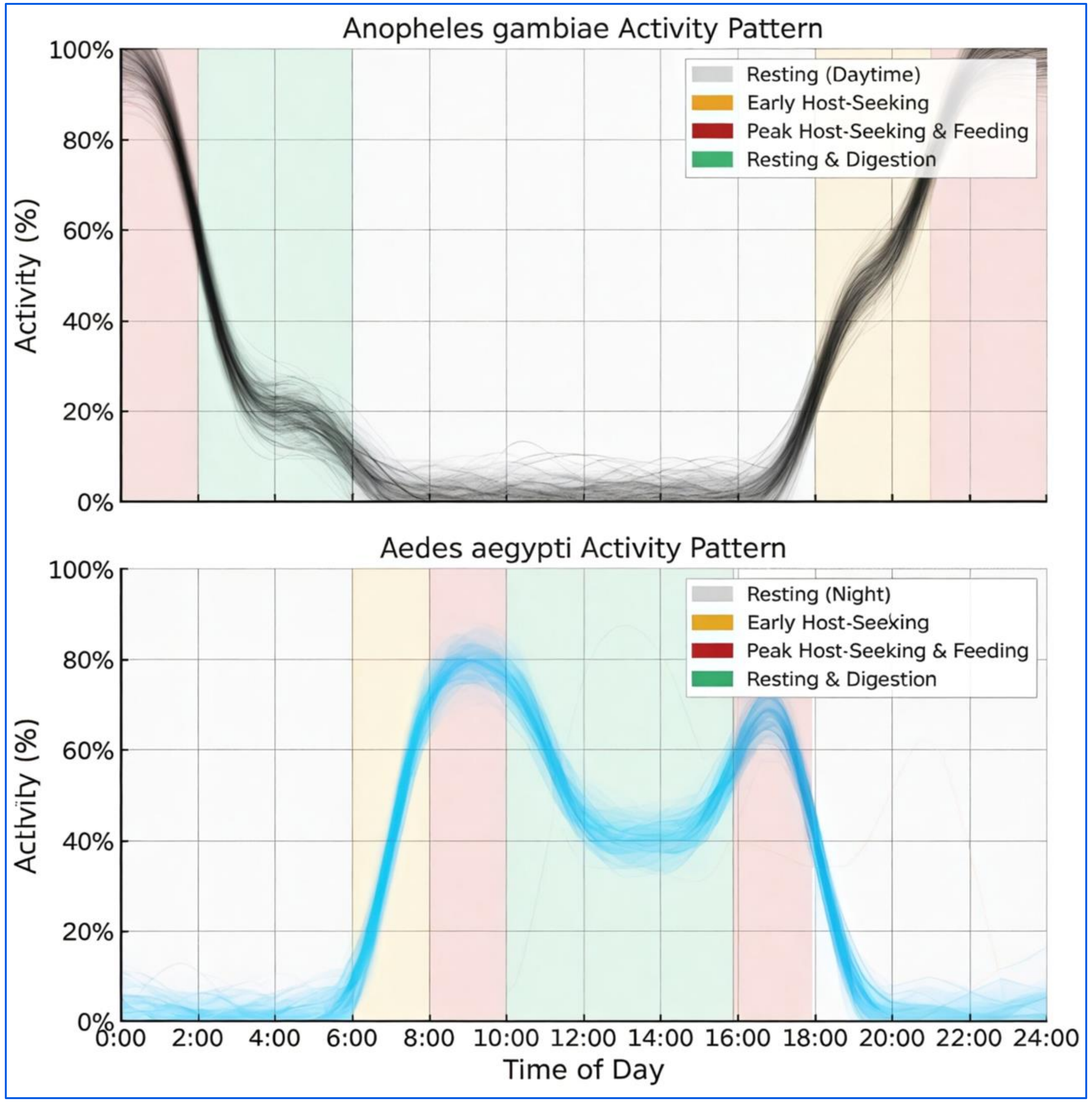
"the **habits** of the **mosquitoes** in question are of prime importance in **determining** the **likelihood** of their being **exposed** to **DDT deposits** for a **sufficient time** to produce **death**"

***Anopheles*, CDC**

The **time** mosquitoes spend **resting, accounts** for a **large portion** of their **daily time-activity budget**

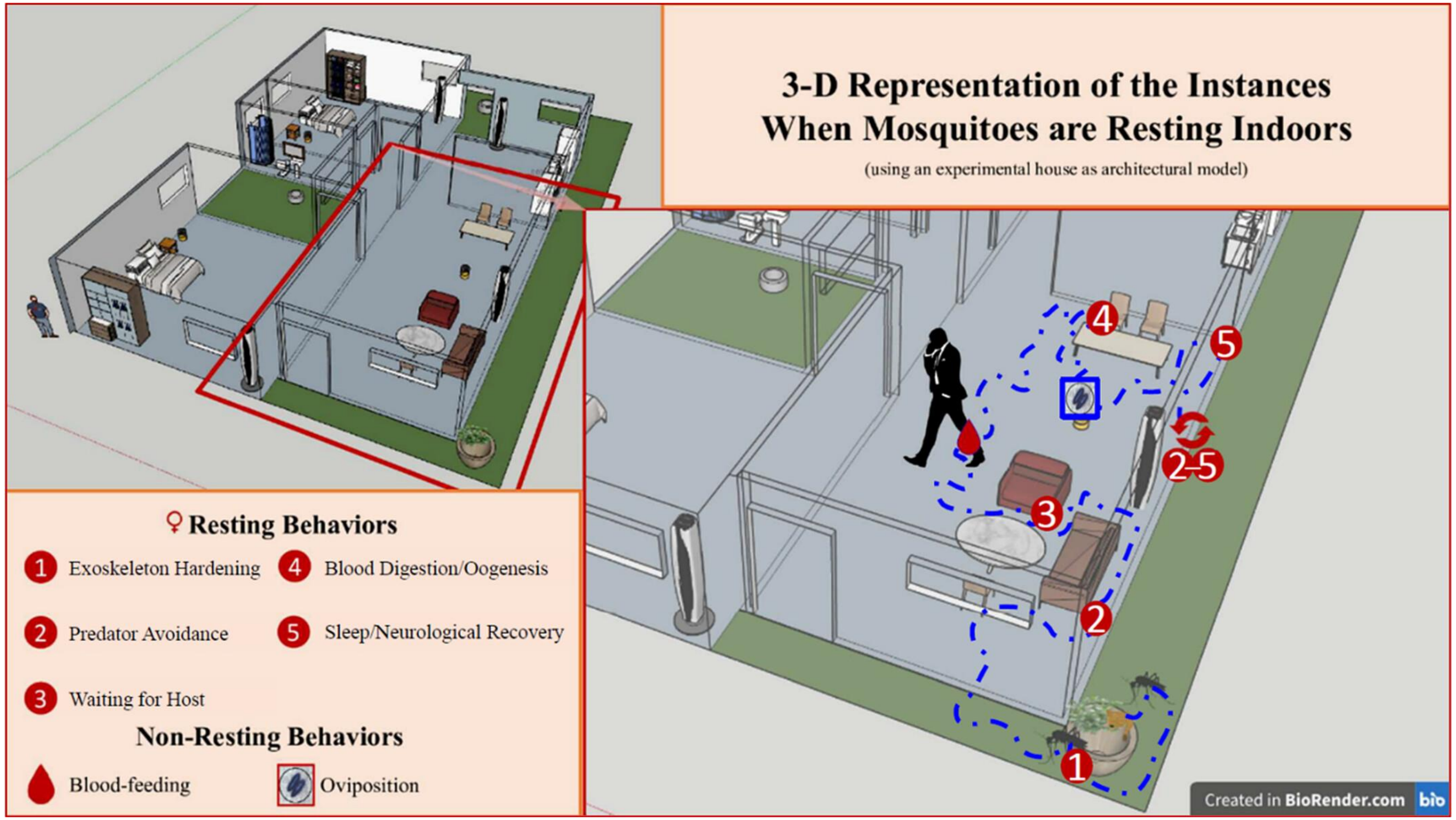
***Anopheles gambiae*, CDC**

***Aedes aegypti*, CDC**



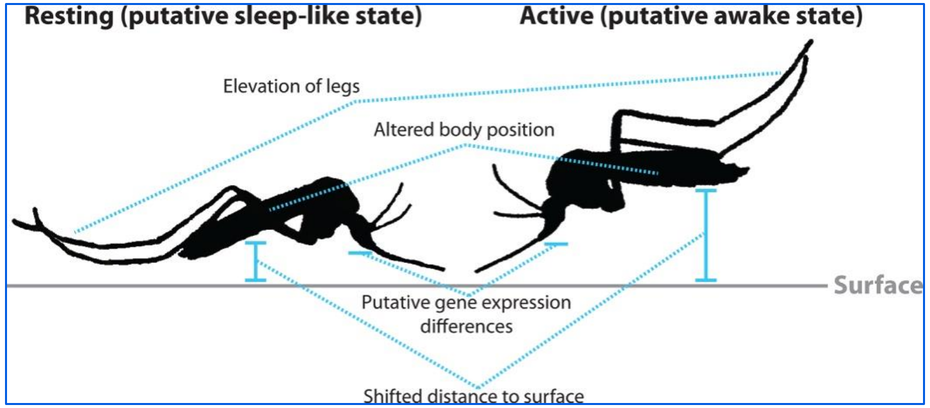
Jimenez-Vallejo et al., 2025

For an **indoor dwelling** species like ***Aedes aegypti***, **resting** is a key **determinant** of **physiology** and **survival**

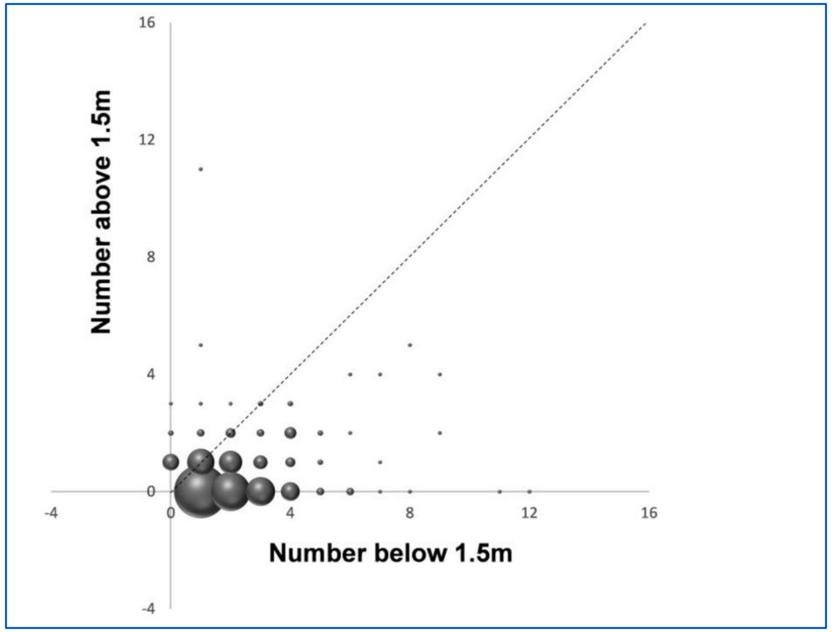


Jimenez-Vallejo et al., 2025

Aedes aegypti, CDC



Ajayi et al., 2021



Dzul-Manzanilla et al., 2016

Aedes aegypti demonstrate a pronounced **preference** to **rest** at **heights** closer to the **ground**

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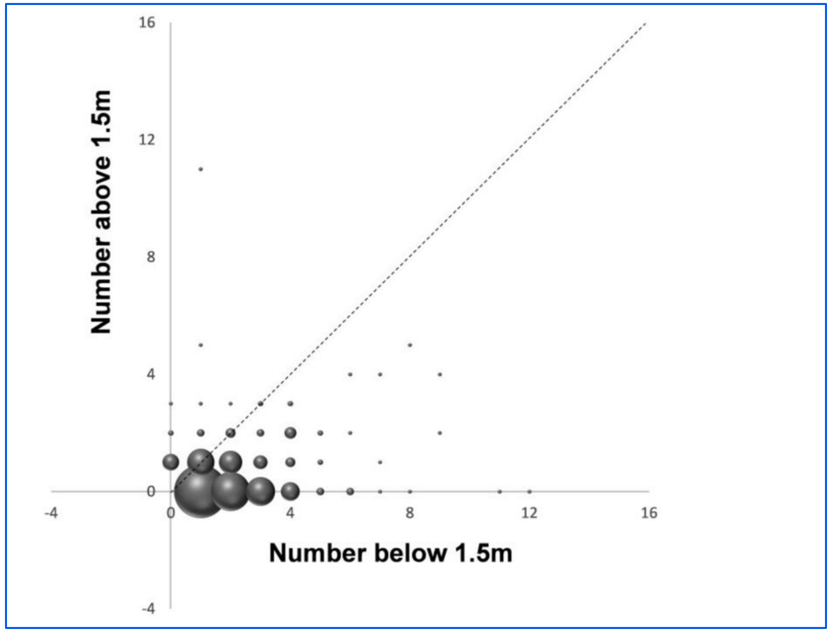

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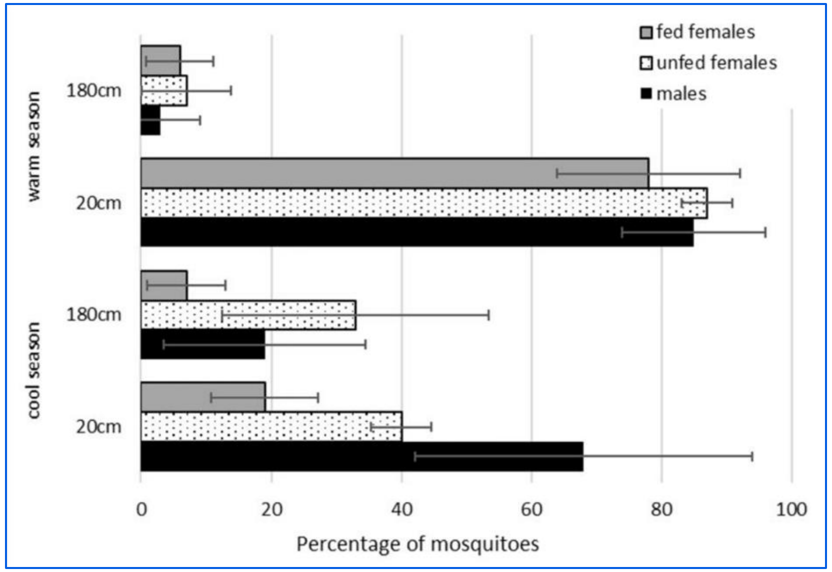

