

Aedes aegypti CONTROLS *Ae. aegypti*: SIT AND IIT—AN OVERVIEW

ROBERT L. ALDRIDGE, SETH GIBSON AND KENNETH J. LINTHICUM

United States Department of Agriculture, Agricultural Research Service Center for Medical, Agricultural,
and Veterinary Entomology, 1600 SW 23rd Drive, Gainesville, FL 32608

ABSTRACT. The sterile insect technique (SIT) and the incompatible insect technique (IIT) are emerging and potentially revolutionary tools for controlling *Aedes aegypti* (L.), a prominent worldwide mosquito vector threat to humans that is notoriously difficult to reduce or eliminate in intervention areas using traditional integrated vector management (IVM) approaches. Here we provide an overview of the discovery, development, and application of SIT and IIT to *Ae. aegypti* control, and innovations and advances in technology, including transgenics, that could elevate these techniques to a worldwide sustainable solution to *Ae. aegypti* when combined with other IVM practices.

KEY WORDS Integrated vector management (IVM), mosquito control, radiation, sex sorting, transgenics, *Wolbachia*

INTRODUCTION

Aedes aegypti (L.) is a prominent worldwide human disease vector that consistently eludes escalating and intensive population reduction and elimination efforts by public health vector control agencies. A principal foundation of the difficulty of control of *Ae. aegypti* populations is the ubiquity of and reliance on traditional integrated vector management (IVM) measures that apply pesticides to control this species. Cryptic peridomestic habitats exploited by *Ae. aegypti* for immature development and adult resting (Harwood et al. 2016) are difficult or impossible to reach with standard adulticide and larvicide applications and can evoke strong opposition to chemical-based control by residents in the intervention area. Skip oviposition behavior (Reiter 2007) and asynchronous egg development or hatching (Gillett 1955) compound and complicate the spatial and temporal scope of larvicide treatments. Furthermore, increasing resistance to pesticide active ingredients approved by the US Environmental Protection Agency (EPA) and escalating deregistration of approved pesticides (Burkett et al. 2013) is rapidly and severely constricting the arsenal of chemical control options available to mosquito control operators. The effects of resistance and deregistration are intensified because of glacial development and registration of new effective chemical control measures, including essential oil and other EPA-exempt active ingredients (Avant 2012), which lag substantially below the rate of expansion and establishment of *Ae. aegypti* populations.

Fortunately, a suite of biological control techniques broadly including the sterile insect technique (SIT) and the incompatible insect technique (IIT) are gaining widespread research attention as well as increasing pilot and operational implementation across nearly every continent (Tables 1 and 2) to control populations of *Ae. aegypti* in support of IVM. The SIT involves what many consider a classical approach of directly sterilizing males, using either irradiation or exposure to a chemical sterilant, damaging gametic tissue. The IIT involves rendering males incompatible through either

genetic manipulation (including transgenics), hybridization, or microbiota (i.e., *Wolbachia*) that doesn't sterilize the males, thus leaving them able to copulate with their conspecifics under the proper conditions. The difference between the two techniques is that for the classical SIT, the males and/or females are rendered sterile from the treatment, which may also damage somatic tissue. For IIT, it is the combination of incompatible heritable factors (i.e., genes or microbiota) that alters female fertility or the successful development of immatures into adulthood.

Both SIT and IIT, with some exceptions, center on releasing overwhelming numbers of colony-reared males that are attractive to wild-type females but have been sterilized (SIT) or are otherwise reproductively incompatible (IIT) with those females, leading to reduction in egg production or production of inviable eggs or offspring, which eventually suppresses or even eliminates the target *Ae. aegypti* population. Conspecific males are used, so the biology of the target organism itself is exploited to locate females specifically. No pesticides are used, so no resistance to pesticide should evolve, although some data exist on evolution of wild-type females to avoid or downselect sterile males (Hibino et al. 1991). Depending on the technique, SIT, IIT, or combined SIT/IIT programs can be developed and implemented with few or no regulatory obstacles, but all techniques share the requirement of a long view (of population elimination or replacement), funding, and support for as long as a defined human population needs to be protected from *Ae. aegypti*. In this overview we present a brief history of the discovery and development of SIT and IIT, their advantages and disadvantages, case studies of historical and recent pilot or operational implementations, and our view of the way forward to elevate these tools to increasingly higher efficacy to complement and address the weaknesses of IVM.

THE STERILE INSECT TECHNIQUE (SIT)

Discovery and early development. The SIT grew from the extension of seminal discoveries on the effect

Table 1. The first area-wide programs, conducted in the 1960s and 1970s, implemented the sterile insect technique (SIT) to control *Aedes aegypti*. These programs failed and were abandoned because of limitations in the understanding of radiation dose and lack of public support.

Year	Location	Sterilizing agent	No. released (total)	No. releases	Estimated success	Reference
1960	Site 1: Pensacola, FL	Gamma: 11,000-18,000 Roentegen	Est. 1,333,567	16	Failed to demonstrate success against control	Morlan et al. 1962
1961	Site 1: Pensacola, FL	Gamma: 11,000-18,000 Roentegen	Est. 2,149,303	27	Failed to demonstrate success against control	Morlan et al. 1962
1961	Site 2: Pensacola, FL	Gamma: 11,000-18,000 Roentegen	Est. 1,287,184	13	Failed to demonstrate success against control	Morlan et al. 1962
1970	Chibarani, Kenya	Sex-linked translocation	63,939	10	Oviposition hatch rate reduced to 36%	McDonald et al. 1977
1971	Model Basti, Delhi, India	Reciprocal translocation/gamma radiation	46,215	4	40.9% mating	Rai et al. 1973
1973	Seahorse Key, FL	Gamma radiation induces translocation	2,900, ¹ 2,900, ² 3,300 ³	1	Fertility reduction (96.8–66.7%)	Seawright et al. 1975
1974	Gainesville, FL	Double translocation	15,000, 15,000, 15,000 × 2	1 × day, 5 days	Fertility reduced by 31.5–34.1%	Seawright et al. 1976
1974	Sonenpat, New Delhi, India	Chemosterilized—theotepa	18,000	6	58–136%; 90% average	Grover et al. 1976
1974	Sonenpat, New Delhi, India	Genetic translocation	11,500	6	28–57%	Grover et al. 1976
1974	Sonenpat, New Delhi, India	Genetic translocation and distorter	11,000	6	155–46%	Grover et al. 1976
1974–1975	Kwa Bendegwa, Kenya	Translocation homozygote—population replacement	45,500	~91	Unable to replace population	Lorimer et al. 1976
1974–1975	Mgandmi, Kenya	Translocation homozygote—population replacement	45,500	~91	Unable to replace population	Lorimer et al. 1976
1974–1975	Kwa Dzivo, Kenya	Translocation homozygote—population replacement	7,000	~14	Unable to replace population	Lorimer et al. 1976

¹ F₁ males (VOYLE × Tt:2-26).

² VOYLE males.

³ VOYLE females.

Table 2. Implemented and planned area-wide programs conducted since 2010 after an approximately 35-yr hiatus that apply the sterile insect technique (SIT) or the incompatible insect technique (IIT) to control *Aedes aegypti* populations around the world.

