Oxitec's Vector Control Solution

A Paradigm Shift in Mosquito Control

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Executive Summary

Effective vector control, and more specifically mosquito control, is a complex and difficult problem as illustrated by the continuing prevalence and spread of mosquito-transmitted diseases globally.

Through the responsible engineering of biology Oxitec has developed a paradigm shift in mosquito control leading to unparalleled levels in the suppression of *Aedes aegypti,* the main vector for several of the world's most damaging viruses including Zika, dengue, and chikungunya. Oxitec's revolutionary and environmentally friendly OX513A male mosquitoes are precisely engineered with a self-limiting gene that expresses a non-toxic and non-allergenic protein to prevent their offspring from surviving to adulthood. In five separate efficacy trials across three different countries, releases of Oxitec OX513A mosquitoes led to a greater than 90% reduction in the local *Aedes aegypti* populations.

A Growing Global Threat from an Invasive Mosquito Species

Difficult diseases attributable to arboviruses transmitted by the *Aedes* mosquito vector, principally *Aedes aegypti,* pose an increasing global threat to human health. *Aedes aegypti* is an invasive species, or a non-native species that causes ecological or economic harm in a new environment, in well over 100 countries. Its passage from West Africa to other continents occurred as a result of early trade between the Old and New Worlds. More recently, the rising trend of globalization coinciding with the halt of *Aedes aegypti* eradication programs roughly four decades ago has led to significant expansion of its territory.

Today worldwide distribution of *Aedes aegypti* extends to all of the continents except Antarctica making it one of the most widespread species globally. It is estimated up to half of the world's population live alongside these dangerous mosquitoes.[1] Globalization has benefitted their success in a number of ways including increasing urban populations as well as climate change expanding their potential habitats. Most relevant to the spread of disease, however, has been the development of modern transportation and means of travel and trade from one country to another.[2]

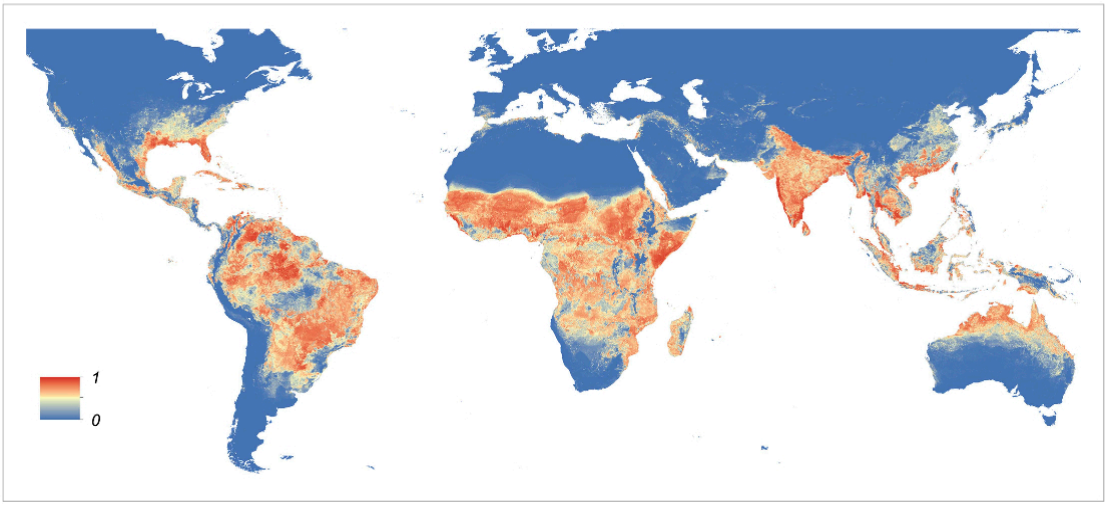


Figure 1. Global map of the predicted distribution of *Ae. aegypti.* The map depicts the probability of occurrence (from 0 blue to 1 red) at a spatial resolution of 5 km × 5 km.[2]

Aedes aegypti – Preference for Human Blood; Numerous Challenges

The *Aedes aegypti* species is anthropophilic and its females' preference for biting humans to secure blood necessary to produce fertile eggs has led to breeding in and around our homes, office buildings, and schools. The domestication of these "container breeders" has made them extremely difficult to control. Among the many challenges associated with *Aedes aegypti* are:

- Zika, dengue and chikungunya viruses are carried and transmitted to humans through female *Aedes aegypti* leading to widespread diseases with no cures.[1]
- Females are aggressive, repeat day-biters increasing the potential spread of diseases.[1]
- Females can be born carrying viruses and therefore have an innate ability to transmit disease.[3-5]
- Females lay 100 to 200 eggs per batch and can produce up to five batches during their two to four week lifetime.[6]
- Females will not lay an entire batch at one site, but rather spread eggs over two or more sites.[7]
- Eggs can stay dormant for over six months and hatch at any time creating issues for conventional vector control approaches.[8]
- Larvae have been found in a host of artificial containers including discarded bottle caps, soda cans, cups and tires creating issues for conventional vector control approaches.[1]
- Estimated annual costs of diseases *Aedes aegypti* transmits continue to rise. Excluding chikungunya and Zika, which the World Bank estimates will have an economic impact of $3.5 billion in Latin America in 2016, the estimated cost for dengue exceeded $39 billion in 2011. [9,10]

In addition, the repeated use of chemical insecticides is leading to rising resistance in *Aedes aegypti* worldwide creating operational challenges for mosquito control programs.[11] Commonly used insecticides including organophosphates and pyrethroids are no longer effective in many areas. There has also been documented resistance in *Aedes aegypti* mosquitoes to the once widely-used DDT (dichlorodiphenyltrichloroethane), a powerful organochloride insecticide.[12]

This insecticide resistance is due to specific genetic traits which enable detoxification of insecticides allowing for the survival of wild mutant mosquitoes which 'naturally' have this genetic code. This genetic programming persists in the environment in subsequent generations of mosquitoes.

Aside from this emerging resistance, many insecticides such as DDT have been banned due to poor environmental safety records.

Fast Growing and Increasingly Challenging Vector-Borne Diseases

Mosquitoes that transmit disease represent only a small fraction of the roughly 3500 mosquito species, yet they render this group of insects the deadliest animal family in the world.[13] Over one million people die each year from the bite of a female mosquito.

After its entry into the Americas from Africa, the invasive *Aedes aegypti* mosquito caused deadly yellow fever epidemics as far north as Philadelphia, Pennsylvania, and as far south as Buenos Aires, Argentina. Yellow fever is now mainly found in tropical and subtropical areas in South America and Africa with an estimated 200,000 cases each year causing 30,000 deaths.[14] While a recent resurgence of yellow fever in Latin America and Africa has led to concerns and presents a potential serious health risk due to large population centers in these areas, this virus, for now, finds itself fourth on the list of dangerous arboviruses transmitted by *Aedes aegypti*.[15,16]

Today, females of the *Aedes aegypti* species are the primary vector for three of the most damaging arboviruses to public health and economies of nations:

1. **Dengue virus is the fastest growing vector-borne disease today. According to the Centers for Disease Control and Prevention (CDC), there are an estimated 400 million dengue infections each year in over 125 countries.[17]**

Dengue fever is caused by any one of four closely related dengue viruses (DENV) or serotypes: DEN-1, DEN-2, DEN-3 and DEN-4. DENV are single-stranded RNA viruses that belong to the genus Flavivirus.[18] As many as 50% of dengue infected individuals are asymptomatic and have no clinical signs or symptoms of disease. The spectrum of illness can range from a mild non-specific febrile syndrome, to classic dengue fever (DF), to the severe forms of the disease dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS).[19]

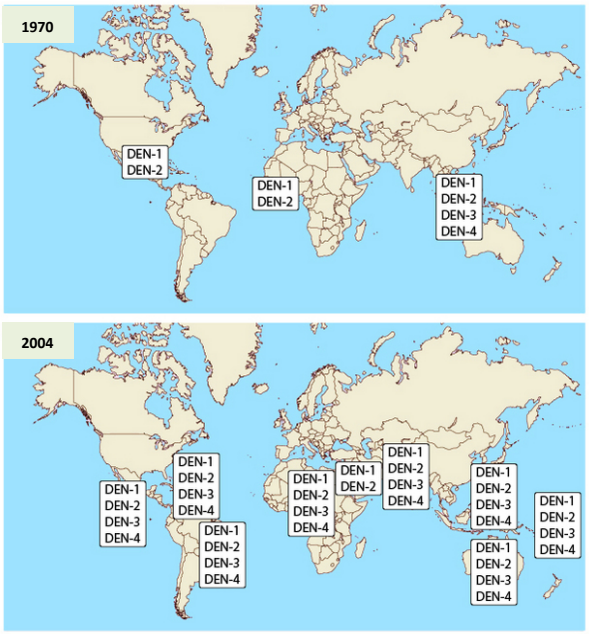
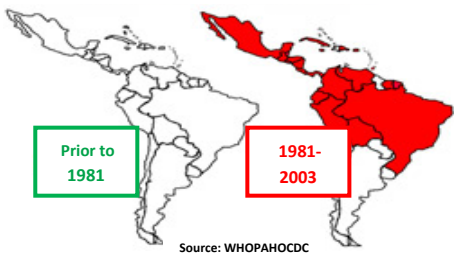


