DENGUE FEVER

The fastest growing mosquito-borne disease

OXITEC

The need for innovation…

…to counter the global dengue pandemic

Dengue is a viral disease transmitted by mosquitoes, sometimes referred to as "breakbone fever". "Classic" dengue is an excruciatingly painful illness with high fever, agonizing bone, muscle and eye pain and an intensely-itching rash that can extend from the head to the soles of the feet. The acute phase lasts less than two weeks but victims may suffer months of post-infection symptoms that include mental depression, physical exhaustion, distressing eye problems and even temporary baldness. About 5% of clinical cases are classed as "severe" and are potentially fatal (dengue shock syndrome/dengue haemorrhagic fever).

The global burden of dengue is formidable: nearly half the world's population lives in dengue-prone regions, there are an estimated 390 million infections per year, and incidence is continually increasing. At present there is no dengue vaccine and there are no effective antiviral drugs; mosquito control is the only available counter-measure.

The principal vector of dengue is the mosquito Aedes aegypti, a species that originated in the African forest but has adopted the human domestic environment. In its original habitat it breeds in natural water-holding containers – holes in trees and rocks, leaf axils, fruit husks, etc. – and feeds exclusively on roaming bands of monkeys. In the peri-domestic environment, we humans are the perfect hosts. Instead of tree holes we provide an abundance of artificial containers: water-barrels, old buckets, flower-pot saucers, discarded tyres and so on. Instead of an environment rich in predators we provide safe, clean quarters for them to hide; clothes cupboards are a favorite. And of course, an abundant supply of fresh blood on tap.

Control of Ae. aegypti dates back to the beginning of the 20th century, when it was demonstrated that yellow fever – a terrifying disease caused by a virus closely related to dengue – is transmitted by the mosquito. The approach, conducted as a military-style operation, was simple and effective: infested containers were either discarded or covered. The first test of the method, in Havana, Cuba, was a resounding success: the disease was wiped out in four months. Within a few years, similar results had been achieved in most major seaports of the Americas.

In the 1950s, "source reduction" was replaced by a new approach: infested sites were treated with a magic powder, DDT. The method was incredibly easy to apply and within 10 years the species was declared "eradicated" in 22 countries of the Americas. Unfortunately, for reasons that are now well-known, DDT fell out of favour and from the mid-1960s onwards there was swift return of the mosquito, quickly followed by dengue, in much of the hemisphere. At the same time, new technology was developed to dispense insecticidal aerosols by hand-held or truck-mounted "fogging machines". These machines are a familiar sight in most regions where mosquitoes are a nuisance or public health problem.

Fogging machines are highly visible, make lots of noise and produce an unpleasant smell. They can be effective against mosquitoes in open areas but have very little impact on Ae. aegypti, mainly because the mosquito rests in sheltered places, inaccessible by the aerosol. Nevertheless, despite their proven lack of efficacy and their high cost, the machines are widely used, particularly during outbreaks of dengue. The best that can be said is that the passing of a machine reassures the public that their government is responding to a problem.

From the 1960s onwards, the burgeoning increase in the size and population density of many tropical cities, coupled with an increasingly pervasive "throw-away society", furnished an expanding paradise for Ae. aegypti and an enormous increase in the prevalence and incidence of dengue. By the 1980s it was clear that conventional interventions were far too costly and impractical for most governments to consider. In response, the idea arose that educational campaigns could be used to transfer responsibility for source-

reduction to the population as a whole; once householders would be aware of their responsibilities, government efforts could be limited to information and enlightenment. The popular phrase was "Bottom Up" rather than "Top Down".

Unfortunately, despite numerous sociological studies and valiant, often highly creative efforts by local communities, there is no evidence that the strategy has ever had a sustainable impact on the mosquito population. Thus, today, we are helpless in the face of an ever-burgeoning mosquito problem, exacerbated by the rapidly increasing mobility of the virus engendered by global air-travel.

The reality is that apart from the early source reduction programmes and the eradication campaign, epidemiologically meaningful control has rarely been achieved and never sustained. Quasi-military strategies are no longer acceptable, the heady days of DDT-optimism have passed, reliance on aerosols has never been the answer and community-based approaches have proven fruitless. To deny all this is ostrich science.