Year started	Location	Sterilizing agent	No. released (total)	No. releases	Estimated success	Reference
2010	Grand Cayman	Oxitec RIDL OX513A-Mexico	3,371,000	56	80% reduction comp. untreated	Harris et al. 2012
2011	Yorkey's Knob, Australia	<i>Wolbachia</i> wMel, population replacement	141,600	10	100% population replacement	Hoffman et al. 2011
2011	Gordonvale, Australia	<i>Wolbachia</i> wMel, population replacement	157,300	10	90% population replacement	Hoffman et al. 2011
2011-2012	Juazeiro, Bahia, Brazil	Oxitec RIDL OX513A	7,205,056	57	95% reduction in population	Carvalho et al. 2015
2012	Bentong, Pahang, Malaysia	Oxitec RIDL OX513A-My	6,045 RIDL, 5,372 My1	1	223 m travel, no difference longevity	Laeroix et al. 2012
2012	Machans Beach, Australia	<i>Wolbachia</i> wMelPop-PGYP	51,600+	6+	>80% replacement, then reduced to below 10%; failed to replace population	Nguyen et al. 2015
2012	Babinda, Australia	<i>Wolbachia</i> wMelPop-PGYP	117,000	15	>80% replacement, then reduced to below 10%; failed to replace population	Nguyen et al. 2015
2012	Juazeiro, Brazil	Oxitec RIDL OX513A	5,900 males/ha/wk (33 ha area)	89 wk	70% reduction in population	Garziera et al. 2017
2013	Jacobina, Brazil	Oxitec RIDL OX513A	14,000 males/ha/wk (31 ha)	117 wk	60% reduction in population	Garziera et al. 2017
2013	Tri Nguyen Island, Vietnam	<i>Wolbachia</i> wMelPop	702,000	23	91.7-96.6% population replacement mid-May 2015	Nguyen et al. 2015; WMP 2022c;
2013	Cairns, Australia	<i>Wolbachia</i> wMel, population replacement	131,420	197 days	Mixed results, no spread of infected <i>Ae. aegypti</i>	Hien et al. 202a Schmidt et al. 2017
2013-2017	Cairns, Australia	<i>Wolbachia</i> wMel, population replacement	Variable (eggs and adults)	Variable	96% reduction in dengue incidence rate (8 yr)	Ryan et al. 2019
2014	Tri Nguyen Island, Vietnam	<i>Wolbachia</i> wMel	12.8 to 93.7 adults/house/wk	27	Up to 96.6% population replacement	Hien et al. 2021
2014	Panama City, Panama	Oxitec RIDL OX513A	4,249 951	81	93% reduction of wild <i>Ae. aegypti</i>	Gorman et al. 2016
2014	Sleman District, Yogyakarta, Indonesia	<i>Wolbachia</i> wMel	25 × 25 m grid; 28-90 adults/site	20 wk	80-90% replacement (3 yr)	Tantowijoyo et al. 2020; WMP 2022h
2015	Bantul District, Yogyakarta, Indonesia	<i>Wolbachia</i> wMel	25 × 25 m grid; 80-120 eggs/site	12 wk	80-90% replacement (3 yr)	Tantowijoyo et al. 2020; WMP 2022h
2015	Bello (Paris), Colombia	<i>Wolbachia</i> wMelRio	Unknown	Unknown	Unknown	WMP 2022i
2015-2017	Niteroi, Brazil	<i>Wolbachia</i> wMelRio	Unknown	25 wk/yr	>80% population replacement	WMP 2022j;
2016	Chachoengsao Province, Thailand	SIT/IIT (gamma [70 Gy]/wAlbA and wAlbB)	437,980	24 wk	97.3% reduced population	Gesto et al. 2021b Kittayapong et al. 2019
2016	Yogyakarta, Indonesia	<i>Wolbachia</i> wMel	9,005-23,756/release	13-15	73% reduction in DHF incidence 2 yr after; >80% population replacement	Indriani et al. 2020; WMP 2022h
2017-2019	Rio de Janeiro, Brazil	<i>Wolbachia</i> wMelRio	Unknown total; 200 adults/vial	Up to 28 wk	30-70% replacement	WMP 2022j;
2017	Fresno, CA	wAlbB <i>Wolbachia</i>	20,000,000	20 wk	68% effective female reduction	Gesto et al. 2021a Gilbert and Melton 2018
2017	Medellin, Colombia	<i>Wolbachia</i>	Unknown	Unknown	Unknown	WMP 2022i
2017	Key West, FL	<i>Wolbachia</i> -MosquitoMate	Unknown	Unknown	Unknown	Schairer et al. 2021
2018	Vinh Luong, Vietnam	<i>Wolbachia</i> wMel	556,461	17 wk	93% population replacement March 2018	WMP 2022c;
2018	Fresno, CA	<i>Wolbachia</i> wAlbB	14,376,511	26 wk	95.55% population reduction	Hien et al. 2021
2018	Selangor, Malaysia	<i>Wolbachia</i> wAlbB	1,662,600	20-57 wk	>90% population replacement	Crawford et al. 2020
2018	Port Vila, Vanuatu	<i>Wolbachia</i>	Unknown	Unknown	Unknown	Nazni et al. 2019 WMP 2022b

Table 2. Continued.

Year started	Location	Sterilizing agent	No. released (total)	No. releases	Estimated success	Reference
2018	Chiapas, Mexico	Gamma sterilization (50 Gy)	1,633,096	11	5.5:1 and 2.8:1 sterile:wild release (ground release and aerial)	Mariana et al. 2022
2018	Tamavua Village, Fiji	<i>Wolbachia</i>	Unknown	Unknown	Unknown	WMP 2022d
2018	Miami, FL	<i>Wolbachia</i> - MosquitoMate	>6,800,000	6-month period	78% reduction in mean number of females	Mains et al. 2019
2018	Queensland, Australia	<i>Wolbachia</i> wAlbB (population suppression)	3,000,000	3×/wk, 20 wk	63–99% population reduction	Beebe et al. 2021
2018–2020	Jacarezinho, Brazil	PTB-1 dsRNA (RNAi) and thiotepa (0.1–0.6%)	First release 10,000,000 estimated; second release 6,000,000 estimated	29 wk, 2018–2019; 20 wk, 2019–2020	Reduced wild population by 91.4%, 13.7–15.9 × lower dengue case rate than nontreatment	de Castro Poncio et al. 2021
2019	Yucatan, Mexico	<i>Wolbachia</i> wAlbB, x-ray irradiation (45 Gy)	1,270,000	24 wk	Reduced egg hatch by 50–90%; 80–90% reduction in population	Martin-Park and Che-Mendoza 2022
2019	Noumea, New Caledonia	<i>Wolbachia</i>	Unknown	Unknown	Unknown	WMP 2022c
2019	South Tarawa, Kiribati	<i>Wolbachia</i>	Unknown	Unknown	Unknown	WMP 2022a
2019	Singapore	<i>Wolbachia</i> wAlbB-SG and x-ray radiation (pupae 30–35 Gy)	Unknown	15 and 31 wk	Reduces both wild-type <i>Aedes aegypti</i> populations [reductions of 92.7% (95% CI: 84.7–95.8%) and 98.3% (97.7–99.8%)] and dengue incidence [reductions of 71% (43–87%) to 88% (57–99%)]	Ng and Wolbachia Consortium 2021
2019	La Paz, Mexico	<i>Wolbachia</i>	Unknown	Unknown	Unknown	WMP 2021
2019	Houston, TX	<i>Wolbachia</i> wAlbB	ca. 1,417,000	3/wk, 11 wk	92% reduction in treatment area	Lozano et al. 2022
2019–2021	Samuel Island, FL	X-ray sterilization (52 Gy)	190,504	7 cycles	1.5% recapture, 3 day majority recapture, 201.7 m mean distance traveled (m)	Carvalho et al. 2022
2020	Colombo, Sri Lanka	<i>Wolbachia</i>	Unknown	Unknown	Unknown	WMP 2022f
2020	Havana, Cuba	Gamma sterilization (80 Gy)	1,270,000	21	Successful suppression	Gato et al. 2021
2021	Cali, Colombia	<i>Wolbachia</i>	Unknown	Unknown	Unknown	WMP 2022i
2021	Florida Keys, FL	Oxitec RIDL OX5034	750,000,000 planned	Unknown	Unknown	Micu 2020
Planned	Vientiane, Laos	<i>Wolbachia</i>	NA	NA	NA	WMP 2022g
Planned	Belo Horizonte, Brazil	<i>Wolbachia</i>	NA	NA	NA	WMP 2022j
Planned	Campo Grande, Brazil	<i>Wolbachia</i>	NA	NA	NA	WMP 2022j
Planned	Petrolina, Brazil	<i>Wolbachia</i>	NA	NA	NA	WMP 2022j
Planned	Sri Lanka	Gamma sterilization	NA	NA	NA	Ranathunge et al. 2022
2016	India	Oxitec RIDL OX513A	NA	NA	NA	Paul et al. 2020, 2021
Planned	Bangladesh	Gamma sterilization	NA	NA	NA	Hossain et al. 2021
Planned	Saba Island	Oxitec RIDL OX513A	NA	NA	NA	Glandorf 2017
Planned	California	Oxitec RIDL	NA	NA	NA	DVM/VCD 2023

