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Entomological impact of targeted indoor residual

spraying on Aedes aegypti density and age structure

By

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Master of Science in Public Health

Environmental Health and Epidemiology

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Committee Chair

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2017

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Abstract

Entomological impact of targeted indoor residual
spraying on Aedes aegypti density and age structure

By Carlos Culquichicon

Background: Targeted indoor residual spraying (TIRS) is an adulticide method of insecticide spraying designed to reduce the time and volume of application, and reaching high efficacy levels when preventatively applied. TIRS have the potential of decreasing *Aedes*-borne virus transmission by decreasing the density of *Aedes aegypti* mosquitoes, and shifting the age structure of the treated mosquito population to earlier stages of development. We aimed to study the efficacy of TIRS on female *Aedes* density, and the physiological age of the mosquito population; and secondarily the proportion of nulliparous, and *Aedes-borne virus* transmission potential.

Methods: After 1, 3, and 6 months of TIRS application, we conducted dissections of a random subset of female *Aedes* routinely collected in entomological surveys of households from a clustered-randomized trial. The Polovodova dissection method was used to identify the number of ovariole dilatations in female *Ae. aegypti*, and classified the egg development using Christopher's growth (F1-F2) and germinative (F3-F5) stages. Female *Ae. aegypti* older than 1 dilatation and germinative stages is a chronological proxy of females with completed extrinsic incubation period (i.e., they have the potential to transmit viruses). Random-effect regression models at the cluster- and household-levels estimated incidence rate ratios for the primary outcomes, and odds ratios for the secondary outcomes in order to determine TIRS efficacy levels.

Results: A total of 324, 102, and 156 dissections (N=582) were conducted at 1, 3 and 6 months post-TIRS, respectively. During post-1, and post-3 evaluations, TIRS efficacy levels on reducing mean female *Ae. aegypti* density per cluster were 60%, and 41%, respectively; but 27% in post-6. Consistently, TIRS efficacy on decreasing the number of ovariole dilatations were 43%, and 46% for post-1 and post-3, but 20% during post-6. Regarding the secondary outcomes, TIRS efficacy on diminishing the proportion of nulliparous *Ae. aegypti* were 37%, and 35% in post-1 and post-3, but 12% in post-6. Distinctly, TIRS efficacy on reducing the proportion of female *Ae. aegypti* with transmissibility potential were 36%, 37%, and 29% for each post evaluation.

Conclusions: TIRS consistently reduced the density of female *Ae. aegypti* and shifted the age structure of the mosquito population towards younger maturation stages, leading to an overall reduction in the presence of female *Ae. aegypti* with the potential to transmit *Aedes*-borne viruses.

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1 Introduction

Historic arboviral diseases such as dengue, and emerging arboviral diseases such as chikungunya and Zika have an increasing global burden due to their impact on endemic regions and newly affected areas (1, 2). *Aedes*-borne viruses are primarily transmitted by *Aedes aegypti* and secondarily *Aedes albopictus*; these dominant vectors impose operational challenges owe to their biology, wide urban infestation, and insecticide resistance in endemic areas (3, 4). Despite efforts of vector-control programs over decades, dengue has increased due to their incidence rates, mortality rates, and years of full health lost due to disease between the 90's and 2017 (1). During the last decade, chikungunya and zika have co-circulated in historically affected areas by dengue (5). In this context, current major approaches to control aedes-borne diseases are conducted not only by using novel biological strategies but also relying on insecticide application methods for larval- and adult-control (6, 7). Thus, an integrated intervention framework which prevents long-term insecticide resistance, optimizes insecticide application, targets adult vectors, and accounts for human movement seems to be the most reliable approach to decrease arboviral burden of disease in local communities (8, 9).

Traditionally, vector-control interventions such as larval elimination, reduction of suitable water-habitats within households, and insecticide spraying have been widely used to control *Ae. aegypti* (10). Generally, these interventions are reactively adopted by local health authorities to control unforeseen peaks of arboviral transmission, or not well articulated in vector-control programs in areas with constant risk of arboviral disease (11). Unfortunately, there is lack of evidence about effectiveness levels of implemented interventions from public health agencies, and scarcity of randomized community trials which evaluate innovative interventions for *Aedes* control (8).

A control method with demonstrated preventative efficacy in other vector-borne diseases, such as malaria, involves spraying long-lasting residual insecticides on mosquito resting surfaces within households, termed indoor residual spraying (IRS) (12, 13). The residual capacity of these insecticides lasts several months, leading to meaningful treatment effects (11, 14, 15). However, the IRS implementation within households of entire cities is challenged by depending on large human capacity, and each household sprayed requires time-demanding efforts (16). In this context, innovative adult control strategies have been designed and validated in both observational and pilot randomized studies, ultimately showing promising results to reduce infection rates and for being implemented in large-scale settings (14, 15, 17, 18). Targeted indoor residual spraying (TIRS) aims to keep the same efficacy levels of IRS but reducing 18% the duration of application and 30% the volume of insecticides used per household, if acceptable coverage levels are achieved (19). TIRS application is focused in mosquito resting surfaces below 1.5 m of height including walls, furniture, wardrobes, and excluding ceilings, kitchen, and door nets (16, 19).

Since using this novel approach, we hypothesize that TIRS efficacy on decreasing *Aedes*-borne virus transmission rates may be accomplished by its double entomological impact: first, reducing *Ae. aegypti* density and, second, shifting the mosquito population to a younger age structure among treated households. This latter hypothesis has been informed by studies highlighting the great impact on reducing transmission capacity of

Aedes if an intervention aims to reduce the age structure of a treated mosquito population (20). The overall impact of determining TIRS efficacy levels on entomological outcomes is to understand the benefits and limits of an adulticide strategy with the potential of scalability and long-term adoption. Here, we present results from a field study conducted in the city of Merida, Yucatan State, Mexico, addressing the efficacy of TIRS on the abundance of female *Ae. aegypti* per cluster, and the age structure of mosquito populations among treated and untreated clusters.

2 Methods

2.1 Study site

Merida is the capital of Yucatan State, and it is also one of the largest cities in southeast Mexico with approximately 892,000 inhabitants (21). Merida has tropical weather with an average annual temperature of 25.9 °C, lowest mean in December with 29 °C and highest mean in July with 34°C; and an annual cumulative precipitation of 1000 mm (22). Due to these environmental favorable conditions, Merida is an endemic area of arboviral circulation, where dengue has been persistently transmitted since 1979. Chikungunya and Zika have been co-circulating since 2015 (23, 24). The arboviral transmission season begins in July and usually peaks in October–November, although cases occur irrespectively all the year (25).

Merida has three major areas of urbanization, the northern area, which is inhabited by people with greater socioeconomic conditions; the ancient downtown, which is in the center of Merida and inhabited by families with fair socioeconomic conditions; and the southern area, which is inhabited by people with low-income conditions. The TIRS trial focused the intervention in households from clusters in the downtown and southern area. Typically, these areas are divided in suburbs called "colonias"; specially in the southern area, the suburbs are densely populated and interconnected only by a main road without pavemented streets within them. In the southern area, the housing architecture are similar among their units, called "fraccionamientos", which typically are 100 m^2 flat households with one or two bedrooms, one bathroom, and a patio which is also used to grow vegetables (21). In the downtown area, the household architecture varies some more but keeps patios for gardener, and expand the number of rooms but not often the levels of the households.

Serology and Aedes-borne diseases transmission

Serological information about arboviral infection rates were collected from a cohort of school-aged children, their caregivers, and relatives living within the same household between 2015 to 2016 in Merida (24, 26, 27). During this period, dengue overall seroprevalence was 70%, which increased by age groups from 31% among 0 to 8 years-old children to 79% in adults above 20 years. The incidence of confirmed cases by laboratory testing, and symptomatic arboviral illness was 14.6, and 3.5 per 1,000 person-years, respectively (24). Besides, most seroconversions occurred in children below 14 years old (24, 26, 27). The incidence rates of symptomatic chikungunya, and zika illness were 8.6, and 2.3 per 1,000 person-years, respectively (24), and zika symptomatic attack rate among pregnant women was 31% (28). The detection of co-circulating *Aedes*-borne diseases were more likely among hotspots with high-constant density of mosquitoes. For instance, in 2008-2016 there were 40,000 dengue illness, 2270 zika illness, and 1100 chikungunya illness cases from constant hotspots in Merida (23). Overall, dengue seroprevalence rates were higher by twofold in hot-spot areas compared to low-density areas (23).