Figure 2. The change in distribution of dengue serotypes between 1970 and 2004[18]

In addition to these well-known manifestations of dengue virus, there are a wide range of atypical multi-systemic manifestations including neurological disorders such as Guillain-Barré syndrome, meningoencephalitis, encephalitis, and encephalopathy, as well as presentations of gastrointestinal, lymphoreticular, cardiovascular, renal, and musculoskeletal conditions.[20-23]

Recovery from infection by one serotype of dengue provides lifelong immunity against it, yet cross-immunity to the other serotypes is partial and temporary. Subsequent infections by other serotypes increase the risk of developing severe dengue due to antibody-dependent enhancement, a known phenomenon in which neutralizing antiviral proteins facilitate virus entry into host cells, leading to increased infectivity and a more severe clinical infection.[24]

Figure 3. Laboratory-Confirmed DHF in the Americas Prior to 1981 versus 1981-2003



Before 1970 only nine countries experienced severe dengue epidemics and they were largely confined to Southeast Asia. This was likely due to the success of the Pan American Health Organization's *Aedes aegypti* eradication program that started in the 1950s which protected the Americas from serious DENV epidemics. Discontinuation of this eradication program in the 1970s allowed the Americas to become re-infested with *Aedes aegypti* mosquitoes, and large numbers of DHF cases began to appear in the Caribbean and Latin America in 1981 (Figure 3).[25]

Severe dengue is now endemic in more than 100 countries in regions of Africa, the Americas, the Eastern Mediterranean, South-East Asia and the Western Pacific. The number of DHF cases has climbed to roughly 500,000 annually resulting in an estimated 22,000 deaths. Severe dengue has become a leading cause of hospitalization and death among children in Asian and Latin American countries.[26]

2. **Zika virus has recently emerged as one of the most challenging threats to human health given its apparent links to microcephaly among infants born to infected women and neurological disorders such as Guillain-Barré syndrome (GBS).[27-29] Since the beginning of 2015, Zika has spread rapidly through the Americas and active transmission of the virus is now present in over thirty countries and territories.[30,31]**

Figure 4. Countries and Territories in the Americas with Active Zika Virus Transmission[30]

Like dengue, Zika (ZIKAV) is a single-stranded RNA virus that belongs to the genus Flavivirus.[32] As many as 80% of Zika infected individuals are asymptomatic and have no clinical signs or symptoms of disease. The most common symptoms of Zika are fever, rash, joint pain, and conjunctivitis, and the illness is usually mild lasting up to a week. These minor reactions had incorrectly given a false sense that Zika was of least concern with respect to the viral diseases spread by *Aedes aegypti*. In 2015 that perception drastically changed.

Just seven months after an initial epidemiological alert in May 2015 that public health authorities in Brazil were investigating a possible autochthonous transmission of the Zika virus, a second epidemiological alert was issued in December by the Pan American Health Organization / World Health Organization (PAHO/WHO).[33] This alert was issued given the increase of congenital anomalies such as microcephaly, GBS, and other neurological and autoimmune syndromes in areas where Zika virus was circulating and their possible relation to the virus. Within just one year of its first reported autochthonous transmission, Brazil is estimated to have between 400,000 to 1,300,000 cases of Zika and over 4,000 suspected cases of microcephaly.[34,35]

In addition to birth defects and neurological disorders, Zika has been associated with meningoencephalitis, a dangerous inflammation of the membranes that surround the brain, as well as acute myelitis.[36,37]

In February 2016, Zika became the first mosquito-borne disease to be declared a Public Health Emergency of International Concern (PHEIC) by the World Health Organization (WHO). They noted the cluster of microcephaly cases and other neurological disorders such as GBS reported in Brazil, following a similar cluster in French Polynesia in 2014, and urged aggressive implementation of vector control measures to manage the Zika outbreak.[38]

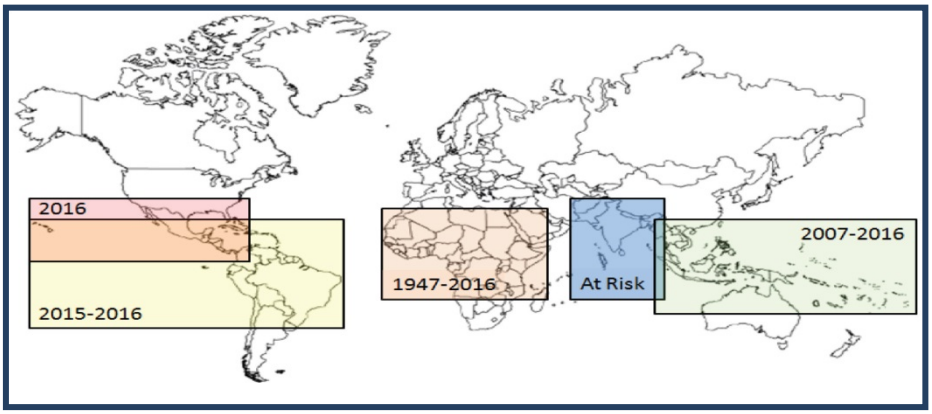


Figure 5. Global Spread of Zika Virus – Geographic zone-specific time ranges of known incidences are shown color-coded according to the year of the earliest reported case (locally-acquired cases or viral isolation)[31]

3. **Chikungunya virus results in numerous difficult disorders, from neurological to rheumatic and beyond. It swept into the Caribbean in 2013 and Central America thereafter. An epidemic soon followed with over 1,000,000 cases occurring within one year.[39] Chikungunya has been identified in over 60 countries in Asia, Africa, Europe and the Americas. Within the United States, chikungunya virus disease became a nationally notifiable condition to CDC in 2015.[40]**

Unlike dengue and Zika, chikungunya (CHIKV) is an RNA virus that belongs to the genus Alphavirus. Approximately 3% to 28% of chikungunya infected individuals are asymptomatic and have no clinical signs or symptoms of disease.[41] It causes fever and severe joint pain, and other symptoms include muscle pain, headache, nausea, fatigue and rash which typically resolve in 7–10 days. The disease shares some clinical signs with dengue, and can be misdiagnosed in areas where dengue is common.[42]

Chikungunya, like Zika and dengue, has resulted in a number of neurologic diseases such as Guillain-Barré syndrome, meningoencephalitis, myelitis, and cranial nerve palsies. Additionally CHIKV has been associated with rheumatic disorders including rheumatoid arthritis, spondyloarthritis, and undifferentiated polyarthritis.[43] Other serious complications that are rare include myocarditis, ocular disease (uveitis, retinitis), hepatitis, acute renal disease, and severe bulbous lesions.[44]

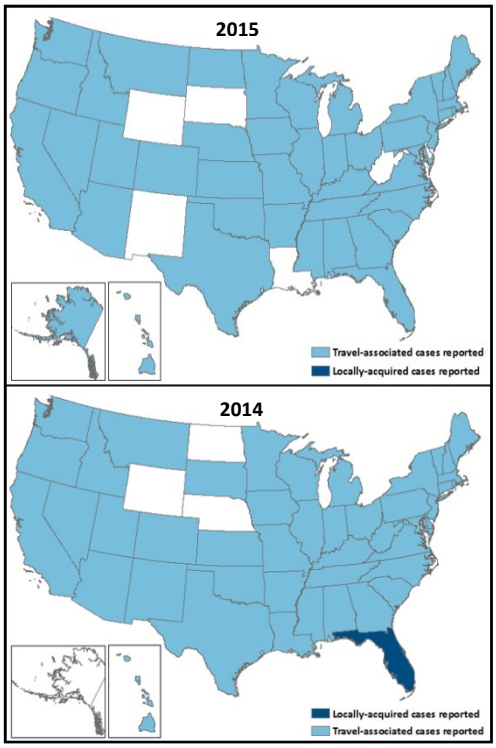


Figure 6. States in U.S. reporting travel-associated and locally acquired chikungunya cases in 2014 and 2015.[40]

Further, intrapartum transmission of CHIKV can result in complications for the baby, including neurologic disease, hemorrhagic symptoms, and myocardial disease.[45] Observations also suggest possible maternal-fetal transmission of CHIKV during early pregnancy, yet risks appear to be very low.[46] After maternal CHIKV infection, there are rare reports of spontaneous abortions.[41]

