

Article

Sterile insect technique: successful suppression of *Aedes aegypti* field population in Cuba

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Simple Summary: The sterile insect technique is a species-specific and environmental friendly method of insect control that relies on the release of large numbers of sterile insects. Mating of released sterile insects with wild females leads to a decrease in the reproductive potential and to the local suppression of the target population. There is increased interest in applying this approach to control disease transmitting mosquitoes. This pilot trial was focused in obtaining evidence of the efficacy of using the sterile insect technique for *Aedes aegypti* control. Two neighborhoods from Havana city were selected as control and release trial sites. Wild population presence, density and fertility were monitored through a network of ovitraps. Approximately 1,270,000 irradiated *Aedes aegypti* males were released in the 50 ha intervention area over a period of 20 weeks. The released mosquitoes showed excellent mating competitiveness, and induced high levels of sterility in the wild population of *Aedes aegypti*. The natural population of the vector was suppressed as reflected in the ovitrap index and in the mean eggs/ trap values that dropped to zero by the last three weeks of the study. We conclude that the sterile males released competed successfully and induced significant infertility to suppress the local *Aedes aegypti* population.

Abstract: Dengue virus infections are a serious public health problem worldwide. *Aedes aegypti* is the primary vector of dengue in Cuba. Since there is no vaccine or specific treatment, the control efforts are directed to reduce mosquito populations. The indiscriminate use of pesticides can lead to increase insecticide resistance as well as adverse effects on human health. The sterile insect technique is a species-specific and environmental friendly method of insect control based on the release of large numbers of sterile males. The success of this technique in sustainable control of agricultural pests has encouraged its evaluation for mosquito control. Here, we describe an open field trial to evaluate the effect of the release of irradiated males on a wild population of *Aedes aegypti*. The case-control study was performed in a suburb of Havana, and compared the mosquito population before and after the intervention, in both control and treated areas. The wild population was monitored by an ovitrap network, recording frequency and density of eggs as well as their hatch rate. A significant induced sterility was observed in the field population, compared to the control. The ovitrap index and the mean eggs/ trap declined dramatically after an expected lag period of twelve and five weeks, respectively. For the last three weeks, no egg was collected in the treated area, evidencing a significant suppression of the wild population. We conclude that the sterile males released competed successfully, and induced enough sterility to suppress the local *Aedes aegypti* population.

Keywords: sterile insect technique; *Aedes aegypti*; suppression study; irradiation; vector control

1. Introduction

Chikungunya, dengue and Zika virus implies serious challenges for global public health. These arboviruses are transmitted by *Ae. aegypti* and *Ae. albopictus* mosquitoes, which are well-established in tropical and subtropical regions. The incidence rate of chikungunya, dengue and Zika virus have increased dramatically over the last 50 years under the effect of expanding vector populations, increasing global travels, human population growth, rapid and unplanned urbanization, and climate change [1]. The urban environment, with its crowded human populations living in unhygienic conditions in intimate association with increased *Ae. aegypti* populations, provided the ideal conditions for dengue transmission [2].

Since there is no vaccine available to prevent dengue disease, the main efforts are directed to avoid the proliferation of the mosquito population. Most of the vector suppression programs rely in pesticides application and reduction of mosquito breeding sites [3]. However, the conventional vector suppression tools have failed to reduce *Aedes* mosquito populations in an effective and sustainable way. Additionally, indiscriminate usage of pesticides may imply several environmental risks as well as adverse effects on human health. Epidemiological evidences revealed the harmful effects of insecticides exposure, including serious and fatal consequences such as cancer [4]. Therefore, the additional use of innovative methods is being considered in many regions, as recommended by WHO [5].

The Sterile Insect Technique (SIT) is an environmental friendly pest control method, with no harmful effect on human health [6]. The technique relies on the mass rearing and release of sterilized male insects that will not produce viable offspring after mating with wild-type females [7]. If there is a sufficiently high number of sterile males, most of the crosses are sterile, and as time goes on, the number of native insects decreases and the ratio of sterile to normal insects increases, thus driving the native population to suppression [8].

The technology has been successfully applied against insect pests of agricultural importance within an area-wide integrated pest management approach [9, 10]. For vectors of human diseases, SIT has not reached the operational level. The feasibility of applying SIT for controlling disease vectors is commonly accepted, but the strategies, logistics and efficiency have yet to be demonstrated, except in tsetse where there are several success records [11, 12]. Pilot trials are ongoing to determine if practical application of the technology is possible to control mosquitoes [13-15].

