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Buzz or Bust for Genetically Engineered Insects?

by Edward Hammond

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Chapter 1

Introduction

The British company Oxitec is perhaps best known for its controversial attempts to release genetically engineered (GE) mosquitoes into the wild. Less well known are the company's efforts to commercialize patented GE strains of at least six different agricultural insect pests. The GE insects have been genetically engineered with reproductive flaws and, according to the company, can be used to reduce natural populations of crop pests.

This paper provides an overview of Oxitec's ventures in agriculture, including its portfolio of patent claims, information on the six GE agricultural pest species it is developing so far, difficulties with the company's plans, and its apparent close relationships with agrochemical giant Syngenta.

Oxitec is virtually alone among companies in its quest to sell genetically engineered insects for field use. In theory, some of the company's GE insects target crop protection markets that are sizable and which could prove profitable for the company.

Oxitec promotes its plan as bold and visionary, but closer analysis shows serious weaknesses, as the safety, regulatory and contamination concerns related to releasing genetically engineered animals, particularly insects, into the wild are quite consequential. The company further must face the reality that many of the genetic engineering "solutions" that it proposes in agriculture can be and are being addressed successfully by non-GE methods.

Chapter 2

Patent Portfolio

Since 2004, Oxitec has sought 12 patents in Europe, the US and elsewhere. Five of these patent applications, published between 2004 and 2008, cover the technology used by the company to create transgenic insects. In addition, Oxitec has access to a sixth patent assigned to Oxford University (where company officials have posts) that was published in 2001.

The remaining six patent applications, published between 2008 and 2012, relate to methods to detect specific DNA mutations in biological samples. These latter applications might have use in detecting the signature of Oxitec's GE insects in the wild, but have broader applicability, including potential use in diagnosing disease. These patents, while assigned to Oxitec, have given rise to at least three spinoff companies involving many of the same investors and staff as Oxitec itself (see box on p20).

The company's patent portfolio for genetically engineering insects is summarized in the following table. The inventor in all of the patents is Luke Alphey, Oxitec's Chief Scientific Officer and co-founder.

PCT Publication Number	**Title**	**Subject Matter**	**National Status**[1]
WO/2007/091099	Gene expression system using alternative splicing in insects	Methods and constructs to transform insects, particularly wherein one gender of a GE type carries a transgene causing its offspring, when bred with wild types, to die before reproducing.	Pending in Europe, the US, Australia and China.
WO/2005/042751	Controlling the spread of infective agents	Creation and release of insects genetically engineered to not transmit parasitic disease.	None reported. The international patent search found the application to lack an inventive step.
WO/2005/012534	Expression systems for insect pest control	Genetically engineered insects with transgenes regulated by tetracycline exposure.	Pending in Europe, the US, Australia, China and South Africa.
WO/2005/003364	Stable integrands	Technique to reduce the problem of GE “jumping genes” (transposons) changing location in an insect genome.	Granted in the US, pending in Europe.

WO/2004/098278	Dilution of genetic traits	Introducing insects susceptible to pesticides into a wild population, in order to retard the spread of pesticide resistance.	Granted in the EU and US, filed in Canada (expired) and China (status unknown).
WO/2001/039599	Biological control by conditional dominant lethal system	Organisms, particularly insects, with a lethal transgene active in natural conditions but suppressible in containment.	Granted in the US, the EU, Australia and New Zealand. Pending in Canada, China, Israel, Mexico and Singapore.

Oxitec's patent claims cover specific genetic engineering techniques but are broad in that they typically are not restricted to a small number of species, instead covering a wide range of insects, and even other types of animals, including mammals. For instance, the genetic engineering method of patent publication WO/2007/091099, while primarily directed toward a variety of insect species, is also claimed "wherein the organism is a mammal, a fish, an invertebrate, an arthropod, an insect or a plant." Similarly, the 2005 patent application titled "Stable Integrands" claims genetic engineering of any organism, including but not limited to any insect.

While, as a practical matter, the company appears to be focused on genetically engineered insects, Oxitec's retention of broader rights to its techniques to introduce reproductive defects into species indicates potential interest in using (or perhaps licensing for a fee) its techniques for the control of other kinds of species. What is certainly the case, however, is that Oxitec holds exclusive rights to its "conditional lethality" and related technologies for all insect species.

Chapter 3

Oxitec and Sterile Insect Technique

In agricultural applications, Oxitec typically proposes its genetically engineered insects as an alternative to, or extension of, an approach called sterile insect technique (SIT). Developed in the southern US, SIT came into practical use in the 1950s, when the US and Caribbean countries successfully used it against screwworm (*Cochliomyia hominivorax*), a larval parasite of cattle.