Mosquito population

In Merida, public health authorities have been conducting vector control with the main objective to target adult *Ae. aegypti*, initially reactive but progressively proactively working on prevention. Typically, ultra-low volume (ULV) spraying with organophosphate insecticides such as chlorpyrifos and malathion is implemented after the detection of a case or increases in the mosquito population. Indoor space spraying is used with pyrethroids as deltamethrin, and organophosphates as malathion in response to reported cases of *Aedes*-borne virus. Merida's *Ae. aegypti* populations have been recently characterized being resistant to type I and II pyrethroids, including deltamethrin, and susceptible to carbamates, including bendiocarb (29, 30).

2.2 *Study design*

We conducted longitudinal entomological surveys in compliant households of randomized clusters at 1, 3, and 6 months after the first TIRS application. Overall, the TIRS trial is a two-arm, parallel, unblinded, cluster randomized controlled trial being conducted in the city of Merida, Yucatan State, Mexico (31). The TIRS trial evaluates the efficacy of the TIRS application within households on the number of *Aedes*-borne symptomatic cases among 2-15 years old children, and involves over 4600 children. The TIRS trial allocates 1:1 TIRS application among the treated clusters vs. untreated clusters. The untreated clusters are not intervened by the TIRS trial, however, the Yucatan Ministry of Health eventually conducts indoor spatial spraying (ISS) when resources are available (31). Our assessment was conducted during the rainy season of Merida which has the highest likelihood of *Ae. aegypti* abundance and disease transmission reported historically over the past decade.

Cluster and household sampling

Overall, the TIRS trial assesses 50 clusters each of them composed by 5 × 5 city blocks under a "fried egg" design. Each 5x5 cluster receives the intervention, but the epidemiological and entomological evaluations will be sampled from households living in the inner 3x3 city block center of each cluster (**Figure 1**). The intervention clusters were randomly allocated (n = 25 per arm) and balanced using census tract-level variables such as population size and density, in addition to the cumulative number of arboviral cases between 2008 to 2016.

Sample size calculation

In order to detect a difference of 25% or greater on the proportion of nulliparous female *Aedes aegypti* between the intervention and control sampled clusters, we collected 6 mosquitoes per cluster in each 25 clusters per arm (total=300), assuming 80% of power and 5% of significance level **(Table 1)**. This sample let us detect Odds Ratio of 0.21 or lower, meaning efficacy levels of 79% or greater for binary outcomes. Besides, let us detect Incidence Rate Ratios of 0.29 or lower, meaning efficacy levels of 71% or greater for count outcomes.

2.3 *Data collection*

The TIRS application used pirimiphos-methyl (Actellic ® 300 CS) produced by Syngenta global, its susceptibility have been previously addressed for *Aedes* population in Merida (32). During May-June 2021, TIRS was applied in approximately 17,000 households from the treatment clusters, while untreated households in the control clusters were under passive surveillance. After the first intervention, monthly collections of indoor adult *Ae. aegypti* have been conducted in 750 households per treatment group (1500 total). The aspirations were conducted by a team of entomologists and biologists using Prokopack® aspirators (33). Each mechanical aspiration lasted 10 min per house, they sampled indoors resting sites such as shaded corners, undisturbed dark places behind furniture, wardrobes, and halls (34). After the mosquitoes were collected from the field, they were transported to the entomology laboratory at UADY-UCBE to identify them by specie and separate female *Aedes aegypti* for further dissection within the first 8 hours of collection in order to prevent follicular development of ovarioles and obtain an unprecise measure.

2.4 *Dissection technique*

Female *Ae.* aegypti were individually dissected to identify the number of dilatations per ovariole and the follicular stage of their ovarioles (35). Ovariole dilatations are structures generated from collapsed egg sacs after a regular ovulation process, in addition a small proportion comes from the condensation of degenerated follicles (36).

Ovarioles are the secondary functional units of an ovary. They are aimed to conduct the gonotrophic cycle starting from germination to oviposition. Each ovariole consists on a primary follicle (PF) and a germarium (G). Ovarioles are attached to the basal body (BB) of oviducts through pedicels (P), where the dilatations (D) will hold after a gonotrophic cycle is completed (36, 37). **Figure 2** shows each of the above-mentioned structures in ovarioles with zero, one, two, and three dilatations (D), from left to right respectively.

The follicular stage of ovarioles reflects the maturation grade of an egg follicle within an individual ovariole. Christophers has described 5 stages of follicular development in female *Aedes aegypti* (35), ranging from growth stages (F1-F2, **Figure 3A-3B**) to germinative stages (F3-F5, **Figure 3C-3E**). The F1 stage is the initial stage of follicular eggs after hatching and they grow until the F2 stage if a female is mated but not fed. Once bloodmeal is completed, the germinated eggs begin the initiation phase (F3) and can complete the rest of trophic phases (F4) until reaching a mature shape (F5) and being positioned (**Figure 3F**). Additionally, after oviposition the saccular stage could be identified **(Figure 3G)** where the ovariole stalk remains slightly swollen until the ovariole dilatations are defined (**Figure 3H**).

Typically, a female *Aedes aegypti* takes 2-3 days to complete a bloodmeal (36). Under ideal temperature conditions of 28.9 °C, the egg development from stage F1 to F5 could take from 1.67 to 5 days after bloodmeal completion (36, 38). Additionally, the egg hatching could take 3 days on average under controlled conditions (35).

2.5 Directed acyclic graph (DAG)

This directed acyclic graph (DAG) represents the causal relationship between treatment strategy (TIRS vs. untreated) and the entomological endpoints (*Ae. aegypti* density and ovariole dilatations), after covariate-constrained randomization of clusters for treatment allocation (**Figure 4**). The *Weather* construct comprises the following variables: 30-day moving average temperature (°C), 7-day cumulative rain (mm). The *Household characteristics* studied were: presence of backyard, windows/door with mosquito nets, and total residents per household. In observational settings, it is expected that household characteristics and weather may confound the effect of TIRS application on the entomological endpoints, by reducing its efficacy. However, achieving balanced clusters for treatment allocation might remove those relationships, allowing us to unconditionally estimate causal treatment effects of TIRS application on entomological endpoints.

2.6 Measures for analysis

The treatment strategies are defined by the *treatment group* which includes the TIRS application group and the untreated group for households within randomized clusters. Among the entomological variables, we studied *parity*, representing the laid eggs status of dissected female *Ae. aegypti; gravity*, physiological status of gonotrophic cycle informed by the blood meal intake of female *Ae. aegypti*; the *follicular stage*, representing the ovariole development stage of dissected female *Ae. aegypti*; and the total number of female *Ae. aegypti* collected in each cluster. *Aedes aegypti with transmissibility potential* was a variable build upon the number of dilatations and follicular stages. Competent Ae. aegypti females need 8-12 days of incubation to become capable to transmit an arbovirus to a susceptible host. The ordered classification level of 1 dilatation and F2 stage serves as a proxy for this timeframe, leading to the classification of dissected Ae. aegypti females as potential arbovirus transmitters or not.

Among the household-level variables, we studied the *floor*, and *roof materials* of households; the presence of *windows or doors with mosquito nets* within households; the presence of household structures as *backyard* and *front yard*; the number of *total residents* living within households, including participants (adults and children) not enrolled in the TIRS trial. Among the weather variables, we studied the *30-day moving average temperature in Celsius degrees (°C)* and the *7-day cumulative precipitation rate (inches)* in order to account for less variance on daily change.

2.7 Statistical analysis

Our primary outcomes were: 1) *Ae. aegypti* density per household, and 2) the number of dilatations detected in female *Ae. aegypti* ovarioles (**Tables 7-8**); both measured as count variables. We analyzed two secondary entomological endpoints: 1) parity, and 2) potential arbovirus transmitters; both measured as binary outcomes (**Tables 9-10**). We described entomological variables by treatment group and post evaluation using frequencies and percentages for categorical variables, and median and range for non-parametric continuous variables. Test of hypothesis with 5% of significance comparing group differences across intervened and control arm included chi-square test for categorical variables, and Kruskal-Wallis test with Dunnet test adjustment for non-parametric continuous variables, respectively (39). Unconditional models were conducted using random-effects Poisson regression to estimate incidence rate ratios (IRR) using the Poisson distribution and log link, and random-effects logistic regression models to estimate Odds

Ratios (OR) using the binomial distribution and logit link. These models considered households and clusters as levels to calculate random-effects levels, and regression estimates included 95% CI limits. Efficacy levels of TIRS application on count outcomes were calculated as: Efficacy= 1- IRR; and for binary outcomes were calculated as: Efficacy= 1-OR (40). Data analyses, and visualizations were conducted in Stata SE 17 (41).