Chikungunya often causes large outbreaks with high attack rates, affecting one-third to three-quarters of the population in areas where the virus is circulating. Outbreaks of CHIKV disease have occurred in Africa, Asia, Europe, South America, islands in the Indian and Pacific Oceans, and islands in the Caribbean.[41,47]

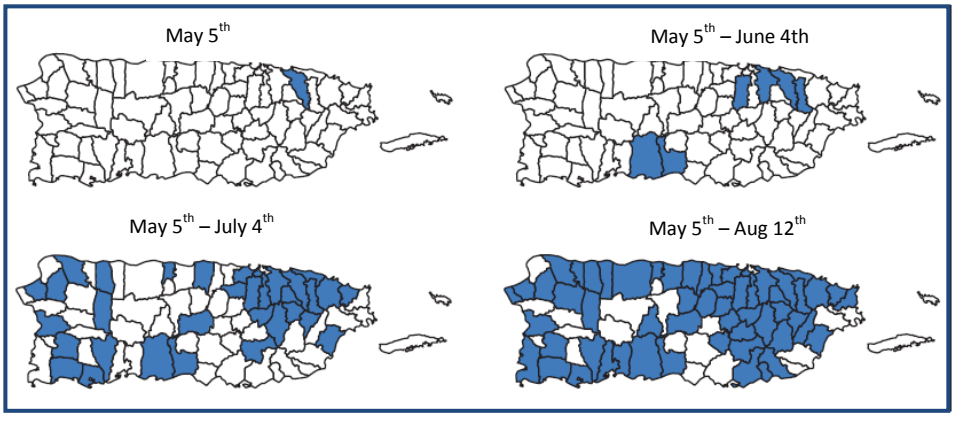


Figure 7. Puerto Rico Outbreak of CHIKV in 2014 – Geographic distribution of laboratory-positive chikungunya cases, May 5th – Aug 12th.[47]

Figure 8. Female *Aedes aegypti* is the primary vector for dengue, Zika, chikungunya and yellow fever

In summary, Zika has generated a significant amount of warranted attention since its linkage with life-changing disorders in late 2015. Yet many years of research on dengue and chikungunya provide evidence these viruses also cause a number of devastating conditions without effective clinical preventions or treatments including neurological and rheumatic disorders.

What is also evident is despite decades of research on arboviruses, we are still learning about their impact on the human system and they are all far from being fully understood. We also have yet to entirely comprehend the impact of co-infection of humans with multiple viruses, yet this is occurring.[48] It is important to note that while we continue to learn more about these viruses and the many non-curable diseases associated with them, they continue to evolve.

What is constant, however, among all of four of these devastating viruses – dengue, Zika, chikungunya and yellow fever – is their primary vector, the female *Aedes aegypti* (Figure 8).

Oxitec's Mosquito Solution: the 'Friendly Mosquito' to Humans and the Ecosystem

Oxitec has been focused on the control of *Aedes aegypti* for over a decade and has pioneered a biological method of vector control to suppress wild populations of this dangerous mosquito species. This novel approach utilizes male mosquitoes which don't bite or transmit disease and live less than a week. These 'OX513A' males search for and mate with wild *Aedes aegypti* females. The offspring of these mating events inherit a self-limiting gene and die before becoming functional adults, thereby reducing the wild population. Importantly this approach leaves no environmental footprint.

Figure 9. In Piracicaba, Brazil, 96% of local residents in CECAP/Eldorado district support the Oxitec program

In addition to OX513A not pursuing humans because males do not bite, the power of this biological solution is evident in its species-specific targeting. Of the ~3500 mosquito species known to exist today, a select few spread disease.[13] *Aedes aegypti* is one of the anthropophilic species transmitting arboviruses to humans. OX513A males control populations of this single dangerous species by mating with *Aedes aegypti* females. The remaining mosquito species, as well as beneficial insects like bees and butterflies, are not impacted by Oxitec's solution. This differs from other conventional approaches that do not discriminate which insects they kill, beneficial or non-beneficial, causing significant effect on ecosystems.

Understanding the potential hazards associated with approaches to mosquito control that change the biology of the insect and persist through subsequent generations, Oxitec intently focused on a self-limiting solution that does not carry that risk. Neither the Oxitec mosquitoes nor their progeny persist over time in the environment.[49]

Figure 10. Brazil press describe Oxitec solution as 'The Friendly Mosquito'

Another design aspect imperative to Oxitec was engineering a built-in biological marker identifiable in the ecosystem enabling superior monitoring. The ability to monitor the deployment and impact of a treatment is important for the implementation of successful vector control programs. Therefore Oxitec's mosquitoes are engineered with a fluorescent color marker, providing a means of both visual and molecular tracking for mosquitoes in the rearing facility and in the field. Through this system it is possible to collect larvae from the area of release and determine the proportion of Oxitec mosquitoes versus wild pest mosquitoes as a measure of population suppression. This built-in marker allows superior traceability, offering a precise, efficient and cost-effective monitoring tool for adaptive management programs, as well as quality control and safety assurance processes.

Efficacy trials of Oxitec's technology all resulted in *Aedes aegypti* population suppression below the threshold designated in a peer-reviewed model for dengue epidemic disease transmission.[49-52] Despite their widespread use insecticides have not achieved this level of vector suppression. Additionally OX513A can control and repress those populations of *Aedes aegypti* that carry insecticide resistance genes, which are leading to a rising tide of ineffectiveness of conventional insecticides.

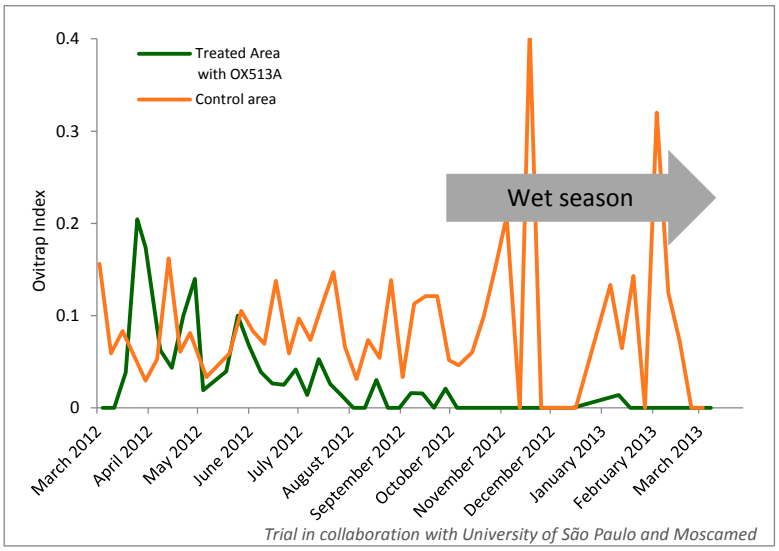


Figure 11. Oxitec's OX513A results in Mandacaru, Brazil suppressed *Aedes aegypti* by >90% and sustained control through rainy season

Furthermore with >150 million Oxitec mosquitoes released to date through use in field suppression programs, no off-target effects have been observed, no evolution of resistance to the self-limiting gene has been seen, and there has never been any assortative mating detected. The solution has been successfully applied alongside other interventions such as insecticide applications and is compatible with all other interventions. OX513A has been approved by Brazil's National Biosafety Technical Commission (CTNBio) for releases throughout the country,[53] received a preliminary finding of no significant impact from the U.S. Food and Drug Administration,[54] and also has been issued a positive recommendation for planned operational deployments by the World Health Organization.[55]

Oxitec's Precise, Regulated Method One of Several Vector Control Approaches Altering the Genetics of *Aedes aegypti*

There are several vector control methods that genetically alter the biology of mosquitoes, yet Oxitec's is the only one currently regulated as a recombinant organism. With this distinction comes a high level of science-based stringent regulatory overview for Oxitec along with a level of community inquiry through non-governmental organizations. These are welcome as safety is of the upmost importance to Oxitec.

With the protection of people in mind, regulatory review of any method to control *Aedes aegypti* that changes the genetics of these mosquitoes at the molecular level should be considered. Approaches that do not trigger the narrow regulatory definition of a genetically engineered (GE) organism frequently fall outside of regulatory examination to ensure they are safe and thoroughly evaluated before use in the field.

Oxitec's Precise, Self-limiting Approach to Control *Aedes aegypti*:

Oxitec's self-limiting approach holds significant advantages over other vector control methods that genetically alter the biology of mosquitoes.