of x-ray radiation on mammalian fertility (Albers-Schönberg 1903) into investigations of the impact of radiation on insects. Early work exposing houseflies, *Musca domestica* L. (Axenfeld 1896) or the European ant, *Formica sanguinea* Latreille (Forel and Dufour 1902) to x-rays resulted in no observed life history effects. Hunter (1912) conducted several experiments exposing a variety of arthropods to x-rays, including vectors of human and veterinary diseases such as *Dermacentor andersoni* Stiles (as *Dermacentor venustus*), *Argas miniatus* Koch, *Rhipicephalus annulatus* (Say) (as *Margaropus annulatus*), and *Culex pipiens* L., which yielded no conclusive evidence that radiation affected fertility as had been observed in mammals. Students of Hunter's continued this work with cigarette beetles, *Lassioderma serricorne* (Fabricius) in Tampa, FL, but similarly found no evidence of reproductive impact (Morgan and Runner 1913). However, a key turning point in the technology was reached when a more stable x-ray tube was used that delivered consistent output and produced sterility in the cigarette beetles (Runner 1916). The mechanism of x-ray-induced sterility was teased out by H. J. Muller, who identified dominant lethal mutations generated in *Drosophila melanogaster* Meigen in somatic cells (Muller 1927) and gametes after exposure to radiation, causing a lack of development in zygotes (Muller 1954). The radiation broke chromosomes and created sticky ends that would reattach and generate deleterious mutations.

Use of radiation for control of insect pests by damaging gametic chromosomes through radiation exposure was first proposed by Serebrovsky (1940); however, he was not able to fully explore the possibility of his hypothesis before his death in 1948.

First application of x-ray sterilization to insect control: The first application of radiation-based sterilization was conducted on Sanibel Island, FL, in 1951 against the New World screwworm fly, *Cochliomyia hominivorax* (Coquerel). However, these efforts fell short and only ca. 80% eradication was achieved (Bushland 1960). The first successful broad application eradication of a population from an area using radiation-based sterilization was conceived of and executed by E. F. Knippling from the US Department of Agriculture (USDA) targeting *C. hominivorax* on the island of Curaçao in 1954 (Baumhover et al. 1955). The concept of this new technique—now called the sterile insect technique (SIT)—consisted of the release of multiple cohorts of colony-reared screwworm flies that had been sterilized by exposure to x-ray radiation per Muller's (1927) findings. The hypothesized outcome of screwworm population reduction and eventual eradication depended on successful and numerically overwhelming competition by these sterile males with fertile wild males for mating opportunities with fertile wild female screwworm flies, thus reducing the number of fertile eggs. In the absence of migration of wild types into the treated area—minimized or eliminated by conducting the study on an island—repeated releases of overwhelming numbers of sterile males were hypothesized

to reduce or eliminate the target population in that area, which was observed during the 1954 investigation (Baumhover et al. 1955). Following this successful demonstration and additional positive results from field releases of sterilized screwworm fly males on Florida's Sanibel Island and Captiva Island (Lindquist 1955), stakeholders in the livestock industry and in the USDA solicited a full area-wide SIT program to eradicate screwworm from the southern USA (Lindquist 1963), which expanded to successful eradication of this severe agricultural pest from the entire USA by 1966 (Baumhover 2001). Following elimination in the USA, plans were put in place to coordinate control efforts with Mexico to eliminate screwworm there and continue pushing the elimination zone farther south, with a new barrier being established at the narrow Darien Gap in the Isthmus of Panama (Baumhover 2001), which has persisted with few breaches to the present day.

In light of the success of area-wide control of the screwworm fly using SIT, the technique was evaluated against *Ae. aegypti* in an effort to expand the arsenal of control options beyond traditional IVM. Early SIT programs targeting *Ae. aegypti* were initiated in the 1960s and 1970s, as outlined in Table 1, but halted because of complications that prevented the success of the programs. Chief among them were lack of public support, ineffective radiation dose calibration, and poor tracking and monitoring of mosquito populations and dispersal of released irradiated males (Dame et al. 2009). It was not until 2016 that SIT was revisited to control *Ae. aegypti* populations (Table 2), which will be discussed later in this review.

Beyond x-rays: Other methods of sterilization: Methods of irradiation of mosquitoes to achieve sterilization are not limited to x-ray exposure. Other methods include exposure of male mosquitoes to ionizing radiation (i.e., gamma radiation) generated from radioactive isotope sources (Darrow 1968, Bourtzis et al. 2016) or exposure to optical radiation such as through the use of an argon laser by focusing 514.5-nm wavelength microbeams for less than a second on the mosquito gonadal region (Rodriguez et al. 1989).

Other means to induce male sterility in mosquitoes such as chemosterilization have been investigated, which consists of exposing mosquitoes to chemical agents such as tepa, thiotepa, and apholate (Weidhaas and Schmidt 1963, Gato et al. 2014). Chemical sterilants function in a number of ways, for example, by causing deleterious mutations in DNA or even internal cellular damage within actively dividing somatic cells (Campion 1972). Chemical sterilization, although unlikely to disrupt male competitiveness, was primarily discontinued following concerns of sterilant bioaccumulation and nontarget sterilization (Bracken and Dondale 1972). However, de Castro Poncio et al. (2021) demonstrated a novel form of SIT that they termed natural vector control (NVC), which combined RNAi and the chemical sterilant thiotepa with the goal of reducing or eliminating the residue of the chemical sterilant in the released adults. Their experiment was successful in

producing thiotepa residue-free, sterile male *Ae. aegypti*. Subsequent field trials in Jacarezinho, a neighborhood of Rio de Janeiro, Brazil, using the SIT NVC process resulted in a 14× reduction of human dengue incidence cases in the area compared to the control.