2.8 Model specification

Let Y_{ijkt} (*dilatations*) denote the t^{th} response (*time*) of the k^{th} subject (*Ae. aegypti*) in i^{th} cluster and j^{th} household. Moreover, X_{ijkt} represent the effect of treatment group (treated vs. untreated), or independent covariates. Define α_i, and σ_{ij} as random effects for clusters (i), and households (*j*), respectively; and account for subject's (k) residuals ζ_{ijk}. Then, we can estimate an unconditional random-effect model for clustered longitudinal data:

Level 1 (Within effects):

$$Y_{ijkt} = \pi_{0ijk} + \pi_{1ijk} * TIME_{ijkt} + \alpha_i + \sigma_{ij} + \varepsilon_{ijkt}$$

Level 2 (Between effects):

$$\pi_{0ijk}: \gamma_{00} + \gamma_{01} * X_{ijk} + \zeta_{0ijk}$$

$$\pi_{1ijk}: \gamma_{10} + \gamma_{11} * X_{ijk} + \zeta_{1ijk}$$

This model specification let us estimate unconditional Incidence Rate Ratios for count outcomes, and Odds Ratios for binary outcomes. Efficacy levels were calculated by taking the complement of the exponentiated estimates as follows:

$$Efficacy_{count} = 1 - \frac{Incidence\ rate_{TIRS}}{Incidence\ rate_{Untreated}}; Efficacy_{binary} = 1 - \frac{Odds_{TIRS}}{Odds_{Untreated}}$$

2.9 Weather data

We studied weather variables such as temperature and precipitation from the city of Merida, Yucatan-Mexico, and measured in March-December, 2021. For the analysis, these variables were handled as 30-day moving average Temperature (°C) and the 7-day cumulative precipitation (mm). These data were retrieved from two weather stations, the first in the UADY's Collaborative Unit for Entomological Bioassays (UCBE), and the second in the international airport of the Merida city (22, 42). Both weather stations are located in the southern side of Merida.

2.10 Ethics

The main study protocol has been approved by the Emory Institutional Review Board (ID: 108666), all collaborative institutions in Mexico, and the US National Institutes of Health. The TRIS trial protocol has been registered on clinicalTrials.gov (NCT04343521). This thesis work was conducted with mosquitoes routinely collected in entomological surveys of the TIRS trial. Also, this thesis protocol was exempted of IRB evaluation, because no extended activities to the main study protocol were conducted.

3 Results

Overall, three entomological evaluations were conducted in 50 clusters, and 357 households (**Table 3**). There was a reduction on positive clusters among treatment groups over follow-ups, 100% vs. 96% in Post 1-3, and 92% vs. 80% in Post 6 (**Table 3**). The expected density of female *Ae. aegypti* per cluster were 26.8 (95% CI: 21.1, 32.7) (untreated) vs. 11.6 (95% CI: 7.8, 15.5) (TIRS) (p<0.001), 14.4 (95% CI: 8.6, 20.2) vs. 9.6 (95% CI: 5.7, 13.5) (p=0.99), and 7.1 (95% CI: 4.0, 10.3) vs. 6.1 (95% CI: 3.2, 9.0) mosquitoes (p=0.99) in Post 1-3-6, respectively (**Figure 3**).

From 310 households with available information, the socioeconomic characteristics of households between study groups were balanced among treatment groups (**Table 4**). Most of the households have backyard (91%) and front yard (88%), 63% of them have mosquito nets and 28% have water tanks. Materials of household structures were concrete for the roof (96%), and floor (53%) (**Table 4**).

From a total of 582 female *Ae. aegypti* dissections, 324 were conducted in Post 1, 102 in Post 3, and 156 in Post 6. The estimated proportion of nulliparous *Ae. aegypti* was significantly different between treatment groups in Post 1 (untreated: 35% vs. TIRS: 59%, p=0.02), higher in Post 3 among intervention group (21% vs. 48%, p=0.11), but not a meaningful difference in Post 6 (78% vs. 81%, p=0.99) (**Figure 4** and **Table 5**). *Ae. aegypti* in late gravid stages (gravid) were lower among TIRS group 54% vs. 46%, 60% vs. 40%, and 78% vs. 22% for Post 1-3-6, respectively. *Ae. aegypti* in egg stages (F5) were lower among TIRS group: 64% vs. 36%, 61% vs. 39%, and 85% vs. 15% for Post 1-3-6, respectively (**Table 5**).

The median number of ovariole dilatations was lower among TIRS group during Post 1: 1 (0-2) vs. 0 (0-1) ($p<0.001$), and Post 3: 1 (0-5) vs. 1 (0-4) ($p=0.002$); but similar in Post 6: 0 (0-2) vs. 0 (0-4) ($p=0.37$) (**Table 5**). The population of female *Aedes* among TIRS group consisted of mosquitoes with less dilatations (#d) and earlier stages of follicular development (F1-F5) in Post 1-3, but virtually no difference in Post 6 (**Figure 5**). Transmissible *Aedes* might be more mature than 1 dilatation and germinative stages (F3-F5), for example, 2d+F1-5, 3d+F1-5, etc. The proportion of transmissible *Aedes* were consistently higher among the untreated group compared to TIRS group (Post 1: 36% vs. 24%, Post 3: 64% vs. 40%, and Post 6: 19% vs. 14%) (**Table 6** and **Figure 6**).

The estimated efficacy of TIRS were calculated for primary and secondary entomological outcomes at one, three, and six months after application (**Figure 7**). Regarding the primary outcomes, TIRS reduced the female *Aedes* density per cluster by 60% (cIRR=0.40, 95% CI: 0.26, 0.61), 41% (cIRR = 0.59, 95% CI: 0.35, 0.97), and 27% (cIRR = 0.73, 95% CI: 0.43, 1.24) in each assessment, respectively (**Table 7**). Besides, the TIRS application decreased the number of dilatations by 43% (cIRR = 0.57, 95% CI: 0.43, 0.77), 46% (cIRR = 0.54, 95% CI: 0.31, 0.94), and 20% (cIRR = 0.80, 95% CI: 0.38, 1.69) (**Table 8**).

Regarding the secondary outcomes, TIRS efficacy on reducing the proportion of nulliparous was 37% (cOR= 0.63, 95% CI: 0.47, 0.84), 35% (cOR= 0.65, 95% CI: 0.46, 0.93), and 12% (cOR= 0.88, 95% CI: 0.46, 1.72) (**Table 9**). Besides, TIRS demonstrated a measurable efficacy level on reducing the proportion of female *Aedes* with transmissibility potential were 36% (cOR= 0.64, 95% CI: 0.40, 1.01), 37% (cOR= 0.63, 95% CI: 0.40, 1.01), and 29% (cOR= 0.71, 95% CI: 0.31, 1.65) over entomological assessments (**Table 10**). Estimates for other covariates by each random-effects model are reported in **Tables 7-10**.

4 Discussion

We studied the efficacy of TIRS application to reduce female *Aedes* abundance, and age structure of mosquito population measured by the number of ovariole dilatations among a population of female *Ae. aegypti* susceptible to pirimiphos-methyl, in Merida-Mexico. TIRS was efficacious in reducing total density and multiparous *Ae. aegypti* among the intervention group for the first 3 months after the application, but null effect upon six months. Moreover, the TIRS application demonstrated to be efficacious at decreasing the number of dilatations especially the first 3 months after the insecticide application, but its effect was null upon 6 months of application. Finally, by considering that female *Ae. aegypti* older than an age grading of one dilatation and germinative follicular stages (F3-F5) are the ones with greater vectorial capacity, i.e. competent *Aedes*, then TIRS showed measurable effects on decreasing competent *Aedes* among the intervention group, especially during the first three months of evaluation.

TIRS demonstrated treatment efficacy to reduce female *Aedes aegypti* abundance during the first three months after application, but null effect at the sixth month survey. Previous studies addressing indoor residual spraying (IRS) efficacy using bendiocarb, a carbamate insecticide, demonstrated 60% reduction in total mosquito population density during 3 months of application (43). and demonstrated an increased occurrence of secondary dengue-infections among untreated households (44). However, the IRS is time-demanding, on average it takes 30-40 minutes per house. Besides, it requires sustained community engagement, which limit its wide-spread adoption in vector-control programs. In contrast, the targeted indoor residual spraying (TIRS), selectively applies residual insecticides below 1.5m in walls, under furniture, and dark areas (19). TIRS provides similar efficacy to IRS, but improving duration of application and optimizes insecticide usage per household (19). Finally, our results validate the expected similarity on efficacy levels between TIRS and IRS on reducing the density of susceptible *Ae. aegypti* population, as previously reported in an observational study in Australia (17).