Modification to the genome of Oxitec's engineered strain has been thoroughly characterized and is genomically stable. Evaluation over 150 generations has shown the self-limiting gene is inherited in a Mendelian fashion with no change in performance.

Furthermore, Oxitec strains have shown strong performance profiles in the assessment of mating fitness against a wild-type comparator strain.[56,57] This is consistent with the field results demonstrating OX513A males can compete for mates well enough to significantly suppress target field populations of *Aedes aegypti*.[49,50,52]

Moreover, after releases are stopped and mosquitoes die, this self-limiting vector control solution does not persist in the environment.[49] For an additional level of safety, Oxitec has engineered a biological fluorescent color marker enabling robust monitoring capabilities and measures of responsiveness built into the system.

As previously mentioned, understanding the potential hazards with approaches to mosquito control that change the biology of the insect that may persist in the gene pool through subsequent generations, Oxitec intently focused on a self-limiting solution that did not carry that risk.

Other Approaches to Mosquito Control That Cause Genetic Changes in *Aedes aegypti*:

Other approaches to *Aedes aegypti* population control lead to genetic changes at the molecular level that in some cases are random or not well defined. Despite not being regulated as GE organisms, several of these methods could lead to mutagenesis in *Aedes aegypti* that may sustain in wild populations with unknown consequences.

1. Chemical Insecticides – The repeated use of chemical insecticides is leading to rising resistance worldwide creating operational challenges for mosquito control programs.[11] Commonly used insecticides including organophosphates (i.e., temephos, malathion, fenitrothion) and pyrethroids (i.e., permethrin, bifenthrin, deltamethrin) are no longer effective for *Aedes aegypti* in many areas.[58-60] Additionally there has also been documented *Aedes aegypti* resistance to the once widely-used DDT.[12]

This resistance to chemical insecticides is due to particular genetic traits that allow for mutant mosquitoes to survive. While the underlying molecular mechanisms, including the identification of enzymes involved in insecticide detoxification, have been studied, they are not completely understood.[60] Molecular screening for common insecticide target-site mutations in *Aedes aegypti* have shown a high frequency mutation of 'knock down resistance' in the sodium channel, as well as significant elevated activities of cytochrome P450 monooxygenases, glutathione S-transferases and carboxylesterases at both larval and adult stages.[58-60]

In addition to insecticide resistance hampering conventional control and eradication efforts, these genetic modifications that arise from insecticide resistance persist in the gene pools of subsequent generations of *Aedes aegypti* mosquitoes.

2. Radiation-based Sterile Insect Technique (SIT) – Regardless of the source of radiation, X-ray or gamma, this form of SIT mosquito control may result in genetic instability of the insect through random and difficult to categorize genomic changes. This damage to, and inexact mutation of, DNA may have unpredictable effects that persist in the environment.

   The basis of radiation sterilization is the induction of random dominant lethal mutations that render males sterile. Studies on radiation impacting the biology of mosquitoes have described a marked species difference in radiation sensitivity, measurable negative effects on insect performance in the laboratory, as well as a need to radio-sterilize males as adults in order to minimize the fitness cost of the radiation.[61-63]

   Utilization of less than fully sterile insects may lead to the release of males bearing unknown genetic changes, including mutations conferring insecticide resistance, which can remain in the gene pool and continue propagating in subsequent generations.[64,65]

3. *Wolbachia* - Another vector control approach that impacts the biology of *Aedes aegypti* is the artificial insertion of *Wolbachia* into the mosquito.[66] *Wolbachia* is an intracellular bacterium that is associated with reproductive alterations of its hosts including parthenogenesis, cytoplasmic incompatibility, and feminization of genetically male hosts.[67]

   While there are numerous strains of *Wolbachia* that are naturally present in a number of insects including mosquitoes (collectively all strains infect up to 40% of insect species), none occur naturally in *Aedes aegypti*.[1,68]

   Various studies on insects and mosquitoes with *Wolbachia*, either artificially-inserted or naturally-occurring, have shown:

   - *Aedes albopictus* mosquitoes can naturally be superinfected with two *Wolbachia* strains (wAlbA, wAlbB), yet are still able to transmit chikungunya and dengue virus;[69,70]
   - *Aedes aegypti* artificially modified with the wMel strain of *Wolbachia* show a reduction in dengue virus replication but virus is still found in the saliva of these engineered mosquitoes which therefore have the capacity, even if reduced, to transmit disease;[71]
   - *Wolbachia* significantly enhances West Nile virus infection in the *Culex tarsalis* mosquito;[72]
   - *Wolbachia* enhances malaria parasite infection in *Anopheles gambiae* and *Culex pipiens* mosquitoes;[73-75]

- Temperature impacts *Wolbachia*-malaria interaction in *Anopheles* mosquitoes suggesting impact of transfection might vary across diverse environments;[76]
- *Wolbachia* infections have demonstrated enhancement rather than suppression of pathogens in insects such as crop pests;[77] and
- Horizontal gene transfer (HGT) between *Wolbachia* and their insect hosts has led to the acquisition of evolutionary innovation as research suggests the transfer of genes to host has functional significance.[78]

HGT is the process whereby *Wolbachia's* genes are transferred to their hosts impacting the biology of the infected insects. Studies have found transfers ranging from nearly the entire *Wolbachia* genome (>1 megabase) to short insertions (<500 base pairs) into the genomes of various insect and nematode species.[79-82]

Despite the fact that through HGT *Wolbachia* could effectively introduce over 1,000 new genes, this vector control method avoids the rigorous regulatory examination for recombinant genetic engineering (which typically introduces only a few genes) because this approach does not fall within the narrow definition of 'genetically engineered' from a regulatory perspective.

4. *Wolbachia* and Insecticide Resistant Mosquitoes - In order to boost invasiveness, it has been proposed to release host mosquitoes infected with *Wolbachia* that also carry insecticide resistance genes for better survival in urban areas.

   Increasing tolerance in mosquito populations by using those with genetic traits that are biologically resistant to insecticides would make them more difficult to control and compound the already prevalent insecticide resistance problem, jeopardizing future vector control efforts.[83]

5. Radiation-based SIT and *Wolbachia* – *Wolbachia* insertion into *Aedes aegypti* is currently being investigated in combination with radiation-induced sterility. Irradiation to sterilize any female mosquitoes would allow for lower sex-sorting stringency, but also expose *Wolbachia* strains and mosquitoes simultaneously to mutagenic radiation with unknown consequences.[84]

6. RNA interference (RNAi) – Some groups have been investigating genetically engineered gene-silencing in mosquitoes to induce sterility by using RNAi. However this technique, which also alters the biology of a mosquito, has only been tested in proof of concept studies in the laboratory, and field efficacy has not been evaluated.[85] Demonstrating potent biological effect to effectively reduce *Aedes* populations with reliable, repeatable male selection and sterility will be vital ahead of broader release of the RNAi treated mosquitoes into the environment.

7. Gene Drive – Another potential genetic engineering strategy for population replacement of disease-carrying mosquitoes that alters the biology of the insect is called gene drive. This approach relies on driving a gene of interest into a wild population to reduce pathogen replication.

   These gene drive systems are designed to establish themselves in the environment permanently, raising concerns regarding irreversible changes to vector and disease dynamics.[86]

In summary, for over ten years Oxitec has been focused on the control of *Aedes aegypti* populations through the responsible engineering of biology. The self-limiting aspect of Oxitec's approach is unique to itself, while all of the other methods referenced here may persist in the environment potentially leading to undesirable consequences.

From the perspective of the public's safety, it is imperative that all approaches resulting in undefined genetic changes, especially those that may persist in subsequent generations of these dangerous disease-transmitting *Aedes aegypti* mosquitoes, go through rigorous safety checks like those in place for solutions defined as 'genetically engineered'. At the moment, the scrutiny given to GE products focuses more on how the product was made, rather than the inherent qualities of the products themselves.

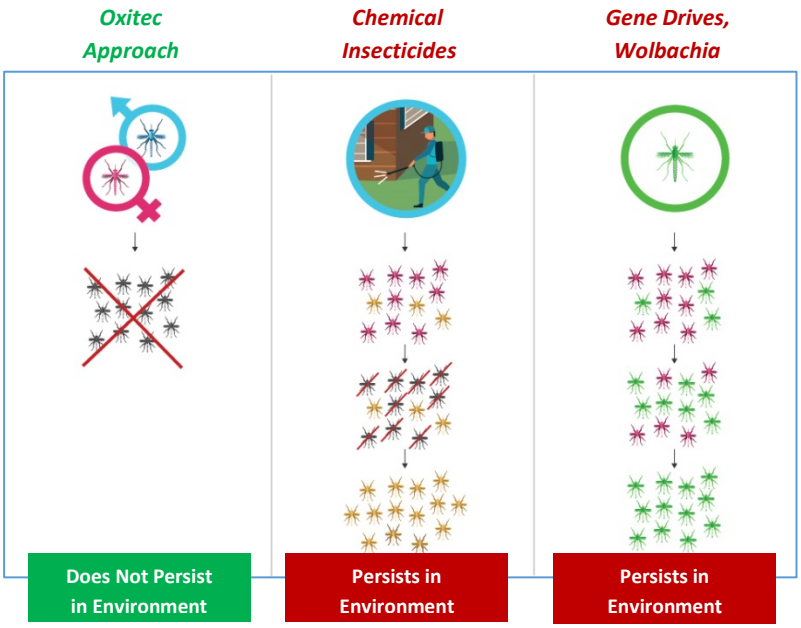


Figure 12. Self-limiting approach of Oxitec that does not persist in the environment compared to other approaches with genetic alterations that may remain in the ecosystem.