Why use SIT to control Ae. aegypti?: Several advantages for incorporating SIT in IVM have been identified. First, males from local *Ae. aegypti* strains may be readily colonized and reared in the laboratory for sterilization and release, and will be more likely to survive in the local environment to compete with nonsterilized males compared to existing laboratory strains that may need to be purchased or otherwise obtained (Oliva et al. 2021). Sterilization of colony-reared mosquitoes is a nonregulated and nonproprietary technology, with perpetual availability limited only by funding, rearing space, and sterilization source, with no need for permits for release as can be the case with genetically modified organisms (Mains et al. 2016). When SIT is implemented with careful and routine surveillance, there is no known nontarget impact or evolution of insecticide resistance.

What factors may limit the efficacy of SIT to control Ae. aegypti?: Several considerations need to be highlighted to understand the limits of SIT in an IVM program. For SIT to be implemented with lasting success, sustained funding and local facilities are essential to generate high numbers of healthy and competitive sterilized males over extended periods of time that are for all intents and purposes indefinite, as exemplified by the screwworm fly SIT program. In the case of radiation-based SIT, a radiation source must be purchased or leased and maintained, permitted, and available throughout the program. In addition, specialized training for operators may also require a personnel screening process for special access to equipment. Also, a hidden cost inefficiency to producing colony-reared males is that of producing and separating out females that are not needed for the next generation and will not be released in the control program. Although sterilization through radiation can be optimized to retain life history qualities of males that increase competitiveness (i.e., survivorship, longevity, energy stores, and fertility), radiation may nevertheless impact male competitiveness through disruption of somatic cells and tissue development (El-Gazzar and Dame 1983). Despite intensive and sustained release of SIT males in a prescribed area, seasonal or point immigration of the target species from adjacent or disconnected untreated areas can occur and reestablish the population (Yasuno et al. 1978).

THE INCOMPATIBLE INSECT TECHNIQUE (IIT)

The IIT may be considered a variation of the SIT, because the objective of both techniques is to force wild-type females to produce unfertilized or inviable eggs, reduced numbers of eggs, or inviable progeny. In contrast to SIT, which relies strictly on the release of males sterilized by radiation or chemicals to interrupt

production of the next generation, IIT relies upon a variety of heritable genetic or biological phenomena that create a barrier to reproduction, such as hybridization and satyrization, cytoplasmic incompatibility, or genetic modification (such as the insertion of dominant lethal genes). Like SIT, a successful IIT program depends on long-term rearing and release of large numbers of colony-produced male mosquitoes; however, the variety of IIT approaches also present a mosaic of advantages and disadvantages that must be considered.

IIT through hybridization: Hybridization-based IIT is accomplished with the release of males of a closely related but reproductively incompatible species capable of competing for females against males of the target species but generating inviable hybrid eggs or offspring, thus reducing the target population over time. In the first successful demonstration of this technique, Vanderplank (1947) was able to control a small population of the tsetse fly *Glossina swynnertoni* Austen by releasing male pupae of the closely related but reproductively incompatible species *G. morsitans morsitans* Westwood in *G. swynnertoni* habitat. This habitat is not ideal for *G. m. morsitans*; therefore the released individuals survived only long enough to mate with *G. swynnertoni* females, which was sufficient to suppress this local population. However, hybridization-based IIT is likely not appropriate for a species such as *Ae. aegypti* because of the challenge of identifying a closely related species that is capable of successful copulation with *Ae. aegypti* females followed by the production of inviable hybrid eggs or sterile hybrid offspring that can also be mass-reared in colony and be sufficiently compatible with the habitat in the intervention area.

IIT through satyrization: Satyrization is a specific form of interspecific reproductive incompatibility in which the act of mating sterilizes females of the target species (Ribeiro 1988). The satyrization effect had been proposed as a mechanism for the displacement of *Ae. aegypti* by *Ae. albopictus* Skuse from the southeastern USA, including Texas, Louisiana, Mississippi, Alabama, and Florida in the 1980s by Nasci et al. (1989) and Tripet et al. (2011). Satyrization was proposed as an enhancement to SIT-based population control by Honma et al. (2019), and later executed by Maiga et al. (2020a) in the release of irradiated male *Ae. albopictus* to reduce both local *Ae. albopictus* and *Ae. aegypti* on La Réunion island. However, closer laboratory analysis revealed that the *Ae. aegypti* population on La Réunion had become resistant to satyrization by male *Ae. albopictus*.

IIT through cytoplasmic incompatibility: The cytoplasmic incompatibility (CI)-based approach to IIT centers on a group of maternally inherited intracellular endosymbiotic bacteria from the genus *Wolbachia* that can be found in approximately two-thirds of insect species (Werren et al. 2008) and has been applied to *Ae. aegypti* population control. *Wolbachia* spp. can cause sterility through CI, a complex bacterial toxin-antidote system that rescues sperm to fertilize eggs harboring the same species and strain of *Wolbachia* (Beckmann et al.

2017). If a female does not have a *Wolbachia* infection or harbors a different *Wolbachia* strain than the male, the sperm is not rescued, and the egg is not fertilized (Chen et al. 2019). Therefore CI-based IIT consists of releasing in the intervention area large cohorts of conspecific males of the target species that harbor an incompatible strain of *Wolbachia* that will overwhelmingly mate with target females and potentially disrupt the target population.

Additionally, *Wolbachia* leveraged in an IIT program can be used for population replacement instead of population reduction. For example, successive releases of both male and female *Ae. aegypti* carrying a novel strain of *Wolbachia* into the Australian towns of Yorkey's Knob and Gordonvale gradually replaced the wild *Wolbachia* strain in the local *Ae. aegypti* population (Hoffmann et al. 2011). As a consequence of this demonstration, the incidence of dengue decreased in and around the release locations because the novel *Wolbachia* strain reduced the vector competence for dengue virus in the local *Ae. aegypti* population (Hoffmann et al. 2011). This same technology and techniques were repeated in Yogyakarta, Indonesia, by Utarini et al. (2021), and the results were similar to those of Hoffman et al. (2011) with a reduction in virologically confirmed dengue by 77% in treatment area populations compared to control area populations. Although population replacement obviously did not eradicate the population of *Ae. aegypti* in the area, it reduced the risk of dengue transmission.

IIT through genetic modification: Genetic modification-based IIT may be used to interrupt the production of a generation in *Ae. aegypti* mosquitoes. The most prominent example of this IIT approach is Release of Insects carrying a Dominant Lethal gene (RIDL). As described by Alphey et al. (2013), RIDL is a genetics-based tool that inserts a dominant lethal transgene that can be artificially repressed—for example, by rearing larvae in the presence of tetracycline—to allow for laboratory colonization, but when allowed to express naturally (i.e., without tetracycline) induces mortality in offspring. For example, when RIDL-modified male or female *Ae. aegypti* mate with wild conspecifics, their offspring inherit the dominant lethal allele, and though eggs develop and eclose, immatures did not survive past the L4 larval stage. However, a new modification of the RIDL technology allows only males to survive to the adult stage without added tetracycline (Spinner et al. 2022). This can be an advantage over traditional SIT—which would not lead to the production of larvae because eggs are inviable—in populations where larval density in immature habitat is a key limiting factor (Alphey et al. 2008).

Another form of IIT that uses genetic modification is the introduction of transgenes into a population that promote the production of homing endonucleases that lead to population elimination by destroying female chromosomes. For example, the ribosomal DNA repeat sequences found on the X chromosome can be targeted by the homing endonuclease *I-PpoI* from

Anopheles gambiae Giles, and, by combining it with a *b2t* promoter and expressed in the testes, all sperm carrying an X chromosome are destroyed, leading to extremely male-biased progeny (Galizi et al. 2014).

