Innovative Techniques for Control of *Aedes aegypti* and *Aedes albopictus* Mosquitoes

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Introduction

Aedes aegypti and *Aedes albopictus* are vectors of mosquito-borne diseases including Zika, dengue, chikungunya, and yellow fever. Both species are highly adapted to humans for survival, and thrive in suburban and urban environments. *Aedes aegypti* and *Aedes albopictus* pose major challenges for effective control because they lay desiccation-resistant eggs in artificial, cryptic water sources (*e.g.,* plastic containers, plant pots and saucers, tires). Current methods used for both immature and adult mosquito control have limitations, and thus several innovative control techniques are under development. These techniques are described below, and may become broadly available with continued research.

Auto-dissemination of insecticide

Auto-Dissemination Augmented by Males (ADAM): The ADAM technique deploys male *Aedes* mosquitoes as vehicles for precise dispersal of larvicides to cryptic larval development sites in the urban and suburban environment. Laboratory-reared, insecticide-dusted males seek and mate with wild females, transferring larvicide particles via physical contact. Contaminated females deposit larvicides in water-holding containers as they lay eggs. Males can also introduce larvicide when they visit small sources of standing water. The chosen pesticide must be active in small doses, and its effect on males must be minimal to not hinder their mating with females or negatively impact their survival. ADAM trials in the United States (US) and other countries have shown promise using the insect growth regulators pyriproxyfen and (S)-methoprene, which are effective by preventing larval development.

In2Care trap: The In2Care trap has been provisionally licensed by the US Environmental Protection Agency (EPA) for use in areas with local Zika virus transmission. This trap is not designed to aid in mosquito surveillance; instead, it is a tool for *Aedes aegypti* and *Aedes albopictus* population reduction through auto-dissemination of larvicides. In2Care traps are composed of a container of water with an inner floating structure that allows females to rest and oviposit (lay eggs). This structure carries gauze impregnated with the insect growth regulator pyriproxyfen and spores of *Beauveria bassiana*, a fungus that slowly kills adult mosquitoes. Resting or ovipositing females pick up both agents on their bodies. In subsequent ovipositions or when touching her legs to water, a “self-treated” female contaminates the water with pyriproxyfen, which will prevent her offspring and any other mosquito larvae already in the water from maturing to adulthood. Pyriproxyfen particles adhere to the female as she flies out of the trap and are carried as she continues to contaminate oviposition sites within her flight range. The In2Care trap takes advantage of female mosquitoes' behavior to seek out cryptic breeding sites. Over a few days, *B*. *bassiana* spores on contaminated females will germinate and kill the female mosquito.

Wolbachia-mediated techniques

Wolbachia are intracellular bacterial symbionts of many invertebrates. When artificially introduced into *Aedes aegypti* or *Aedes albopictus* populations, select strains of *Wolbachia* bacteria can either disrupt mosquito reproduction or reduce vectorial capacity, which are the principle objectives of Incompatible Insect Technique (IIT) and population replacement strategies, respectively. Both strategies require releases of large numbers of laboratory-reared, *Wolbachia*-infected mosquitoes into target areas.

In IIT, *Wolbachia*-infected males are released to mate with females of the wild population. Wild females that mate with *Wolbachia*-infected males are unable to produce viable offspring (due to incompatability), thus interrupting the production of the next generation of mosquitoes. In this manner, IIT aims to reduce and eliminate *Aedes aegypti* or *Aedes albopictus* populations. This technique is self-limiting and requires repeated releases of IIT males for continued population reduction. The exclusion of females from releases is critical. If *Wolbachia*-infected females are released alongside males due to sorting error, a viable population of infected mosquitoes can become established, thus defeating the population reduction goal of IIT. As a safeguard, IIT mosquitoes can be treated prior to release with low-level radiation that sterilizes any female mosquitoes accidentally included in release cohorts. The treatment ensures that any laboratory-reared females released into the environment will not contribute to *Aedes* populations in the wild.

In contrast, population replacement strategies aim to replace wild, uninfected populations of *Aedes aegypti* with *Wolbachia*-infected males and females. This *Wolbachia* infection blocks arboviral infection in females, thereby reducing their ability to transmit viruses such as dengue and chikungunya. *Wolbachia*-infected females have a reproductive advantage over uninfected females, because they can mate with both uninfected and infected males in an existing population and still produce viable offspring. An uninfected female can only mate with an uninfected male to produce offspring. This 2:1 reproductive advantage allows the *Wolbachia* infection to spread into an uninfected population. Once sufficient numbers of *Wolbachia*-infected males and females have been released, they will establish themselves and replace the wild population. Additional releases are typically not needed. The objective of this strategy is to halt virus transmission by *Aedes aegypti* within the release area rather than reduce mosquito abundance.

Field trials of IIT have been conducted within the US, and IIT and population replacement have been implemented in other parts of the world. Trials have shown promise in combating *Aedes aegypti* and *Aedes albopictus* and their associated pathogens, such as dengue virus.

Sterile insect technique (SIT)

In mosquito control, SIT traditionally refers to the release of large numbers of radiation-sterilized males to overwhelm and outcompete wild males for mates in a target area. Potential drawbacks to this method include (1) diminished competitiveness of released males against wild males, (2) a proportion of radiation-treated males may retain a residual level of fertility upon release, and (3) some released males may recover fertility over time. For these reasons, SIT is currently used in combination with IIT to minimize the potential for these undesirables.

Genetically modified mosquitoes

Advances in genetic techniques have allowed Oxitec Ltd. to produce a strain of *Aedes aegypti* carrying a dominant lethal gene. Males carrying this gene are released to mate with wild females; however, the lethal gene causes their offspring to die during development. The technique, known as Release of Insects carrying a Dominant Lethal (RIDL), requires the dominant lethal gene to be (1) inactivated during mass rearing of RIDL mosquitoes in the laboratory through the use of tetracycline, (2) activated in offspring in the environment, and (3) not hinder the ability of released males to compete for and mate with wild females. As all mosquito offspring carrying the gene fail to reach adulthood, this technique is self-limiting and requires repeated releases of RIDL males for continued population reduction.

Population reduction via *Aedes*-targeted traps (trapping out)

Several *Aedes*-specific traps such as the Biogents Gravid *Aedes* Trap (BG-GAT), CDC-Autocidal Gravid Ovitrap (CDC-AGO), and Springstar Trap-N-Kill are designed to reduce mosquito populations by attracting and killing ovipositing females. These traps take advantage of *Aedes* oviposition behavior by providing dark, enclosed water sources attractive to gravid females. The inside surfaces of BG-GAT and CDC-AGO traps are treated with an adhesive coating to capture mosquitoes when they rest; mosquitoes entering a Springstar Trap-N-Kill are exposed to an insecticide-treated velour strip that provides rapid knock-down. This method of population reduction is effective when many traps are deployed per parcel in a mosquito-infested area, and is most effective in conjunction with elimination of other water sources in the environment.