Significant Scalability; Expansion of Production Capacity Underway

The power and scalability of biological solutions that utilize mosquitoes can be relatively easy to see from a production point of view. Oxitec's biological engineered approach is embedded from the outset within each egg from its OX513A strain. Given a female mosquito can yield approximately 250 male mosquitoes within five weeks, by month five almost four billion OX513A male mosquito eggs can be bred from that single female and her offspring.

To give perspective on yield from egg production to final product here is a brief overview of a factory with capacity of four million eggs per week. Approximately three and a half million are hatched for egg colony and production with the remainder stored for backup. Eggs can be stored for up to 6 weeks. Two million larvae are reared every week for the Release Generation colony producing around 571,000 male pupae. After taking into account pupae and adult mortality during emergence and releases, approximately 543,000 adult OX513A male mosquitoes are available for release per week. (Figure 13).[87]

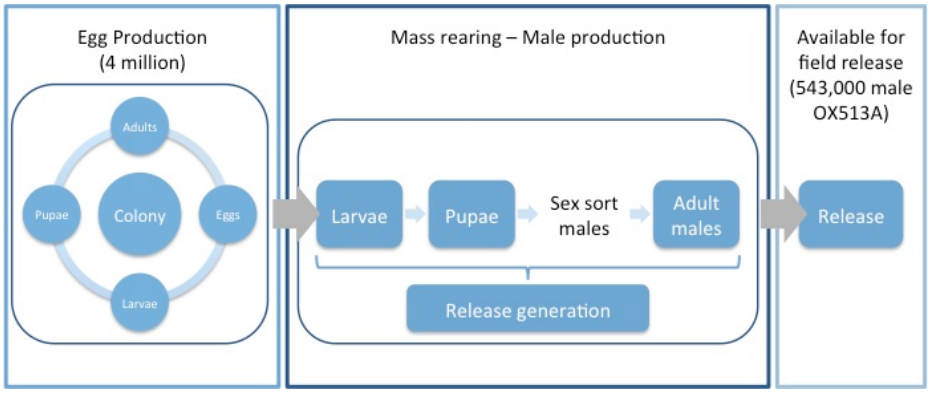


Figure 13. Small scale factory production schematic for OX513A

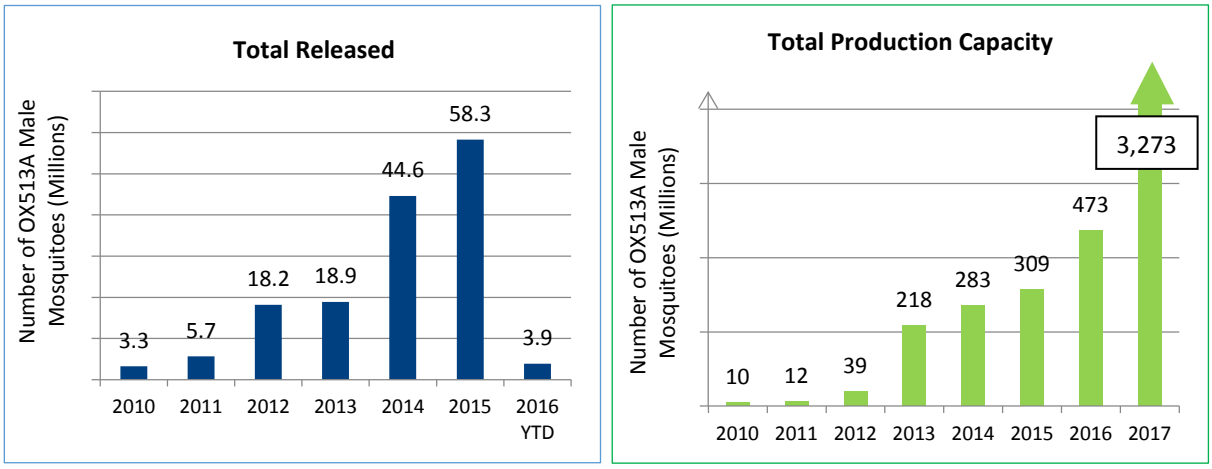


Figure 14. Total OX513A releases from 2010 through 2016 and estimated total OX513A production capacity from 2010 through 2017. For 2016 and 2017 estimates include existing and planned production facilities.

To date over 150 million Oxitec mosquitoes have been released through use in field suppression programs across Brazil, Panama, Malaysia, and the Cayman Islands (Figure 14). The solution has been successfully applied alongside other interventions such as insecticide applications. OX513A is compatible with all other conventional approaches.

The production capacity for Oxitec's pioneering solution has been steadily increasing since the first releases in 2010. In preparation of increasing demand for its proprietary vector control solution, Oxitec is initiating a new mosquito production facility in Piracicaba that will have capacity to protect over 300,000 people. With its existing and planned production facilities, total production capacity is expected to grow significantly in 2016 and 2017 (Figure 14).

Oxitec's Unparalleled Control of the *Aedes aegypti* Vector

Using a biological approach in which self-limiting genetic programming is installed from the outset in the offspring of these damaging *Aedes aegypti* is paramount to effectively reach and reduce their populations.

To date, suppression programs with Oxitec mosquitoes have delivered superior results to any other known intervention for *Aedes aegypti*.[88] Oxitec's species-specific biological approach has been used to successfully decrease wild populations of *Aedes aegypti* in five efficacy trials across Brazil, Cayman Islands and Panama.[42,44,53] Following the release of sufficient numbers of OX513A to target suppression, the local *Aedes aegypti* populations were reduced by >90% (Figure 15) within four months in one trial, six months in three trials, and nine months in the remaining trial. Appendix 1 ('Field Data Summaries') has details on three of these trials.

Figure 15. Suppression results of *Aedes aegypti* from five separate outdoor efficacy trials using OX513A

Through the responsible engineering of biology, Oxitec has developed a new paradigm of self-limiting, species-specific vector control resulting in dramatic reductions of dangerous *Aedes aegypti* mosquitoes, without persistence or harm to the ecosystem, representing a major scientific and environmental advance.

Appendix 1

Field Data Summaries for the OX513A *Aedes aegypti* Suppression Projects:

1. East End, Grand Cayman, Cayman Islands

From May 2010 to October 2010 a demonstration of OX513A *Ae. aegypti* took place in East End, on the Southeast coast of Grand Cayman, one of the three islands that form the Cayman Islands. The area covered was 16 hectacres with 291 residents. The suppression of wild *Ae. aegypti* achieved equaled 96%.

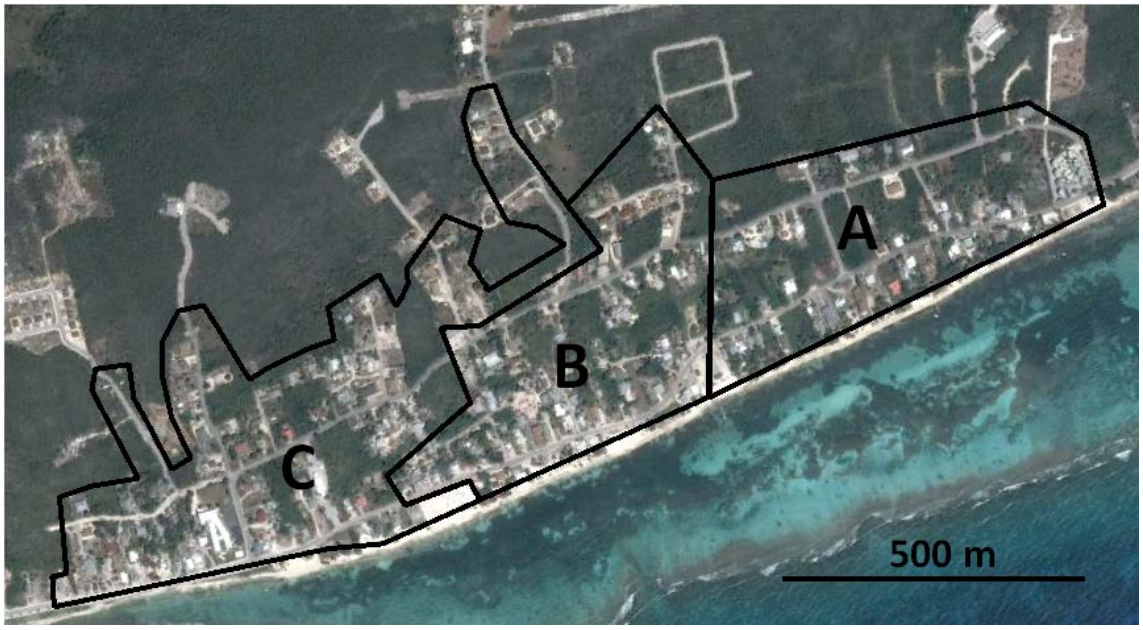


Figure 1. *Aerial photograph of East End showing the areas A (treated with OX513A), B (buffer zone), C (untreated control). Lat: 19.297, Long: -81.109.*