Facchinelli et al., 2023

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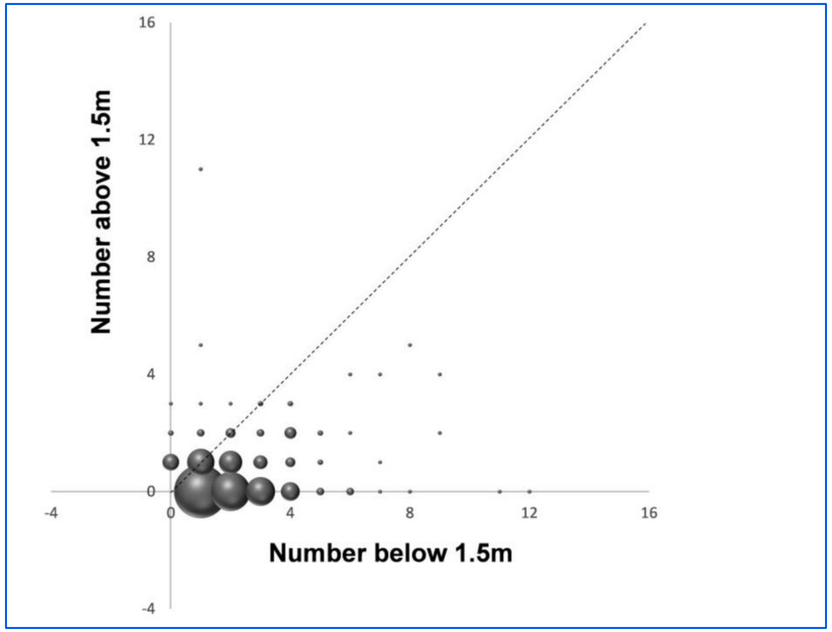

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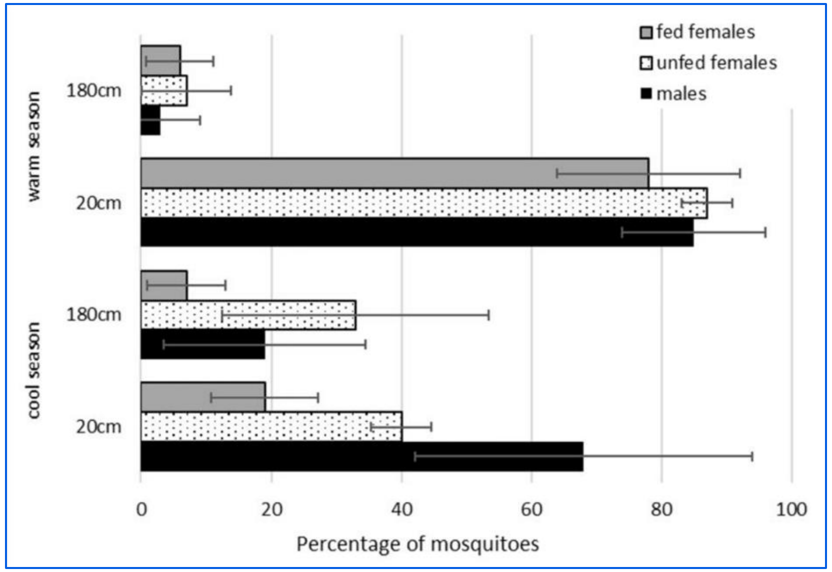

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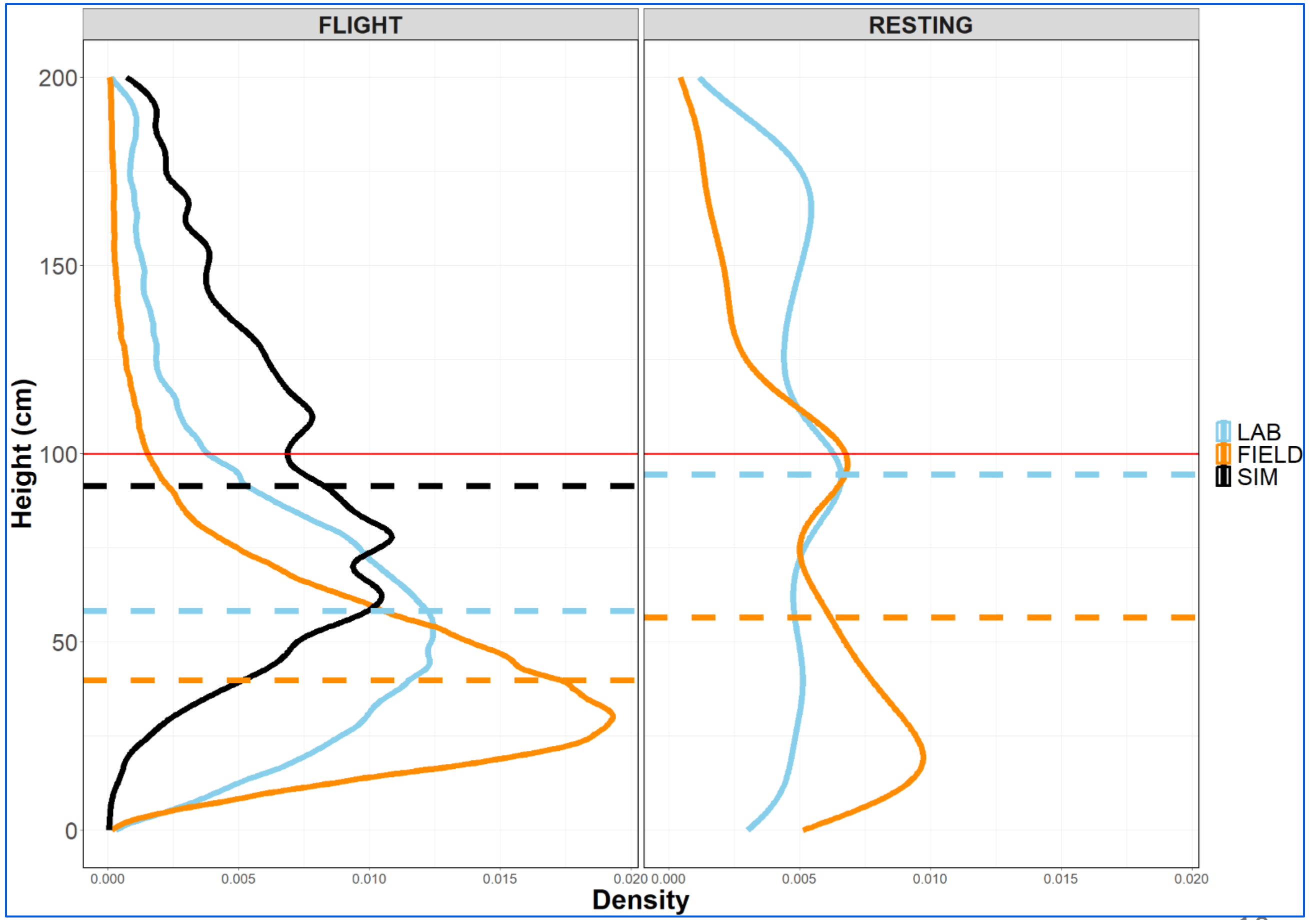
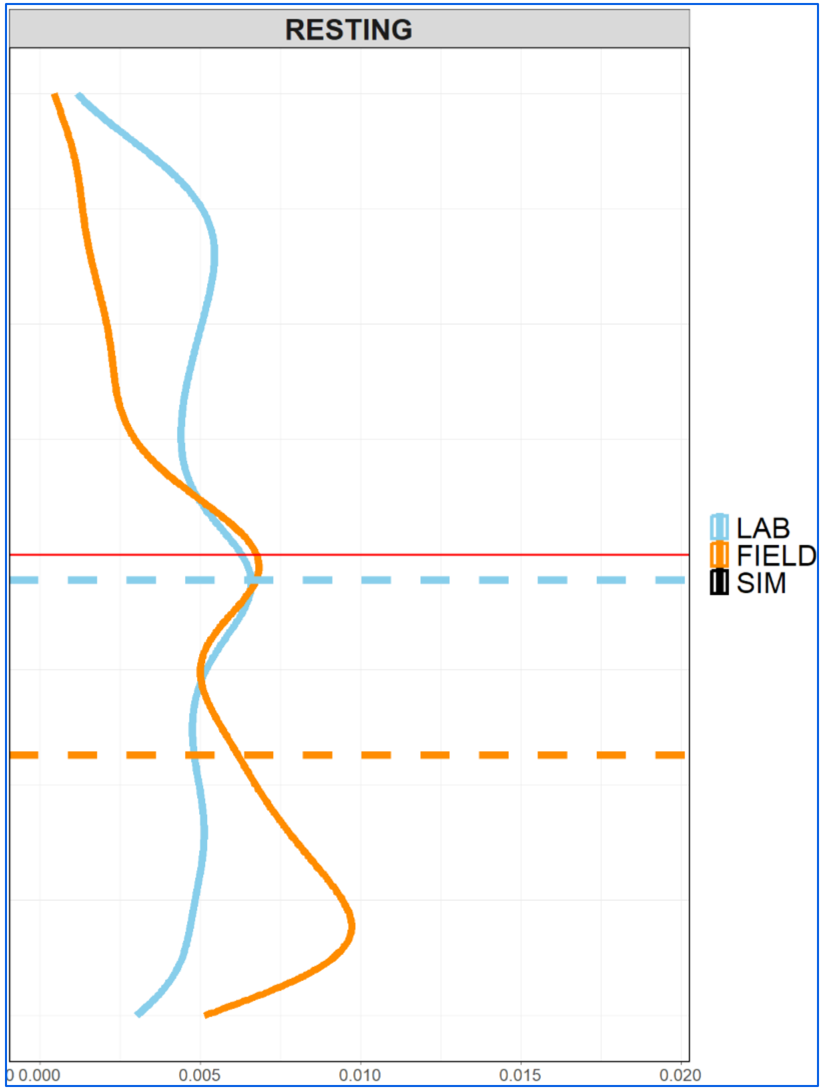


Jimenez-Vallejo et al., in prep

Reciprocal **crosses** suggest this **preference** for **lower heights** may be **modulated** by **(epi)genetic interactions**



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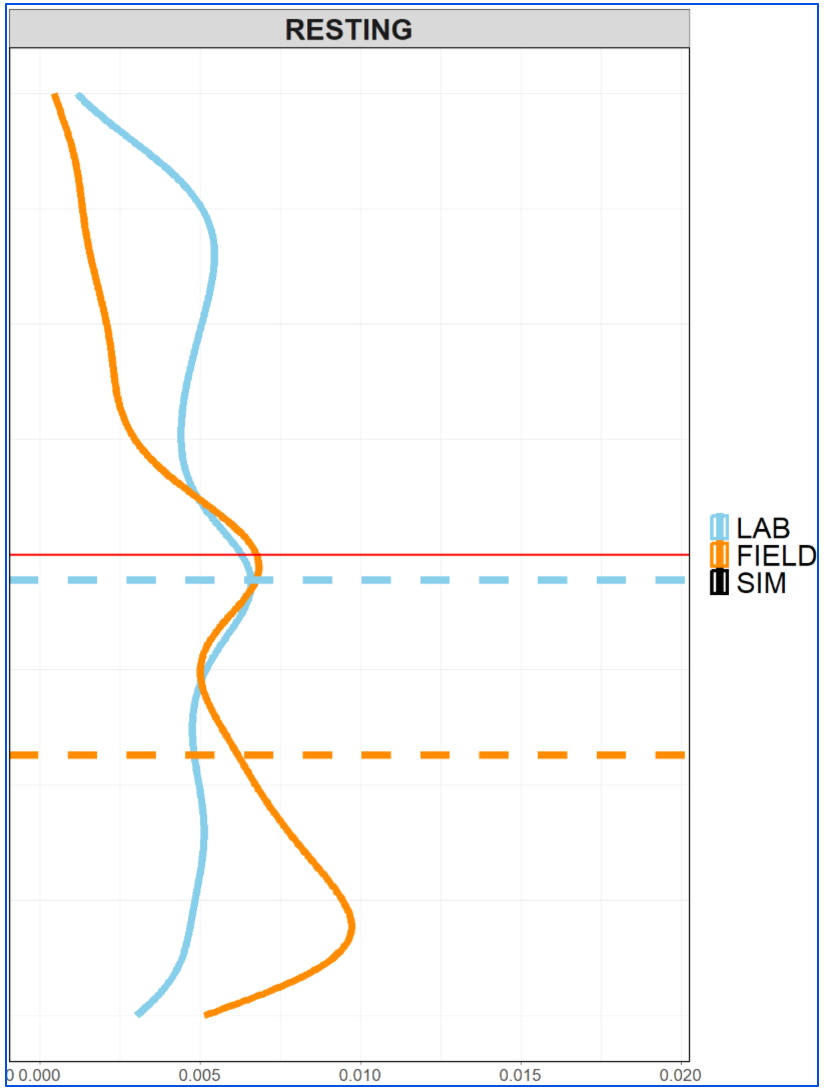

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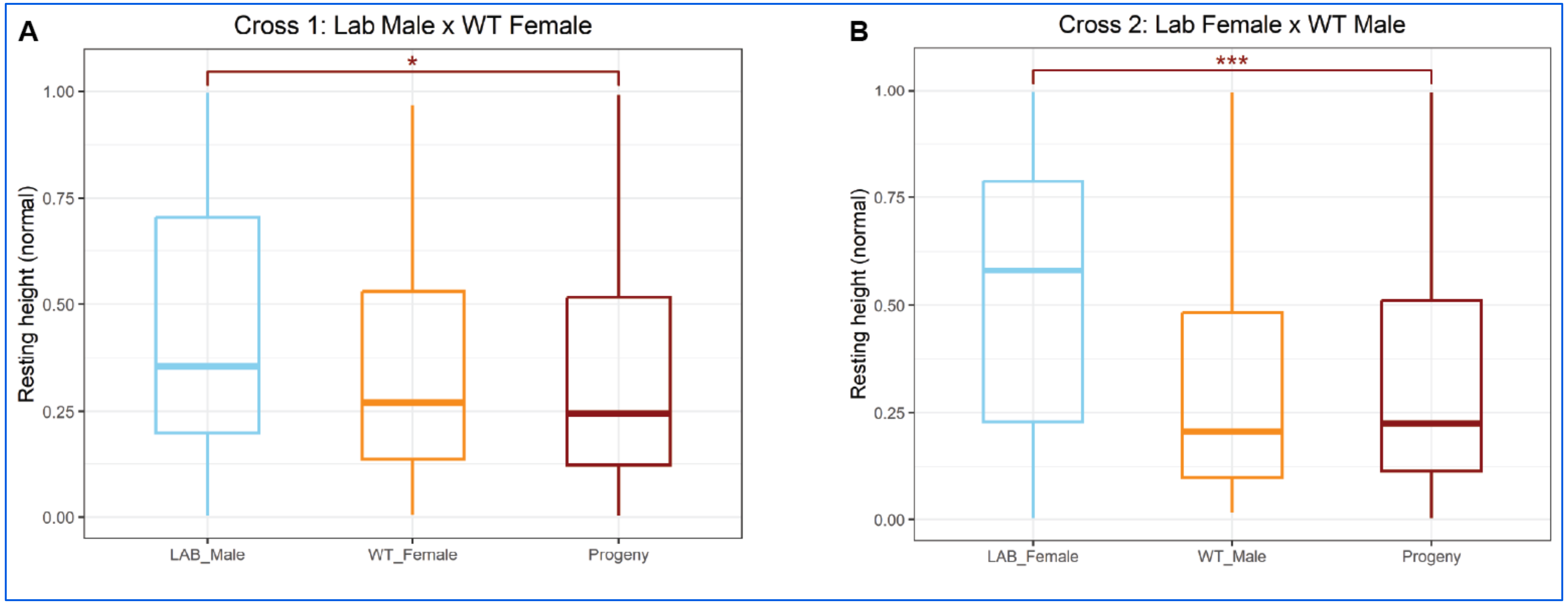


Jimenez-Vallejo et al., in prep

Selectively spraying insecticides according to the **behavior** of ***Aedes aegypti*** can **improve efficacy** and **reduce costs**

Insecticide Residual Spraying has evolved to be **"TARGETED"**; aka **TIRS**

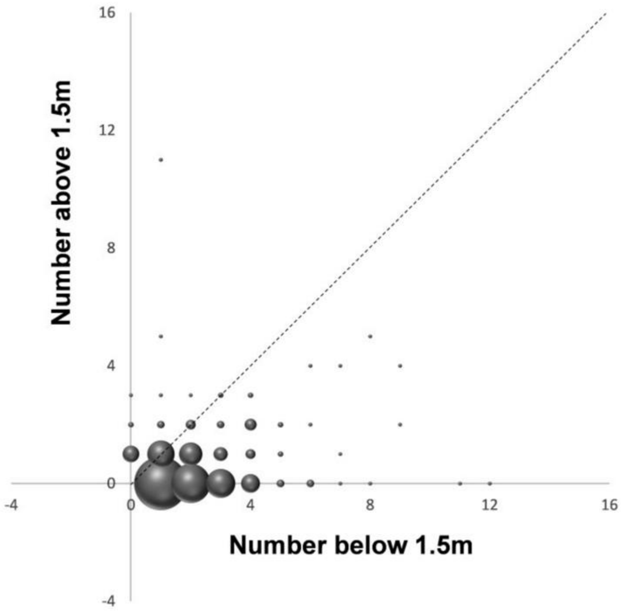


Fig. 1. Distribution of paired *Ae. aegypti* abundances inside houses stratified by collection height. Each symbol indicates an abundance occurrence (e.g., the point at [x = 12, y = 0] shows a house that had 12 *Ae. aegypti* below 1.5 m and none above 1.5 m). The size of the spheres is proportional to the number of houses with a given *Ae. aegypti* abundance above and below 1.5 m (e.g., the largest sphere indicates that 205 houses had the values [1,0], whereas the smallest spheres represent a single house). The dashed line indicates the point of indifference, where abundance is the same above and below 1.5 m.