Once we confront this reality we can accept the need to innovate, to explore new and imaginative approaches, from the simplest, low-tech ideas to novel methods of using insecticides, transgenics, cellular pathogens and other pioneering technology. None of these approaches is likely to succeed on its own: we need to devise ways to synergise them with one another. Moreover, to develop these synergies we must eliminate important gaps in our knowledge of mosquito biology and our understanding of dengue epidemiology

Finally, dengue vectors will never be controlled on a shoe-string. Even if the community assumes a major portion of responsibility, support is still needed at various levels of government. For this there must be continuity in management, immune from frequent and abrupt changes in leadership. The need for high-quality vector control personnel implies a sound career structure and the recognition that continued vigilance can never be terminated; as a colleague once remarked, we would never think of stopping rubbish collections. These requirements may seem impracticable or impossible when ranked against many other disease priorities but when set against the cost of repeated dengue and dengue haemorrhagic fever epidemics in terms of morbidity, mortality, medical resources, work absenteeism, impact on tourism and other criteria, the benefits of effective prevention are clear.

Paul Reiter, MPhil, DPhil, FRES

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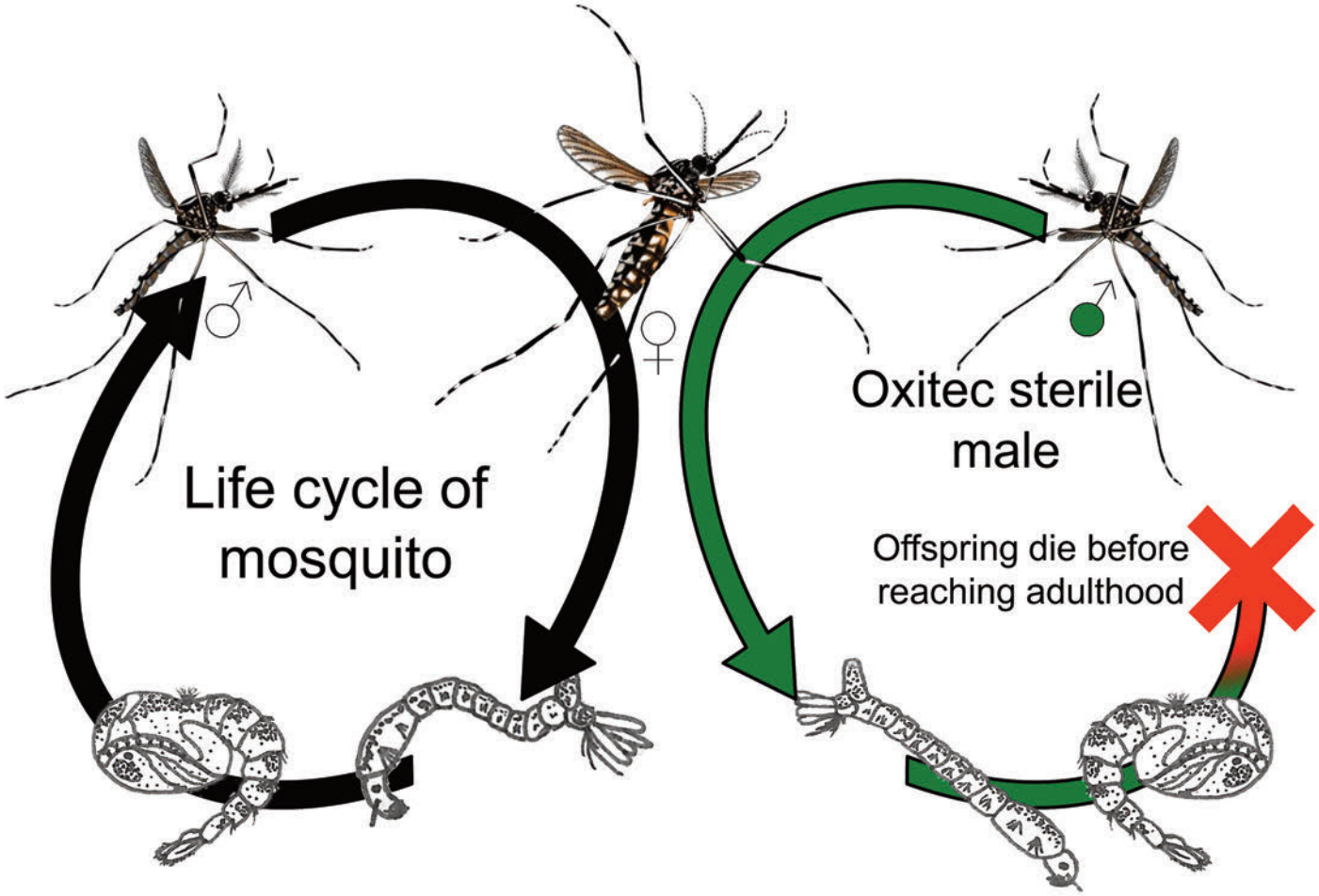
A new solution