Pros and cons of IIT: Similar to SIT, implementing IIT allows specific targeting of a mosquito species such as *Ae. aegypti* with no impact on nontargets. However, unlike SIT, IIT can be developed to induce mortality in a specific life stage, as seen with RIDL, which targets late-stage larvae, allowing larval competition among conspecifics (Phuc et al. 2007, Alphey et al. 2013), or as seen with *Wolbachia*, which can target adult survivorship to allow procreation supporting population replacement with reduced vector competence (Hoffmann et al. 2011), and it can disrupt or limit the development of pathogens commonly transmitted by mosquitoes (Moraireira et al. 2009).

Some disadvantages to the use of IIT include the difficulty of winning public support and obtaining regulatory agency licensing for genetically modified organisms or introduced novel microbiota (Alphey et al. 2002, Mains et al. 2016). Additionally, the relatively higher complexity of IIT modifications to colony-reared mosquitoes compared to those in an SIT program may require commercial production at distant facilities that do not incorporate strains local to the intervention area. Nonlocal laboratory strains may not express the same feeding, mating, and resting behaviors as the local population, thus compromising their competitiveness (Carvalho et al. 2022). Furthermore, transport of adult IIT mosquitoes from a centralized production location to distant release locations may compromise survivorship and competitiveness (Sasmita et al. 2021).

A BRIEF HISTORY OF SIT AND IIT CONTROL OF *AE. AEGYPTI*

Early investigations and applications: *The 1960s and 1970s:* The earliest recorded effort to apply SIT to control local populations of *Ae. aegypti* was conducted by Morlan et al. (1962), when they irradiated colonized male *Ae. aegypti* with 11,000–18,000 roentgen (equal to 96–158 Gy) from a cobalt 60 gamma source and released them in Pensacola, FL, in 1960. In 1961 Morlan et al. (1963) repeated the experiment, this time with a locally colonized *Ae. aegypti* strain. Unfortunately, both attempts failed to demonstrate a difference in the number of mosquitoes collected between the treatment and control sites from 1960 and 1961. It is possible that the attempts made by Morlan et al. (1962) may have been successful had they used a lower radiation exposure level, given that a dose of 50 Gy returned 99% sterile but more competitive males (i.e., longer survival rates) than those irradiated at higher doses (Chen et al. 2023).

Following the failed attempt by Morlan et al. (1962), Fay and Craig (1969) released *Ae. aegypti* genetically marked with autosomal variants in Meridian, MS, to investigate dispersal of released males and the

acceptance of released males by wild females. Similar additional studies were conducted by Bond et al. (1970) and Hausermann et al. (1971) to investigate dispersal of genetically marked male and female *Ae. aegypti*. These studies were important in understanding *Ae. aegypti* dispersal and how to exploit their movement behavior to better release SIT mosquitoes. Bond et al. (1970) and Hausermann et al. (1971) examined the same mosquito strain in Meridian and identified socioeconomic and topographical factors in dispersal patterns of male and female *Ae. aegypti* documenting their propensity both to fly between 100 and 200 m and to disperse to the edge of the release area rather than remain at the release site.

Asman et al. (1981) reviewed early efforts to control wild *Ae. aegypti* populations using the SIT and IIT from three separate projects. The first and largest was led by the World Health Organization (WHO) and the Indian Council for Medical Research (ICMR) in New Delhi, India. The second project was led by the International Centre for Insect Physiology and Ecology (ICIPE) in Mombasa, Kenya, and the third by the USDA Agricultural Research Service laboratory in Gainesville, FL.

The ICMR program consisted of several project releases. The first release was conducted in August 1971 with colony-reared male *Ae. aegypti* carrying a dominant mutant marker expressing a *silver lyre* marking to assess whether a genetic variant strain could be successfully introduced and propagated in the field (Rai et al. 1973). Positive results from the first release supported execution of a second release later that month of the first set of heterozygous translocated male *Ae. aegypti* in an effort to control the population. Further attempts by the ICMR program evaluated chemo-sterilized males and males from additional transgenic strains for their mating competitiveness (Grover et al. 1976): In 1974, 18,000 chemosterilized males were released over six nights (3,000 per night), and chemosterilized males accounted for 90% of mates for the wild female population. However, although mating success of genetically translocated males was lower than the chemosterilized males (28–57%), males with a population distorter gene had higher mating success (46–155%) than only genetically translocated males. Overall, males with a population distorter gene and those males that were chemosterilized were as competitive with respect to mating as wild males, unlike the genetically translocated males.

The IIT program initiated by ICIPE in Kenya engineered *Ae. aegypti* to carry a heritable genetic translocation mechanism induced by radiation exposure and maintained by crossing to a multiple marker stock which yielded a fertility rate of 25% (McDonald et al. 1977). Several releases were carried out across three villages totaling nearly 64,000 pupae, resulting in a dramatic drop in fertility rates of eggs from 97% to 36% in one village. Lorimer et al. (1976) released *Ae. aegypti* with a T3–sex-linked translocation homozygote on chromosomes 1 and 3 induced from a population originating from New Delhi, India, for population replacement in Kenya in 1974–75 at the same locations

investigated by McDonald et al. (1977). Lorimer et al. (1976) demonstrated released female populations of *Ae. aegypti* T3 had a significantly lower egg hatch rate (75%) versus the wild-type females (93%), and that the T3 females preferred to oviposit in ovitraps placed to monitor oviposition rather than within the clay water storage pots in homes in the intervention area. However, the T3 homozygote compared unfavorably with the wild-type *Ae. aegypti* in several measures of fitness and was unsuccessful in replacing the local population and in establishing itself in a village without wild-type *Ae. aegypti* (Lorimer et al. 1976).

The two investigations conducted by the USDA in Florida tested competitiveness in the field of male *Ae. aegypti* carrying a T1:2–26–male-linked translocation carrying markers for *red eye* (*re*), *spot* (*s*), and *black tarsi* (*blt*) on chromosomes 1, 2, and 3, respectively. The first trial was conducted in July 1972 on an island artificially populated with the VOYLE strain, a wild-type stock generated from hundreds of field-collected mosquitoes from Gainesville, FL (Seawright et al. 1975). The T1:2–26 *Ae. aegypti* strain was found to be competitive against VOYLE males in mating, indicated by the reduction fertility in VOYLE females from 96.8% to 36.2% (Seawright et al. 1975). The second trial also used males carrying a single or double genetic translocation released in a cypress/pine forest 10 km north of Gainesville, FL against VOYLE *Ae. aegypti* (Seawright et al. 1976). The latter trial yielded similar results to Seawright et al. (1975), indicating that males carrying genetic translocations could be competitive against a form of wild-type males and reduce fertility in a form of wild-type females.

Efforts to control Ae. aegypti using SIT and/or IIT from 2010 to the present: Following an approximately 35-yr gap, efforts resumed starting in 2010 to attempt to control *Ae. aegypti* using SIT and/or IIT approaches. A RIDL-based IIT investigation was conducted on Grand Cayman Island in 2010 with transgenic OX513A *Ae. aegypti* developed by Oxitec (Oxitec Ltd, Abingdon, United Kingdom). Approximately 3.3 million transgenic male mosquitoes were released over 56 releases and reduced the *Ae. aegypti* population in the intervention area by 80% compared to the control plot. Following the positive results from the investigation on Grand Cayman Island, Oxitec released RIDL *Ae. aegypti* (OX513A) in Brazil, Malaysia, Panama, and India (Table 2).