Dzul-Manzanilla et al., 2016

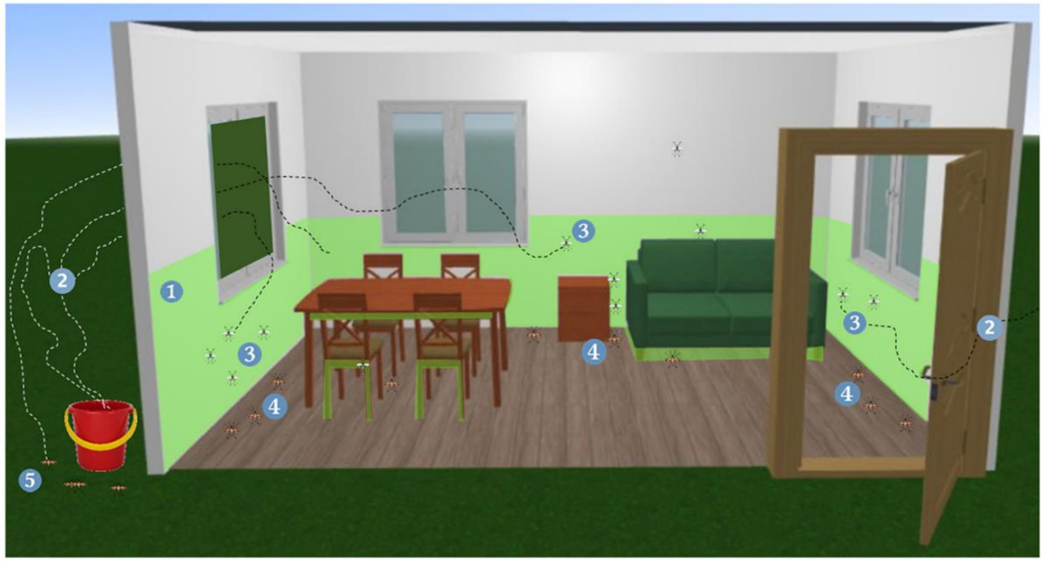


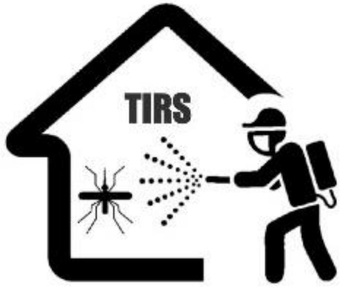
Fig. 1 Targeted indoor residual spraying (TIRS) to control *Ae. aegypti*. In urban environments, houses are primarily built of brick and cement, and *Ae. aegypti* rests preferentially below 1.5 m of height. Spraying residual insecticides in walls below 1.5 m and in key resting sites such as under furniture (#1 in figure, represented in green) will eventually kill *Ae. aegypti* that may be emerging from immature larval habitats outdoors (2) and rest indoors on treated surfaces (3). After exposure to the residual insecticide, mortality can occur immediately (4) or after several hours/days (5)

Manrique-Saide et al., 2020

Similar entomological **impact** (~70% reduction in adult abundance) using a **fraction** of **application time** (<18%) and **insecticide volume** (<30%)

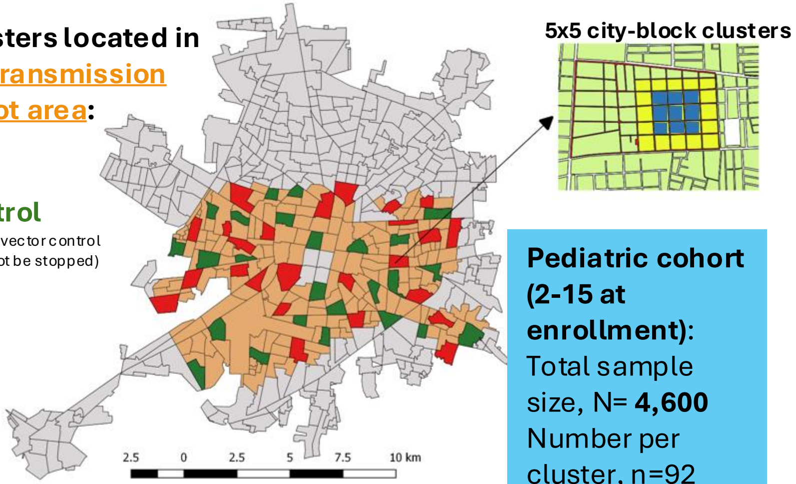
A **two-arm, parallel, unblinded, cluster randomized controlled trial** in **Yucatan** quantified the **efficacy** of **TIRS** to **reduce disease burden**

TIRS trial Design
Merida, Mexico



50 Clusters located in DENV transmission hot-spot area:

25 TIRS
25 Control
(regular MOH vector control actions will not be stopped)



Pediatric cohort (2-15 at enrollment):
Total sample size, N= **4,600**
Number per cluster, n=92

Manrique-Saide et al., 2020

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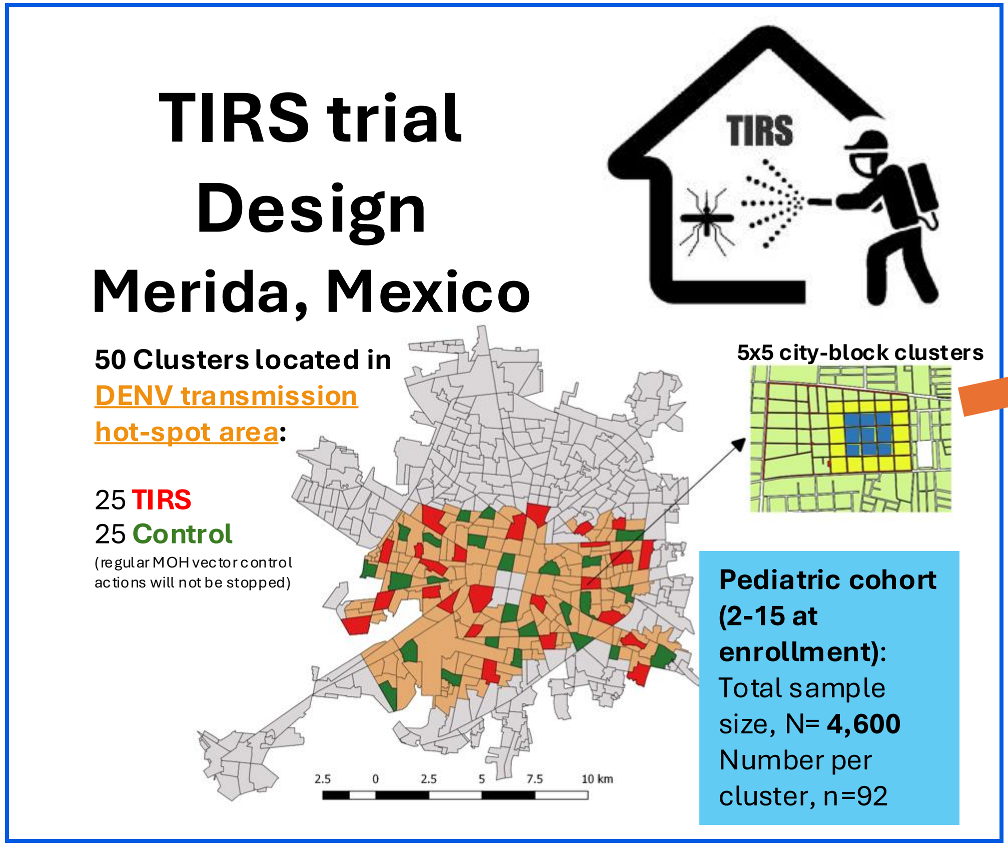

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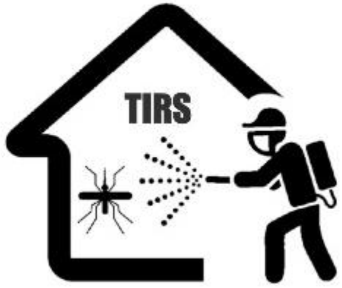

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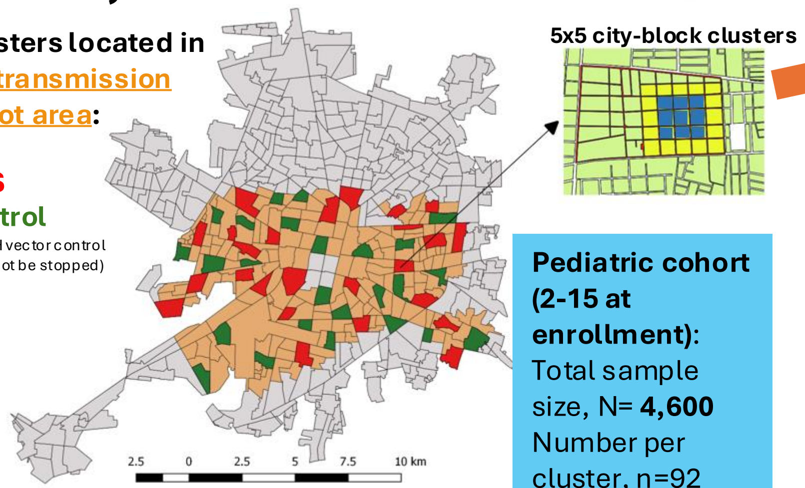

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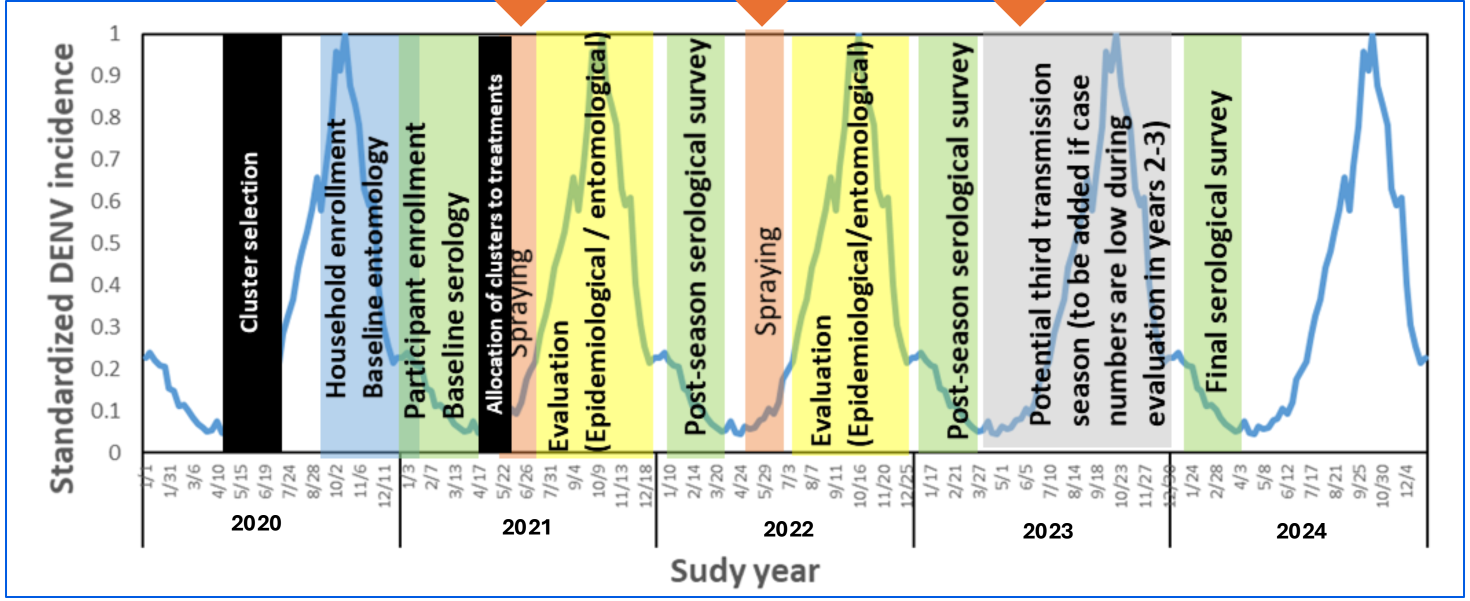
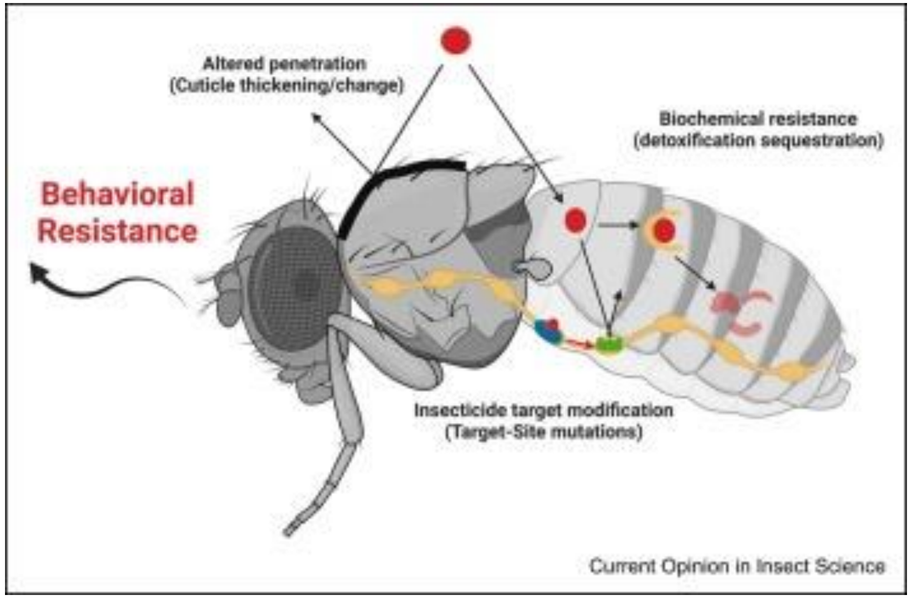
Manrique-Saide et al., 2020

