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Research

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Preparation for targeted sterile insect technique to control invasive *Aedes aegypti* (Diptera: Culicidae) in southern California: dose-dependent response, survivorship, and competitiveness

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Aedes aegypti is fast spreading across California, with over 300 cities within 22 central and southern counties being infested since its introduction in 2013. Due to its cryptic breeding habitats, control efforts have not been successful so far. This calls for innovative tools such as sterile insect technique (SIT) to reinforce the existing integrated pest management (IPM). Here, we assessed fitness, survivorship, and dose response of X-ray irradiated male *Ae. aegypti* in California. Locally acquired *Ae. aegypti* eggs were hatched and reared in temperature-controlled laboratory setting at the West Valley Mosquito and Vector Control District in Ontario, California. Freshly emerged adult male mosquitoes were manually separated using motor-operated aspirators and treated with X-ray radiation at different dosage (42–60 Gy). Dose response of irradiated males was analyzed and induced sterility determined. Survivorship of males treated with different X-ray doses was compared. Fecundity of females that mated with irradiated males at different X-ray doses was generally comparable. Overall, induced sterility increased with higher X-ray doses. Nulliparous females that mated with male *Ae. aegypti* treated with 55–60 Gy laid eggs with over 99% sterility. Non-irradiated male mosquitoes had higher survivorship (mean = 0.78; $P = 0.0331$) than irradiated mosquitoes (mean range = 0.50–0.65). The competitiveness index of irradiated males decreased with increasing X-ray treatment doses, 1.14 at 55 Gy and 0.49 at 60 Gy, and this difference was significant ($P < 0.01$). Irradiated males showed high survivorship and competitiveness—key for the anticipated SIT application for the control of invasive *Ae. aegypti* in California.

Key words: sterile insect technique, *Aedes aegypti*, X-ray irradiation, survivorship, competitiveness

Introduction

The urban-adapted, daytime-biting *Aedes aegypti* is the primary mosquito vector of dengue and has the potential to transmit several other arboviruses, including Zika, chikungunya, and yellow fever (Kraemer et al. 2019). In California, this vector has spread to over 300 cities within 25 central and southern counties in less than a decade since its introduction (CDPH 2023a). Historically associated with tropical and subtropical regions, the spread of *Ae. aegypti* to California highlights the complex interplay of factors such as global travel (Leta et al. 2018), urbanization (Wilke et al. 2021), and climate change (Iwamura et al. 2020), which facilitate the expansion of its geographic range. In 2023, two locally acquired dengue human cases were reported in Southern California (CDPH 2023b). With the rapidly expanding populations of *Ae. aegypti* in California (Metzger et al. 2017) and throughout the United States (McGregor and Connelly 2021), these mosquitoes have posed a real and increasing threat of local spread of *Aedes*-borne tropical diseases. This has spurred intensive efforts to curb its population, with a range of control strategies employed. However, due to its cryptic breeding habitats, *Aedes* control efforts have been limited so far. This urgently calls for the need for innovative tools to strengthen the existing integrated vector management (IVM) strategies.

The sterile insect technique (SIT) has garnered attention for its potential to suppress mosquito populations by introducing sterile males into the wild, thus reducing the reproductive success of the target species. SIT is not a new tool (Proverbs 1969). Several countries have used SIT and demonstrated suppression and even elimination/eradication of a target mosquito population, e.g., elimination of *Anopheles albimanus* in El Salvador (Lofgren et al. 1974), and suppression of *Ae. aegypti* in Cuba (Gato et al. 2021). The fact that SIT is self-limiting, unlike population replacement using Wolbachia or genetically engineered transgenic traits, is one of its advantages. This targeted approach has no collateral damage to non-target species and reduces the overall environmental impact of control efforts. In addition, no biological agent is introduced into the environment when applying SIT using X-ray irradiation. Radiation causes overt lethal mutations that lead to embryonic death after fertilization (Robinson 2005).