Dr Renaud Lacroix describes Oxitec's new approach to combat Aedes mosquitoes

The principal vector of dengue fever, Aedes aegypti, has only recently adapted its lifestyle from the forests of Africa to now living in close proximity to humans in urban and semi urban areas across the world. During its aquatic larval stage, it thrives in small, frequently transient containers filled with rain water – common around our homes and in disposed rubbish – and in stored water when tap water is not available. Aedes aegypti is an anthropophilic mosquito: the female almost exclusively bites humans when taking a blood meal, dramatically increasing rates of virus transmission between people. Only female mosquitoes bite – they need the blood proteins for egg maturation – whereas males only require carbohydrates, which they obtain from natural sugar sources such as nectar from plants and flowers.

The female mosquito that takes a blood meal from a dengue virus-infected person becomes itself infected with the virus. About 8-10 days later, the virus spreads to the mosquito's salivary glands and is subsequently released into its saliva while biting another host. The female mosquito remains infected for life and can transmit the virus to a new person each time she bites.

In the absence of an effective vaccine or specific



The Oxitec approach. Females that mate with wild males can produce up to 500 offspring during their life. However, if they mate with an Oxitec male the offspring die before adulthood.

Oxitec release male mosquitoes as males do not bite

therapeutic or prophylactic drugs against dengue, the only way to tackle dengue transmission is by targeting its vector, the mosquito. Conventional mosquito control relies largely on insecticides: sprayed or fogged insecticides to kill adults, larvicides spread in breeding sites against larvae. As described by Professor Paul Reiter in the accompanying article, these strategies do not reach all of the breeding and resting sites that Aedes aegypti favours, and have off-target ecological effects. Targeting the larval breeding site of Aedes aegypti is a major challenge, as they are so dispersed. Widespread resistance to insecticides further limits their effectiveness. These strategies have not been able to stem the rise in dengue cases over the years. Novel control methods are desperately needed to fight this burden.

The "gold standard" mosquito control method would have several attributes:

- **Ubiquitous:** ability to reach every corner where mosquitoes breed and rest, i.e. hidden discarded containers, humid resting places in houses, etc…
- **Safe to people:** it will mostly be used in residential areas.
- **Species-specific:** no off-target effects.
- **Environmentally friendly:** no long-lasting side effects on the environment.
- **Effective:** suppress Aedes aegypti population below the disease transmission threshold.
- **Sustainable:** maintain the level of control at a reasonable cost.

Recent breakthroughs in genetic engineering have made possible new control methods. Oxitec's Aedes aegypti product is an advancement of the Sterile Insect Technique (SIT), in which released radiation-sterilised male insects mate with wild females and thereby reduce the population in the next generation. SIT has been widely used since the 1950s against a number of major agriculture pests and has enabled control at a continental level of the New World screwworm and Mediterranean fruit fly. Unfortunately, mosquitoes have proven too vulnerable to the irradiation process – required for sterilisation – to compete effectively against the wild males for mates.