25 random "yolks" across Merida received annual TIRS spraying once every THREE years



Manrique-Saide et al., 2020

Like other **mechanisms** of **resistance**, **behavioral adaptations** against **insecticides** can **decrease efficacy** of interventions



Hubbard and Murillo., 2024

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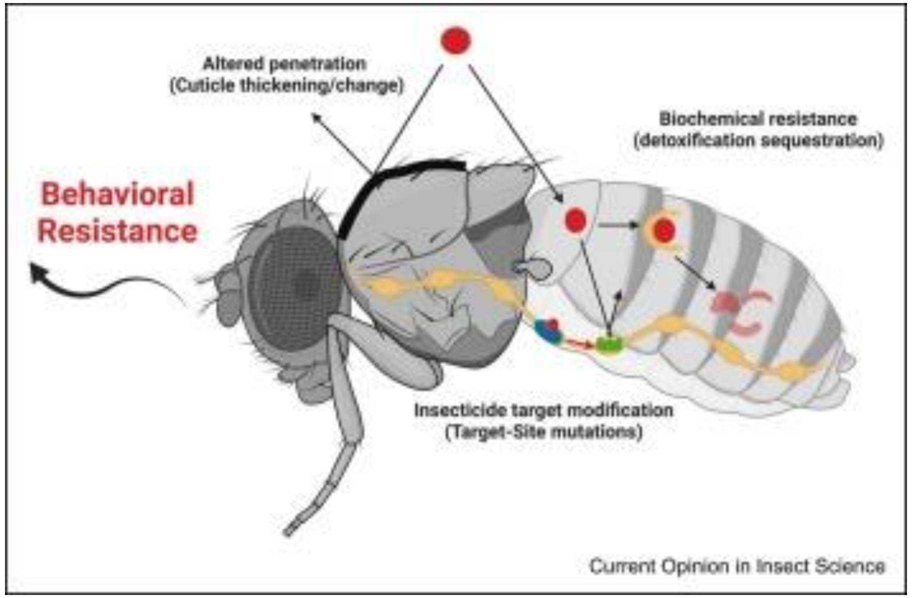

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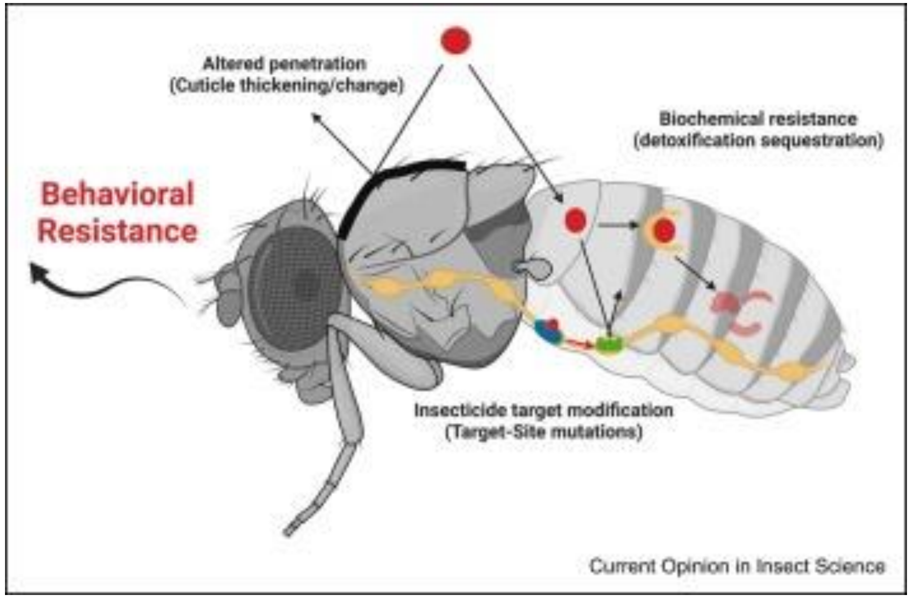

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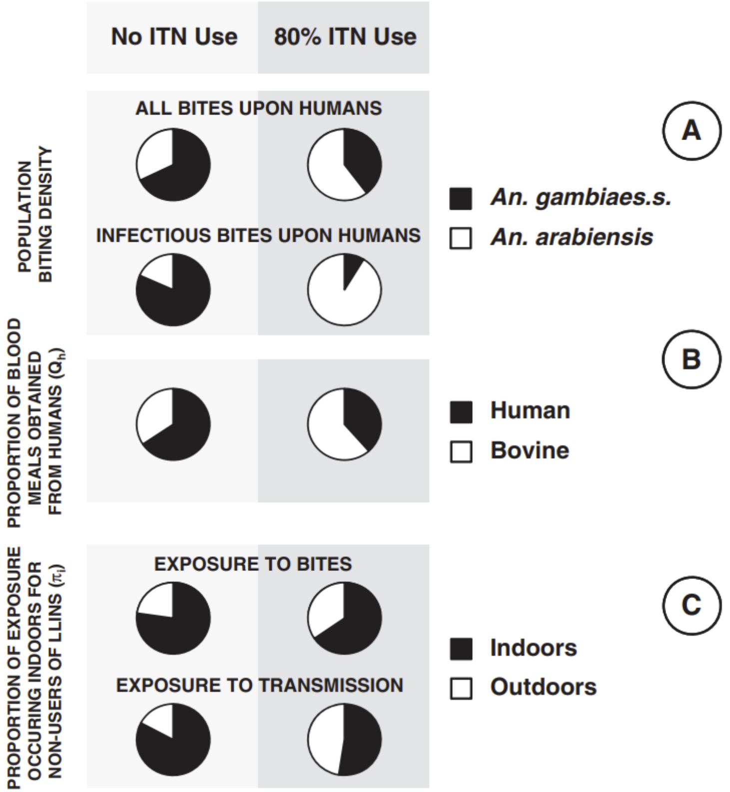

Figure 2 The impact of high ITN use on the behavioural characteristics and population composition of a transmission systems comprised of a mixed population of *An. gambiae* and *An. arabiensis*. The relevant summary outcomes presented reflect the means weighted by relative population size or the relative contributions of these two vector species: **A**; Sibling species composition, **B**; Proportion of blood meals taken from humans, and, **C**; Proportion of human exposure to mosquito bites and malaria transmission occurring indoors.

Govella et al., 2013

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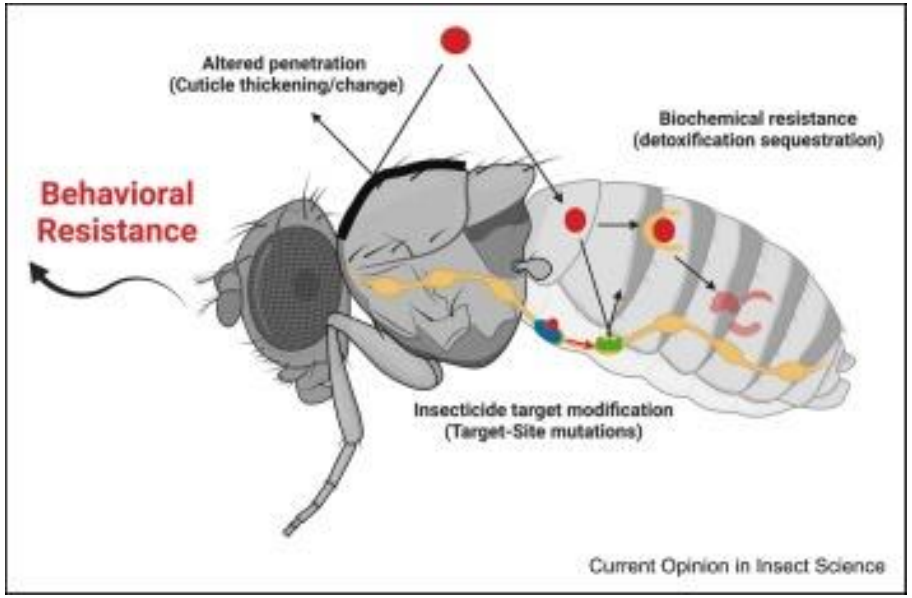

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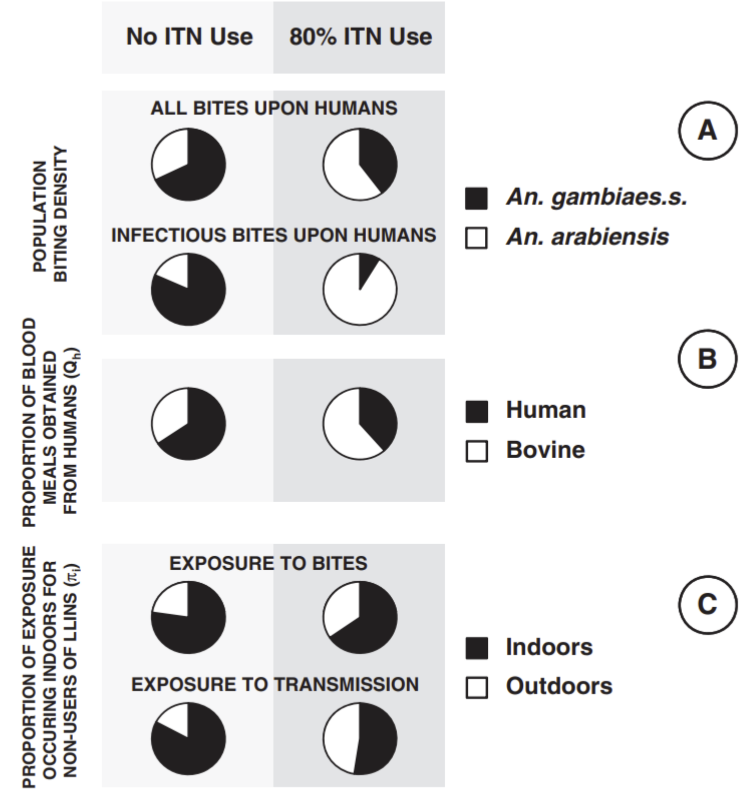

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The **targeted focus** of TIRS **shifts** the **selective force** experienced by mosquitoes in **3D space**

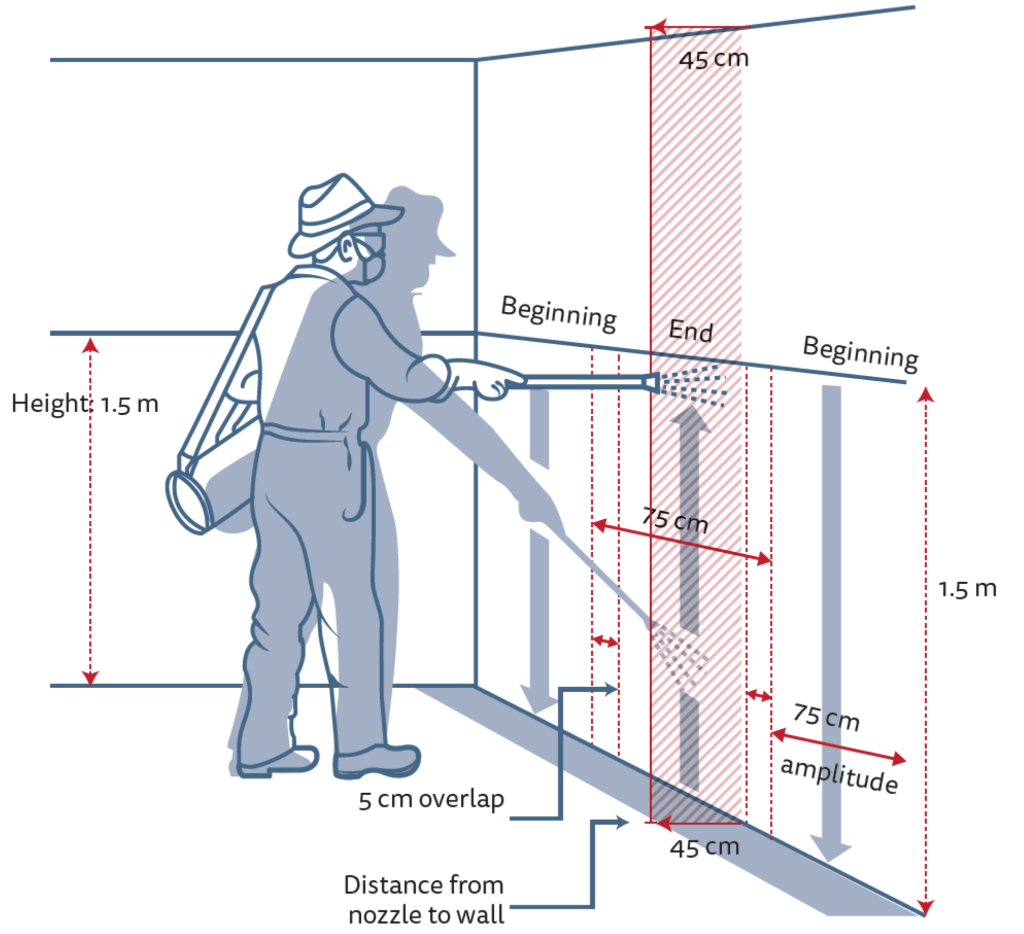


Figure 5. Illustration of the IRS technique for urban *A. aegypti* control (adapted from WHO, 2015).

Manrique-Saide et al., 2020

- A **fraction** of mosquitoes are still choosing to **rest > 1.5m**
  - **Escape mortality**
    - Across scales component
  - **Selection** can **favor** this **fraction** (if resting behavioral traits are heritable)
    - Behavioral resistance evolves
      - TIRS is ***jeopardized***

My **dissertation** looks to deeply **characterize** the factors driving **resting** and **assess** evolution of **behavioral resistance**

A. Which intrinsic and extrinsic factors drive resting behavior?

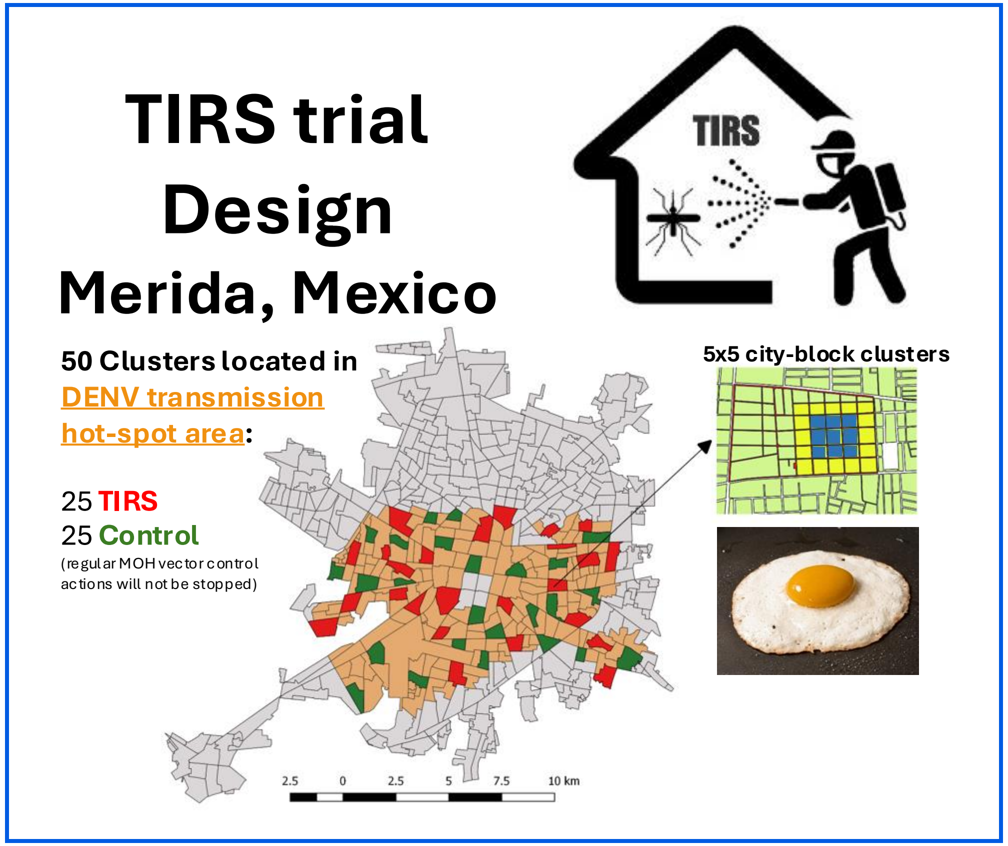
B. What impact does TIRS have on the resting behavior of *Ae. aegypti*?