Ae. aegypti is the major vector of arboviruses in Cuba. This mosquito is characterized by its biting pattern, which consists of multiple blood meals during each gonotrophic cycle, and its ability to grow in diverse water reservoirs during its immature stages, associated with human domestic activities. These features make it an ideal vector for dengue virus transmission, especially in large urban areas where the human population density is high and provides abundant artificial and cryptic water containers [16]. The vertically-structured national program of *Ae. aegypti* control demand substantial economic resources [17].

Progressive assessment of SIT from laboratory to large cages performed prior to the present study is valuable because it allows for systematic assessment of possible effects on mosquito survival and performance under conditions increasingly more natural [18, 19].

The growth in scientific articles on the development of SIT and related technologies for mosquito control has encouraged the interest of decision-makers from public health. The objective of this pilot trial was to evaluate the efficacy of using SIT to suppress a field population of *Ae. aegypti* in real conditions, as an evidence for potential further scale-up.

2. Materials and Methods

2.1. *Ae. aegypti* strain

The mosquitoes for open-field releases were colonized from eggs collected in ovitraps deployed within the study area in 2018. The colony was maintained under standard controlled conditions $28 \pm 2^\circ\text{C}$, $80 \pm 10\%$ RH and 8h light: 16h dark.

2.2. Mass rearing

The adults for egg production were reared in 61 x 61 x 61 cm aluminum cages (BioQuip, USA) at a density of one mosquito per cm² of resting surface, and ratio 1 male: 2 females. A 50 x 50 cm gauze panel was hung inside the cage to limit the flight of mosquitoes, and to provide a higher resting surface. Adult colonies were maintained for three gonotrophic cycles. Sterilized gauze strips soaked with 10% honey and dechlorinated water were hung inside the cages for feeding/hydrating adult mosquitoes, and were daily replaced to prevent fungi growth. The honey-soaked strips were removed 12 hours before blood-feeding. Defibrinated porcine blood was provided weekly to females in collagen casings (Fibran, Spain) during four hours. The blood casings (10 mL) were heated in a warm water bath at 38°C, placed on top of the cages, and covered with warm rice-bags. Casings and bags were re-heated every fifteen minutes. After blood-feeding, the water-soaked gauze strips were removed to prevent oviposition. Plastic trays (20 x 10 x 8 cm) with inside walls lined with filter paper strips and filled with 300 mL of dechlorinated water were used for oviposition, and as water source. The trays were placed inside the cages two days after blood-feeding to induce synchronous oviposition and were removed 24 hours later. Eggs in filter papers were allowed to mature in a wet environment for three days. The filter papers were dried in air-conditioned room; the eggs were gently brushed, and stored in plastic containers for up to three months. A larval rearing unit was used for immature rearing [20], at a density of 2 larvae/mL in 4 L of deionized water per tray (100 x 60 x 3 cm). The larvae density was achieved by using eggs quantity-weight regression curves described by Zheng M. et al. [21]. Plastic flasks with the desired quantity of eggs per tray were filled with 100 mL of 36°C de-oxygenated water, and kept under vacuum for 10 minutes for synchronous hatching. The newborn larvae were left overnight in clean water without food to induce homogeneous development. First instar larvae were transferred to the trays. IAEA standard diet (50% tuna meal, 36% bovine liver powder, and 14% brewer's yeast) was provided daily at the rate of 0.2, 0.4, 0.8 and 0.6 mg/ larvae in larval instars I, II, III and IV, respectively. After pupation onset, the immatures were collected by tilting the trays in the rack, at convenient intervals to achieve the desirable range of pupal age. The collected biological material was sorted by mean of a Fay-Morlan apparatus (John W. Hock Co, USA). The remaining larvae were restored to the rearing trays, and the female pupae discarded. Male pupae were dosed by volume in 10 mL plastic tubes graduated for approximately 500 individuals. Male pupae in batches of 6,000 individuals were kept in 1 L flat tissue-culture flasks (Thermo Fisher, USA) filled with 250 mL of dechlorinated water, until the optimal age for irradiation was reached. The flasks without lids were placed horizontally to provide wide water surface.