Location and project

East End is a sparsely populated residential area that has a tropical climate with distinct seasonality of *Ae. aegypti*. Populations are lower during the dry season (November to April; 30˚C) and increase markedly during the rainy season (May to October; 26˚C). This was a challenging coastal environment with a high population of the vector and where no other control methods had been used.

The primary objective of the project was to make sustained releases of OX513A males at a level sufficient to suppress the local *Ae. aegypti* population. The population suppression was demonstrated through a comparative assessment of a single area treated with OX513A and an untreated control area (Figure 1).

Results achieved

Throughout the study changes in release rate preceded corresponding changes in the percentage of progeny obtained from ovitraps that carried the fluorescent marker (Figure 2A). This value is indicative of the proportion of local females mating OX513A males (mating fraction), a factor that ultimately guides the rate of population suppression. Behavioural and mating competitiveness studies were conducted during preparation phase from which the release rate necessary to achieve suppression could be established. OX513A numbers sufficient to target suppression of the local *Ae. aegypti* population began early July. Substantial suppression of the local population followed within four months.

A number of different metrics were used to assess local *Ae. aegypti* populations, before and after suppression, allowing the percentage suppression to be ascertained. Ovitrap surveys were the principal monitoring tool deployed in both treated and untreated sites. Any change in the local population of the OX513A-treated area was quantified relative to the untreated area. This was done by dividing the values for the treated area by those of the untreated area to give relative mean number of eggs caught per trap and relative ovitrap index (Figure 2B).

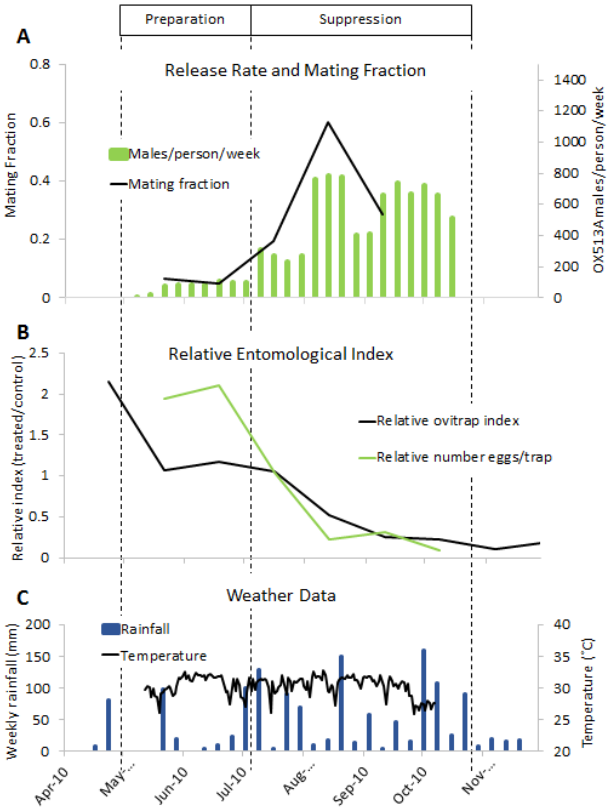


Figure 2. *(A) Number of OX513A males released per person per week and estimated mating fraction during the study. (B) Relative number of eggs per trap and ovitrap index. (C) Average daily temperature and weekly rainfall during the study*

Adult population sampling in areas receiving treatment provided a ratio of recaptured OX513A to local *Ae. aegypti* males, allowing the population density of adult local *Ae. aegypti* to be estimated. Pupae per person were calculated according to the method described in Focks *et al.* with site-specific parameters for the temperature and human population density in the treated area.[51] For all metrics, a substantial suppression of local *Ae. aegypti* population was observed following treatment with OX513A males, including a 96% reduction in the number of mosquito eggs recovered from ovitraps (Appendix 1).

Commentary

A primary consideration for public health agencies is maintaining the vector population below the level required for sustained disease transmission. Disease transmission thresholds are dynamic and dependent upon multiple factors. These factors vary spatially and temporally making predictions for

specific localities difficult. However, using the temperature dependent model proposed by *Focks et al.*, a generic prediction for dengue transmission thresholds that are related to initial serotype prevalence (also termed herd immunity) is possible.[51]

Despite the potential negative effects imposed by migration (as outlined below), during this demonstration study the local *Ae. aegypti* population was reduced to below the transmission threshold predicted for when two-thirds of the population possesses immunity (seroprevalence rate of 67%), thereby demonstrating the potential for this technology to have a positive impact upon dengue transmission rates (Figure 3).

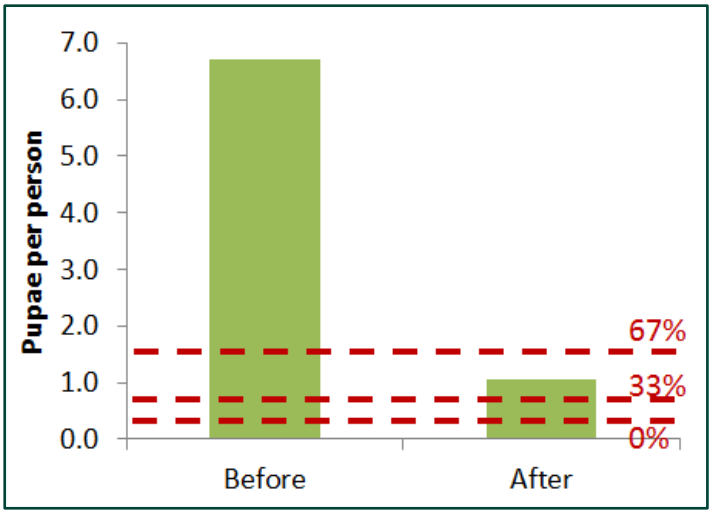


Figure 3. *Suppression of* Ae. aegypti *population in pupae per person relative to predicted dengue transmission thresholds at three initial seroprevalence rates of 0 (0.42 pupae per person), 33 (0.61), and 67% (1.27).*

The treated area was not isolated with regard to the *Ae. aegypti* populations as it was adjacent to the untreated area (area B) where populations of *Ae. aegypti* remained high. The relatively small size of the treatment site meant it was subject to migration of local *Ae. aegypti,* which can typically disperse about 100 metres in its lifetime (5-10 days), from neighbouring area.

Releases of OX513A were stopped in October 2010, but monitoring continued for a further 9 months. During this time the *Ae. aegypti* population remained suppressed relative to the untreated control site. This was despite the fact that the site was relatively small, and susceptible to migration as discussed above.

Related publication

Harris *et al.* (2012) Successful suppression of a field mosquito population by sustained release of engineered male mosquitoes. Nature Biotechnology 30(9).[52]

2. Itaberaba neighbourhood, Juazeiro (Bahia), Brazil

From May 2011 to October 2012 Moscamed and the University of São Paulo conducted a project to suppress local *Ae aegypti* using the strain OX513A in Itaberaba, a neighbourhood in the southeast of the city of Juazeiro, Bahia. The area covered was 5.5 hectacres with 900 residents. The suppression of wild *Ae. aegypti* achieved equaled 93%.

Figure 4. *Aerial photograph of Itaberaba showing the areas A (treated with OX513A), B (buffer zone), C (untreated control). Lat: -9.450, Long: -40.482.*

Location and project

Itaberaba is a densely populated urban setting with a semi-arid climate and limited seasonality of *Ae. aegypti*. Populations of the mosquito are present all year round although lower during the dry season (May to October; 25˚C) and higher during the rainy season (November to April; 28˚C). The site was identified by the local vector control agency as a disease hotspot with a consistently high vector population. The primary objective of the project was to achieve substantial suppression of the local *Ae. aegypti* population in this challenging area. The population suppression was demonstrated through a comparative assessment of a single area treated with OX513A and an untreated control area (Figure 4).