Gamma-ray irradiation was frequently used in the past to irradiate insects for SIT releases (Aldridge et al. 2020, Silva et al. 2022). However, as the control of radioisotopes has become increasingly strict, it became difficult to purchase, transport, or reload gamma-ray radiators (Wang et al. 2023). Therefore, low-energy X-ray irradiation systems are a good alternative, as they present easy accessibility, discontinuous emission of radiation, low harmfulness (self-contained), simple operation, and low cost (Aldridge et al. 2020).

Central to the success of SIT is the fitness and survivorship of released males. Fitness encompasses various factors, including mating competitiveness, flight ability, and longevity, all of which influence the efficacy of SIT in suppressing target populations. Studies have shown that X-ray sterilization does not significantly compromise the fitness of *Ae. aegypti* males (Yamada et al. 2014).

Survivorship is another critical aspect of SIT, as released males must survive long enough to compete for mates and suppress wild populations. While irradiation can impact survivorship, the dosage of radiation plays a crucial role in determining its effects. Research has indicated a dose-dependent response in *Ae. aegypti* survivorship following irradiation, with higher doses leading to increased mortality (Aldridge et al. 2020). However, careful optimization of radiation dosage can mitigate these effects, ensuring the production of viable sterile males with adequate survivorship for successful population suppression. Therefore, before putting X-ray into use for mosquito SIT, tests should be carried out to determine effective X-ray dosage with least fitness cost and ensure its efficiency and reliability in terms of processing capacity, induction of sterility in the insects, ease of handling, and long-term durability.

This work aimed to assess the fundamentals in the application of targeted sterile insect technique using X-ray radiation, focusing on its impact on fitness, survivorship, and radiation dosage response on male *Ae. aegypti* mosquitoes. Understanding these characteristics of irradiated mosquitoes is crucial for the success of the SIT applications. Furthermore, developing a tailormade standard operation procedure is required to roll out effective vector control intervention.

Methods

This study was conducted in the laboratory of the West Valley Mosquito and Vector Control District located in Ontario, California. The District serves residents in 6 cities in southern San Bernadino County: Chino, Chino Hills, Ontario, Upland, Montclair, and Rancho Cucamonga, with a total population of over 600,000, covering an area of 544 sq km. *Aedes aegypti* has been prevalent in the District since 2015 (Mullens et al. 2021). The region is considered as hot semi-arid, characterized by dry summer months (June–September) with temperatures ranging between 32 and 43°C. (www.visualcrossing.com/weather/weather-data-services/). *Ae. aegypti* eggs were locally obtained and mosquito colonies were established under temperature-controlled laboratory setting for over 12 months prior to running this experiment, with continual addition of field collected *Ae. aegypti* larvae reared to adults and then introduced into the colony.

Egg Hatching and Larval Rearing

Eggs were stored at room temperature for 1 month before hatching. Hatching (500 eggs/hatching flask) was done in a flask containing 2 g of brewer's yeast in 400 ml water under negative pressure (600 mmHg) for 1 h in climate-controlled room at a constant temperature 29°C ± 3.0°C, relative humidity 75% ± 1%, and photoperiod of 12L: 12D day length. Then, the flasks were emptied into large tubs (29 cm × 16 cm × 11 cm) containing alfalfa infused water (4 alfalfa pellets in 2L of water). After 4 days, larvae were transferred to smaller tubs (15 cm × 20 cm × 10 cm) and kept at a density of ~300/tub. Larvae in each tub were fed every other day with 5 g of alfalfa. Late instar larvae and pupae were counted and recorded. All pupae were collected in small containers with two-thirds of water and placed inside cages (30 cm × 30 cm × 30 cm) until adult emergence.

Daily adult emergence was counted and sorted out by sex using morphological features. Battery operated handheld aspirators (Clarke, St. Charles, IL) were used by trained technicians to sort out male mosquitoes from the cages within 24 h after emergence. Freshly emerged male *Ae. aegypti* were placed in a small cup for X-ray treatment.