2.3. Sterilization and packing

Mosquitoes were irradiated at pupal stage at age as old as possible, to reduce somatic damage. The minimal age for irradiation was established at 30 h. A cobalt 60 Gammacell irradiator was used (Isogamma LLC, Hungary).

An irradiation dose of 80 Gy was applied with a dose rate of 8 kGy/h. The irradiation canisters consisted of cylindrical plastic tubes (120 mm height, 45 mm diameter) which are commonly used for adult tests with insecticide impregnated papers. The mesh in the lid allowed the drainage of the water, and the easy handling of the pupae. Three tubes containing 6,000 pupae each were placed vertically in the irradiation chamber, without water.

After irradiation, the pupae from tubes were returned to the culture-flasks for transport and emergence. Adult containers consisted of cardboard boxes of 15 x 15 x 60 cm placed horizontally. For air flow and mosquito release, square holes of 10 x 10 cm were cut-off the two smaller sides of the box and covered with a fine mesh fixed with a rubber band. Additionally, one 3 cm diameter hole was cut-off from one of the 15 x 60 cm sides of the box. The neck of a culture-flask with 6,000 irradiated male pupae was introduced through this hole. The newborn mosquitoes tend naturally to escape from light through the neck and rest in the boxes. After complete emergence, the flasks were removed and the holes were covered with a 50 mL plastic tube, coated with honey-soaked filter paper. The adults were additionally provided with 10% honey solution and dechlorinated water in soaked cotton pads of 15 x 20 x 1 cm placed inside.

2.4. Field trial design

A case-control and before-after SIT-intervention study was conducted in two comparable neighborhoods belonging to southwest suburb of Havana city. The intervention consisted in the release of non-marked sterile male mosquitoes. Eggs collected from the traps were counted under a stereomicroscope and hatched to assess the species identification and fertility. Trapping was initiated eight weeks before the intervention for baseline data collection, and ended four weeks after the last release. The outcomes were the ovitrap index, egg density and egg hatch rate. The time unit was week. Ovitrap index is the proportion of ovitraps with at least one *A. aegypti* egg after one week in the field. Egg density was calculated as the weekly mean number of *Ae. aegypti* eggs per ovitrap.

2.5. Study sites

Potential sites for pilot study were considered against a predefined set of entomological, ecological, sociological and logistical criteria [22, 23]. Arroyo Arenas ($23^{\circ}02'47.1''N$ $82^{\circ}28'01.9''W$) and El Cano ($23^{\circ}01'59.8''N$ $82^{\circ}27'32.9''W$) were definitely selected for open-field trial (Figure 1). The communities are isolated from each other, and from the core metropolitan area of Havana by non-residential areas including forest, rivers, agricultural land, railway and national highway, which is expected to minimize mosquito migration.



Figure 1. Satellite images showing the study sites. **(A)** Location in southwest of Havana city. **(B)** Details from control (Arroyo Arenas) and SIT-treated (El Cano) sites; yellow dots indicate the ovitrap location. Image courtesy of Google Earth (January 21, 2021).

There is history of continuous infestation by *Ae. aegypti* in both areas in recent years. The socioeconomic characteristics are similar with highly diverse occupational profile and households with a modest standard of living. The typical houses are relatively small, one floor, two or three bedrooms and courtyard in the back; with running water, electricity, sewerage and regular rubbish collection.

Each community is around 50 ha, and they are linearly separated by 1,200 m. El Cano has 3,805 inhabitants and 906 houses distributed in 20 blocks, while Arroyo Arenas has 3,726, 890 and 23, respectively.

2.6. Intervention management

The release parameters were frequency, location and release rate. They were set up based on the mosquito average-life-expectancy, flight-range, and wild-male-abundance, respectively, that were estimated by a mark-release-recapture trial. The release rate was initially restricted by production capacity. Approximately 40,000 males were released initially for three consecutive weeks. The release rate was increased with the production capacity to 50,000, 60,000, 70,000, and 80,000 males per week.

for 3, 5, 2 and 5 weeks, respectively. Due the low wild-density in the last three weeks, the release rate was reduced to 60,000, and 50,000 males per week for 1, and 2 weeks, respectively. The intervention began in April 5th 2020 (week 15), corresponding to the beginning of summer, when wild mosquito populations tend to be in a growing phase. The latest release was performed on August 29th 2020 (week 35). Cuba has two distinct seasons, a wet/hot one from late April to October and a dry/fresh one from November to early April.

