Institution: University of Oxford		
Unit of Assessment: 5 – Biological Sciences		
Title of case study: Oxitec: pest and pathogen control with genetic modification technologies		
Period when the underpinning research was undertaken: 2000-2007		
Details of staff conducting the underpinning research from the submitting unit:		
Name(s):	**Role(s) (e.g. job title):**	**Period(s) employed by submitting HEI:**
Professor Luke Alphey	Professor in Genetics	1997-2008
Period when the claimed impact occurred: August 2013 – December 2020		
Is this case study continued from a case study submitted in 2014? Y		

1. Summary of the impact

Research at the University of Oxford led to the invention of self-limiting genetic modification tools for insects and the establishment of a successful spin out company, Oxitec Ltd, to develop techniques for applying these tools for control of disease vectors and insect pests. Since 2014, Oxitec has undertaken several successful trials in Brazil of two strains of GM mosquitoes that have reduced the wild populations of mosquitoes in those areas. In one of these field sites, a reduction in the number of cases of dengue fever was also recorded. These technological developments and the success of the early trials led the National Biosafety Committee in Brazil to develop a regulatory framework to evaluate similar products in the future, and informed the development of the Guidance Framework issued by the Special Programme for Research and Training in Tropical Diseases on behalf of the WHO. Oxitec was sold to US company Intrexon in 2015 for USD160,000,000.

2. Underpinning research

Insect pests have long caused huge economic and health impacts. Among many techniques developed to combat them, releasing sterile adults (the Sterile Insect Technique, or SIT) to control populations has historically been favoured as a targeted intervention. Traditionally, irradiation or chemosterilants have been used to generate chromosomal aberrations in the sperm of adult male insects, which are released in large quantities to mate with wild females; over a sustained period the wild population can be suppressed or eliminated. Conventional SIT has major disadvantages: the techniques used to induce sterility damage the whole insect and can affect competitiveness against non-sterilised insects; SIT is difficult to use in some insect species (notably mosquitoes); and it does not enable easy sex-separation.

Research undertaken by Professor Luke Alphey at the University of Oxford developed a novel approach to SIT in a technique called ‘Release of Insects carrying a Dominant Lethal’ (RIDL) [1]. A dominant lethal gene, repressible in the lab by use of the antibiotic tetracycline, is introduced into an insect’s DNA to make a new transmissible modified strain. When the genetically modified (GM) adult insects are released, males carrying the mutation mate with wild females and offspring inherit the lethal gene, which is no longer supressed. The genetic modification can be sex-specific (only female offspring die) or non-sex-specific (all offspring die).

This was the first time that transgenic methods had been developed for SIT, and the research demonstrated that both methods worked in Drosophila [1]. Their models predicted that RIDL would be at least as effective as SIT and would have additional benefits: transgenic males were likely to have a fitness advantage over irradiated males, and sex-separation could be facilitated by the use of female-specific lethality [1]. Oxitec Ltd was founded as a University of Oxford spin-out in 2002 to enable scale-up of the technology, regulatory approval and large-scale pilot studies.

Further research at the University of Oxford culminated in the first use of the RIDL technique in a serious insect pest: the Mediterranean Fruitfly (Medfly). In this case the dominant lethal gene killed both male and female offspring. As Medflies cause most damage as larvae, the gene was designed to cause lethality at an early stage in development. A fluorescent genetic marker causing was included to allow discrimination of wild type and engineered insects – a key component of effective monitoring of populations. The research showed that RIDL could provide a replacement or back-up for radiation sterilisation [2]. Concurrently, Alphey and colleagues were working on *Aedes aegypti*, the key vector worldwide of the yellow fever and dengue fever viruses, and specifically OX513A, a GM mosquito again designed to carry a dominant lethal gene [3]. The research investigated how late-acting lethality could be used to offset the tendency for surviving offspring to survive better owing to reduced competition for resources ('density-dependent' effects). Conventional SIT induces lethality at the embryo stage; this paper showed how RIDL could allow the timing of the lethal phase to be tailored to improve cost-effectiveness.

This is the same body of underpinning research as set out in the REF2014 case study "An innovative GM approach to the control of insect pests and mosquito vectors of human disease".