Dose-dependent Response

Freshly emerged males (< 1-day old) *Ae. aegypti* mosquitoes were placed in a small cup (266 ml) (capacity of 200 mosquitoes per cup) to be treated at different radiation dosages using Rad Source X-ray Machine (Rad Source RS 1800·Q, Rad Source Technologies, Buford, GA), with a dose rate of 13.89 Grey (Gy)/min. At this constant dose rate, the amount of irradiation dose was time-dependent, ranging from 6.02 min at 42 Gy to 8.41 min at 60 Gy. The machine auto-calibrates the time required based off the dosage requested. Dosimeter measurements were taken before and after the treatments indicating the amount of absorbed radiation at different parts of the loading rack. We consistently placed the treatment cups in specific (front central) part of the canister where the amount of radiation released was consistently 13.89 Gy/min. Irradiated males were allowed to rest for an hour before transferring them to mosquito cages. In each cage, 15 irradiated male and 30 non-irradiated female (1:2 ratio) *Ae. aegypti* mosquitoes were kept. Mosquitoes were provided with sugar solution (10%) ad libitum. After a week, allowing enough time for mating, each cage was blood fed (bovine whole blood with 3.8% NaCit) (Animal Technologies, Tyler, TX) for 2 days (each day for 6 h) using glass feeders (see Supplementary File 1). Mosquitoes were blood fed only once. A plastic cup (200 ml) with filter paper and half-filled with tap water with 1 g of brewer's yeast was placed inside the cages for egg laying. The number of eggs laid by female mosquitoes in each cage was compared across the different X-ray treatment cohorts to evaluate fitness of the sterile male mosquitoes. All eggs collected were also hatched to determine induced sterility.

Radiation-induced Sterility

Induced sterility rendered by the X-ray treatment was evaluated to determine effective X-ray dosage with the least cost on fitness. All

Table 1. Mean number of eggs laid by female *Ae. aegypti* mosquitoes that mated with X-ray treated and untreated (control) male mosquitoes

	Mean no. of eggs				
X-ray dosage	Per cage (± SE)	Per female (± SE)	Mean no. larvae hatched	% egg hatched	% unhatched egg
42 Gy	1,289 ± 62.1	85.9 ± 12.2	184.0	14.3	85.7
45 Gy	1,224 ± 43.7	81.6 ± 9.8	118.7	9.7	90.3
47 Gy	1,167 ± 38.6	77.8 ± 13.5	92.8	7.9	91.1
50 Gy	1,142 ± 47.2	76.1 ± 19.8	59.1	5.2	94.8
52 Gy	1,037 ± 28.8	69.1 ± 15.2	38.4	3.7	96.3
55 Gy	1,168 ± 36.9	77.9 ± 12.7	12.4	1.1	98.9
58 Gy	1,037 ± 41.8	69.1 ± 18.4	5.3	0.5	99.5
60 Gy	1,018 ± 48.4	67.9 ± 21.6	3.0	0.3	99.7
Control (unsterilized)	1,591 ± 59.8	106.1 ± 32.9	1,556.0	97.8	2.2

eggs laid by females that mated with treated and untreated (control) males were hatched using the procedure discussed above. The number of larvae hatched was counted in each tub. Each treatment cohort was run in triplicates. X-ray radiation induced sterility was calculated as (h-u) where h is hatching rate of the eggs from treatment cohort; u is the unhatched proportion (%) in the control (non-irradiated) group (Yamada et al. 2014). Data were compared among treatment cohorts (42 Gy, 45 Gy, 47 Gy, 50 Gy, 52 Gy, 55 Gy, 58 Gy, and 60 Gy).

A group of 30 freshly emerged female mosquitoes were also treated with 42 Gy, 45 Gy, 47 Gy, 50 Gy, 52 Gy, 55 Gy, 58 Gy, and 60 Gy, and kept in cages with untreated males ($n = 15$). Ten percent sugar solution was provided ad libitum. Mosquitoes were blood fed for egg collection.