2.7. Mosquitoes releases

The sterile mosquitoes were released shortly after sunrise (around 7:00 a.m.), when *Ae. aegypti* have a peak of fly activity in nature [24], and while the values of temperature and humidity are usually favorable for mosquitoes. Sterile non-marked males were released as three days old adults, by opening the lid of the boxes from a vehicle moving slowly throughout the intervention area.

2.8. Monitoring system

Wild population was monitored through a network of ovitraps deployed in both SIT-target and control areas, in around one ovitrap per block density. The ovitrap consisted of a black 300 mL plastic cup lined with filter paper. Ovitraps were filled with tap water on site. The filter papers were weekly collected, and transferred to lab in plastic boxes. The eggs in papers were allowed to mature in wet conditions for three days, dried, counted, and classified as field-hatched or non-hatched eggs. Papers with non-hatched eggs were immersed in hatching containers made from pipette-tip boxes (transparent, hinged lid), half-filled with dechlorinated-water, and tuna meal as food. The containers were daily checked; immature were counted as third instar larva and allowed to reach the adulthood. Adults were freeze-killed and were morphologically classified by species.

2.9. Estimation of parameters for SIT-releases settings

A mark-release-recapture trial was performed two weeks before the start of the intervention. For the marking procedure, boxes with two days old adult sterile males were individually placed in a fridge at 4°C for 15 minutes. Immobilized mosquitoes were transferred in batches of around 3,000 specimens to 1 L plastic containers containing 12.5 mg of fluorescent powder (DayGlo® Color Corp., USA). The containers were gently rotated for ten seconds to achieve the contact of every mosquito with the pigment [25]. Marked mosquitoes were transferred into 30 x 30 x 30 cm metallic cages (BioQuip, USA). The easy-open top side of these cages allowed the insects release in the field. Mosquitoes were provided with water and honey.

About 10,000 yellow-marked sterile males were released by ground from a single point in the center of the community "El Cano".

The adult mosquito monitoring network consisted in 21 BG-Sentinel traps baited with BG-lures (Biogents, Germany). The traps were assigned to buildings located in concentric rings of 50, 100, 150, 200, 250, 300 and 400 m each 3, 3, 3, 4, 2, 1 and 5 traps, respectively. Adult traps were placed indoor at ground level in quietest sites of habited buildings, and checked daily during two weeks.

The biological material was daily collected and transferred to the lab in plastic containers to prevent mosquitoes from being crushed. Insects were killed at -20°C and mosquitoes were identified morphologically by species and sex under stereoscope. Males were also classified as wild or marked under ultraviolet light.

The density of wild males was estimated using the Lincoln index [26]. The probability of daily survival (PDS) for sterile males was estimated by fitting the exponential model to log-transformed data for recaptured males against the day of collection. The antilogarithm of the slope of the regression line gives an estimate of PDS. The average life expectancy of sterile males was calculated from the PDS as 1/-log_e PDS [27]. The flight behavior of released males was identified by dispersal measures: mean distance travelled and flight range [28].

2.10. Ethics statement

The open field mosquito's releases were approved by the government, the national health authorities and the regulatory agency for biological safety. All the activities of the national program

of surveillance and vector control remained under normal operation in both SIT-treated and control areas. There were no mosquito-borne diseases outbreak reported during this study.

2.11. Social issues

A community communication campaign was conducted, encouraged by the family doctors and social leaders from the own study sites, highlighting the safety of the release of sterile male mosquitoes. In order to avoid an additive intervention, no active community participation in mosquito control activities was promoted.

2.12. Data analysis

Statistical analyses were performed using R Software version 3.5.2 (R Development Core Team, Vienna) [34].

The frequency (ovitrap index) and density of eggs of *Ae. aegypti* per trap (eggs/ trap) were averaged per time unit (week). The induced egg sterility percentage was calculated by the Equation (1), proposed by Bellini et al. [29, 30].

$$S = 1 - ((Eh_I/E_I)(E_C/Eh_C)), \quad (1)$$

where S is the percent egg sterility, Eh is the mean number of hatched egg per ovitrap per week, E is the mean number eggs per ovitrap per week, I is the intervention area, and C is the control area.

Competitiveness index as defined by Fried [31] was calculated using egg hatch rate from control and intervention areas (Equation (2)).

$$\text{Fried Index} = (W/S) ((Pw - Ps) / (Ps - P_{RS})), \quad (2)$$

where W and S are the number of wild and sterile males, respectively; Pw is the percentage egg hatch in the control area; Ps is the percentage egg hatch in the release area; and the assumption that residual fertility of sterile males (P_{RS}) is 3%.