Oxitec's solution overcomes this limitation using genetically engineered sterile male mosquitoes

The Moscamed production unit in Juazeiro, Brazil. Courtesy of Moscamed

(males can't bite or transmit diseases) whose offspring will die. The Oxitec males, which to wild females are indistinguishable from wild males, are released in large numbers to out-number the wild males. Consequently, a majority of females will encounter and mate with Oxitec males and produce no viable offspring. Sustained releases over several months will cause the local Aedes aegypti population to collapse. The releases need to be carefully planned so that the Oxitec males are evenly distributed in the area; highly infested areas will initially require higher numbers to be released – or other control methods can be employed prior to the releases to provide an initial knock-down. Oxitec males provide a means of reducing the population much further than conventional methods, as they are able to spread in the treated area and find the females wherever they hide: mosquito biology turned against the mosquitoes themselves. The releases are adaptable and can be conducted by hand, truck or even boat, depending on the local situation.

The Oxitec lethal gene can be suppressed by a chemical antidote, tetracycline, which is not significantly present in the environment. The males can therefore be mass-produced at an affordable cost in a dedicated production facility, which can be established locally either in dedicated refurbished buildings or in mobile rearing units developed by Oxitec. The technology has already been transferred successfully in Brazil, Cayman Islands and Malaysia and only a few months are required to deliver the facility and train the new staff.

A fluorescent marker gene is also integrated in the Oxitec insects to provide easy and efficient monitoring. Indeed, a simple fluorescence microscope can be used to monitor the presence of the gene in mosquito larvae collected in the environment, thus providing information on the proportion of mated females, dispersal of the Oxitec males, or disappearance of the gene when releases are stopped. Oxitec technology is self-limiting as, like wild mosquitoes, the males can't survive for long in the environment. The marker also enables in-programme monitoring to ensure releases are tailored to the local population level of Aedes aegypti.

After gaining regulatory approval, Oxitec male mosquitoes were first released in the Cayman Islands in 2009. The experience was a success and demonstrated that they mated with wild females and competed well against wild males. Subsequently, sustained releases of males in the Cayman Islands and in two locations in north-eastern Brazil have achieved reductions in local Aedes aegypti population of over 80%. In the last of these studies, in the village of Mandacaru in Bahia, the mosquito population was reduced by 96% (see graph). These levels of effectiveness contrast with those of conventional strategies, offering a tool that is uniquely effective against Aedes aegypti.

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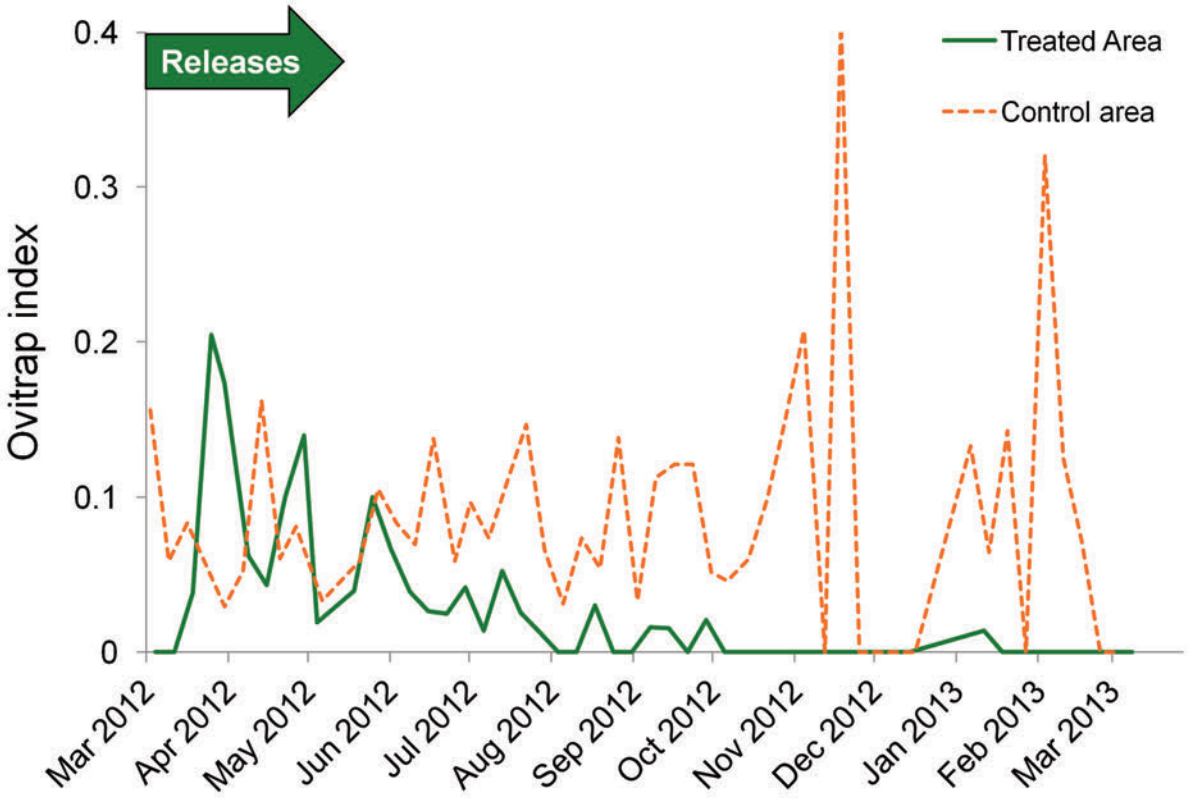
A vital component of Oxitec's mosquito control programmes is interaction with local communities, which needs to be adapted to local cultures and social structures. The projects mentioned above included various vehicles for the dissemination of information. In Brazil, where Oxitec's partner Moscamed led the projects, after being advertised by flyers, posters, radio broadcasts and newspapers, open meetings with the local residents were organised with the local authorities to answer all questions regarding the technology. In the same location, house-to-house visits were also conducted to inform and discuss with the locals without the pressure of talking in front of a crowd: 88% of the houses were visited in one area and none of the householders opposed the technology. In general, the local response has been very positive and communities show great support for the technology.