Survivorship

Understanding the fitness and survivorship of X-ray sterilized *Ae. aegypti* is crucial to the effectiveness and feasibility of SIT program for vector control. Therefore, the number of eggs laid by female mosquitoes was taken as a proxy to measure fitness. The number of eggs laid by female mosquitoes that mated with males treated with different doses of X-ray radiation were compared to evaluate sterility offered by this technique. Freshly emerged male *Ae. aegypti* were X-ray irradiated at 47 Gy, 50 Gy, 52 Gy, 55 Gy, 58 Gy, and 60 Gy. X-ray doses of 42 Gy and 45 Gy were not included in this experiment because of low egg sterility rate. For each dose, 15 irradiated males and 30 non-irradiated nulliparous females were kept in cage with 10% sugar solution with cotton wick. Cages were made in triplicates and kept for 50 days to monitor mosquitoes' survivorship. Sugar water solution was provided ad libitum. Dead mosquitoes were counted daily, except during weekends.

Competitiveness

Competitiveness of sterile males and non-sterile males for mating with female mosquitoes was evaluated by determining the effect observed through proportion of females producing sterile eggs. Treatment doses of 55 Gy, 58 Gy, and 60 Gy were selected for this experiment because of their high sterility rate (> 98%) and survivorship. With 3 replicates for each treatment dose, each cage contained a total of 15 sterile males and 15 non-sterile males with 30 female *Ae. aegypti*. The competitiveness index (C) was calculated as ((Hn-Ho)/(Ho-Hs)) × (N/S), where Hn and Hs were respectively the hatch rate from eggs of females mated with untreated or sterile males, Ho was the observed egg hatch rate in the experiment and N and S were the numbers of untreated and sterile males, respectively (Oliva et al. 2012).

Statistical Analysis

Data were processed and charts were plotted with Microsoft Excel 2010 (Microsoft Corp., Redmond, WA, USA). Radiation effects on egg hatch, induced sterility, and survivorship were analyzed using 1-way ANOVA and Tukey's post-hoc tests.

Hatch rates, adult survivorship, and induced sterility were arcsine transformed to achieve normal distribution before analysis. The proportion of eggs laid by female mosquitoes was corrected for female mortality. Comparisons between treatments were made using 1-way ANOVA and Tukey's post-hoc test. Kaplan–Meier survival analyses were conducted to determine relative differences in adult survivorship between treatment cohorts. Survival curves were compared using the survdiff function in the R Survival package (Zheng et al. 2015). All data are presented as mean ± SE.

Results

Fecundity

The mean number of eggs laid by female *Ae. aegypti* mosquitoes that mated with males treated with different X-ray doses demonstrated a dose-dependent response (1-way ANOVA, $F_{(8, 26)} = 0.98$; $P = 0.081$) (Table 1). Female mosquitoes in the control cages (1,500 eggs per cage; 106.1 eggs/female) laid 20–50% more eggs than the treatment cages (1,018–1,289 eggs per cage; 67.9–85.9 eggs/female). Overall, females that mated with males treated with higher X-ray doses laid relatively fewer eggs than those mated with males treated with lower X-ray doses. The hatching rate of eggs from control cages was 98% while overall hatching rate of treatment cohorts ranged from 0.3 to 14.3% for eggs collected from cages with irradiated males treated with 60 and 42 Gy, respectively.

Radiation-induced Sterility

Induced sterility was compared among treatment cohorts (Fig. 1). Higher X-ray doses were associated with higher sterility (1-way ANOVA, $F_{(7, 21)} = 3.04$; $P < 0.001$). Doses between 55 and 60 Gy resulted in over 98% induced sterility. The highest sterility (98%) was achieved when male mosquitoes were treated with 58 and 60 Gy while males treated with 42 Gy yielded only 84% sterility. Overall, induced sterility increased with higher X-ray doses. All treated female mosquitoes did not lay any eggs.