The effect of the intervention was examined by an interrupted time series analysis with a control group. A common trend model was used [32, 33]. The explanatory variable (x_t) was the egg density. The model allowed comparison in both pre- and post-intervention data and data for intervention vs. control. We implemented a linear estimating equation regression model, as follows.

$$d_t = y_{I_t} - y_{C_t} = \alpha_d + \beta_1 x_{1t} + \beta_2 x_{2t}(t - T) + \epsilon_{dt} \quad (3)$$

where $\alpha_d = \alpha_I - \alpha_C$ and $\epsilon_{dt} = \epsilon_{I_t} - \epsilon_{C_t}$. Thus, the intervention effect, β , can be estimated by performing a regression where the difference, d_t , is the outcome and x_t is the explanatory variable. ϵ is an error term, t is the time unit, T is the time elapsed since the start of the study, I is the intervention, C is the control, β_1 is the effect in the roll out period, β_2 represents the change in the intervention effect for each unit increase in time, x_{1t} is an indicator for the roll out period, x_{2t} is an indicator for the intervention period. Confidence intervals were calculated using Newey-West standard errors.

3. Results

Similar values of the ovitrap index, mean eggs/ trap, and hatch index were observed in both, "El Cano" and "Arroyo Arenas" prior to the start of releases ($P>0.05$). "El Cano" neighborhood was assigned randomly as treatment (under SIT) while "Arroyo Arenas" remained as control.

The mark-release-recapture trial performed prior to releases revealed a mean distance traveled by marked mosquitoes of 77.31 m. The flight range showed values from 43.24 m (50%) to 110.52 m

(90%), and the average life expectancy was 3.76 days. The relative abundance of male wild population at this initial time was 130.3 males/ha.

According to this results the releases were planned as twice per week, minimum of 40,000 males/week and distance between release points of 200 m.

By the time the releases were initiated for SIT, the wild mosquito populations were low, because the seasonal fluctuation. Approximately 1,270,000 irradiated *Ae. aegypti* males were released in the pilot trial site during 21 weeks. The release of 40,000 sterile males/week for three consecutive weeks represented an initial sterile/wild ratio of 6.43: 1. However, this ratio was increased along the intervention period as a result of a substantial increase in the release rate from 800 to 1,600 sterile males/ha/week.

The mean ovitrap index in the first eight weeks (baseline) was similar in SIT-treated and control areas (0.41 and 0.37, respectively) ($P>0.05$). In the control area, the ovitrap index showed an increasing trend throughout the study time as expected by season fluctuation, with a mean of 0.49 in the last three months (Figure 2).

In the treated area, after the intervention with SIT, the ovitrap index initially fluctuated under 0.5, but there was an abrupt and consistent decline after the week 29, reaching zero positive ovitraps during three weeks at the end of the study.