After these initial successes, the Brazilian state of Bahia commissioned Moscamed to conduct a pilot-scale control programme in the city of Jacobina, home to 50,000 people. For this purpose, Moscamed is expanding its production facility for Oxitec mosquitoes, aiming to produce several million males per week. The releases started in the north-west of Jacobina in June 2013 and the programme will roll out across the entire city over two or three years. This programme offers new hope for populations throughout the tropics that live with the daily threat of dengue infection and provides authorities with a new tool in their fight against disease vectors like Aedes aegypti.



Oxitec releases in Mandacaru resulted in a reduction in the dengue mosquito population by 96%. Reduced release maintained this control even into the rainy season when neighbouring areas experience a huge increase

Squaring the circle in pest control

Protecting people while preserving our environment

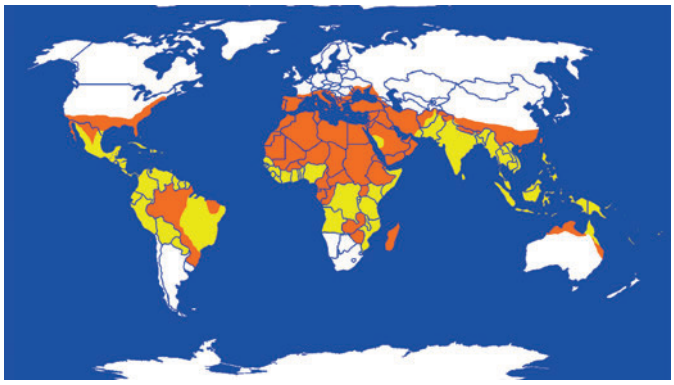
In 1992, at the Earth Summit in Rio de Janeiro, the world's nations were for the first time united in their recognition that mankind's activities were having a potentially irreversible impact on the environment and biodiversity. More than two decades later, the United Nations announced that the years 2011-2020 would be the UN Decade on Biodiversity, reflecting a broad international consensus that the decline and loss of species remains one of the most pernicious threats to our natural environment.

Of course, since people first developed the capacity to manipulate the world around them, our attempts to shape our environment according to our own needs and comforts have engendered tensions between our lifestyle and industry, and the welfare of species who share that environment. And nowhere are these battle lines more clearly drawn than in our attempts to contain, control, or altogether eradicate species that many of us feel we might be better off living without.

Mosquitoes are the deadliest animal on the planet. Each year, they claim the lives of some 2 million people: victims of mosquito-borne diseases such as malaria, yellow fever and dengue fever. From earliest times, the war that humanity has waged against these traffickers in pestilence has had a profound impact on our natural environment; from the drainage of the malaria-infested marshes of ancient Rome to the widespread use of DDT around much of the tropical world this century.