Survivorship

The survivorship of male *Ae. aegypti* treated with different doses of X-ray radiation were compared (Fig. 2). Non-irradiated male mosquitoes had higher survivorship (mean = 0.78; 95%CI = 0.75–0.81; $F_{(8, 18)} = 4.81$; $P = 0.0331$) than irradiated mosquitoes (mean

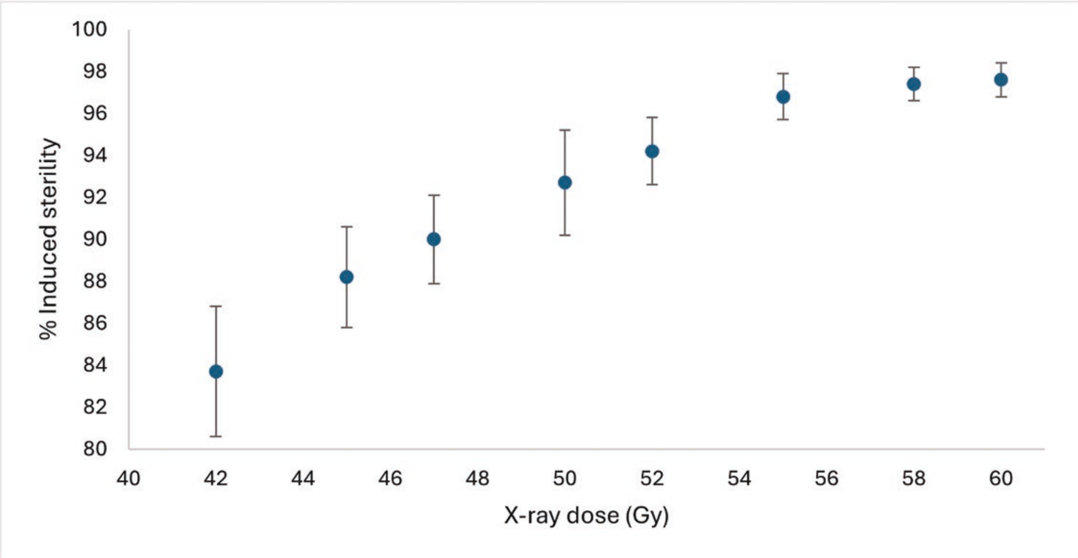

Fig. 1. Mean (± SE) induced sterility of male *Ae. aegypti* treated with different doses of X-ray radiation.