3. References to the research

Oxford authors in bold. Authors in bold and italics had dual University of Oxford and Oxitec affiliations at the time of publication.

1) **Thomas DD**, Connelly CA, Wood RJ and **Alphey LS** (2000). Insect population control using a dominant, repressible, lethal genetic system. *Science* **287**(5462): 2474-2476.
DOI: 10.1126/science.287.5462.2474
[614 citations, Google Scholar 31/12/2020]

2) **Gong P**, **Epton MJ**, Fu G, Scaife S, **Hiscox A**, Condon KC, Condon GC, ***Morrison NI***, ***Kelly DW***, Dafa'alla T, Coleman PG and ***Alphey L*** (2005). A dominant lethal genetic system for autocidal control of the Mediterranean fruitfly. *Nat Biotechnol* **23**(4): 453-456.
DOI: 10.1038/nbt1071
[232 citations, Google Scholar 31/12/2020]

3) **Phuc HK**, **Andreason MH, Burton RS, Vass C, Epton MJ, Pape G,** Fu G, ***Condon KC***, Scaife S, Donnelly CA, Coleman PG, **White-Cooper H** and ***Alphey L*** (2007). Late-acting dominant lethal genetic systems and mosquito control. *BMC Biol* **5**: 11.
DOI: 10.1186/1741-7007-5-11
[430 citations, Google Scholar 31/12/2020]

4. Details of the impact

Pathway to impact

Oxitec was spun out from the University of Oxford in 2002 to develop and commercialise the use of engineered sterile males through large-scale production and release. As reported in the REF2014 case study, Oxitec raised substantial investment from 2008 onwards, enabling early trials of the OX513A GM mosquito strain in the Cayman Islands in 2009, and in various locations in Brazil from 2011 onwards. These were highly successful, reducing the wild populations of *Ae. aegypti* by up to 98% and confirming the effectiveness of the approach [A]. During the REF2021 impact period the research has resulted in new impacts with global reach, including large-scale trials with public health benefits, further development of the technology and regulatory framework, and realisation of major commercial impact.

Further trials of the OX513A strain

A four-year programme of releasing the OX513A mosquitos took place in the Brazilian city of Piracicaba starting in July 2015, initially treating an area with 5,000 residents, but subsequently expanding to include 11 additional neighbourhoods, home to a further 60,000 residents. The releases achieved 83% or greater suppression of wild *Ae. aegypti*, and up to 98% suppression, compared to untreated sites [Bi].

Development of new strain, OX5034, and new delivery methods

Building on the original University of Oxford research [1,2,3], Oxitec subsequently developed an improved 2nd generation strain, OX5034 which offers more sophisticated genetics using sex-specific alternative splicing. Here, the male mosquitoes carry only two introduced genes; the male-selecting self-limiting gene and a simple marker gene that allows easy tracking in the field.

A first pilot study of OX5034 took place in four suburbs of Indaiatuba, Brazil, between 2018 and 2020. The trial demonstrated an average of 89% peak suppression of wild *Ae. aegypti* populations across the two communities treated with a low release rate of mosquitoes and an average of 93% across two communities treated with a higher release rate compared to the untreated control site [Bi].

Oxitec also developed a new delivery method for releasing the GM mosquitos, via soaking an egg-filled mini capsule in water. The Friendly™ technology (trade name *Aedes do Bem*™ in Brazil) was successfully deployed in trials in the city of Indaiatuba, Brazil, in October 2019 and extended to three different mosquito vectors of disease (*Aedes aegypti*, *Anopheles albimanus* and *Anopheles stephensi*) opening the potential to tackle a number of vector-transmitted diseases such as dengue fever, zika and malaria [Bii]. Following the trial, the Health Secretary of Indaiatuba said "*After the success of the first pilot with Oxitec's Aedes do Bem™, we are eager to explore the benefits that technology can offer to our city and other communities affected by the mosquito*" [C].

Following the successful trial and final approval of biosafety by CTNBio, the national regulatory authority for biosafety in Brazil in May 2020 [D], Oxitec and the Dengue Control Programme of the City of Indaiatuba rolled out further releases of the Friendly technology ("Aedes do Bem!") in November 2020 in preparation for the 2020-2021 high mosquito season [F]. The Health Secretary of Indaiatuba emphasised the significance of the project in terms of public health management: *"Indaiatuba is privileged to have Aedes do Bem!™, because without this restraint we could have two epidemics together, Covid-19 and Dengue"* [E].