C. Is *Ae. aegypti* capable of evolving behavioral resistance?

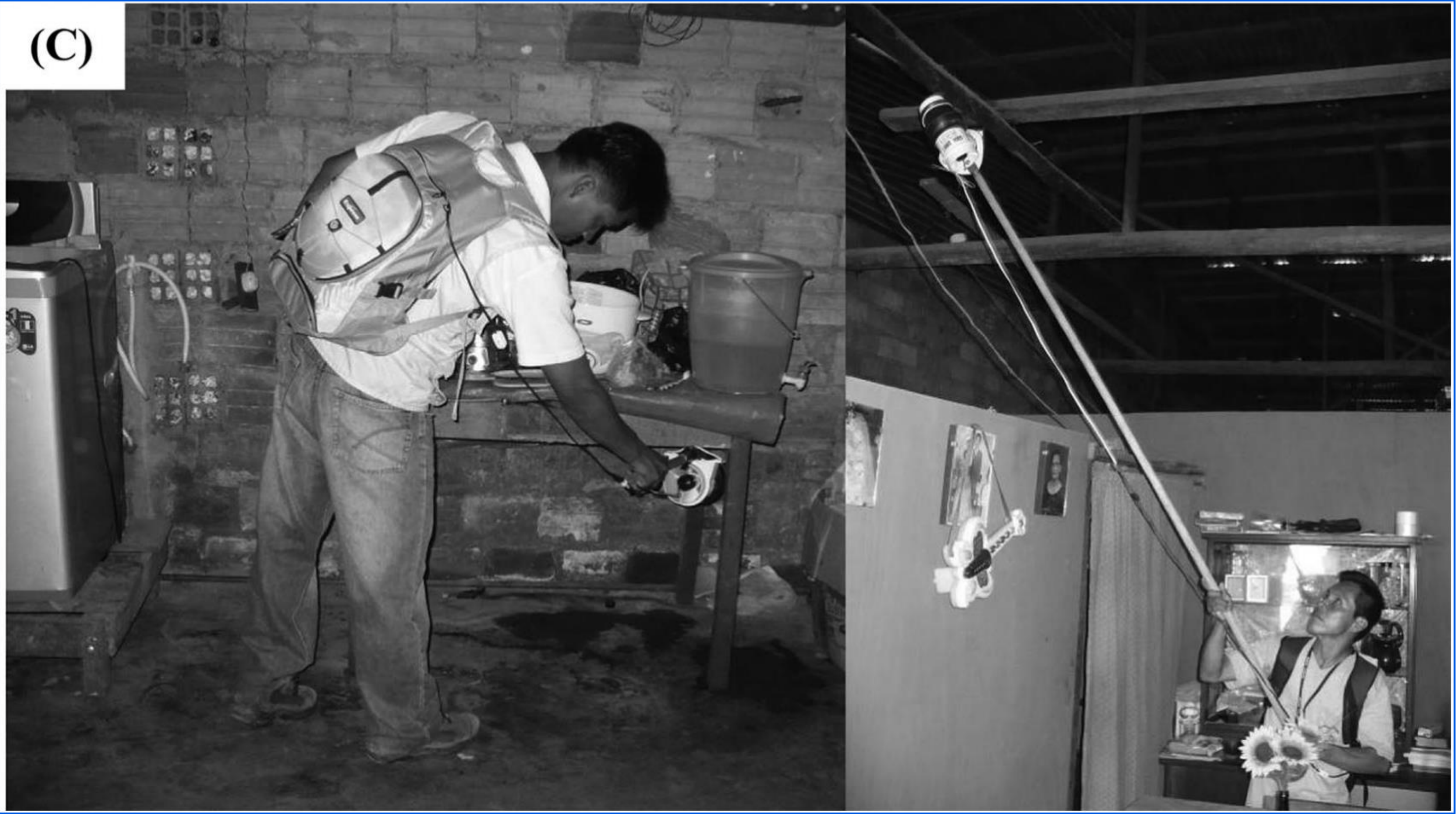
Aim 1:	Aim 2:	Aim 3:
Assess and **quantify** the **individual** and **combined impact** of **intrinsic** & **extrinsic** factors driving resting	**Evaluate** the impact of **TIRS** on **factors** assessed in Aim 1, and on **development** of **behavioral resistance**	**Assess** resting preferences of **natural populations** exposed to **TIRS**, and **evaluate** if **behaviorally resistant**

- **Hypothesis**
  - After **3 years** of **exposure**, if ***Aedes aegypti*** can develop **behavioral resistance** to **TIRS**, then a **higher number** of mosquitoes would be **collected resting** at heights **above 1.5 meters** in **TIRS-treated** households **vs Control** households

Stratified collections (above vs below) were done **2mo**, **4mo**, and **6mo after** third round of TIRS **spraying** in **2023**



Manrique-Saide et al., 2020

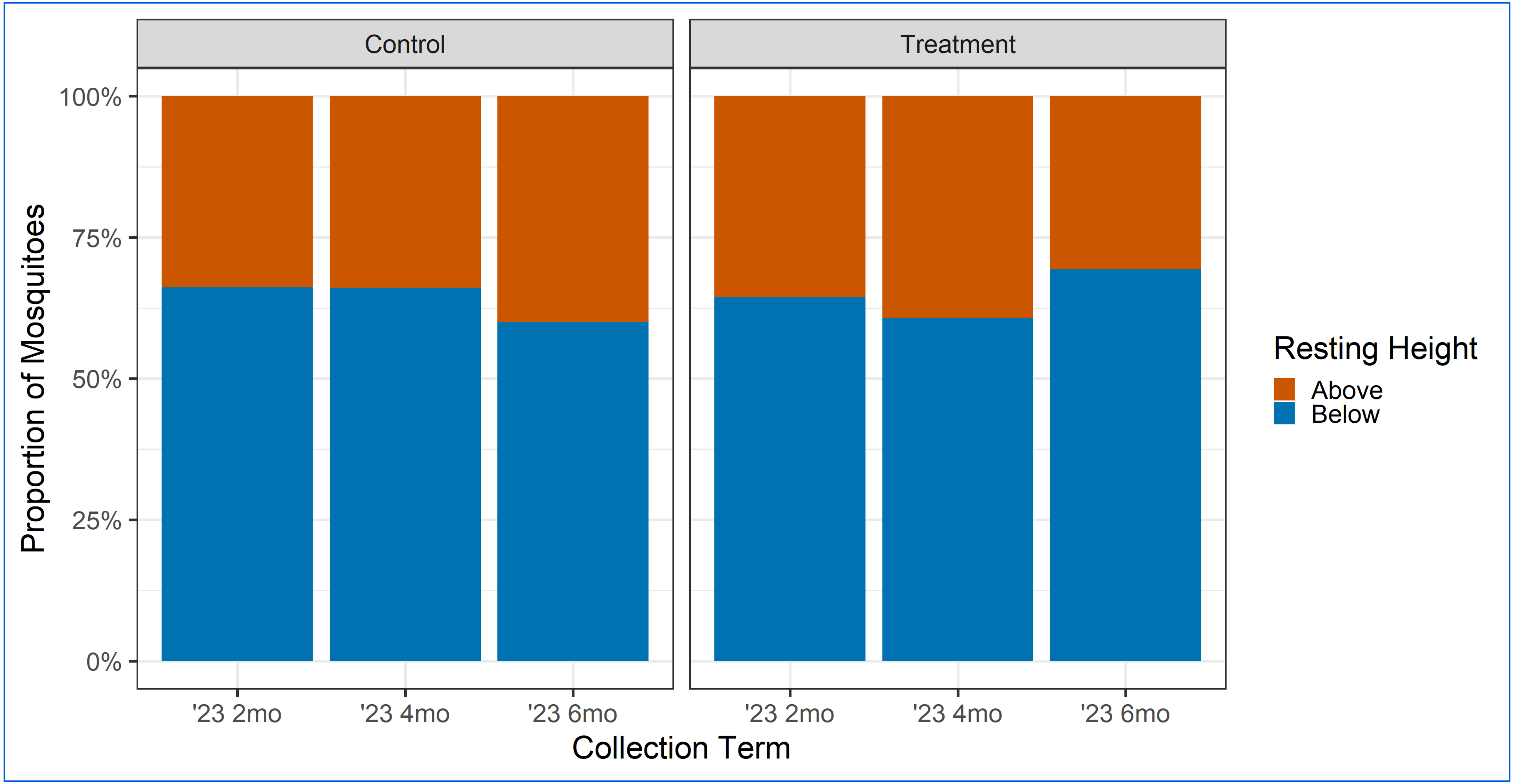


Vazquez-Prokopec et al., 2009

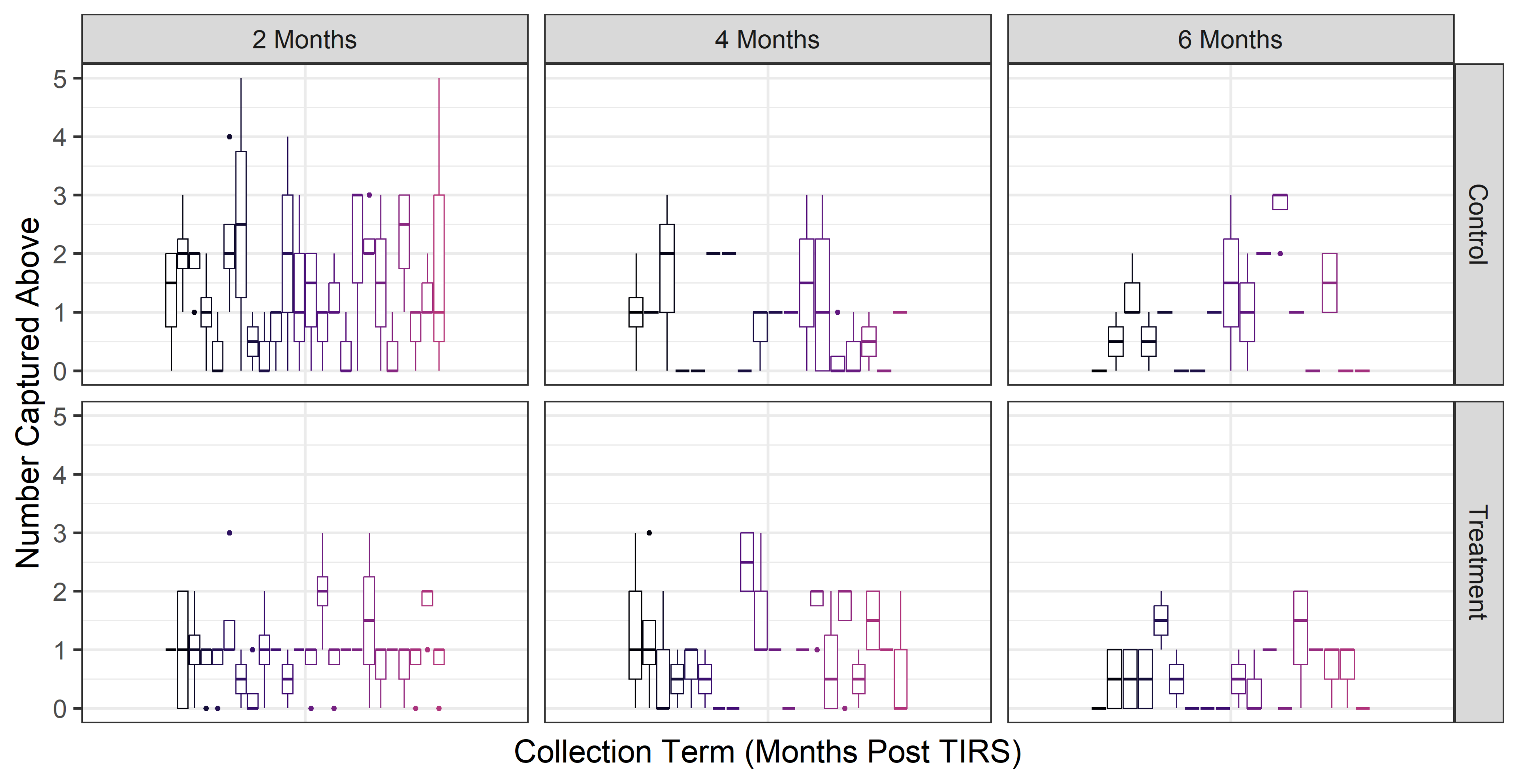
For each collection:

- **A random sample of 10% of the houses (~1350) located in the "yolk" of each cluster (treatment vs control)**
- **Collections took 10 minutes using Prokopack aspirators**
    - **Stratified by height: one tech collects above 1.5m and the other below 1.5m**