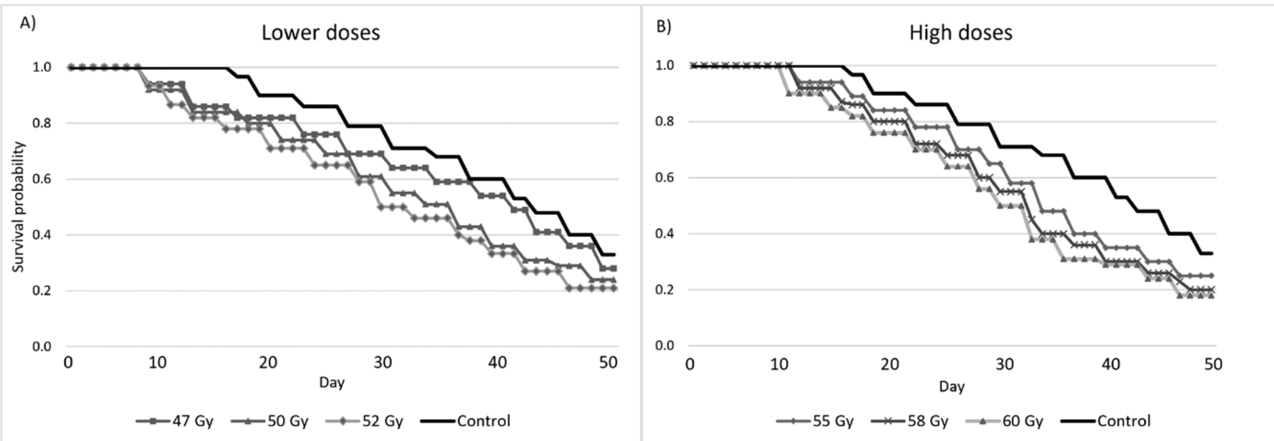

Fig. 2. Kaplan–Meier estimates of survivorship for X-ray treated and untreated (control) male *Ae. aegypti* mosquitoes under climate-controlled laboratory conditions. A) lower doses (47–52 Gy); B) Higher doses (55–60 Gy).

range = 0.50–0.65). Overall, male mosquito's survivorship decreased with increasing X-ray dosages, and the differences were statistically significant (ANOVA, Tukey's post-hoc test, $P < 0.05$) (Fig. 2). Daily survivorship of male *Ae. aegypti* treated with 60 Gy (mean = 0.51) was 50% lower than the non-sterilized males (mean = 0.78) (Fig. 3).

Competitiveness

Competitiveness was compared by placing irradiated and non-irradiated males with normal female mosquitoes in a cage in 1:1:2 ratio (Table 2). Three X-ray doses (55, 58, and 60 Gy) were assessed. The competitiveness index (c) lowered with increasing X-ray treatment doses, 1.14 at 55 Gy and 0.49 at 60 Gy, and this difference was significant ($F_{(2, 7)} = 12.65$; $P < 0.01$).

Discussion

Dose response, fitness, and survivorship are key characteristics that determine the success of sterile insect technique. Our results indicated that when nulliparous females mated with male *Ae. aegypti* mosquitoes treated with 55–60 Gy of X-ray radiation, over 99% of their eggs would not hatch. Since the calculations of induced sterility often leave unaccounted for the number of eggs unhatched due to defect or inefficiency of the hatching technique, the actual induced sterility is often lower than the empirical hatching rate. It can also be argued that similar factors in the natural environment such as predation and weather factors influence the success of hatching (De Majo et al. 2017). Overall, over 99% sterility was achieved by irradiating mosquitoes at 55 Gy dose. All females treated with 42–60 Gy did not lay any eggs—indicating complete infertility of females subjected to radiation treatment in case accidentally treated and released with males.

The present study showed that the daily survivorship of male mosquitoes decreased with increasing X-ray dose. The mean survivorship of unirradiated mosquitoes reported here (0.78) was higher than previous studies (Tussey et al. 2023). Among the X-ray doses tested, male mosquitoes irradiated with 55 Gy showed a better survivorship with 99% sterility and low fitness cost. A recent study that utilized gamma rays for mosquito sterilization indicated that exposing late-stage pupae to 50 Gy of radiation yielded 99% male sterility while maintaining similar survival of pupae to