Yet it is an often under-appreciated fact that this most ancient of mankind's enemies (and mosquitoes were plaguing the dinosaurs long before people were added to their list of victims) is nevertheless a relative newcomer in many of the areas where it is today proving most troublesome.

Take the dengue mosquito, Aedes aegypti, as a prime example. Thought to have originated in central Africa, its global spread over the last few decades has been remarkably rapid. An urban-dweller, which thrives in densely-populated human habitation, its fortunes have risen as inexorably as the high-rise apartment blocks in rapidly-expanding towns and cities; so much so that it is now endemic throughout the world's tropical regions (see below).



Aedes aegypti has spread from Africa around the world, aided mainly by the movement of humans and freight. The dengue virus has followed though not all countries report dengue cases. The WHO estimate that 2.5bn people are at risk from dengue.

Aedes aegypti is not alone in embarking on a global march. The Asian tiger mosquito, Aedes albopictus – aegypti's partner-in-crime where dengue transmission is concerned – has likewise been spreading at an alarming rate, capitalising in particular on the global trade in used tyres to colonise territories as diverse as Russia, La Réunion and Italy. Environmentalists in Europe face a deluge of rogue immigrants, which are threatening native species and the livelihoods of farmers. A changing climate, and an era of global trade and travel, has resulted in the unprecedented mobility, not only of our own species, but that of many others who have hitch-hiked upon our transport networks. These 'rogue' species add a new dimension to the challenge of protecting human health and welfare, alongside that of preserving natural ecosystems.

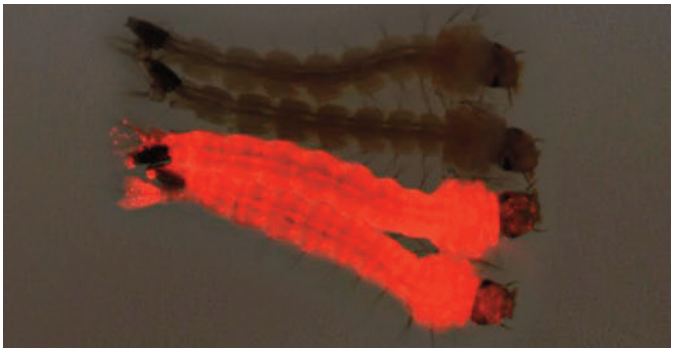
Faced with a threat as severe as that of the dengue mosquito, inaction is unthinkable. Recent estimates

"Today, dengue ranks as the most important mosquito-borne viral disease in the world. Everywhere the human and economic costs are staggering"

Dr Margaret Chan, 2012 Director General, World Health Organization

place the number of annual dengue cases at 350 million[1]; more than three times the figure previously estimated by the World Health Organization. With increased consciousness of the importance of biodiversity, however, comes a need for interventions that are precise, targeted, limited, and reversible; calculated to minimise wider species impacts, and cause the smallest possible ecological disturbance.

Evaluated against this scorecard, Oxitec's novel approach to pest control is uniquely well-suited. It involves the release of male mosquitoes modified to pass on a lethal gene to their offspring. Any wild female that mates an Oxitec male will fail to produce progeny capable of surviving and breeding, thus reducing the population. Only male mosquitoes are released, which don't bite. Crucially, from the perspective of environmental impact, the males will only mate with females of their own species. In doing so, they deliver a precisely targeted form of control.



Larvae that have an Oxitec male as a parent display a fluorescent colour when viewed under a fluorescent microscope. Using the marker allows Oxitec scientists not only to monitor the efficacy of the approach but to demonstrate there is no persistence of the released males or gene in the environment

After mating with a female, the released males will die, as will their offspring: there is no persistence in the environment. If it remains true that any attempt to control or contain invasive species risks leaving holes in the delicate fabric of a habitat's ecosystem, then Oxitec's solution nonetheless provides a means to elegantly un-pick the rogue thread, with minimum risk of unravelling the complex weave of wider ecological networks.