The Bill and Melinda Gates Foundation awarded a grant of USD5,812,666 to Oxitec in 2018 to further develop this technology for malaria control, to transfer a self-limiting genetic platform into the malaria vector for future application in Meso-America and the Caribbean to reduce or eliminate this mosquito where it transmits malaria [Fi]. The director of the malaria program at the Gates Foundation said Oxitec's approach would complement other types of interventions being used to stop malaria's spread. "*Vector control has played a critical role in reducing cases and deaths due to malaria over the past 15 years. With further progress stalled at present, continued innovation of new and transformational interventions is critical to realizing the goal of a world free of malaria*." [Fii].

Impact on disease control and public health

Results from the early field trials demonstrated the technique was effective at suppressing the wild *Ae. aegypti* and this had a significant impact on the spread of dengue fever in the trial sites [A]. According to the Piracicaba Department of Health, in the year immediately preceding the trial release of the OX513A GM mosquito strain from July 2014 to July 2015, 133 cases of dengue were recorded in the region. In the period after the release of "Aedes do bem", from July 2015 to May 2016, there were only seven cases of the disease in the same area [G]. As a result, the Mayor of Piracicaba announced that the transgenic Aedes would be a fundamental part of the city's strategy in combating dengue, zika and chikungunya: *"Piracicaba is investing in the expansion of a solution that has already shown itself to be able to control a serious public health problem. And the best part is that we are doing this with innovative, clean, environmentally friendly and sustainable technology"* [G].

In the United States there have been several outbreaks of dengue, which is spread by *Ae. aegypti* - an invasive species in the United States - particularly in Florida, which experienced 62 locally acquired cases in one county in 2020 [Hi]. As such the Florida Keys Mosquito Control District

formed a partnership with Oxitec as part of its commitment to explore novel, safe and effective ways to keep mosquito populations at low levels and stop the spread off dengue, as well as an emerging threat from the zika virus. Oxitec received approval from the U.S Environmental Protection Agency (EPA) in May 2020 [Hii] to carry out pilot projects to try and control the spread of dengue via this vector, although activities have been delayed to 2021 due to the COVID-19 pandemic.

Regulatory Impacts

As highlighted in the 2014 impact case study, the University of Oxford's research was the first to result in the development of GM insects ready to for release into field trials. At the time there were few regulatory frameworks in place to manage these activities, prompting the World Health Organisation, the US Department of Agriculture and other national bodies to develop relevant guidance.

The Special Programme for Research and Training in Tropical Diseases (TDR) published its Guidance Framework for testing genetically modified mosquitoes in June 2014 [I]. The Framework extensively quotes the research undertaken at the University of Oxford by Alphey, including [1].

In April 2014, the National Biosafety Committee in Brazil (CTNBio) gave their technical approval for commercial release of Oxitec's OX513A strain of GM mosquito [J], primarily to try and control the spread of the dengue virus. In doing so, Brazil was the first country in the world to approve the unconstrained release of a genetically modified mosquito [J]. In April 2016 the National Health Surveillance Agency (Anvisa) of Brazil announced that following the approval by CTNBio of commercial release of OX513A, it would grant Oxitec a special temporary registration for OX513A. Anvisa also announced that it would "*create a regulatory framework capable of evaluating this and other similar products that may be developed*" and that "*the Agency has already been drafting new rules, under the theme 54.1 of the Regulatory Agenda 2015-2016, 'Evaluation of Macroorganisms for biological control of vectors and pathogens in urban environment*'" [K]. The new strain, OX5034, was reviewed by CTNBio and on 25 May 2020 "*the Commission concluded that this request complies with CTNBio standards and the relevant legislation aimed at ensuring the biosafety of the environment, agriculture, human and animal health*" [D].