A new version of *Ae. aegypti* OX513A was later developed, termed the “friendlyTM mosquito,” *Ae. aegypti* OX5034, that unlike its predecessor was designed to mate with wild female cohorts allowing only male offspring surviving to adulthood (Spinner et al. 2022). The original OX513A strain males could not produce offspring that survive to the adult stage unless tetracycline was added to the water to rescue the larvae (Berube 2020, Spinner et al. 2022). In 2021 Oxitec cooperated with the Florida Keys Mosquito Control District to release *Ae. aegypti* OX5034 to evaluate its efficacy and public reception (Micu 2020); however, the results have not yet been published.

The World Mosquito Program (WMP; formerly Eliminate Dengue: Our Problem) organized a release of *Ae. aegypti* infected with a novel *Wolbachia* endosymbiont in Yorkey's Knob and Gordonville, Australia, in 2011 for control of dengue transmission by *Ae. aegypti* through population replacement as described earlier (Table 2), (Moreira 2019). Colonized male and female *Ae. aegypti* infected with *Wolbachia* wMel, isolated from *Drosophila melanogaster* and transferred into embryos of *Ae. aegypti* (Walker et al. 2011), were released to mate with wild cohorts and drive the endosymbiont into the local population by exploiting CI. Their efforts were successful and led to 100% and 90% population replacement in Yorkey's Knob and Gordonville, respectively (Hoffman et al. 2011). These successes spurred the World Mosquito Program to develop additional novel *Wolbachia* infection lines in *Ae. aegypti* to drive pathogen-refractory *Ae. aegypti* into more communities to reduce disease transmission. The World Mosquito Program has collaborated with organizations and governments for additional releases in Australia, Vietnam, Indonesia, Colombia, Vanuatu, Mexico, Fiji, New Caledonia, Kiribati, and Sri Lanka and plans to release in Laos (Table 2; WMP 2021, 2022a–j). Overall, their ongoing efforts have resulted in effective population replacement followed by disease suppression (Table 2; WMP 2021, 2022a–j).

Efforts by additional groups continue to develop IIT approaches to protect public health through suppression of *Ae. aegypti*. MosquitoMate (MosquitoMate, Inc., Lexington, KY) partnered with Verily (South San Francisco, CA) in 2017 to develop a *Wolbachia* wAlbB-infected *Ae. aegypti* strain for population suppression in Fresno, CA. Twenty million colony-reared *Wolbachia*-infected male *Ae. aegypti* were released, leading to a 68% reduction in wild-type *Ae. aegypti* (Table 2; Gilbert and Melton 2018). The trial was repeated in 2018 with nearly 14 million males released, leading to a 95.5% reduction in wild-type female *Ae. aegypti* (Table 2; Crawford et al. 2020). The MosquitoMate technique experienced additional successes in Miami, FL (Table 2; Mains et al. 2019), Key West, FL (Schairer et al. 2021), and Houston, TX (Table 2; Lozano et al. 2022).

A new SIT approach called boosted SIT, or bSIT, discussed further below, was developed for a 2016 *Ae. aegypti* control effort in Chachoengsao Province, Thailand (Table 2; Kittayapong et al. 2019). Briefly, the bSIT approach in this investigation consisted of releasing 437,980 males treated with a combination of gamma radiation and *Wolbachia* wAlbA and wAlbB over 24 wk, which led to a 97.3% reduction in the wild population (Table 2). Additional variations of bSIT-treated males targeting *Ae. aegypti* were developed and deployed in Yucatan, Mexico, as well as Singapore (Table 2; Ng 2021, Martín-Park et al. 2022), both projects returning successful vector control or reduction in pathogen transmission.

RECENT ADVANCES IN SUPPORTING TECHNOLOGIES FOR SIT AND IIT

Mass production technology: Both SIT and IIT depend on extensive production of quality incompatible or sterile males, and sometimes females, to be released in intervention areas. In the past, mosquito colony production demanded vast amounts of lab space and water (Morlan et al. 1963). However, the IAEA developed a protocol (Maiga et al. 2020b) and a larval-rearing apparatus to generate large numbers of mosquitoes in less space (Mamai et al. 2020). Each IAEA larval-rearing apparatus occupies 0.6 m² and can rear approximately 300,000 ± 20,000 male mosquitoes across 5 d of pupation (Mamai et al. 2020). By contrast, an equivalent number of mosquitoes reared in a standard laboratory colony would require more than 200 41 cm × 30 cm × 8 cm plastic trays, each with 3.0 liters of deionized water and 5 m² of floor space (Balestrino et al. 2014).

Sexing technology: A significant and heavily targeted bottleneck of SIT and IIT programs is separation of males from females. Traditional separation techniques exploit the sexual dimorphism of *Aedes* spp. pupae to separate them into small (male) and large (female) cohorts using a variety of mechanisms. One separation instrument designed by Sharma et al. (1972) that consisted of square holes of a fixed width of 1,400 μm was used by Bellini et al. (2013) to separate *Aedes albopictus* at 24–30 h after pupation. Male *Ae. aegypti* have been separated by size using a device with adjustable louvered slits of aluminum sheets to allow only pupae of a certain size or smaller to pass through (McCray 1961). Another design consists of flat plate glass panels that create an adjustable wedge-shaped space capturing smaller male pupae in the narrow portion and larger female pupae in the wide portion (Fay and Morlan 1959). Importantly, variables such as larval-rearing density, temperature, or food can affect pupal cephalothorax size, making it critical to follow strict culture conditions so a given device can consistently separate males (Papathanos et al. 2009).

Sex separation of colony-reared mosquitoes has also been achieved by exploiting adult behavior. For example, adult female mosquitoes can be eliminated from a cohort by being presented a toxic (or “spiked”) blood-meal containing boric acid or ivermectin that males will not be able to consume (Yamada et al. 2013, Gunathilaka et al. 2019). However, separation at the adult stage using this method may be compromised if pupae are irradiated because several strains of irradiated female *Ae. aegypti* pupae do not readily blood feed (Aldridge et al. 2020, Cunningham et al. 2020, Chen et al. 2023), although other strains may have increased blood-feeding behavior following irradiation (Moretti et al. 2022).

Computer techniques for discrimination between male and female pupae could be applied to sex-specific dimorphism separation methods (Zacarés et al. 2018). However, combining computer supported sorting with genetically modified strains shows enormous

potential in production of male mosquitoes for SIT and IIT programs. For example, generation of transgenic lines containing a fluorescent protein marker linked to sex chromosomes as demonstrated by Catteruccia et al. (2005) has allowed separation of male and females visually at any stage: first instar *An. gambiae* larvae with a sex-linked green fluorescent protein have been sorted by sex with 100% accuracy using the complex parametric analyzer and sorter system with a flow cytometry machine, abbreviated as COPAS (Marois et al. 2012).

Genetic sorting systems have also been developed to produce strains of female-specific flightless adults that allow flight-capable males to be automatically separated from females (Fu et al. 2010). This technique was used by Labbé et al. (2012) to generate flightless *Ae. albopictus* and by O'Leary and Adelman (2020) to generate flightless *Ae. aegypti*.