The TIRS treatment effect was consistent on increasing the proportion of nulliparous *Aedes aegypti* in the intervention group during Post 1-3 but not in Post 6. The collected nulliparous female *Ae. aegypti* haven't completed one oviposition already, regardless of their follicular stage. *Aedes aegypti* host-seeking behavior and blood-meal occur during the first 2-3 days after emergence, simultaneously with mating (45), and by 3 days intervals after each oviposition (36). During this timeframe, females are prone to rest on sprayed surfaces, decreasing their survival rates of aging and shifting the age structure of the mosquito population to a larger proportion of nulliparous. Our findings are consistent with TIRS efficacy simulations, the TIRS treatment effect might reduce its effectiveness starting from 6 months of application onwards likely due to a reduction on the abundance of mosquito population, even having the same TIRS efficacy and coverage levels than in previous entomological assessments (14). Moreover, not even an outdoor treatment may explain the increase on nulliparous during Post 6 because it might be detected in a non-null difference between TIRS and untreated groups.

A reduction of the number of ovariole dilatations after TIRS application was consistent during the first 3 months, but not in the 6-month evaluation. The ability to identify the number of ovariole dilatations, give us information about the biological age of mosquitoes, and provide us a proxy for their chronological age (37, 46). After 8-12 days of virus incubation into female *Ae. aegypti*, the vector becomes capable of transmitting the virus strain and ultimately infect susceptible humans (36). By approximate determination of the chronological age of mosquitoes, we could infer about the age-structure and vectorial capacity of the mosquito population and evaluate vector control interventions (20). In the context of TIRS, it is expected to reduce not only the abundance of mosquitoes but also to reduce the age structure of the remaining low-density mosquito population. Ultimately, this means that the remaining young female *Ae. aegypti* are unable to incubate the virus within them, which leads to have a reduced risk of transmitting arboviral diseases (20). This expectation holds during the first three months of entomological assessments, but not in the sixth month.

It is important to still recognize the utility of age-grading using the Polovodova method and its potential for evaluating vector-control interventions in low-income settings. In the 1960s, entomologists developed this method aiming to determine the age-structure of important *Anopheles* species populations (47, 48), and extended to Culicidae species (49). Despite its great value on providing information of age-structure and vectorial capacity, this method is time-consuming, and needs trained entomologists or technicians (50). Overall, this method should not be used in outbreaks given the large amount of time needed per dissection and an overloaded response capacity. However, it might be used at strategic cut-off points to evaluate the impact of vector-control interventions on reducing the population of older females capable to transmit diseases. In this way, we can identify mosquito survival rates, and longevity to understand dengue transmission dynamics due to specific vector-control interventions such as TIRS.

The proportion of transmissible mosquitoes was higher among the untreated group during the first three months but virtually not different in the sixth month after TIRS application. The vectorial capacity describes the expected number of infected humans resulting from one single initial case, and takes into account host, virus, and vector parameters (51). An increase in vectorial capacity drives implies greater transmission chains of arboviral disease among a human population (36, 51). This might be observed in endemic areas, and when outbreaks occur. Some crucial and measurable parameters include biting rates, the number of mosquitoes per host, and the survival rates of mosquitoes (51). Several vector control interventions have been developed to target some of these parameters, for instance, repellents and insecticide treated nets reduce biting rates, and have demonstrated a moderate reduction on transmission of vector-borne diseases (52). Other adulticides interventions aiming to reduce mosquito survival rates, such as insecticide spraying, have demonstrated greater efficacy levels to reduce arboviral disease transmission (52-54). In our context, the TIRS application targets to decrease the survival rates of mosquitoes, meaning that the longevity of the mosquito population in the treatment group is lower than the control group. The lower mosquito's number of dilatations and earlier follicular stages among the treatment group reflects that the age structure is younger, as well as there is a lower proportion of mosquitoes capable of transmitting disease.

Our study should be interpreted under the light of the following considerations. First, precision of mixed effects model estimates relies on the number of primary sample units, i.e. clusters. There was a reduction in sampled clusters which may impact in the precision of our efficacy estimates, especially in the six-month entomological assessment among the treatment group. However, we believe this was due to a decrease on the positive cluster availability due to a reduction in the overall household density of female *Ae. aegypti*. It could naturally occur that the TIRS treatment effect on clusters with low-density might reduce to virtually zero mosquitoes available indoors. Then, this event is a genuine representation of this complex phenomenon, and does not imply any bias. Second, in order to comprehensively understand arbovirus transmission dynamics, we might be interested to measure the entomological inoculation rate (EIR). This evaluates the risk of exposure to infectious mosquitoes, usually interpreted as the number of infective bites from a given vector, received by one individual. In a previous study (18), the EIR was estimated along with vectorial capacity. We could not conduct RT-PCR to quantify infected mosquitoes, then measure EIR. However, our results are consistent to show that VC is increased by the greater number of *Ae. aegypti* collected per cluster, which implies that our measure of VC can adequately inform the potential of disease transmission after TIRS application.

Third, there is still debate to recognize a reliable, reproducible and accessible age-grading method (20). Drawbacks of dissection methods, such as the Polovodova method, include its dependence to trained personnel, which interpretations sometimes could be subjective by untrained technicians; labor-intensive, were each dissection take on average 25-30 minutes, and did not allow for its use on large-scale studies. However, our dissection team was led by a senior entomologist (JCC), after carefully following dissection prompts (36, 37, 55, 56), and using *Ae. aegypti* growth under bloodmeal controlled conditions in chambers. Besides, dissection methods for age-grading are valuable because their easy implementation in low-income settings. However, modern age-grading methods such as near- and mid-infrared spectroscopy, biochemical methods, and genetic profiling might provide more precise estimations but they lack of validation with empirical data, and are inaccessible for vector control programs in low-income settings (20).

5 Conclusions and recommendations

Pirimiphos-methyl TIRS has demonstrated its efficacy on a consistent reduction of several entomological measures relevant to vector-control interventions after three months of application. These entomological measures include the total mosquito population, the proportion of nulliparous *Aedes*, the number of ovariole dilatations from female *Aedes*, and ultimately the proportion of mosquitoes with transmissibility potential. Overall, TIRS can reduce the total density of a mosquito population, shift the age-structure of a mosquito population to one with a greater proportion of nulliparous, which ultimately reduce the potential of arboviral disease transmission. Age-grading provide useful insight to understand the potential transmission from a mosquito population after implementing a vector-control intervention. Dissection methods such as Polovodova, could be implemented by a well-trained personnel and standardized practices in resource-limited settings, but using modern such as spectroscopy, biochemical or genetic methods could improve accuracy when resources are available.

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7 Tables

Table 1. Sample size calculation assuming $\alpha = 0.05$, $\beta = 0.8$, and rho $= 0.5$

Difference in proportions	Aedes per Treatment cluster (m1)	Aedes per Control cluster (m2)	Treatment clusters (k1)	Control clusters (k2)	N	Odds Ratio (m1/m2)	Incidence Rate Ratio (m1/m2)
25.97%	4	4			200	0.198	0.278
25.34%	5	5			250	0.203	0.283
24.91%	6	6	25	25	300	0.207	0.286
24.37%	8	8			400	0.212	0.291
24.04%	10	10			500	0.215	0.294

Table 2. Measures for analysis

Measures	Definition	Type	Values
Treatment group	Clusters receiving TIRS application (intervention group) and untreated clusters (control group)	Categorical	0, Control 1, Intervention
Entomological			
Parity	Females having already positioned a batch of eggs.	Categorical	0, Nulliparous 1, Multiparous
Gravity	Physiological status of gonotrophic cycles characterized by blood intake	Categorical	0, Unfed 1, Blood fed 2, Half-gravid 3, Gravid
Follicular stage	Maturation degree of eggs within ovarioles, follicular stages could be classified as growth stages (F1-F2) and germinative stages (F3-F5)	Categorical	1, F1 2, F2 3, F3 4, F4 5, F5
Dilatations (*)	Compressed egg sacs after completed oviposition which are located in pedicels of ovarioles.	Numeric	[0, +inf.]
Female Aedes per cluster	Number of collected female *Aedes* by Prokopack aspirations per treatment cluster.	Numeric	[0,+inf.]
Aedes with transmissibility potential	Aedes with ovarioles being orderly classified as 1 dilatation and F3 follicular stage or later stages, e.g. 1 dilatation and F5 stage, 2 dilatations and F2 stage, 2 dilatations and F4 stage, and so on.	Categorical	0, No (<1d+F2) 1, Yes (>=1d+F3)
Household-level			
Floor material	Household characteristics measured at baseline evaluations of the TIRS trial	Categorical	0, Concrete 1, Ground
Roof material		Categorical	0, Concrete 1, Metal
Windows/doors with mosquito nets		Numeric	[0,+inf.]
Backyard		Categorical	0, No 1, Yes
Front yard		Categorical	0, No 1, Yes

Water tank		Categorical	0, No 1, Yes
Computer		Categorical	0, No 1, Yes
Internet connection		Categorical	0, No 1, Yes
Total residents per household		Numeric	[0,+inf.]
Weather			
30-day moving average Temperature (°C)	Average temperature of previous 30 days for a given date.	Numeric	[15,45]
7-day cumulative Precipitation rate (inches)	Sum of precipitation rates of previous 7 days for a given date.	Numeric	[0,+inf.]