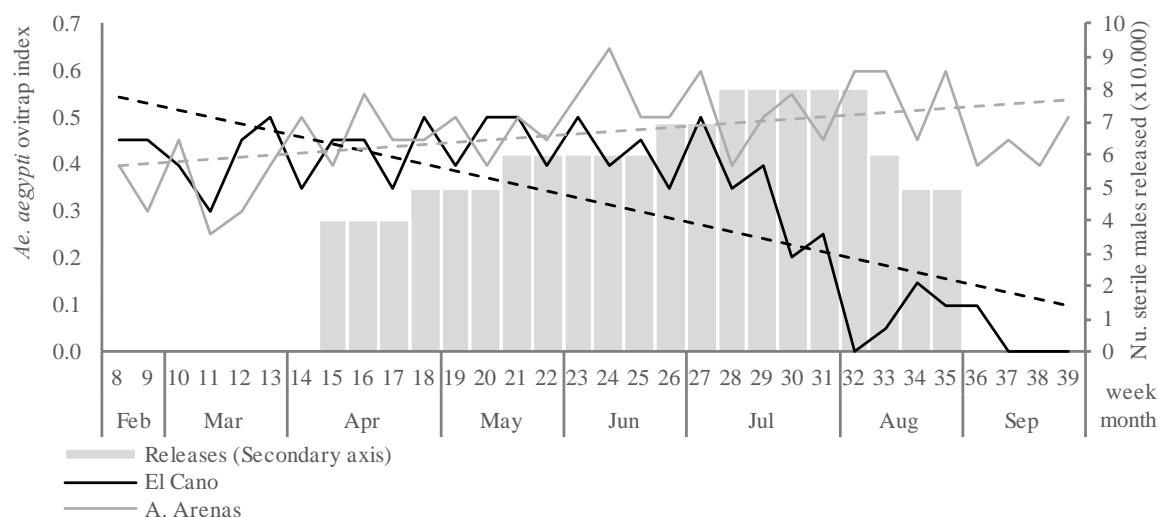


Figure 2. Ovitrap index of *Ae. aegypti* (solid lines) for the weeks 8-39, 2020 in treated (El Cano) and control (Arroyo Arenas) areas, and number of sterile males released (weeks 15-35) in the neighborhood "El Cano" (bars). Dashed lines represent the linear trend.

The decrease in fertility of *Ae. aegypti* eggs became evident by week five post-release in the treated area relative to control (Figure 3). In subsequent weeks, the induced sterility increases notably, and no viable eggs were collected for up to 6 weeks.

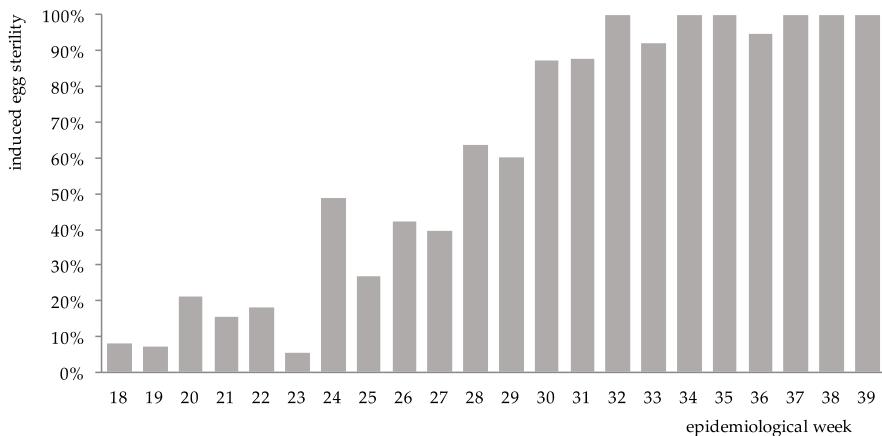


Figure 3. Weekly mean of induced egg sterility (%) in SIT-treated area "El Cano", from epidemiological weeks 18 to 39, 2020.

The mean number of eggs/trap in control and treated areas were similar during the pre-intervention period. There was a significant drop in the mean egg collections per trap after five weeks of releases.

The egg density from "El Cano" became zero by week 17 post-release. During the subsequent four weeks, there was a fluctuation below a mean value of five eggs per trap, dropping to zero by the last three weeks, indicating a positive impact of the sterile males on the wild population reduction. In contrast, the mean eggs/trap in the control area showed a trend to increase across the study duration.

During weeks 37 to 39, the values from the SIT-treated area remained null, whereas in the control area, the mean collections were 32.75, 28.05 and 32.2 eggs/trap, respectively (Figure 4).

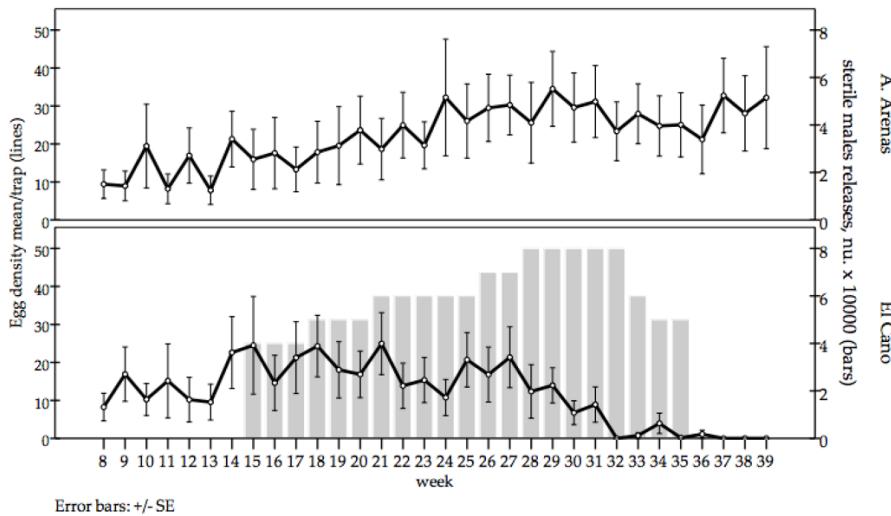


Figure 4. *Ae. aegypti* mean eggs/trap in treated (El Cano) and control (Arroyo Arenas) areas, from epidemiological weeks 8 to 39, 2020.