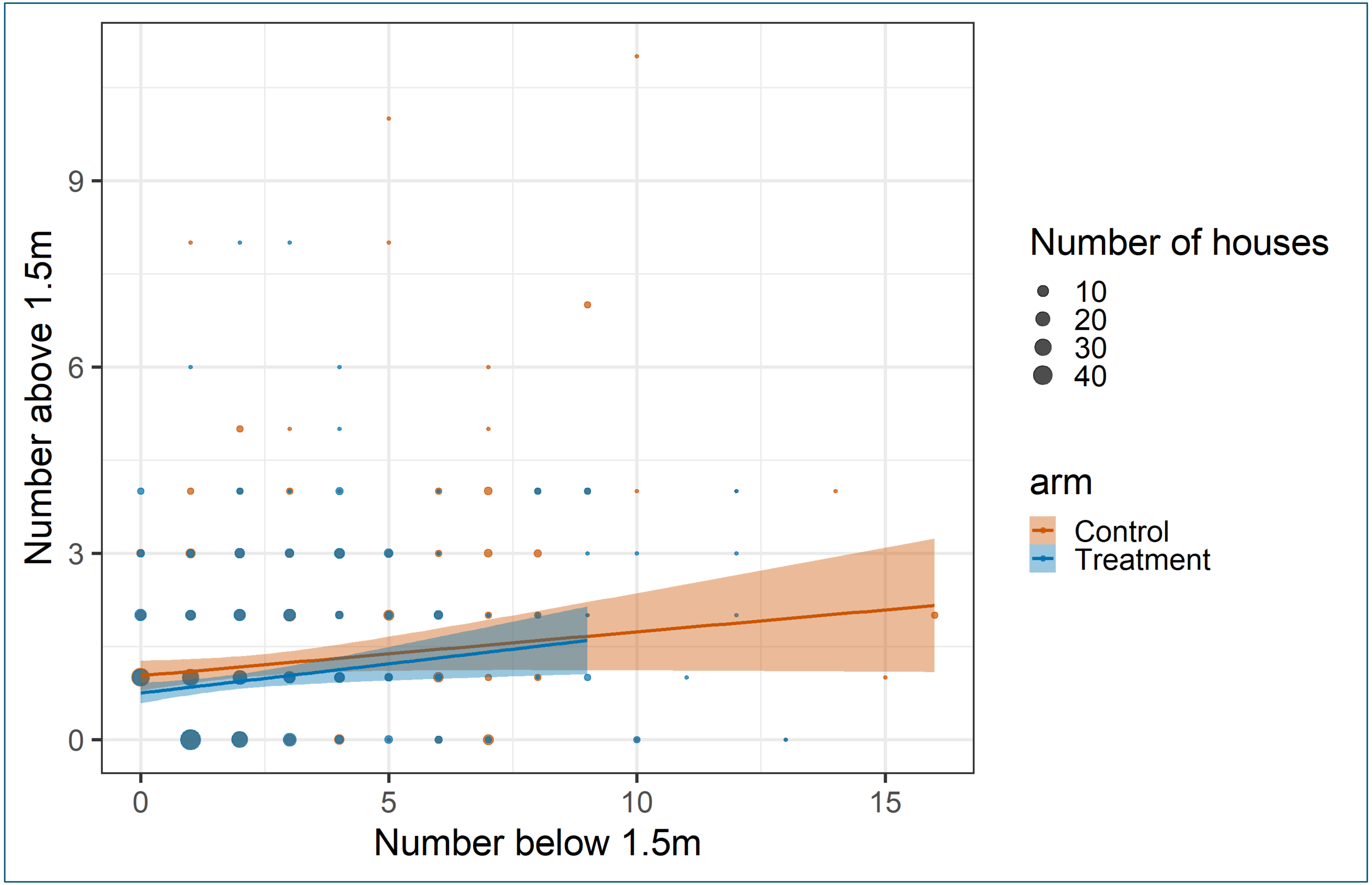
After three years of **exposure** to **TIRS**, the **proportion** of mosquitoes collected **below** stays close to **~65%** for **both TIRS** and **Control** arms



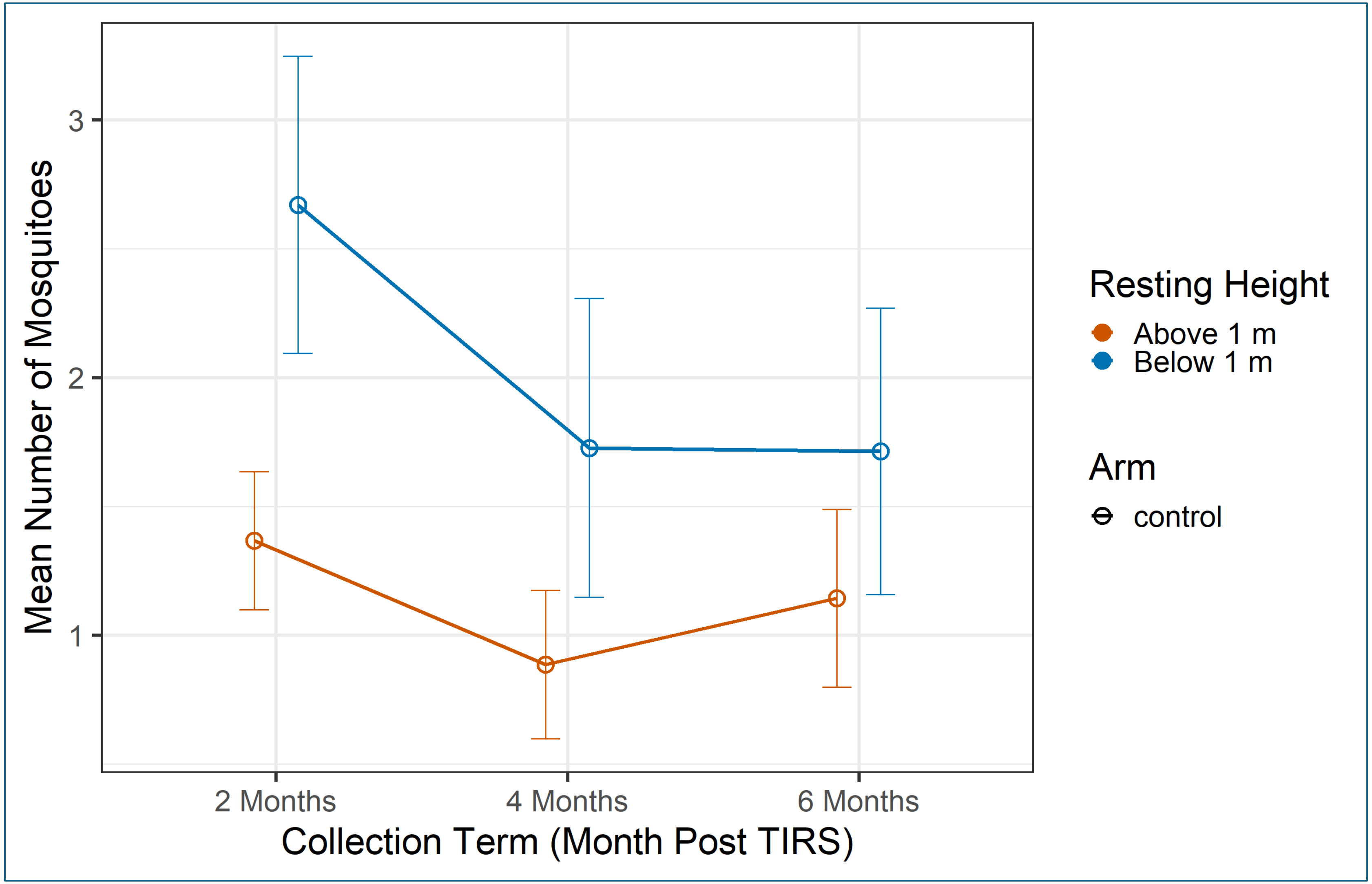
On a **cluster scale**, we **observe no** significant **difference** in the **number** of **mosquitoes** collected **above** 1.5m **between arms** across time



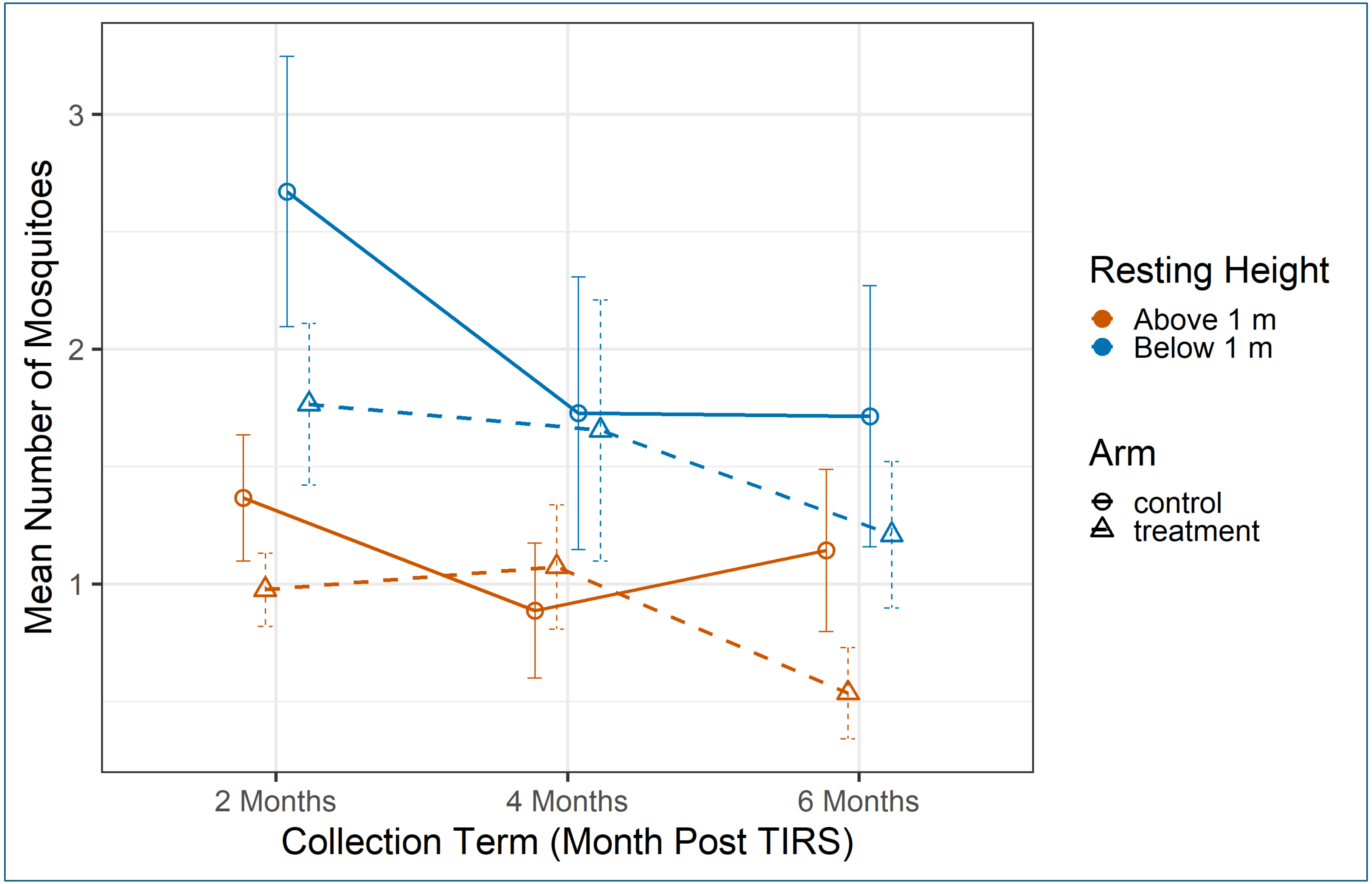
On a **house scale**, we observe **higher abundance** of **low resting** mosquitoes **both** in the **TIRS** arm and the **Control** arm



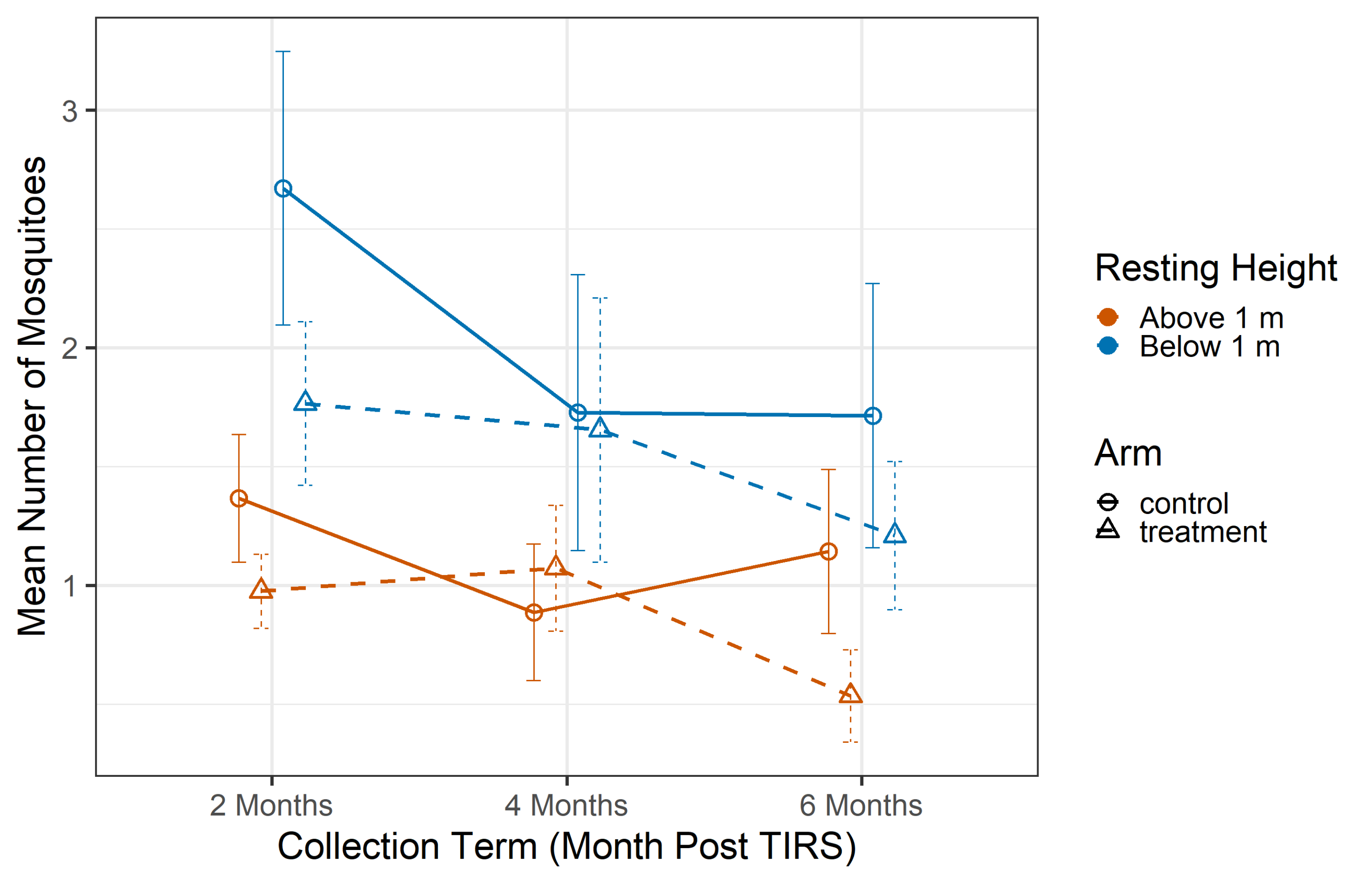
Averaging at a **house-scale**, a **higher number** of mosquitoes is **collected below** in **Control houses** across time