Table 3. Cluster characteristics and entomological characteristics by households

Characteristics	Total					Post 1					Post 3					Post 6				
	Control		Intervention		*P* value	Control		Intervention		*P* value	Control		Intervention		*P* value	Control		Intervention		*P* value
	N	%	N	%		N	%	N	%		N	%	N	%		N	%	N	%	
Number of clusters	25	50.0	25	50.0		25	50.0	25	50.0		16	51.6	15	48.4		22	53.7	19	46.3	
Number of households	212	59.4	145	40.6		111	55.5	89	44.5		42	54.5	35	45.5		82	67.8	39	32.2	
Positive cluster						25	100.0	24	96.0	0.31	25	100.0	24	96.0	0.31	23	92.0	20	80.0	0.22
Female Aedes per cluster	5691	(18.48±15.16)	2314	(10.10±10.27)	<0.001	4189	(26.68±14.93)	1571	(12.57±12.85)	<0.001	778	(14.41±11.67)	344	(7.32±3.13)	<0.001	724	(7.46±7.44)	399	(7.00±5.08)	<0.001
Fed female Aedes *	1157	(0.51±1.07)	641	(0.28±1.07)	<0.001	683	(0.91±1.47)	339	(0.45±1.47)	<0.001	337	(0.45±0.81)	215	(0.29±0.88)	<0.001	137	(0.18±0.58)	87	(0.12±0.65)	<0.001
Unfed female Aedes *	273	(0.12±0.54)	101	(0.04±0.25)	<0.001	158	(0.21±0.80)	47	(0.06±0.31)	<0.001	88	(0.12±0.43)	36	(0.05±0.25)	<0.001	27	(0.04±0.19)	18	(0.02±0.18)	0.08
Male Aedes*	1889	(0.84±2.06)	889	(0.40±1.42)	<0.001	1199	(1.60±3.12)	536	(0.71±2.05)	<0.001	540	(0.72±1.30)	236	(0.31±1.00)	<0.001	150	(0.2±0.55)	117	(0.16±0.83)	<0.001

* Per household. N, mean and standard deviation reported. Kruskall wallis test of hypothesis.

Table 4. Socioeconomic and infrastructure characteristics of households assessed overall entomological surveys in Merida, Mexico, 2021.

	Total		Control		Intervention		*P* value*
	N=310	%	N	%	N	%	
Backyard							0.41
Yes	281	90.7	162	57.7	119	42.4	
No	29	9.4	19	65.5	10	34.5	
Front yard							0.78
Yes	272	87.7	23	60.5	15	39.5	
No	38	12.3	158	58.1	114	41.9	
Windows/doors with mosquito nets							0.18
0 nets	114	36.8	61	53.5	53	46.5	
1+ nets	196	63.2	120	61.2	76	38.8	
Floor - concrete							0.70
Yes	165	53.2	98	59.4	67	40.6	
No	145	46.8	83	57.2	62	42.8	
Roof - concrete							0.23
Yes	296	95.5	175	59.1	121	40.9	
No	14	4.5	6	42.9	8	57.1	
Roof - metal							0.17
Yes	30	9.7	14	46.7	16	53.3	
No	280	90.3	167	59.6	113	40.4	
Water tank							0.87
Yes	88	28.4	52	59.1	36	40.9	
No	222	71.6	129	58.1	93	41.9	
Computer							0.37
Yes	175	56.5	106	60.6	69	39.4	
No	135	43.6	75	55.6	60	44.4	
Internet connection							0.02
Yes	258	83.2	158	61.2	100	38.8	
No	52	16.8	23	44.2	29	55.8	

* Chi-2 test

Table 5. Descriptive and bivariate statistics of dissected female *Aedes aegypti*

Characteristics	Total					Post 1					Post 3					Post 6				
	Control		Intervention		P value **	Control		Intervention		P value **	Control		Intervention		P value **	Control		Intervention		P value **
	N=334	%	N=248	%		N=168	%	N=156	%		N=54	%	N=48	%		N=112	%	N=44	%	
Parity					0.002					<0.001					0.006					0.65
Nuliparous	161	51.6	151	48.4		61	39.9	92	60.1		12	34.3	23	65.7		88	71.0	36	29.0	
Multiparous	173	64.1	97	35.9		107	62.6	64	37.4		42	62.7	25	37.3		24	75.0	8	25.0	
Gravity					<0.001					<0.001					0.45					0.27
Gravid	126	64.3	70	35.7		40	54.1	34	46.0		32	60.4	21	39.6		54	78.3	15	21.7	
Half-gravid	74	51.4	70	48.6		47	49.0	49	51.0		11	44.0	14	56.0		16	69.6	7	30.4	
Blood fed	57	72.2	22	27.9		31	96.9	1	3.1		5	41.7	7	58.3		21	60.0	14	40.0	
Unfed	77	47.2	86	52.8		50	41.0	72	59.0		6	50.0	6	50.0		21	72.4	8	27.6	
Follicular development					0.001					0.12					0.39					0.08
F1	83	50.9	80	49.1		55	47.0	62	53.0		16	59.3	11	40.7		12	63.2	7	36.8	
F2	60	58.3	43	41.8		29	54.7	24	45.3		5	50.0	5	50.0		26	65.0	14	35.0	
F3	75	56.8	57	43.2		34	54.8	28	45.2		9	37.5	15	62.5		32	69.6	14	30.4	
F4	16	38.1	26	61.9		14	38.9	22	61.1		1	33.3	2	66.7		1	33.3	2	66.7	
F5	100	70.4	42	29.6		36	64.3	20	35.7		23	60.5	15	39.5		41	85.4	7	14.6	
Vectorial capacity*	0.16 (0-3.65)		0.12 (0-5.43)		0.06	0.10 (0-3.26)		0.06 (0-3.26)		0.07	0.44 (0-3.65)		0.22 (0-1.31)		0.07	0.17 (0-3.48)		0.14 (0-5.43)		0.46
Adjusted vectorial capacity*	0 (0-1.74)		0 (0-1.31)		0.04	0 (0-1.74)		0 (0-0.52)		0.05	0.16 (0-1.74)		0 (0-1.31)		0.03	0 (0-1.31)		0 (0-0.87)		0.26
Number of dilatations*	1 (0-5)		0 (0-4)		0.001	1 (0-2)		0 (0-1)		<0.001	1 (0-5)		1 (0-4)		0.002	0 (0-2)		0 (0-4)		0.37

* Median and range, Kruskall-Wallis test of hypothesis.

** Chi-2 test

Table 6. Female *Ae. aegypti* with potential for transmitting *Aedes*-borne viruses by treatment groups and entomological survey.