The difference in egg density was evidenced before-after intervention (interrupted time series analysis) and also by comparing intervention-control time series (common trend model). The roll out period was from week 15 to 19. The value of the outcome of the common trend model was 1.5.

The competitiveness index (Fried) calculated with the hatch rates from weeks 17 to 20 was of 0.56, assuming a residual fertility of 3% in released mosquitoes (table 1).

Table 1. Hatch rate per week of *Ae. aegypti* field collected eggs in treated (El Cano) and control (Arroyo Arenas) areas and Fried index of sterile males released, weeks 17-20.

	Hatch rate per week		Sterile: wild ratio	Fried index
	Mean (%)	SD		
Treated area	79.77	7.30	6.43: 1	0.56
Control area	86.47	4.13		

4. Discussion

This pilot study demonstrates the effectiveness of the sterile insect technique to suppress an *Ae. aegypti* population in real field conditions. The intervention with SIT started at the end of winter, corresponding with naturally low wild population density. Thus, it was not considered necessary to suppress the population with another control method before applying SIT.

The density of 130.3 wild males/ha found in the mark-release-recapture trial is considered very high in Cuba, according to the standards of the national program of vector control. These standards are based on human bites, a subjective method with poor accuracy.

The selection of the pilot study site is critical for obtaining solid evidences [22, 35]. Despite the logistical complexity associated with metropolitan areas, two urban neighborhoods belonging to Havana were selected, since *Ae. aegypti* is predominantly an urban species [36]. "El Cano" and "Arroyo Arenas" are partially ecologically isolated between them and from the surrounding neighborhoods. The size, shape, architectural and social structure were relatively similar between both sites. These areas also had a history of habitual presence of *Ae. aegypti* for years, corroborated in this study by monitoring the mean egg density per trap during the pre-release phase.

The ovitrap resulted a simple and effective method for monitoring. Ovitrap surveillance data correlated well with other calculated indices which are used to estimate seasonal population dynamics of *Ae. albopictus* in Italy [37]. The adult mosquito traps are labor intensive, as pointed by Reiter [38], and has several inconveniences. The BG-Sentinel traps are to be installed indoor for safety, and the typical houses in the study area are small. The BG-lure releases a strong smell, and the deployment of a network of traps requires daily visits for collecting the catches for a long period. In this study, the sterile males for SIT releases were not marked to prevent damage which could compromise their competitiveness. Therefore, the adult wild population was not monitored directly. The major inconvenient was the impossibility of estimate the sterile: wild ratio and, consequently, the competitiveness across the whole study.

The initial sterile: wild ratio was below optimal values due to low mass rearing capacity. Nevertheless, a previous study performed in our lab showed that the weekly release of chemosterilized males at a 5:1 ratio with the fertile males was sufficient to eliminate a caged population of *Ae. aegypti* within 15 weeks [18]. Certainly, the trials of SIT suppression of mosquito-caged populations should be interpreted carefully, but gives gross evidences of the efficacy of the technique, as part of the progressive, stepwise evaluation of sterile mosquitoes [39]. We presume higher flooding ratio after week 17, since the release number was noticeably increased from 50,000 to 80,000 males per week once the mass rearing was improved, whereas we observed a reduction in the number of eggs collected per trap from the field.

In a similar way, Zheng et al. settled sterile to wild male ratio (5:1) at the beginning of the pilot study in China. Their mosquito releases (*Ae. albopictus* irradiated plus *Wolbachia* infected) around this ratio, appeared to be able to induce high degrees of sterility [40].

In our facility, we achieved significant enhancement in efficiency of the mass rearing by week 28, allowing obtaining over 120,000 males/week. However, the releases were intentionally restricted up to 1,600 mosquitoes per ha, to prioritize the assessment of a realistic flooding ratio for further extended scenarios.