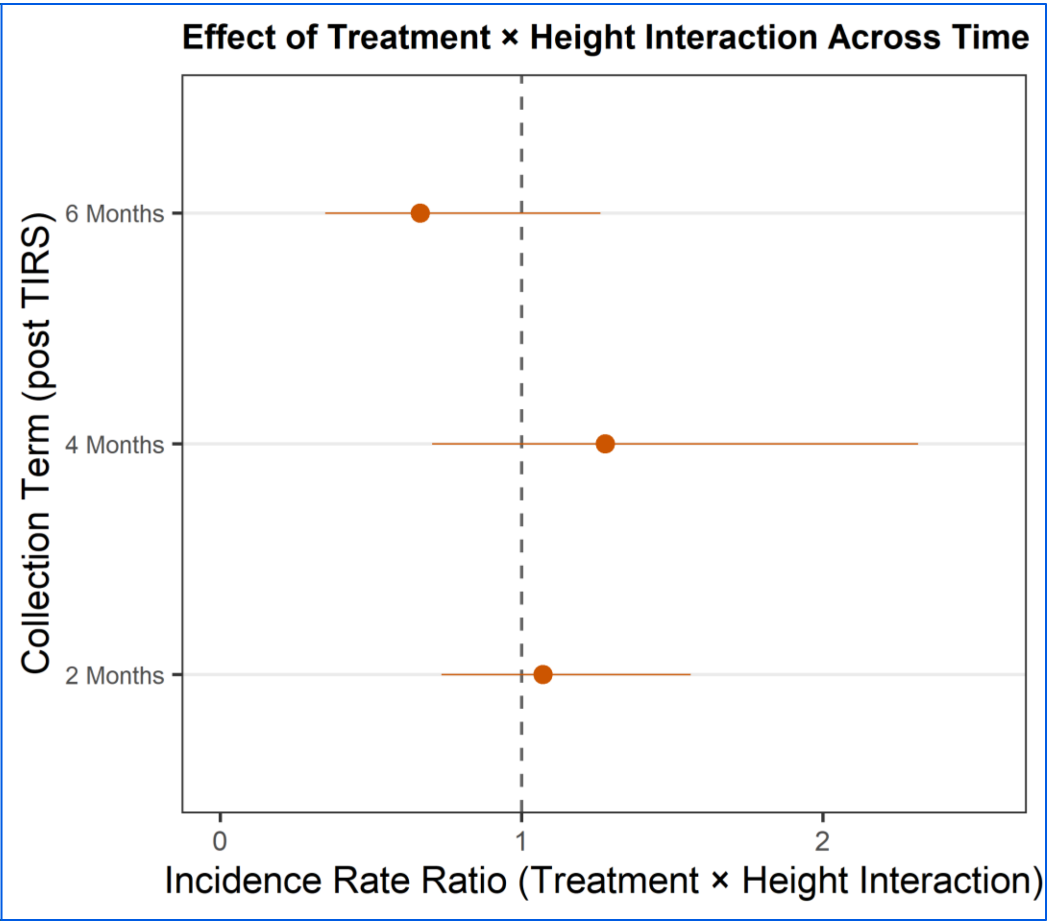


The **same** is **observed** in **TIRS-treated houses**, with a **sustained reduction** in the **number** collected at **either height across time**



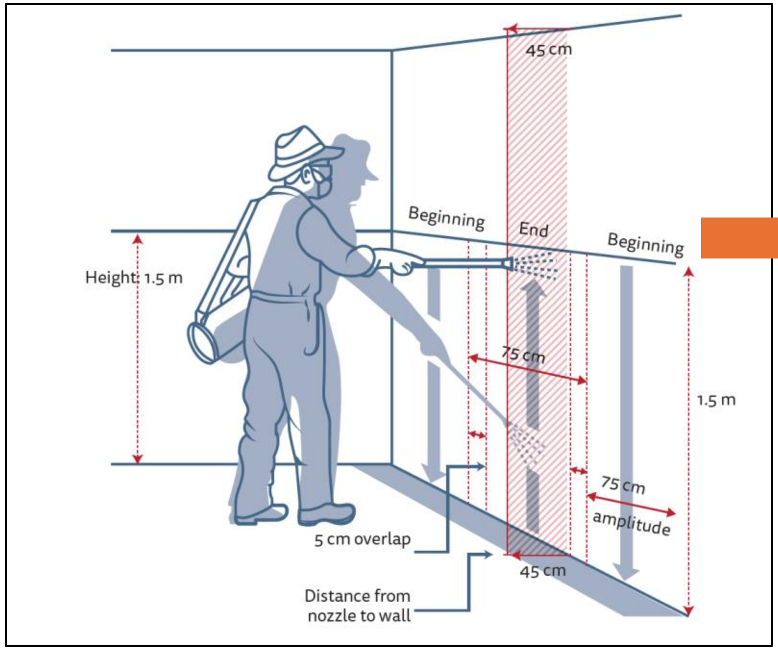
Across **all** post-intervention **periods** in **2023**, there is **no evidence** that **TIRS** treatment **altered** the **number** of mosquitoes **resting above 1.5m compared** to **Control** houses

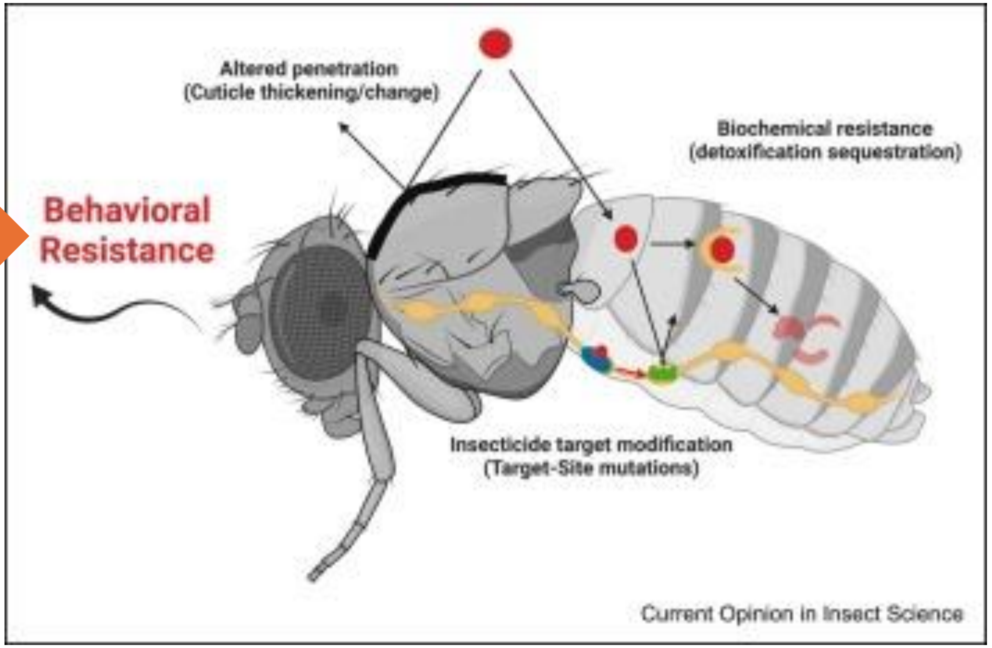




GLMM: Count ~ Arm * Height + (1 | House_ID)

Conclusions





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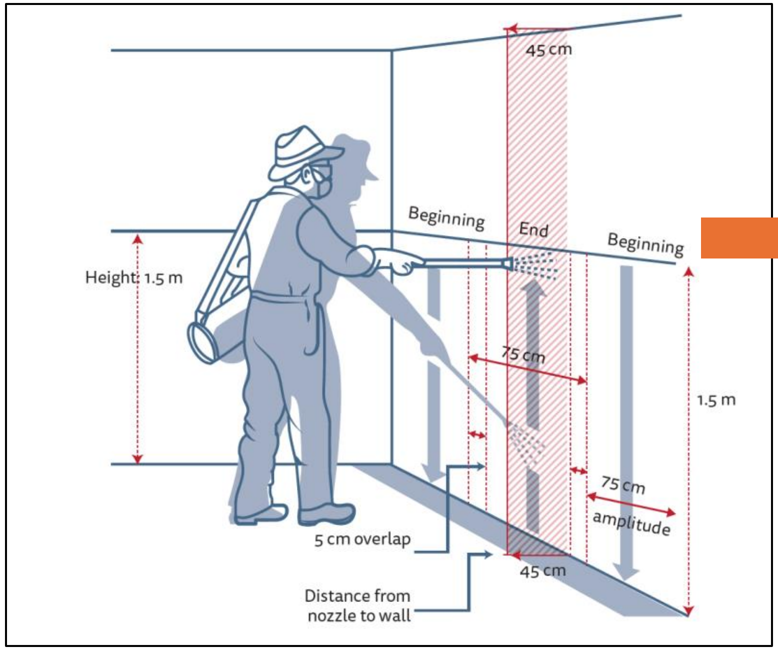

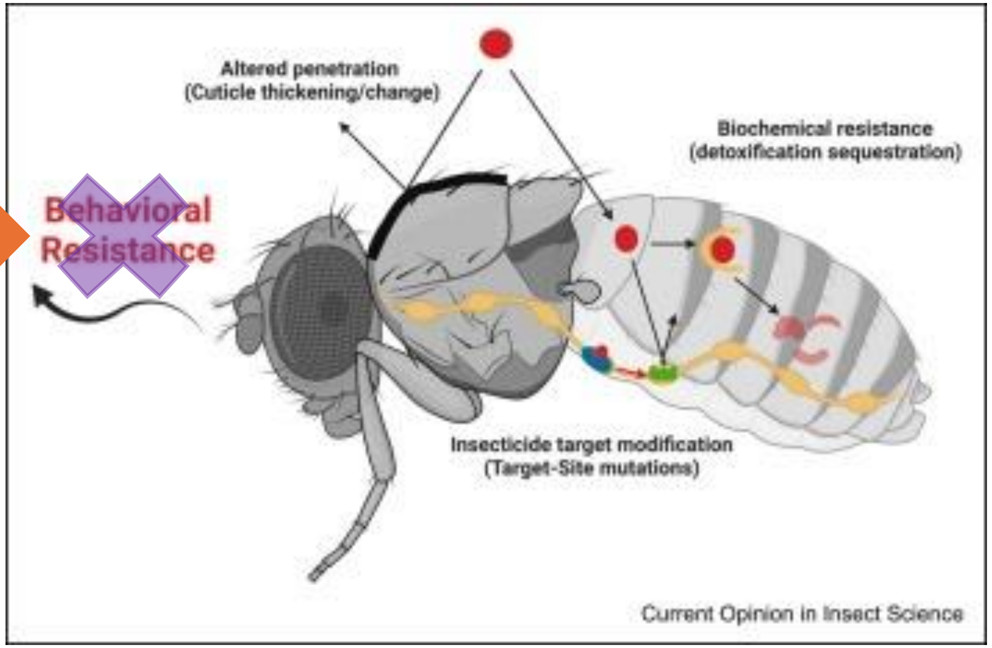

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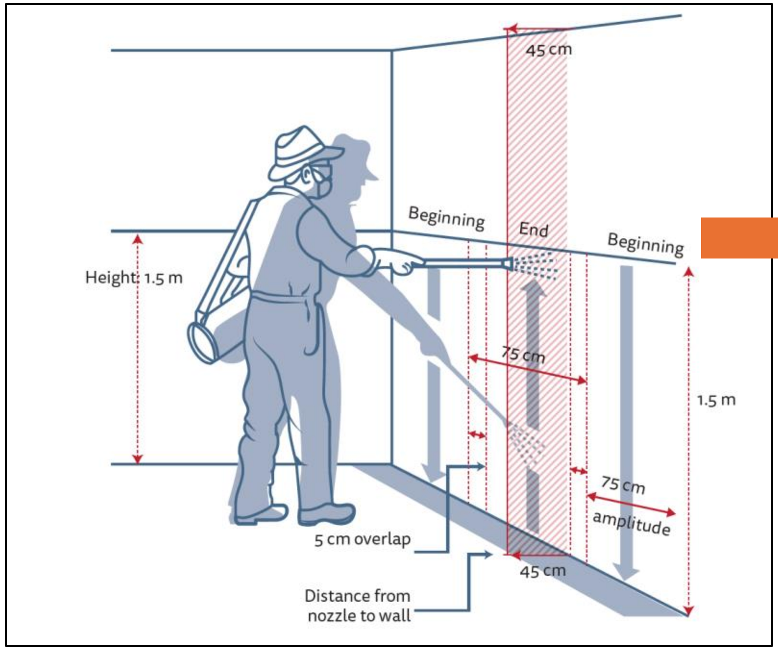

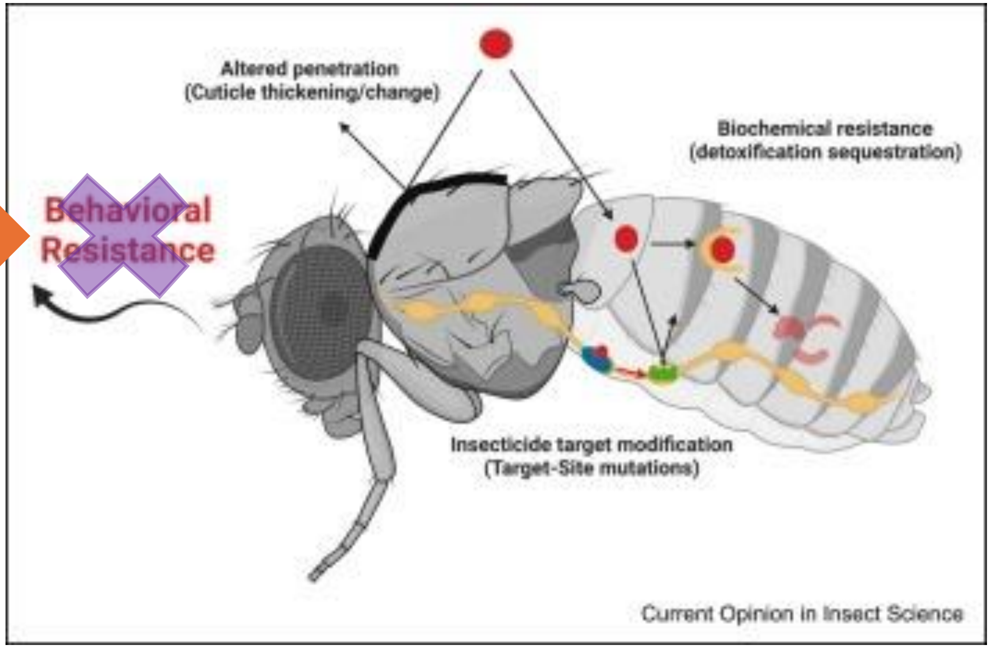

Across three years of continuous exposure, *Aedes aegypti* did not shift resting preferences

- **Not enough time?**
- **Not enough heritability in resting behavior traits?**
- **Shifting from indoor to outdoor?**
- **Other resistance mechanisms favored**

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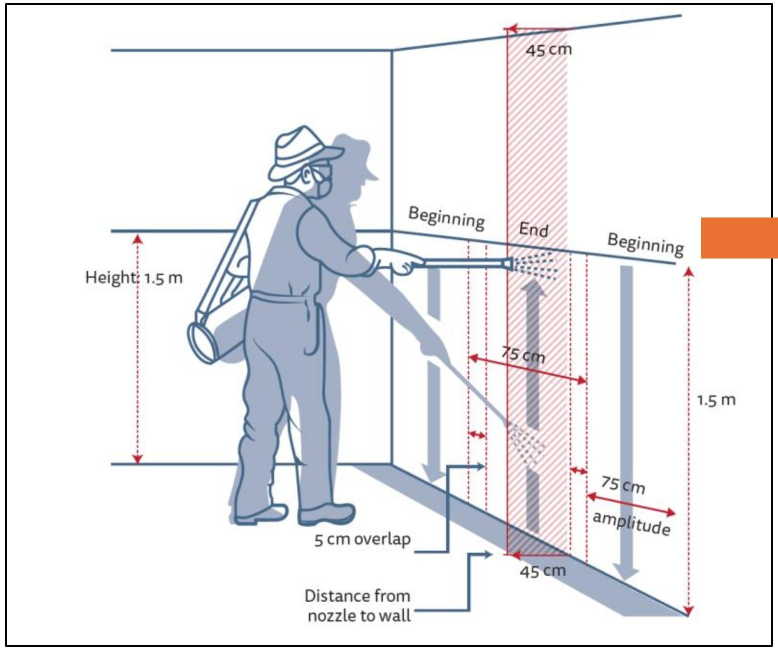