	Non-transmissible		Transmissible		P value*
	N=403	%	N=179	%	
Total					
Control	203	64.0	114	36.0	0.003
Intervention	200	75.5	65	24.5	
Post 1					
Control	104	63.4	60	36.6	0.01
Intervention	122	76.3	38	23.8	
Post 3					
Control	20	36.4	35	63.6	0.02
Intervention	28	59.6	19	40.4	
Post 6					
Control	79	80.6	19	19.4	0.37
Intervention	50	86.2	8	13.8	

* Chi-square test

Table 7. Crude regression models* for TIRS application and covariates on the number of female *Ae. aegypti*.

	cIRR	95% CI	*P* value
Study group - intervention			
Total	0.97	0.52 - 1.79	0.92
Post 1	0.40	0.26 - 0.61	<0.001
Post 3	0.59	0.35 - 0.97	0.04
Post 6	0.73	0.43 - 1.24	0.25
Gravity - >24 hours of blood meal			
Total	1.18	0.05 - 29.96	0.92
Post 1	1.00	0.99 - 1.01	0.76
Post 3	1.08	1.01 - 1.15	0.02
Post 6	0.99	0.95 - 1.03	0.62
Follicular development - F3-F5 germination/eggs stage			
Total	1.02	0.03 - 30.35	0.99
Post 1	1.01	1.00 - 1.02	0.05
Post 3	1.04	0.97 - 1.11	0.27
Post 6	1.00	0.96 - 1.05	0.85
Backyard			
Total	0.02	0.00 - 6.94	0.20
Post 1	1.00	0.98 - 1.01	0.89
Post 3	1.04	0.96 - 1.13	0.36
Post 6	0.99	0.94 - 1.04	0.70
Windows/doors with mosquito nets			
Total	0.32	0.01 - 10.91	0.53
Post 1	1.01	1.00 - 1.01	0.28
Post 3	0.98	0.93 - 1.03	0.44
Post 6	1.00	0.95 - 1.05	0.97
Total residents - 5+ persons			
Total	0.42	0.02 - 9.81	0.59
Post 1	1.00	0.99 - 1.01	0.70
Post 3	1.00	0.97 - 1.03	0.99
Post 6	0.99	0.96 - 1.02	0.56
30-days moving average Temperature °C **			
Total	3.86	1.26 - 11.79	0.02
Post 1	3.85	0.86 - 17.13	0.08
Post 3	1.58	0.76 - 3.28	0.22
Post 6	1.76	0.90 - 3.45	0.10
7-days cumulative rain rate (10 inches) **			
Total	0.19	0.04 - 0.82	0.03
Post 1	1.00	1.00 - 1.00	<0.001
Post 3	1.00	0.99 - 1.00	0.51
Post 6	0.40	0.23 - 0.69	0.001

* Multilevel Poisson regression with random effects to estimate Incidence Rate Ratios (IRR) nested in clusters and neighborhoods.
** Per 10 mosquitoes increase

Table 8. Crude regression models* for TIRS application and covariates on the number of ovariole dilatations of female *Ae. aegypti*.

	cIRR	95% CI	*P* value
Study group - intervention			
Total	0.81	0.40 - 1.67	0.58
Post 1	0.57	0.43 - 0.77	<0.001
Post 3	0.54	0.31 - 0.94	0.03
Post 6	0.80	0.38 - 1.69	0.57
Gravity - >24 hours of blood meal			
Total	3.84	1.53 - 9.62	0.004
Post 1	1.18	0.88 - 1.59	0.27
Post 3	1.09	0.72 - 1.64	0.69
Post 6	3.90	1.55 - 9.81	0.004
Follicular development - F3-F5 germination/eggs stage			
Total	1.37	0.52 - 3.59	0.52
Post 1	1.25	0.94 - 1.66	0.13
Post 3	1.35	0.73 - 2.50	0.34
Post 6	1.58	0.71 - 3.54	0.27
Backyard			
Total	0.45	0.18 - 1.11	0.08
Post 1	1.29	0.83 - 2.01	0.26
Post 3	0.58	0.29 - 1.18	0.13
Post 6	0.40	0.16 - 1.02	0.06
Windows/doors with mosquito nets			
Total	2.11	0.94 - 4.75	0.07
Post 1	1.31	0.98 - 1.75	0.07
Post 3	0.92	0.66 - 1.29	0.63
Post 6	2.26	0.98 - 5.20	0.06
Total residents - 5+ persons			
Total	0.87	0.41 - 1.85	0.72
Post 1	0.92	0.71 - 1.18	0.50
Post 3	0.84	0.60 - 1.16	0.29
Post 6	0.84	0.40 - 1.77	0.64
Female Aedes per cluster (100 units)			
Total	0.01	0.00 - 0.78	0.04
Post 1	2.27	1.01 - 5.09	0.05
Post 3	16.14	3.21 - 81.25	0.001
Post 6	0.02	0.00 - 1.92	0.09
30-days moving average Temperature °C			
Total	0.66	0.21 - 2.10	0.49
Post 1	5.27	1.40 - 19.79	0.01
Post 3	2.00	1.02 - 3.91	0.04
Post 6	0.66	0.21 - 2.06	0.47
7-days cumulative rain rate (10 inches)			
Total	2.44	0.89 - 6.73	0.08
Post 1	1.07	1.03 - 1.10	<0.001
Post 3	1.04	1.00 - 1.08	0.05
Post 6	2.28	0.86 - 6.06	0.10

* Multilevel Poisson regression with random effects to estimate Incidence Rate Ratios (IRR) nested in clusters and households. Deviance: 497.14, degrees of freedom: 495.

Table 9. Crude regression models* for TIRS application and covariates on the proportion of multiparous *Ae. aegypti*.

	cOR	95% CI	*P* value
Study group - intervention			
Total	0.70	0.56 - 0.89	0.003
Post 1	0.63	0.47 - 0.84	0.001
Post 3	0.65	0.46 - 0.93	0.02
Post 6	0.89	0.46 - 1.72	0.72
Gravity - >24 hours of blood meal			
Total	1.25	1.01 - 1.55	0.04
Post 1	1.17	0.88 - 1.56	0.28
Post 3	1.14	0.82 - 1.59	0.44
Post 6	3.78	1.59 - 9.02	0.003
Follicular development - F3-F5 germination/eggs stage			
Total	1.28	1.00 - 1.64	0.05
Post 1	1.29	0.96 - 1.71	0.09
Post 3	1.25	0.82 - 1.89	0.30
Post 6	2.17	0.97 - 4.85	0.06
Backyard			
Total	0.98	0.72 - 1.34	0.90
Post 1	1.21	0.78 - 1.89	0.40
Post 3	0.91	0.52 - 1.58	0.73
Post 6	0.45	0.19 - 1.07	0.07
Windows/doors with mosquito nets			
Total	1.16	0.93 - 1.44	0.18
Post 1	1.30	0.99 - 1.73	0.06
Post 3	0.78	0.58 - 1.06	0.11
Post 6	1.76	0.82 - 3.80	0.15
Total residents - 5+ persons			
Total	0.86	0.71 - 1.04	0.11
Post 1	0.84	0.66 - 1.05	0.13
Post 3	0.87	0.66 - 1.16	0.36
Post 6	1.00	0.54 - 1.86	1.00
Female Aedes per cluster (100 units)			
Total	3.79	2.02 - 7.10	<0.001
Post 1	2.25	1.05 - 4.84	0.04
Post 3	5.88	2.33 - 14.85	<0.001
Post 6	0.08	0.00 - 3.64	0.19
30-days moving average Temperature °C			
Total	1.34	1.23 - 1.46	<0.001
Post 1	6.27	2.14 - 18.41	0.001
Post 3	1.98	1.31 - 2.98	0.001
Post 6	0.76	0.30 - 1.91	0.558
7-days cumulative rain rate (10 inches)			
Total	1.06	1.05 - 1.07	<0.001
Post 1	1.06	1.02 - 1.09	0.001
Post 3	1.04	1.02 - 1.07	<0.001
Post 6	1.43	0.61 - 3.38	0.41

* Multilevel Logistic regression with random effects to estimate odds ratios (OR) nested in clusters and neighborhoods.

Table 10. Crude regression models* for TIRS application and covariates on the proportion of transmissible female *Ae. aegypti*.

	cOR	95% CI	*P* value
Study group - intervention			
Total	0.71	0.31 - 1.65	0.43
Post 1	0.64	0.40 - 1.01	0.057
Post 3	0.64	0.40 - 1.01	0.06
Post 6	0.71	0.31 - 1.65	0.43
Gravity - >24 hours of blood meal			
Total	5.55	1.99 - 15.50	<0.001
Post 1	1.30	0.81 - 2.07	0.28
Post 3	1.57	1.08 - 2.28	0.02
Post 6	5.55	1.98 - 15.54	0.001
Backyard			
Total	0.45	0.17 - 1.17	0.10
Post 1	0.83	0.46 - 1.50	0.53
Post 3	0.71	0.39 - 1.29	0.26
Post 6	0.45	0.17 - 1.17	0.10
Windows/doors with mosquito nets			
Total	1.34	0.61 - 2.94	0.46
Post 1	1.44	0.93 - 2.23	0.11
Post 3	0.88	0.58 - 1.34	0.55
Post 6	1.34	0.61 - 2.94	0.46
Total residents - 5+ persons			
Total	0.80	0.38 - 1.69	0.56
Post 1	0.83	0.58 - 1.20	0.33
Post 3	1.05	0.74 - 1.49	0.79
Post 6	0.80	0.38 - 1.68	0.56
Female Aedes per cluster (100 units)			
Total	0.11	0.00 - 8.40	0.31
Post 1	4.40	1.87 - 10.35	0.001
Post 3	14.46	4.04 - 51.78	<0.001
Post 6	0.11	0.00 - 8.48	0.32
30-days moving average Temperature °C **			
Total	1.02	0.36 - 2.89	0.96
Post 1	21.04	5.18 - 85.53	<0.001
Post 3	1.85	1.03 - 3.32	0.04
Post 6	1.02	0.36 - 2.89	0.96
7-days cumulative rain rate (10 inches) **			
Total	1.02	0.41 - 2.55	0.97
Post 1	1.00	1.00 - 1.00	0.14
Post 3	1.00	0.99 - 1.00	0.21
Post 6	1.02	0.41 - 2.55	0.97

* Multilevel Logistic regression with random effects to estimate Odds Ratios (OR) nested in clusters and neighborhoods.
** Per 10 mosquitoes increase

8 Figures

Figure 1. Cluster design and distribution of households from the TIRS trial.