The existence of *Ae. albopictus* in the pilot site was a challenge for monitoring since their eggs are morphologically indistinguishable from those of *Ae. aegypti*. It was managed by allowing the mosquitoes to reach adulthood for identification. However, it is a time and space consuming task, and the need of a different approach for wider studies is clear.

The competitiveness index was calculated for weeks 17 to 20 based on an accurate wild population abundance estimation. Further estimations were not feasible, since the sterile males were not marked. The Fried index of 0.56 reflected the excellent ability of male mosquitoes to induce sterility in the wild population. This was higher than the only other estimated field competitiveness of *Ae. aegypti* in field conditions in Brazil, estimated to 0.26 (95% confidence interval, 0.05 to 0.72) [41]. There is a recent study in Mexico where the competitiveness of 70 Gy-irradiated *Ae. aegypti* males ranged from 0.09 to 0.46, but the experiments were carried out in field cages [42]. Results from mating competitiveness trials in cages have generally been found to underestimate the performance of irradiated mosquitoes in the field [43, 44]. In Cayman Islands (2012) and Brazil (2015), the genetically modified OX513a strain from Oxitec showed competitiveness values of 0.059 (0.011–0.210) and 0.031 (0.025–0.036), respectively, during field trials [45, 46].

There is a report from Thailand about a high competitiveness of *Ae. aegypti* in cages. They observed a high Fried index of 0.86 when the sterile: fertile male ratio was 5:1 [47]. In Italy, the field competitiveness of irradiated *Ae. albopictus* was estimated through the weekly capacity to induce sterility. The authors reported strong negative correlation between the field competitiveness of the *Ae. albopictus* males released and the ratio of sterile to wild males [29].

In our study, the wild population suppression in the treated area was evaluated by monitoring the mosquito eggs presence, density and hatching rate by the ovitrap network. The interrupted time series analysis evidenced that the reduction in the mean eggs/trap after the week 19 was caused by the intervention. The common trend model displays a reliable confirmation of the effect of the intervention by comparing time series in treated versus control area. Assuming the common trend model, we eliminate the effect of the unobserved confounders (the trend) by subtracting the control series y_{C_t} from the intervention series y_{I_t} . The outcome of the model suggests that the intervention reduced the average of mosquito eggs per trap by 1.5 (95% CI: -1.76 - 1.39) for each unit (week) increase in time. In a previous laboratory study of caged *Ae. aegypti* population, we described a finite rate of natural increase of 2.92, and an intrinsic rate of natural increase of 1.07 [19]. Therefore, the common trend model seems to be plausible. The model also evidenced a time lag between the beginning of the intervention and the observed effects on indicators. These roll out period ranged from five weeks for mean eggs/trap to 12 weeks for the ovitrap index.

As far as we know, this is the first study that reports the suppression of a field population of *Ae. aegypti* by applying classical SIT in real conditions. However, similar studies have been conducted for *Ae. aegypti* control by means of SIT-related technologies.

Recently, Mains et al. achieved significant reduction in the number of *Ae. aegypti* in an urban neighborhood within a metropolitan area of Miami, USA, by releasing *Wolbachia*-infected males [48]. In this case, the cytoplasmic incompatibility was used as sterilization method [49], but the rest of the practices are similar to SIT. Previous studies comprising genetically modified mosquitoes, also reported success in *Ae. aegypti* reduction in Cayman Islands (80%), Brazil (85%) and Panama (93%) [45, 46, 50]. In Thailand, Kittayapong et al. demonstrated the reduction of natural populations of *Ae. aegypti* in a semi-rural village, but combining SIT with *Wolbachia*-induced incompatibility (SIT/IIT) [47]. Bellini et al. obtained success in controlling the related species *Ae. albopictus* in Italy [30, 51]. In China, Zheng et al. reported the largest open field trial ever conducted using SIT/IIT. As a result, the population of *Ae. albopictus* in the residential areas of two islands in China were successfully suppressed [40].

5. Conclusions

We conclude that the irradiated males released competed successfully with the wild males, and induced sufficient infertility to suppress the local *Ae. aegypti* population. The findings from this study provide optimism to move to larger trials directed to estimate the impact of SIT on epidemiological outcomes. The area-wide sustained release of irradiated males is a promising tool to be integrated with existing control methods for the management of the diseases transmitted by *Ae. aegypti*.

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