Solutions like this are urgently needed if we are to combat the dual threat of invasive pest species and the decline in biodiversity. More traditional approaches, such as the use of insecticides, will continue to play an important role. But chemical controls almost invariably have 'off-target' effects on other species, and are faced with concerns over human health impacts: for this reason many jurisdictions, such as the EU, are re-evaluating which agents and what manner of use they consider acceptable. Biological controls, too, such as deployment of natural predators, can have unintended consequences for the habitats in which they are deployed. Two decades of study on biodiversity teaches that the use of all pest control approaches should be governed with caution; moreover, that traditional methods may be both safer and more effective when used in combination with alternative approaches.

This is the niche in which Oxitec's method can play a vital role. Other alternative approaches – trapping, breeding site removal, community interventions – need also be brought into play. Above all, effective environmental monitoring to assess the impact of any intervention will be a central part of a comprehensive and more environmentally conscious ethos of pest control.

Today, the global disease-burden resulting from mosquito-borne illnesses may already exceed 600 million people each year; by the close of the UN's Decade of Biodiversity, that number could be considerably higher. There are no straightforward solutions to meeting the threat of invasive, disease-carrying pests while managing the parallel challenges to biodiversity that might result. Yet neither are these challenges insoluble: by embracing new technology, making use of the full range of tools at our disposal, and by judging potential solutions without prejudice, there is no reason why the needs of humanity and those of our environment cannot find common ground – in this decade and beyond.

[1] Bhatt et al. 2013 The global distribution and burden of dengue. Nature.

Michael Conway, PhD student

A holistic approach to vector management

Dr Kevin Gorman describes the implementation of contemporary vector control

Dengue is currently the fastest-spreading human disease, driven by increasing levels of urbanisation and international trade that facilitate the proliferation of the disease vector mosquitoes. This fuels demand for effective and environmentally acceptable mosquito control solutions.

However, conventional insecticides are being withdrawn due to environmental or human health concerns and resistance is developing in the target insect populations. In fact, for the dengue vector Aedes aegypti, current control options are simply not effective enough. We find ourselves in a position of increasing need with decreasing solutions. There is now the real prospect of being left 'empty-handed' with no viable alternative for controlling some of the world's most devastating pest species.

The pressure is on to find novel technologies that can deliver long-term protection from vector-borne diseases, while minimising the ecological footprint associated with pest management practices. Oxitec have developed a control strategy that offers levels of Aedes aegypti control that have not been possible through conventional means.

To appreciate the context and significance of this new tool, it helps to understand a little of the evolution of pest control approaches.

Historically, agricultural pest management practices have focused almost exclusively on efficacy and economics, with compromises between 'how much it costs' and 'how well it performs'. Over-reliance on favoured insecticides has inevitably shortened their effective life-span through the development of resistance. Contemporary approaches attempt to combine complementary management techniques (chemical, biological, physical, cultural and genetic) into a more effective and sustainable systems approach, known as integrated pest management.

For control of vectors of human disease, such as Aedes aegypti, the situation is altogether different. New, effective active ingredients have been few and far between, dramatically limiting the control options available. Many control programmes still rely on conventional chemistries, such as synthetic pyrethroids, some of which have been in use since the 1970s. Chemical applications targeting adult life-stages frequently come from these older, broad-spectrum chemical classes with poor environmental profiles. At the same time, the efficacy of products acting on water-borne larvae is limited by our inability to find and treat a high enough proportion of breeding sites. To date, the desperately narrow range of tools that have been available to vector control teams has made effective long-term control of Aedes aegypti practically impossible.

For the success of future Aedes aegypti control it will be important to maintain a focus on community engagement that seeks to educate and empower the public, reducing the number of potential breeding sites within local environments. Although sustaining impetus and public activity levels has previously been a significant hurdle to providing long-term benefits in this manner, community involvement offers a firm foundation for an integrated approach to disease vector control.

The current toolset of insecticides and public education has been insufficient to halt the global proliferation of Aedes aegypti and the associated increase in dengue fever to over 350 million cases per year. A holistic approach can deliver sustained, environmentally friendly control and Oxitec's technology is the final piece of the mosquito control puzzle.

Oxitec has conducted a series of suppression trials against Aedes aegypti, to reduce wild populations in areas of dengue incidence by 80% to 96%, and recently sustained this level of population suppression through an entire high season, with minimal releases of Oxitec male mosquitoes.