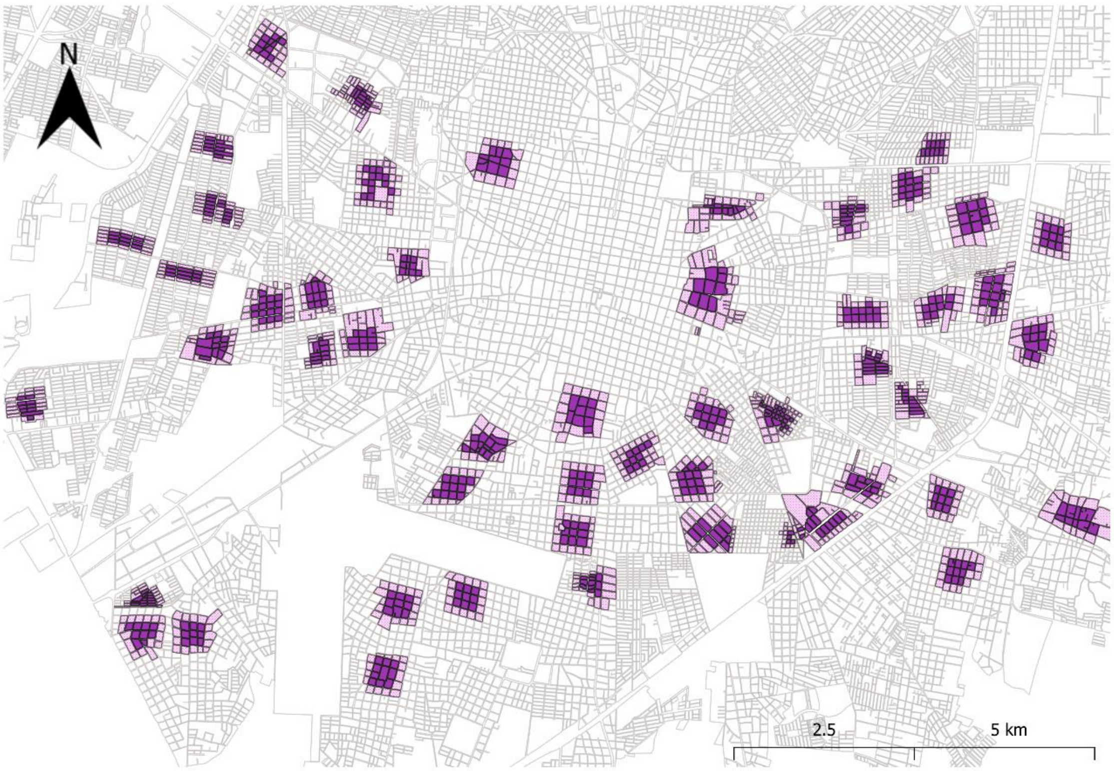


Figure 2. Identification of dilatations in pedicels of ovarioles from *Ae. aegypti*. Image from Foster et. al. (36)

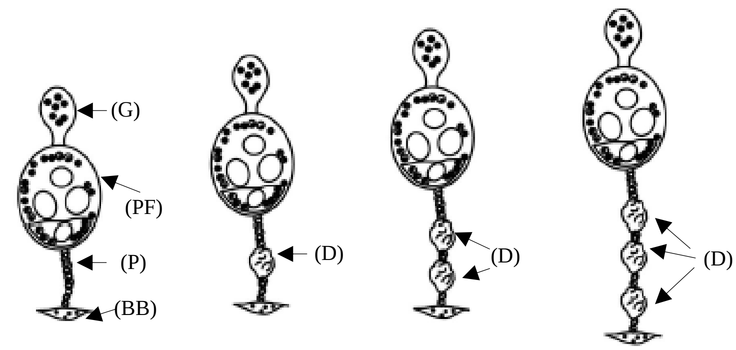


Figure 3. Follicular stage of ovarioles from *Aedes aegypti*. Image from Foster et. al. (36)

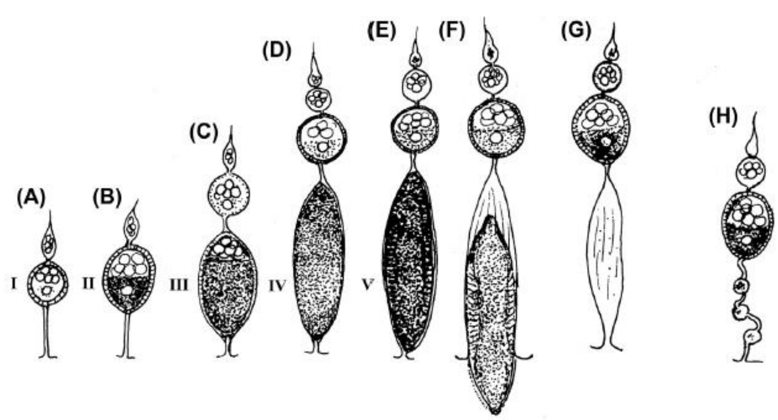


Figure 4. Directed acyclic graph showing TIRS and entomological outcomes relationship, and other covariates

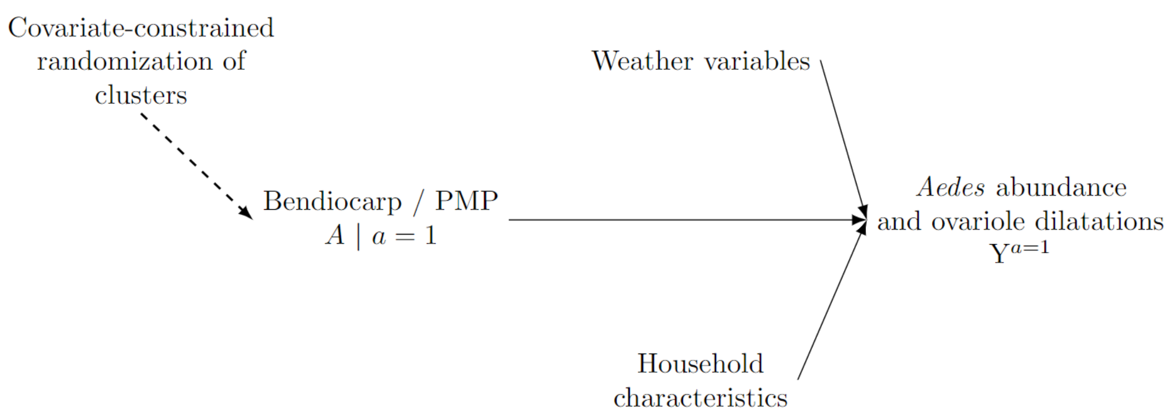


Figure 3. Female *Aedes aegypti* density per clusters by treatment groups and entomological surveys.