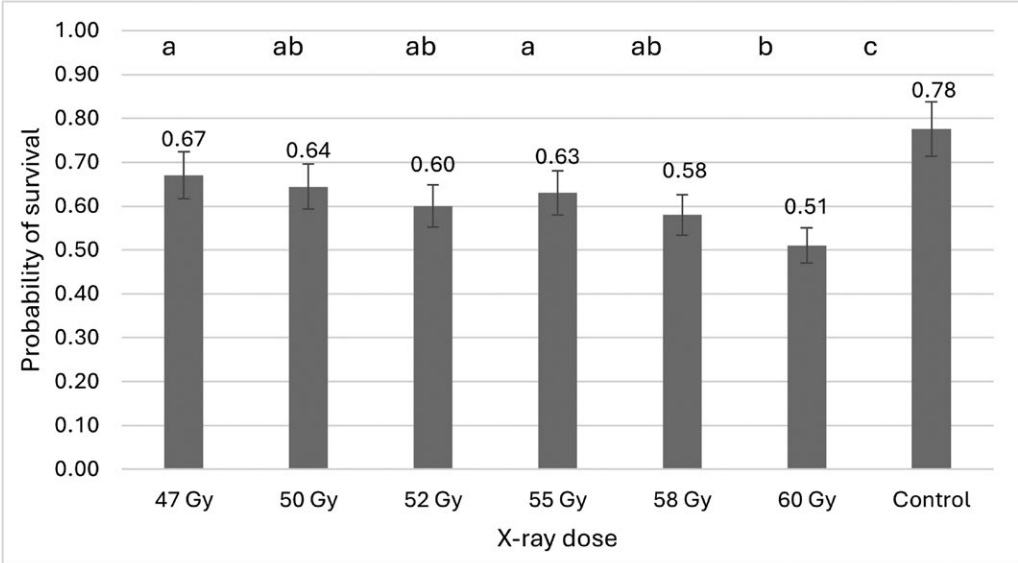

Fig. 3. Comparison of mean daily survivorship of irradiated and non-irradiated (control) male *Ae. aegypti* mosquitoes. (Different letters indicate significant differences in the mean survivorship, $P < 0.05$).

Table 2. Competitiveness (c) of irradiated male *Ae. aegypti* treated with 55, 58, and 60 Gy when placed with non-irradiated (control) males in a cage with female mosquitoes in 1:1:2 ratio in laboratory condition. SE refers to standard error

	55 Gy	58 Gy	60 Gy
No. replicates	3	3	3
Mean no. eggs laid per cage	1,061	989	911
Mean hatching rate (%) (± SE)	62.2 ± 5.8	58.1 ± 8.3	41.7 ± 12.4
Mean competitiveness (c) (± SE)	1.14 ± 0.18	0.72 ± 0.11	0.49 ± 0.08

adult emergence, adult longevity and male mating competitiveness compared to unirradiated males (Chen et al. 2023). Similarly, in Mexico, Bond et al. (2019) reported that irradiation of *Ae. aegypti* males at a dose of 50 Gy resulted in 99% sterility.

The comparable mean number of eggs laid by female *Ae. aegypti* mosquitoes that mated with irradiated males treated with different X-ray doses indicated the fitness of sterile males. A previous study indicated that the average number of eggs laid per female was significantly reduced in *Ae. aegypti* at lower doses and a 100% sterility was achieved at 70 Gy but with a cost on fitness (Bond et al. 2019). While a 100% sterility can be achieved by increasing the X-ray dose, the trade-off between sterility and fitness is often key. In the present study, a high survivorship was reported for males treated with 55 Gy as compared to previous studies (Zheng et al. 2019, Tussey et al. 2023).

Since SIT relies on the releases of sterile (male) insects in overflooding numbers to mate with wild females, and thereby suppress the insect population in the targeted area (Vreysen et al. 2021), mating competitiveness with the wild population is critical. The competitiveness index reported in this study (1.14 at 55 Gy and 0.49 at 60 Gy) was higher than the 0.26 (95% CI = 0.05–0.72) that was recorded during a field study with *Ae. aegypti* in Brazil (Bouyer et al. 2020). A recent study in Mexico reported a competitiveness index between 0.09 and 0.46 for 70 Gy-irradiated *Ae. aegypti* males, but the experiments were carried out in field cages (Bond et al. 2021). A high competitiveness index of 0.86 was also found when the sterile: fertile male ratio was 5:1 in another cage study with *Ae. aegypti* in Thailand (Kittayapong et al. 2019). At Lee County in Florida (Tussey et al. 2023), male *Ae. aegypti* irradiated as adults at 50 Gy were found to be significantly more competitive than males irradiated as pupae at 45 Gy.