Economic and commercial impact of Oxitec Ltd

At the start of the impact period in mid-2013, Oxitec Ltd had 37 staff all based in Oxford. Over the period 2014-2019 staff numbers averaged 56, peaking at 70 in 2017 [L]. In July 2014 Oxitec opened its first factory in Brazil and now has a large facility there - Oxitec do Brasil, Campinas, Sao Paolo, a subsidiary of Oxitec Ltd. Oxitec was purchased in September 2015 for USD160,000,000 [M] by a US company, Intrexon Inc, realising significant returns for its investors, and representing foreign direct investment into the UK. Oxitec Ltd was the Winner of Social Impact Investment of the Year**,** at the UK Business Angels Association Awards in 2016 [N].

5. Sources to corroborate the impact

[A] Carvalho et al (2015) Suppression of a Field Population of Aedes aegypti in Brazil by Sustained Release of Transgenic Male Mosquitoes. *PLoS Neglected Tropical Diseases* 9(7) DOI: 10.1371/journal.pntd.0003864

[B] Oxitec announcements: i) Field trials in Brazil https://www.oxitec.com/brazil
ii) Details of Friendly mini-capsule technology https://www.oxitec.com/en/news/oxitecs-friendly-mosquito-technology-receives-us-epa-approval-for-pilot-projects-in-us-c2943

[C] News article from Paran@shop 28/09/2019 'Biotechnology eliminates up to 98% of Dengue mosquitoes' (in Portuguese) https://paranashop.com.br/2019/09/biotecnologia-elimina-ate-98-dos-mosquitos-da-dengue/

[D] Approval by CTNBio of the commercial release of OX5034, May 2020 (in Portuguese). https://www.in.gov.br/web/dou/-/extrato-de-parecer-tecnico-n-6.946/2020-258262552

[E] News article 23/11/2020 'Oxitec Expands Aedes do Bem! Technology in Indaiatuba for the 2020/2021 Dengue season' (in Portuguese) https://www.indaiatuba.sp.gov.br/relacoes-institucionais/imprensa/noticias/28997/

[F] Award from Bill and Melinda Gates Foundation i) details of grant awarded in 2018 https://www.gatesfoundation.org/about/committed-grants/2018/06/opp1181812 and ii) news article about award 19/06/2018 https://www.geekwire.com/2018/gates-foundation-teams-oxitec-new-breed-malaria-blocking-mosquito/

[G] News article 31/05/2016 'After testing, Piracicaba expands use of transgenic Aedes aegypti mosquito' (in Portuguese) http://g1.globo.com/sp/piracicaba-regiao/noticia/2016/05/apos-teste-piracicaba-amplia-uso-de-mosquito-aedes-aegypti-transgenico.html

[H] Florida Keys project i) Centres for Disease Control and Prevention: Dengue in USA https://www.cdc.gov/dengue/statistics-maps/2020.html and ii) EPA Issuance of an Experimental Use Permit 27 May 2020. Federal Register Vol 85 No 111 https://www.federalregister.gov/documents/2020/06/09/2020-12372/issuance-of-an-experimental-use-permit

[I] The Special Programme for Research and Training in Tropical Diseases (TDR) The Guidance Framework for testing genetically modified mosquitoes (June 2014) WHO framework Report, available from: https://www.who.int/tdr/publications/year/2014/guide-fmrk-gm-mosquit/en/

[J] Paes de Andrade et al (2016) Use of transgenic Ades aegypti in Brazil: risk perception and assessment. *Bulletin of the World Health Organization* 2016;94:766-771. DOI: 10.2471/BLT.16.173377

[K] National Health Surveillance Agency – Anvisa. "Anvisa decides that transgenic mosquito is subject to health regulation" May 2016 (in Portuguese) https://www.gov.br/anvisa/pt-br/assuntos/noticias-anvisa/2016/anvisa-decide-que-mosquito-transgenico-e-objeto-de-regulacao-sanitaria

[L] Oxitec reports and accounts filed with Companies House, available from: https://find-and-update.company-information.service.gov.uk/company/04512301/filing-history?page=1. Report ending December 2017 provided.

[M] Case study of Oxitec on gov.uk website February 2016 "Oxitec: business accelerates its fight against dangerous viruses" https://www.gov.uk/government/case-studies/oxitec-business-accelerates-its-fight-against-dangerous-viruses

[N] News article 15 July 2016: Oxitec wins UK Business Angels Association Social Impact Investment of the Year Award 2016 https://ukbaa.org.uk/oxitec-wins-social-impact-investment-of-the-year-award-2016/