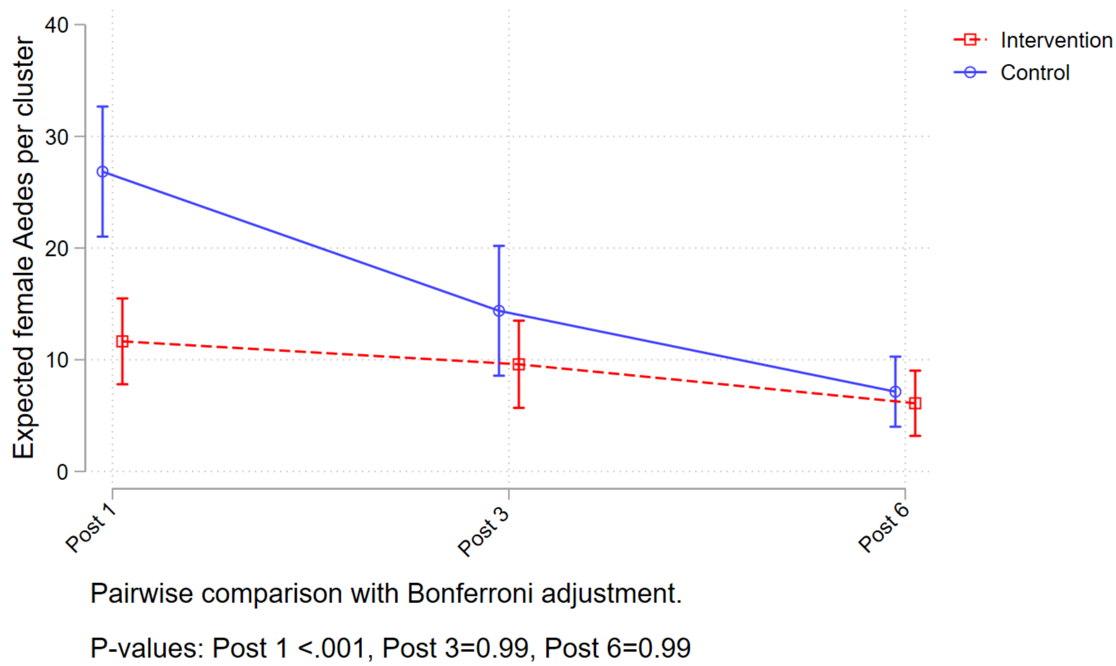
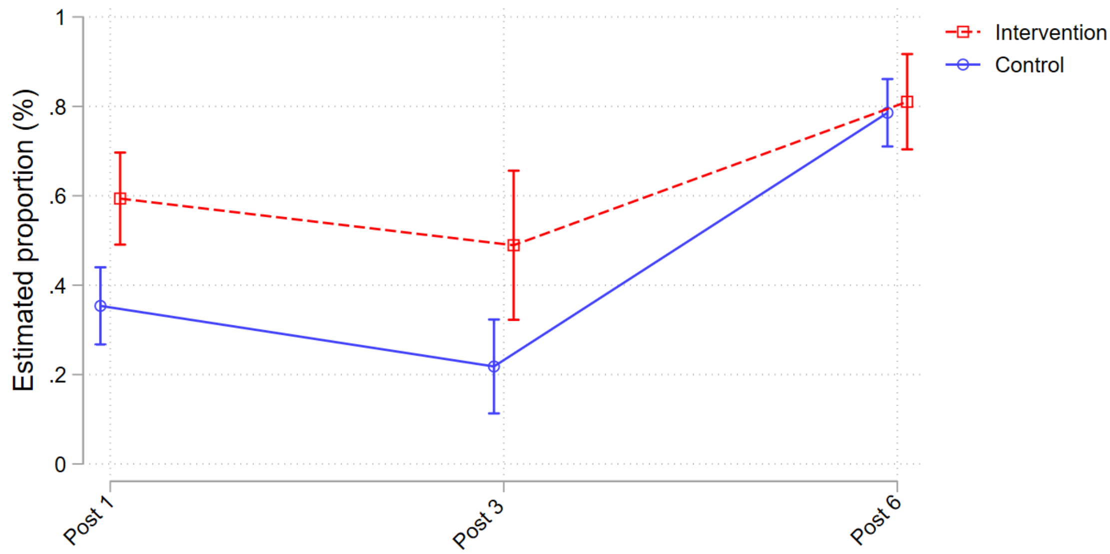


Figure 4. Nulliparous female *Aedes aegypti* by study groups and entomological surveys



Pairwise comparison with Bonferroni adjustment.

P-values: Post 1= 0.02, Post 3=0.11, Post 6=0.99

Figure 5. Dilatations and follicular stage by study groups and entomological survey.

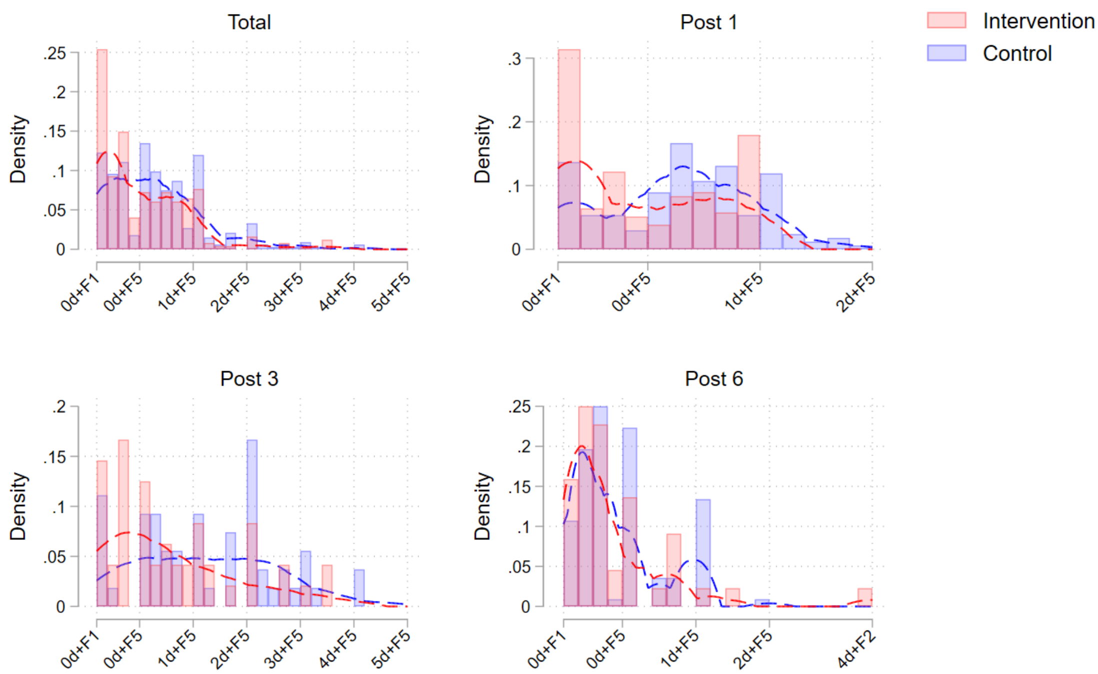


Figure 6. Follicular stages of *Aedes aegypti* by entomological surveys, number of dilatations and treatment group.

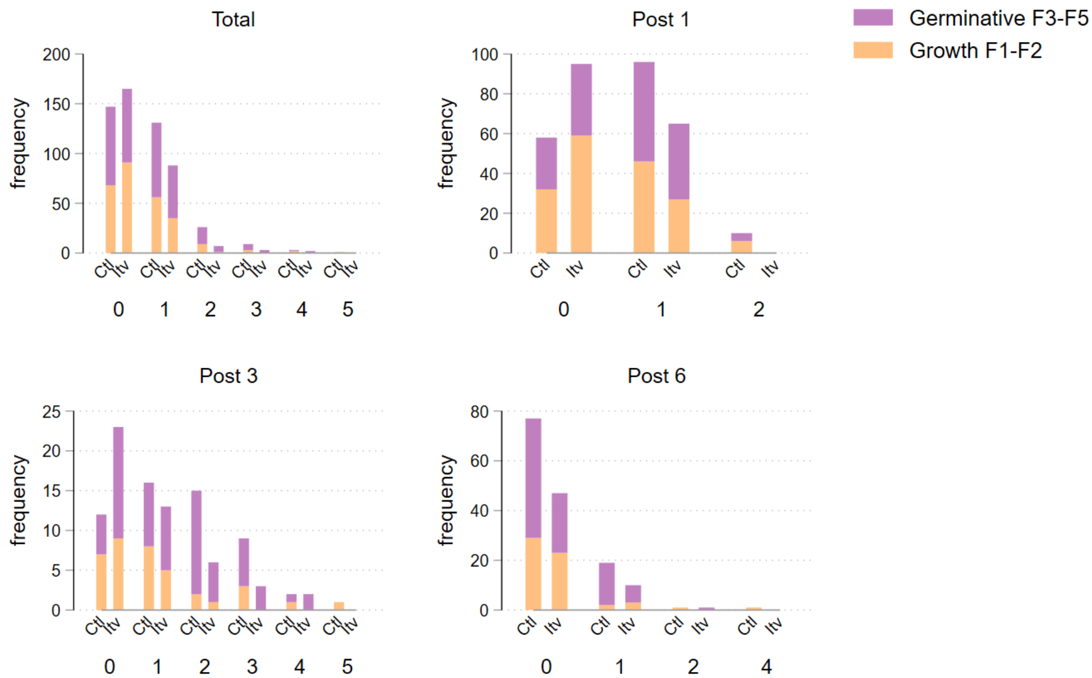


Figure 7. Efficacy levels for primary and secondary entomological outcomes after TIRS application by entomological surveys.