Drug-induced separation of transgenic mosquitoes is another method of sex separation that we mentioned earlier: the RIDL technique (Thomas et al. 2000). Here we go into greater detail. A successful example of this technique is described by Phuc et al. (2007) where *Ae. aegypti* are engineered to carry a dominant, repressible, non-sex-specific, late-acting, and heritable lethal genetic system, together with an Act5C-DsRed2 fluorescent marker. If reared in the presence of 30 µg/ml tetracycline the lethal gene is repressed; tetracycline therefore acts as an "antidote" or repressor of the lethal system to allow the RIDL strain to be reared under defined conditions (Massonet-Bruneel et al. 2013) so that they can be released and mate with wild types and produce offspring that will develop without tetracycline in the wild and die in the larval stage. In the wild, tetracycline does not occur naturally and therefore would not rescue the developing larvae from the expression of the lethal gene. More detailed information on sex sorting technologies and techniques is provided in reviews by Gilles et al. (2014) and Papathanos et al. (2018).

Drone dispersal: Uncrewed aircraft systems (UAS) or drones have already been operationalized by mosquito and vector control districts for mosquito surveillance and applications of adulticide space sprays and solid and liquid larvicides (Aragao et al. 2020, Carrasco-Escobar et al. 2022). These devices have recently been investigated and are beginning to be operationalized for dispersal of mosquitoes for SIT and IIT programs (Bouyer et al. 2020) for both areawide and pinpoint (or "hotspot") targeting (Garcia et al. 2022). SIT or IIT mosquitoes may be compressed into a dense puck, allowing a greater payload for release (Culbert et al. 2017). Drone dispersal of SIT and IIT mosquitoes overcomes a major barrier to ground-level dispersal of *Ae. aegypti*, a species that will not readily fly across roads (Russell et al. 2005, Hemme et al. 2010). Despite the many advantages of incorporating drones into SIT and IIT programs, more work needs to be done identifying, for example, optimal height for releases of mosquitoes, optimal rearing conditions to support the unique stressors of this method of dispersal,

and understanding effects of drift at various heights, wind speeds, and flight velocities.

Chilling and compaction: Optimization of packaging and preparation of mosquitoes prior to release can improve both survival during transport and performance upon release. *Aedes albopictus* that were chilled and compacted prior to transport showed differences in mating performance and survivorship: chilling to 5°C or 10°C for >3 h resulted in lowered competitive performance against wild males, and males chilled for >3 h demonstrated lower levels of glucose than those chilled for <3 h (Zhang et al. 2020). One strategy to mitigate the impact of chilling, compaction, and transportation is to provide a sugar meal prior to these processes (Bellini et al. 2014).

Hormesis: Hormesis is a biological dose-response phenomenon in which exposure to a small quantity of a stressor improves subsequent performance, whereas exposure to larger quantities of the same stressor will decrease subsequent performance (i.e., low dose stimulation, high dose inhibition; Costantini et al. 2010). Furthermore, mild exposure to one stressor can induce greater protection to subsequent exposure of other stressors, known as cross-tolerance (Boardman et al. 2011). Hormesis and cross-tolerance can be leveraged to improve males reared for irradiation in an SIT program: severe hypoxia of *Ae. aegypti* can lead to increases in longevity following radiation exposure by reducing the number of free radicals generated due to the low oxygen atmosphere, an increase in the amount of radiation needed to achieve >95% sterility, and significantly increased competitiveness compared to wild, nonirradiated males (Tussey et al. 2022). Unfortunately, Tussey et al. (2022) did not examine hormetic effects on female *Ae. aegypti*; however, we speculate that female *Ae. aegypti* would have greater median survivorship following anoxic treatment prior to irradiation due to the disposable soma hypothesis (Kirkwood 2002), where cellular resources are diverted from reproduction to survivorship in the event reproduction has been compromised as observed in the female Caribbean fruit fly *Anastrepha suspensa* (Loew) (López-Martínez and Hahn 2014), the codling moth *Cydia pomonella* (L.) (White and Hutt 1970), and the lubber grasshopper *Romalea microptera* (Palisot de Beauvois) (Hatle et al. 2008).

Boosted SIT (bSIT): SIT used in conjunction with either an enhancement of the SIT-treated organism or with another management strategy, or both, is called boosted SIT (bSIT). One example of bSIT with enhancement of the SIT organism is rearing irradiated sterile males with a probiotic diet to enhance competitiveness. Although this enhancement has not yet been applied to mosquitoes, probiotic diets that supplement gut microbiota have improved competitiveness of irradiated Tephritid fruit flies (Kyritsis et al. 2017). An example of bSIT centered on enhancing the effect of SIT-treated males with a layered management strategy is to coat males with a juvenile hormone analogue larvicide such as pyriproxyfen. Matings with wild females will hypothetically contaminate

them with the larvicide, passively disseminating the formulation to cryptic immature habitats to disrupt the growth of immature mosquitoes (Bouyer and Lefrancois 2014), which models suggest could result in rapid reduction of the population (Pleydell and Bouyer 2019).

An SIT program centered on irradiated males can also be boosted by combining it with an IIT program. For example, given that female *Ae. aegypti* have a higher sensitivity to radiation than males (Aldridge et al. 2020), Kittayapong et al. (2019) and Zheng et al. (2019) combined SIT/IIT programs by applying radiation to sterilize mosquitoes reared for an IIT program so that incidentally released females would be more likely to be sterile. Also, as the density of mosquitoes to be irradiated increases, the amount of radiation necessary to penetrate the mass and successfully sterilize males on the interior of the irradiator chamber increases, rendering males on the exterior less competitive due to a higher dose of radiation (Yamada et al. 2019). Therefore, combining IIT males (unirradiated) with SIT irradiated males in a program potentially provides a numerical buffer for sufficient competitive males (Baton et al. 2021).

Microbiota and diet: Variation in time to pupation, adult size, and longevity are factors documented to be influenced by microbiota of developing larvae (Souza et al. 2019). Mosquito larvae possessing no microbiome (axenic) reared in an axenic environment did not survive more than 5 days after hatching (Coon et al. 2014). By supplementing developing or adult mosquito diets with nutrients such as vitamins and minerals (Phasomkusolsil et al. 2017) or microbiota as seen in Mediterranean fruit flies (*Ceratitis capitata* [Wiedemann]) released for SIT (Ami et al. 2010), male performance could be optimized for SIT as mentioned earlier, providing a competitive advantage over wild-type males. Moreover, supplementing adult mosquito colony diet with a multivitamin may enhance longevity as observed in *Anopheles campestris* Reid, *An. dirus* Peyton & Harrison, and *An. sawadwongporni* Rattananarithikul & Green by Phasomkusolsil et al. (2017). Furthermore, microbiota have been demonstrated to indirectly influence vector competence by potentially influencing the *Toll* pathway tied to the innate immune system of the mosquito (Xi et al. 2008). Consequently, a notable feature of *Wolbachia* is that in some instances it can disrupt infection by viruses such as dengue, chikungunya, Zika, West Nile, and yellow fever viruses in the mosquito host as observed by Moreira et al. (2009), Van Den Hurk et al. (2012), and Dutra et al. (2016) and reviewed by Caragata et al. (2019), which is the primary motivation behind population replacement. But *Wolbachia* has also been shown to positively influence infection of West Nile virus in *Culex tarsalis* Coquillett (Dodson et al. 2014).

Improving SIT with models: Wave/pulse releases and buffer/corridor zones: Optimization to a threshold level of both the number of release sites and frequency of releases can have a mathematically profound

influence on the predictive efficacy of SIT programs for *Ae. aegypti* (Oléron Evans and Bishop 2014), due to balances among forces of recolonization of low-density mosquito areas from high-density mosquito areas and density-dependent mortality associated with large groups of released sterile males. One model suggests distribution of large single batches of sterile males into multiple smaller release sites could be beneficial, but releasing above the optimal ratio of sterile:wild males provided no benefit to control and instead leads to costly and ineffective overproduction of sterile males (Oléron Evans and Bishop 2014).