Dengue mosquitoes can breed in any water containing vessel, community participation is essential to reduce the number of breeding sites that are available.

Furthermore, the Oxitec mosquito is safe, environmentally friendly and practical to use:

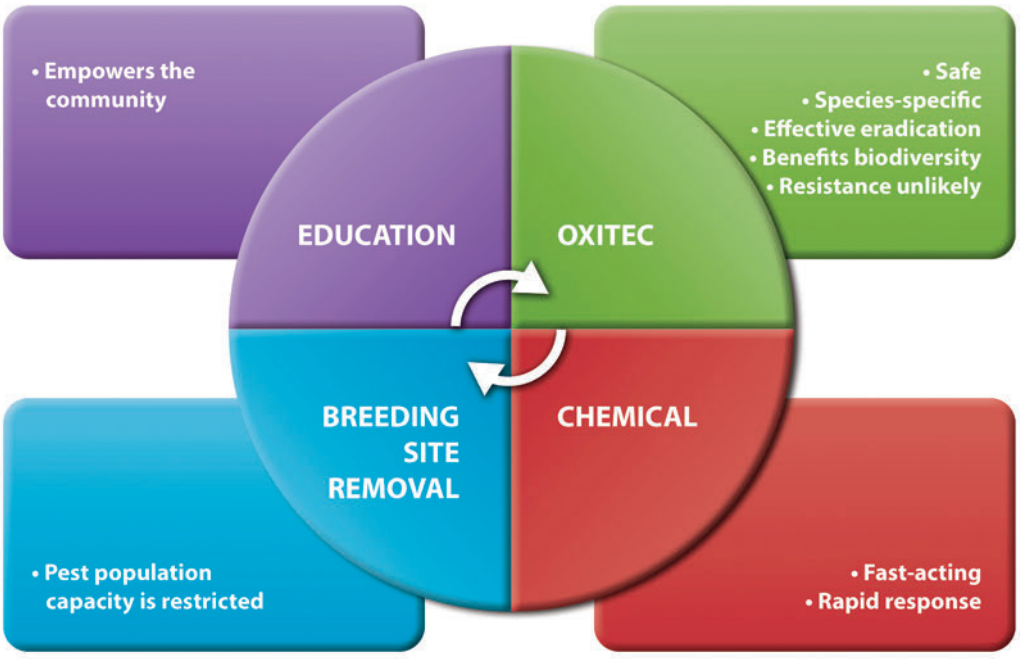
- The males cannot bite and so do not transmit disease;
- Targets only Aedes aegypti;
- Does not persist in the environment;
- Built-in marker for simplicity of monitoring;
- Ability to tailor to pest infestation level.

These strengths come together with existing tools in a complementary system that offers a sustainable solution to public health problems that have previously been unmanageable, revolutionising vector control.

Truly sustainable management of a pest, whether in a public health, agricultural or veterinary context, has repeatedly proven problematic without a cohesive approach. Adaptation of pests to challenging environments is an on-going process and, in response, management strategies need to be flexible and dynamic. Oxitec's approach is tailored to pest infestation levels so areas of greatest infestation receive correspondingly higher rates of control, minimising redundancy and maximising efficiency.

Oxitec has developed a programme of control for Aedes aegypti that is safe, widely applicable, environmentally friendly, cost-efficient, and highly effective. The approach is underpinned by area-wide mosquito monitoring based upon retrieving samples of eggs from the wild population; the data from which are used to assess and adjust the range of control methods being employed. A fundamental component is community-based breeding site removal, which not only helps to minimise mosquito habitats but also raises levels of public awareness. For large mosquito populations, initial short-term use of insecticides can be utilized to provide a rapid 'knock down'. Long-term suppression of the population is then provided by repeated releases of Oxitec's genetically sterilised male insects that are capable of sustained and substantial suppression of the pest population, with potential for local elimination and protection from re-infestation.

Invasive species such as Aedes aegypti are generally highly adaptable and prolific when conditions are favourable, which makes them such successful colonisers and renders them difficult to control. The prospect of making headway against this globally important disease vector is now a genuine one, with potential for enormous societal benefits. Best use of current and new vector control tools will be to employ them in strategic combinations to exploit the strengths of each.



Holistic Approach different but complimentary features are provided by the four central elements

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