Results achieved

Throughout the study changes in release rate preceded corresponding changes in the percentage of progeny obtained from ovitraps that carried the fluorescent marker (Figure 5A). This value is indicative of the proportion of local females mating OX513A males (mating fraction), a factor that ultimately guides the rate of population suppression. An extended preparation phase of releases below the threshold needed to achieve suppression enabled extensive behavioural and mating competitiveness studies, building detailed knowledge of the release numbers required for suppression.

The high and relatively stable year round *Ae. aegypti* population provided an ideal setting for these studies. OX513A numbers sufficient to target suppression of the local *Ae. aegypti* population began

in early 2012. Substantial suppression followed within six months (Figure 5). The local population was then maintained at very low levels despite the treated area being relatively small and susceptible to migration of *Ae. aegypti* from adjacent untreated areas where the *Ae. aegypti* population remained high.

A number of different metrics were used to assess local *Ae. aegypti* populations, before and after suppression, allowing the percentage suppression to be ascertained. Ovitrap surveys were the principal monitoring tool deployed in both treated and untreated sites. Any change in the local population of the OX513A-treated area was quantified relative to the untreated area. This was done by dividing the values for the treated area by those of the untreated area to give relative mean number of eggs caught per trap and relative ovitrap index (Figure 5B).

Adult population sampling in areas receiving treatment provided a ratio of recaptured OX513A to local *Ae. aegypti* males, allowing the population density of adult local *Ae. aegypti* to be estimated. Pupae per person were calculated according to the method described in Focks *et al.* with site-specific parameters for the temperature and human population density in the treated area.[51] For all metrics, a substantial suppression of local *Ae. aegypti* population was observed following treatment with OX513A males, including a 93% reduction in the number of mosquito eggs recovered from ovitraps (Appendix 1).

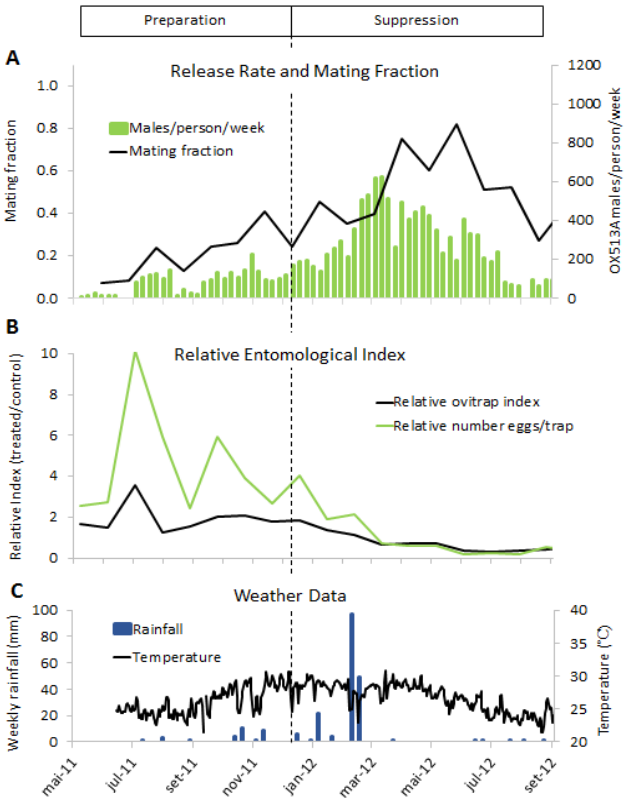


Figure 5. *(A) Number of OX513A males released per person per week and estimated mating fraction during the study. (B) Relative number of eggs per trap and ovitrap index. (C) Average daily temperature and weekly rainfall during the study.*

Commentary

A primary consideration for public health agencies is maintaining the vector population below the level required for sustained disease transmission. Disease transmission thresholds are dynamic and dependent upon multiple factors. These factors vary spatially and temporally making predictions for specific localities difficult. However, using the temperature dependent model proposed by *Focks et al.*, a generic prediction for dengue transmission thresholds that are related to initial serotype prevalence (also termed herd immunity) is possible.[51]

Despite the potential negative effects imposed by migration (as outlined below), during this demonstration study the local *Ae. aegypti* population was reduced to below the transmission threshold predicted for when there is no previous immunity in the population (seroprevalence rate of 0%) (Figure 6). This demonstrates the strong potential for this technology to reduce dengue transmission rates, even in areas where the human population is totally susceptible to the disease.

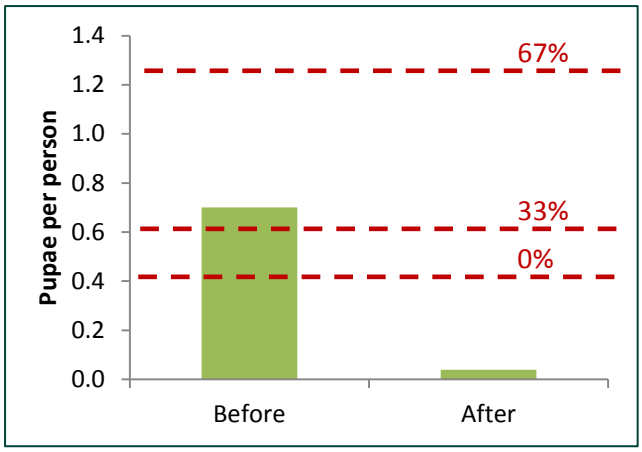


Figure 6. *Suppression of* Ae. aegypti *population in pupae per person relative to predicted dengue transmission thresholds at three initial seroprevalence rates of 0 (0.42 pupae per person), 33 (0.61), and 67% (1.27).*

The treated area was not isolated with regard to the *Ae. aegypti* populations as it was a suburb within a larger city where populations of *Ae. aegypti* remained high. The relatively small size of the treatment site meant it was subject to migration of local *Ae. Aegypti,* which can typically disperse about 100 metres in its lifetime, from neighbouring areas.

The results of this study show an excellent degree of control and suggest that, even in small areas with a high degree of migration, sustained releases of OX513A can offer outstanding reductions in the local *Ae. aegypti* population. This indicates that Oxitec's technology can be used to combat *Ae. aegypti* hotspots as well as for wide area control strategies in which the impact of migration is reduced.

Related Publication

Carvalho *et al.* (2015) Suppression of a Field Population of *Aedes aegypti* in Brazil by Sustained Release of Transgenic Male Mosquitoes. PLoS Negl Trop Dis 9(7): e0003864.[50]

3. Nuevo Chorrillo, Arraijan, Panama

From April 2014 to October 2014 the Gorgas Institute conducted a project to suppress local *Ae. aegypti* using the strain OX513A in Nuevo Chorrillo, a neighbourhood west of Panama City. The area covered was 10 hectacres with 900 residents. The suppression of wild *Ae. aegypti* achieved equaled 93%.

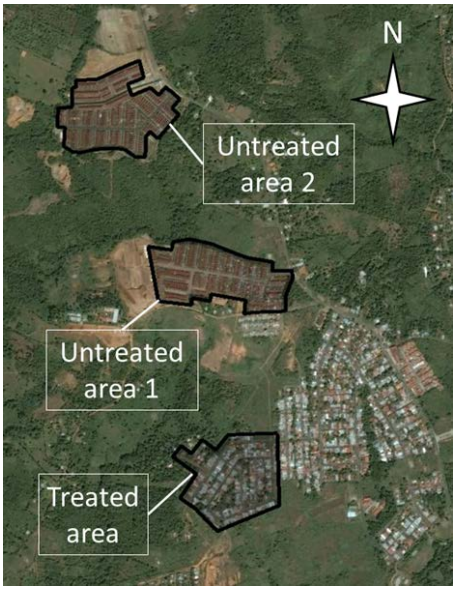


Figure 7. *Aerial photograph of neighbourhoods in Arraijan, showing the OX513A treated site and both untreated control sites.*
Lat: 8.952355; Long: -79.699245.

Location and project

The neighbourhood of Nuevo Chorrillo has a moderate human population density, a tropical maritime climate, and marked seasonality of *Ae. aegypti*. Populations of this mosquito are present all year round although are lower during the dry season (December to April; 26˚C) and higher during the wet season (May to November; 26˚C). Vector surveillance over several years confirmed a predominating *Ae. aegypti* population with an increasing presence of *Ae. albopictus*. The primary objective of the project was to assess whether releases of OX513A prevented the annual surge in local *Ae. aegypti* that accompanies the increased rainfall of the wet season. Performance was measured by comparing the abundance of *Ae. aegypti* relative to two nearby untreated sites (Figure 7). This study was the first OX513A suppression trial to be completed in an area where both *Ae. aegypti* and *Ae. albopictus* co-exist.