Mathematically modeled releases of mosquitoes for SIT have also identified that releasing massive numbers of sterile males within a corridor between the intervention area and an adjacent nonintervention area, while maintaining small releases within the intervention area, will mitigate immigration of wild populations that would enter the targeted intervention area (Anguelov et al. 2020).

Education/community engagement: Successful SIT or IIT programs depend on effective engagement and education of the community where the intervention will take place (Dame et al. 2009). Without community involvement SIT or IIT programs can rapidly fail because community opinion and perception can positively or negatively shape the outcome of the program. For example, a news article authored by Dr. K. S. Jayaraman in India in 1974 accused the World Health Organization (WHO) and the US government of conducting germ warfare under the guise of scientific research in vector control through SIT (Oh, New Delhi; oh, Geneva 1975). This accusation fomented political backlash by the Indian government, and local communities were no longer receptive to the SIT projects, and the WHO did not renew tenure of the Genetic Control of Mosquitoes Research Unit facility (Tomiche 1975).

Although some programs invested heavily in community involvement and engagement to win community stakeholders, there are unfortunately examples in which successful practices for community engagement in one country may not carry over to another country and lessons from the past need to be revisited. For example, Oxitec successfully employed a community engagement program prior to the release of genetically modified *Ae. aegypti* in Brazil (Capurro et al. 2016), but when releases were initially attempted in Key Haven, FL, the release program stalled because of intense public pressure and had to be revised as explained by Taylor (2020). However, this project has been restarted in the Florida Keys, approved by a majority of the precincts of Monroe County (31 out of 33) and permitted by the EPA for 2021, with additional projects approved by the EPA for Florida and California in 2022 (Waltz 2022).

The priority in an SIT or IIT program is community engagement (Moreira 2019) and should be accounted for in developing the program budget. SIT program leaders should interact with the affected population, their local institutions including health clinics and

schools, and their community leaders for disseminating accurate, transparent, and easily accessible information about the program. Transparency should be exemplified by a frank discussion of advantages and disadvantages compared to other approaches, as well as benefits, and risks, of the proposed program. Initially, surveys should be conducted to measure existing understanding of and acceptance by the local population. In addition, a community reference group should be organized: a local committee that monitors all actions conducted by the program and is the first to be informed by SIT/IIT program leaders so that communication channels established with the community, including telephone, e-mail, face-to-face interactions, and social media, can be engaged (Moreira 2019). Finally, a postrelease survey or questionnaire should be distributed to allow the community to provide feedback on the performance of the program (Capurro et al. 2016).

Traditional IVM and other optimizations prior to SIT/IIT release: Proper preparation of the target area must be part of the SIT/IIT program. For example, some programs may pretreat the target area through conventional adulticide or larvicide actions prior to initial SIT/IIT releases, to bestow released cohorts the greatest chance to numerically outcompete surviving wild-type males. Another mode of optimization is to leverage findings from long-term and ongoing mosquito surveillance by local mosquito and vector control districts so that the SIT/IIT program can be ramped up and implemented early in the anticipated season for the target species, when populations are at their lowest and the released mosquitoes can be the most numerically overwhelming. However, consideration of seasonal conditions should also inform release timing and balance findings from target population level monitoring so that colony-reared mosquitoes are not negatively stressed by the release environment. It should be emphasized that given the current capabilities and ongoing development of SIT and IIT—i.e., they are not perfected and may not be successful for all targets in all places—these biological control approaches should be integrated into the full picture of IVM and not considered a panacea to mosquito control (Pleydell and Bouyer 2019, Martín-Park and Che-Mendoza 2022).

SIT AND IIT FOR *AE. AEGYPTI* CONTROL: THE WAY FORWARD

The inadequacy of traditional chemical-based IVM control techniques targeting *Ae. aegypti* means that it is critical we continue to develop and refine novel control strategies such as SIT and IIT. Deregistration of pesticides (Rose 2001, Usta 2013), slow development of new pesticides (Roush and Tabashnik 2012), evolution of pesticide resistance (Vontas et al. 2012), loss of funding for traditional control programs (NACCHO 2017), hesitancy or lack of support from the public toward pesticide use (Piltch-Loeb et al. 2019), and the difficulty of finding, accessing, and eliminating oviposition sources (Reiter et al. 1995) are a constellation of factors

that will limit the role of traditional IVM in long-term sustainable control of *Ae. aegypti* populations. However, the increasing attention focused on SIT and IIT as a potential alternative or supplement to traditional IVM may steer more funding and research towards improving the techniques and expanding their operational use. In 2020 a joint commission by the IAEA and WHO generated a comprehensive guidance framework for preparing a complete SIT program and evaluating its performance for suppression of *Aedes*-borne diseases (WHO and IAEA 2020).

The 2010 revival of investigations and operations focused on SIT and IIT for the control of *Ae. aegypti* is very timely because of new technology and a public receptive to alternatives to traditional chemical control (Alphey et al. 2008, 2010). Public support can be better developed by including specific budgets for coordinated outreach and education in SIT programs (Alphey et al. 2010). Better quality radiation doses are now possible because of more efficient irradiators and calibration equipment (Klassen and Curtis 2005). Capabilities for tracking and monitoring *Ae. aegypti* populations and dispersal of released males in the intervention area may now be greatly enhanced with GPS and GIS technology (Klassen and Curtis 2005). Also, radiation-based SIT releases can be enhanced by combining with IIT-based strains of *Ae. aegypti* designed to reduce disease transmission and/or populations of the vector (Alphey et al. 2010). In addition to these new capabilities, a suite of innovations—some of which have yet to be adapted to target *Ae. aegypti*—is positioned to further optimize SIT and IIT approaches to the point of widespread operational implementation.

CONCLUSIONS

Aedes aegypti is a prominent worldwide disease vector threat that consistently eludes formidable efforts by public health vector control agencies because of the difficulty of aligning expected pesticide-based control efficacy with the unique bionomics and evolving resistance of this species. Fortunately, biological control techniques such as SIT and IIT can be used in place of or alongside pesticides and/or other traditional IVM techniques to significantly improve *Ae. aegypti* control programs. Both SIT and IIT are species-specific, exploiting conspecifics to seek out females, and so may overcome the limitations of pesticide applications that attempt to target *Ae. aegypti* in cryptic habitat or resistant populations. Release of mosquitoes sterilized by radiation does not require EPA approval and cannot be deregistered. Technological advancements continue to improve the efficacy of SIT and IIT through, for example, advancements in genetic modifications, sex sorting, movement and dispersal tracking using GIS and GPS data, refinement in radiation calibration to preserve male competitiveness, optimization of mosquito rearing conditions to increase production, performance, and survivorship, and timing releases to exploit environmental conditions that maximize performance of *Wolbachia* endosymbionts.

SUMMARY POINTS

1. Early development of radiation-based SIT was hindered by problems with radiation dose calibration and irradiation equipment malfunction.
2. SIT has been used extensively to control a variety of veterinary and medically significant vector and pest species. With continued development, we hypothesize that additional species such as *Ae. aegypti* can be controlled.
3. Bionomics, resistance, and standard IVM challenges to *Ae. aegypti* control may be mitigated with the SIT; however, technological and scientific breakthroughs are needed for 1) more efficient, consistent, and benign sterilization of *Ae. aegypti* males, 2) better tracking of dispersal and survival of released sterile males, and 3) more precise monitoring of target population dynamics, which includes detecting immigration from adjacent or disparate locations.

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