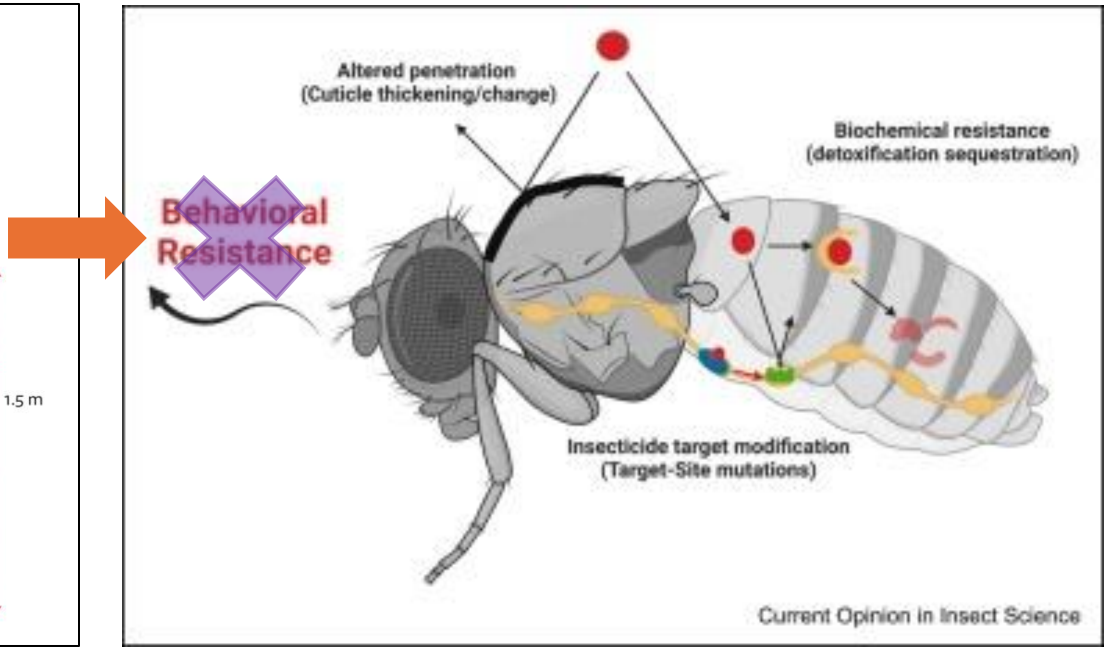

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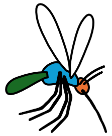
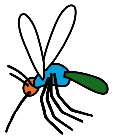
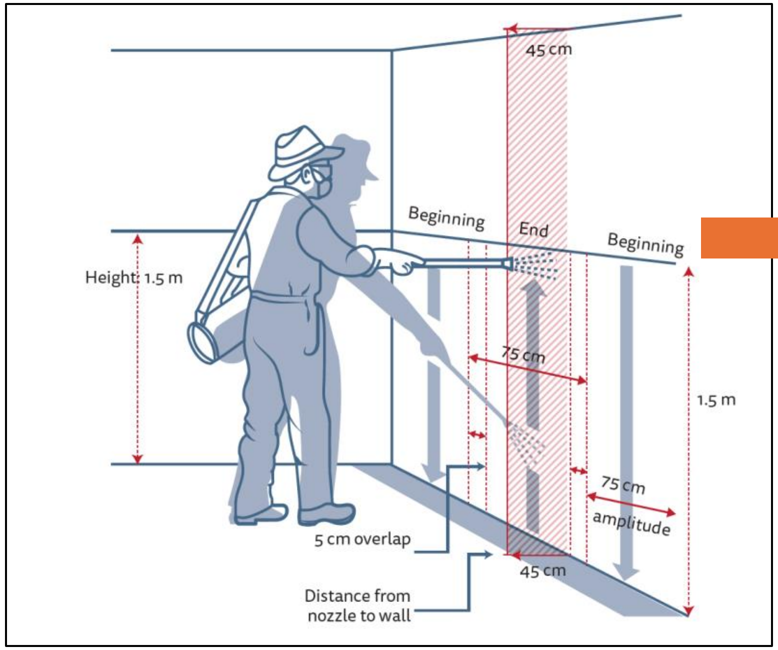
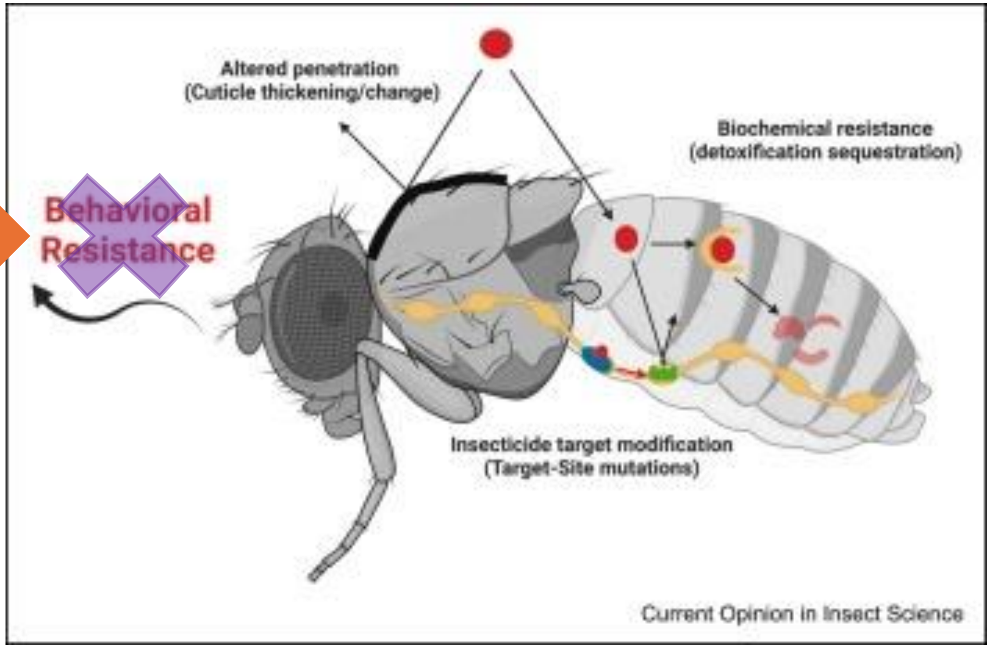
Next Steps



Conclusions







Across three years of continuous exposure, *Aedes aegypti* did not shift resting preferences

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- **Not enough heritability in resting behavior traits?**
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Evaluate the impact of **TIRS** on **factors** assessed in Aim 1, and on **development** of **behavioral resistance**

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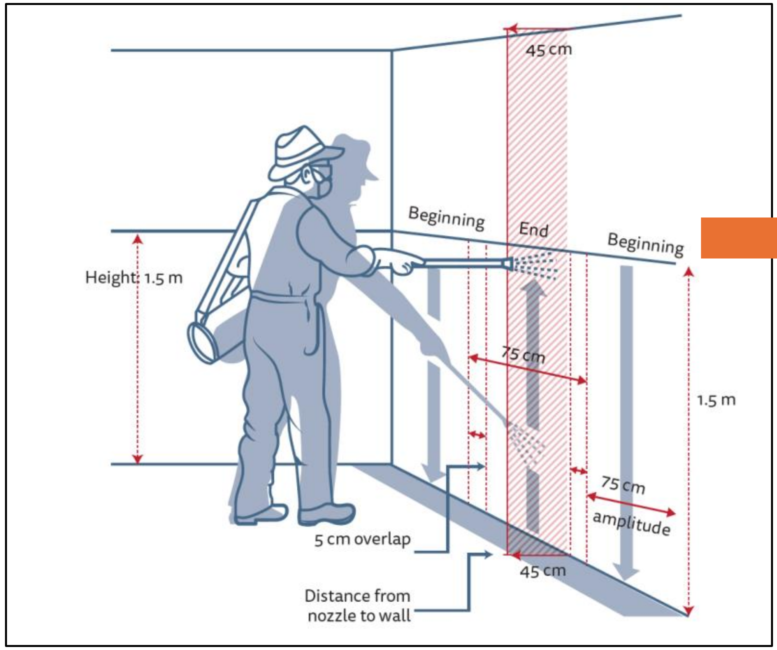

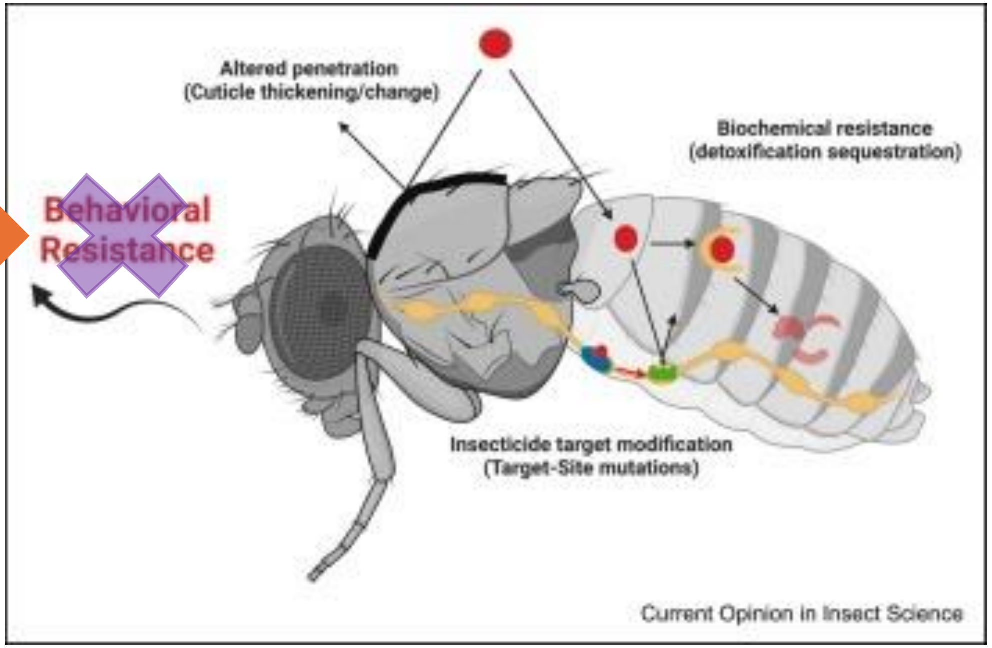

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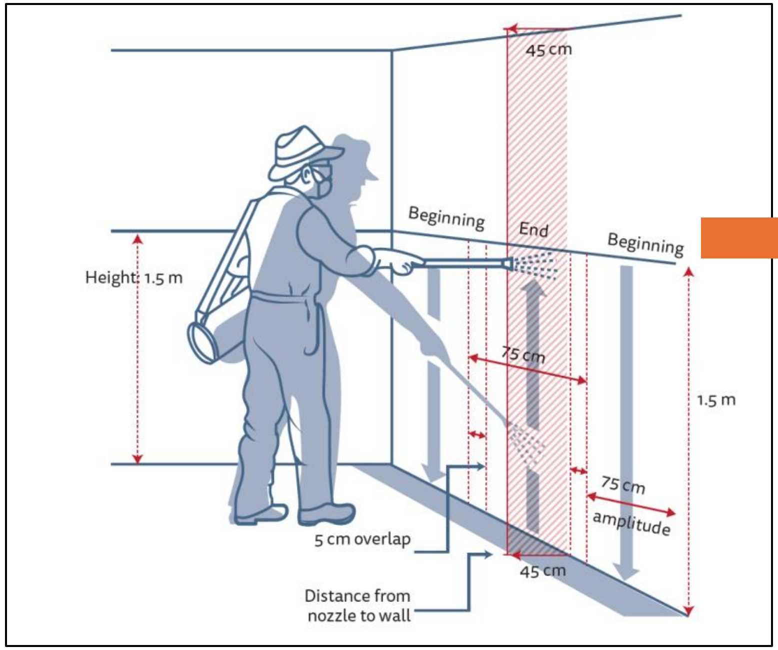

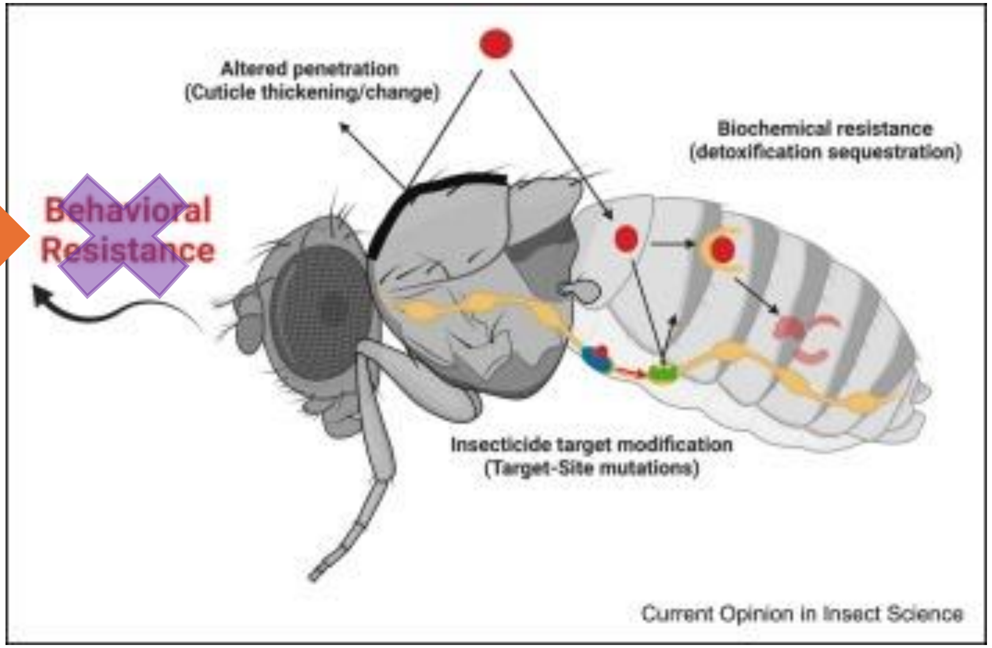

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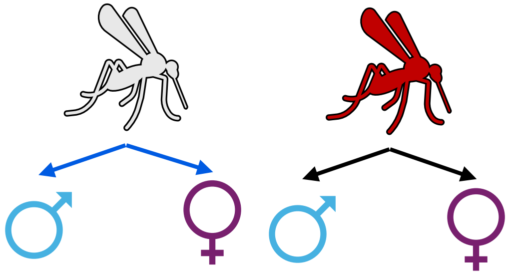
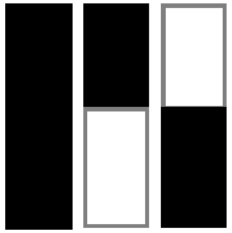


Sticky sheet treatments



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