By combining better sterilization technique with minimal impact on fitness and survivorship, SIT technique could offer a sustainable and effective solution to mitigate the spread of mosquito-borne diseases when used along with other existing IVM strategies. Continued research into the optimization of radiation dosage and integration to existing control strategies will further enhance the efficacy and scalability of this innovative approach, ultimately contributing to effectively combat vector-borne diseases.

The findings from this study provide optimism to initiate targeted SIT application in California. In SIT simulation experiments, a 5:1 sterile to wild male *Ae. aegypti* ratio allowed a 2-fold reduction of the wild population's fertility (Oliva et al. 2012). Successful suppression of *Ae. albopictus* in China (Zheng et al. 2019) and Italy (Bellini et al. 2013) was achieved using lower wild to sterile mosquito ratios in SIT. Unfortunately, most SIT applications that utilize X-ray radiation target pupae for radiation treatment (Bellini et al. 2013, Tussey et al. 2023). However, it has been reported that irradiating pupae could cause somatic damage (Yamada et al. 2014). Irradiation at the adult stage can be beneficial in terms of minimizing somatic damage. In Florida, Tussey et al. (2023) indicated an increased longevity in male *Ae. aegypti* irradiated as adults compared to males irradiated as pupae. As the result, they are switching from pupal to adult irradiation.

One of the challenges of maintaining area-wide SIT-based mosquito control is logistics and scalability. For instance, a large-scale trail in Fresno County in California released 14.4 million sterile male *Wolbachia*-infected *Ae. aegypti* mosquitoes to target 2.93 sq km control area (Crawford et al. 2020). This could be extremely challenging for smaller mosquito control agencies as they can neither afford the budget to establish an insectary that could harvest millions of mosquitoes every week nor be able to run it for a considerable number of years. To address this challenge, we are proposing a targeted SIT approach based on counts from weekly mosquito surveillance across the district and releasing sterile males at these identified hotspots. A 100-times the number of female mosquitoes in the traps will be released at these targeted sites bi-weekly and mosquito population will be monitored using weekly mosquito surveillance. By releasing sterile males only in areas predominately infested by *Ae. aegypti* will save resources and benefit budget-constrained districts. The naturally short-flight range of *Ae. aegypti* would have an added benefit to the targeted SIT releases. Moore and Brown (2022) estimated the mean distance traveled by *Ae. aegypti* by pooling data from 27 experiments and confirmed the short-flight range of this mosquito (mean = 105.69 m). This means released sterile mosquitoes will likely remain in the targeted neighborhood to bring the anticipated impact.

In conclusion, this study documented a high survivorship and high competitiveness of irradiated *Ae. aegypti* males which are beneficial for the success of the anticipated SIT program in the District. At West Valley Mosquito and Vector Control District, targeted use of SIT at historical *Ae. aegypti* hotspots is underway. As an alternative to area-wide treatment, our District is attempting to selectively release sterile males at sites over a set threshold. SIT should be considered as an additional tool, not a silver bullet, to be utilized alongside the existing IPM strategies for effective control of invasive mosquitoes.

Supplementary data

Supplementary data are available at *Journal of Medical Entomology* online.

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Author contributions

Solomon Birhanie (Conceptualization [Lead], Formal analysis [Lead], Investigation [Lead], Methodology [Lead], Supervision [Lead], Validation [Lead], Visualization [Lead], Writing—original draft [Lead], Writing—review & editing [Lead]), Jennifer Thieme (Data curation [Lead], Formal analysis [Supporting], Investigation [Equal], Methodology [Equal], Supervision [Supporting], Validation [Supporting], Visualization [Supporting]), Ale Macias (Data curation [Supporting], Investigation [Supporting], Methodology [Equal]), Rubi Casas (Data curation [Supporting], Investigation [Supporting]), and Michelle Brown (Conceptualization [Equal], Formal analysis [Equal], Funding acquisition [Lead], Methodology [Equal], Project administration [Lead], Supervision [Equal], Validation [Equal], Visualization [Equal], Writing—original draft [Equal], Writing—review & editing [Equal])

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