Results achieved

The first fluorescent larvae were observed just one week after releases began, demonstrating the inherent mating compatibility of OX513A males with females of the local strain. Over the course of the treatment period, the majority of progeny collected from ovitraps carried the fluorescent marker. This reflected the proportion of local females successfully mated by OX513A males (also termed mating fraction), a factor that ultimately guides the rate of population control (Figure 8A).

Several different metrics were used to assess mosquito abundance both before and after suppression, allowing the levels of *Ae. aegypti* control to be determined. To accommodate the presence of *Ae. albopictus*, all eggs collected were hatched and reared to the late-larval stage so that species identification of every individual could be completed. Egg trap (ovitrap) data are therefore presented as larvae (as opposed to eggs) to accurately reflect the life-stage assessed.

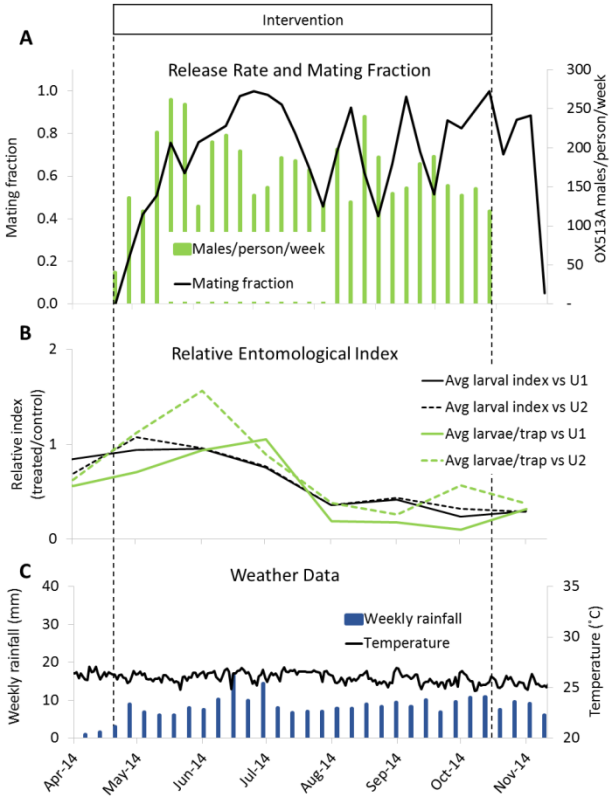


Figure 8. *(A) Numbers of OX513A males released per person per week and estimated mating fractions (proportions of local female Ae. aegypti mated by OX513A males). (B) Relative numbers of eggs per trap and relative ovitrap indexes. U1: untreated area 1; U2: untreated area 2. (C) Mean daily temperatures and weekly rainfall.*

Weekly ovitrap surveys were the principal monitoring tool deployed in both treated and untreated sites. Any changes over time in the local populations were quantified by dividing the number of *Ae. aegypti* caught in the treated area by those caught in the untreated areas. This produced relative mean numbers of larvae caught per trap and relative ovitrap indexes (Figure 8B). Surveillance of adult populations, using commercially available BG Sentinel® traps (Biogents, Germany) in the treated site and untreated area 1, provided a ratio of recaptured OX513A males to local *Ae. aegypti* males. These data were used to estimate the population densities of local *Ae. aegypti* adults and corresponding pupae per person values according to the method described by

Focks *et al.*, with site-specific parameters for the temperature and human population density in the treated area.[51]

For all metrics, a substantial suppression of the local *Ae. aegypti* population was observed following treatment with OX513A males, including a 93% reduction in the number of larvae per trap (Appendix 1). Substantial control of the local *Ae. aegypti* population had been achieved within six months after releases began; the population was then maintained at this low-level in the treated area despite the intense pest pressures in both untreated areas as the wet season progressed.

Commentary

A primary consideration for public health agencies is maintaining vector populations below the levels required for sustained disease transmission. Disease transmission thresholds are dynamic and dependent upon multiple factors. These factors vary spatially and temporally making predictions for specific localities difficult. However, using the temperature dependent model proposed by *Focks et al.*, generic predictions for dengue transmission thresholds that are related to initial serotype prevalence (also termed herd immunity) are possible.[51] Mark-release-racapture statistics and known numbers of OX513A males released at the treated site were used to estimate local population sizes.

Despite the potential negative effects imposed by migration (as outlined below), in the treated area the local *Ae. aegypti* population was maintained below the transmission threshold predicted for a population without prior immunity (seroprevalence rate of 0%) (Figure 9). In contrast, the untreated areas showed significantly increased infestation during the wet season to the point that there was a risk of a dengue epidemic even with 33% serotype prevalence. This demonstrates the potential for this technology to hold vector populations below the dengue transmission threshold, even in areas where the human population is fully susceptible to the disease.

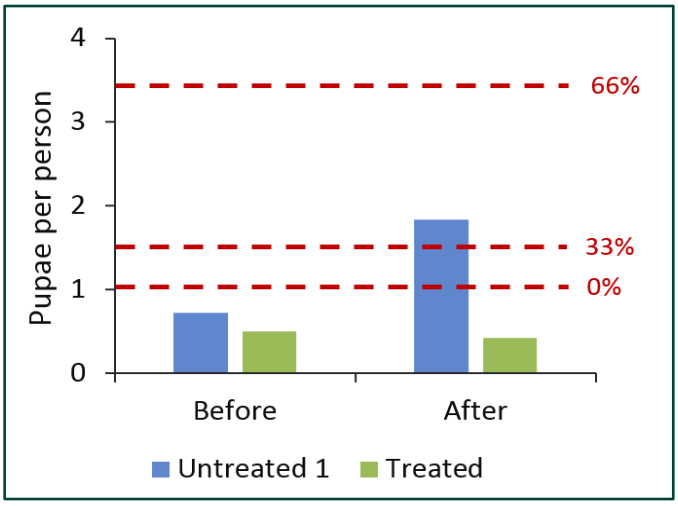


Figure 9. *Suppression of Ae. aegypti population in pupae per person relative to predicted dengue transmission thresholds at three initial seroprevalence rates of 0 (1.05 pupae per person), 33 (1.55), and 67% (3.41).*

The treated site was within a wider suburb where populations of *Ae. aegypti* remained high and was therefore subject to migration of mosquitoes from these adjacent areas.

These results demonstrate the ability of Oxitec technology to efficiently contain *Ae. aegypti* even in difficult areas, mitigating against dengue outbreaks before the disease has established.

Related publication

Gorman *et al.* (2016). Short-term suppression of *Aedes aegypti* using genetic control does not facilitate *Aedes albopictus*. Pest Management Science, DOI: 10.1002/ps.4151.[49]

Appendix 2: Tables

	Eggs/trap (treated area)	Relative eggs/trap (treated/control)	Ovitrap index (treated area)	Relative ovitrap index (treated/control)	Local *Ae. aegypti* (adults/ ha)	Local *Ae. aegypti* (pupae/person)
1. East End, Grand Cayman, Cayman Islands						
Before suppression	7.91	1.70	30.2%	1.36	1047	6.72
After suppression	1.10	0.07	5.4%	0.23	61	1.09
Reduction	**86%**	**96%**	**82%**	**83%**	**84%**	**84%**
2. Itaberaba neighbourhood, Juazeiro (Bahia), Brazil						
Before suppression	20.5	2.85	36.7%	1.61	418	0.7
After suppression	1.71	0.21	6.3%	0.35	20	0.04
Reduction	**92%**	**93%**	**83%**	**78%**	**95%**	**95%**

3. Nuevo Chorrillo, Arraijan, Panama				
	Larvae per trap		Larval index	
	Relative to U1	Relative to U2	Relative to U1	Relative to U2
Before suppression	0.93	1.41	0.90	1.34
After suppression	0.08	0.07	0.19	0.16
Reduction	**93%**		**84%**	

Summary of observed reductions in abundance of *Ae. aegypti*. Relative values were calculated by dividing those for the treated area by those of the relevant untreated area. Larval reductions are presented as mean values across both untreated areas. T = treated; U1 = untreated 1; U2 = untreated 2.

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Appendix 4: Oxitec Publications

Description of the OX513A strain and production

- Phuc HK, Andreasen MH, Burton R, Vass C, Epton MJ, Pape G, Fu G, Condon KC, Scaife S, Donnelly CA, Coleman PG, White-Cooper H, Alphey L. (2007) Late-acting dominant lethal genetic systems and mosquito control. BMC Biology 5: 11.
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Studies on OX513A phenotype

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