Now used against a number of other insect pests, especially species that attack crops, SIT involves irradiation of large populations of insects reared in confinement. The irradiated insects are released into the field, where they seek out mates. But because the captive-reared insects' genetic material is scrambled by the radiation exposure, they cannot successfully reproduce.[2] Nevertheless, the captive-reared insects mate with wild partners. Since these pairings do not produce viable offspring, the overall population of the target species declines and, in some cases, can even be eliminated.

In its patents and patent applications, Oxitec claims any insect genetically engineered by its methods; however, for practical purposes, the company must focus on species already used in the field of SIT. This is because Oxitec needs the knowledge and skills in insect rearing, sorting and release that have been developed for SIT, many of which are species-specific and have been made possible as the result of a significant research effort over many years.

Indeed, the challenges of captive-rearing large populations of insects that effectively compete for mates against their wild counterparts can be substantial. For instance, SIT for tsetse fly (carrier of African trypanosomiasis, or sleeping sickness) has long been studied, and used in limited situations, but cost-effective rearing systems that can be used on a large scale have yet to be achieved.

Publications by Oxitec authors appear to systematically muddle distinctions between SIT and the company's genetic engineering approach, characterizing releases of GE insects as a logical and practical extension of SIT, including in cases where no fundamental problem has been identified in existing SIT programmes.[3]

In reality, rendering insects sterile through genetic engineering is a radically different technology than using SIT. In Oxitec's approach, the sterilization mechanism (transgenes) is released into the wild, whereas with SIT, sterilization is accomplished within the physical containment of rearing facilities. Potentially dangerous radioactive materials stay at the facility and are not released into the environment.

Oxitec arguments casting genetically engineered insects as a logical and practical extension of SIT are disingenuous and appear intended to be disarming in much the same way that some proponents of biotech crops argue, without credibility, that genetically engineered seeds are little different than conventionally bred ones. And by incorrectly framing its fundamentally different GE technology as an extension of

SIT, the company also hopes to capture the support and interest of the SIT field (frequently a public effort), which the company needs in order to be successful.

But in truth, while Oxitec needs SIT, SIT doesn’t need Oxitec.

Chapter 4

Oxitec's GE Insects: Worms, Flies and Miners

Oxitec has six different agricultural pests that it is targeting. Into one or more strains of each species, the company has genetically engineered reproductive defects that it calls RIDL ("Release of Insects Containing a Dominant Lethal"). "Dominant lethal" refers to an introduced gene that, when expressed, has the effect of killing the insect.

The company's GE insect strains are dependent on the presence of a chemical – typically an antibiotic – that captive insects are fed. Without this addition to their food when released into the environment, a lethal gene becomes active, causing the insects to die. This defect can be introduced such that it renders one or both sexes sterile, or such that the lethal gene is heritable, resulting in the death of offspring (or one gender thereof). The result is to diminish an insect population's success at reproduction, particularly pairings between wild and captive-bred insects.

According to the company, the introduced genetic deficiencies offer no selective advantage, are self-limiting in nature, and would be naturally selected out of existence in the wild within a short time (typically a few generations, depending on the specific case). This company claim, based on theory and small-scale contained experiments, remains unproven in the large, natural environments where the company's GE insects would be released.

In one variant or another, the GE technology has been used in the following agricultural pest species:

Diamondback moth: Thought to have originated in Europe, the diamondback moth (*Plutella xylostella*) can now be found worldwide. It lays its eggs on plants in the *Brassica* genus, which includes a number of row and vegetable crops, such as rape (canola), broccoli, radishes, mustard and collards. The larvae cause significant crop damage. Options for controlling the moth are many, including chemicals, Bt toxins, SIT, biological control (with moth parasites) and trap crops.[4] Oxitec's GE diamondback moth would work by release of male insects that pass a lethal trait on to female offspring. Oxitec has recently proposed testing of this insect in the UK (see below).

Pink bollworm: The cotton pest pink bollworm (*Pectinophora gossypiella*) is an Asian native that has spread worldwide.[5] As the name suggests, bollworm larvae burrow into cotton bolls, feeding on the seeds contained inside. In the process, the larvae cut and stain cotton fibres, and provide an avenue for microbial infections of the host plant. The result of pink bollworm infestation is poor cotton quality. Methods to control pink bollworm include chemical pesticides, Bt toxins and other biological controls, as well as SIT.

There are two GE pink bollworms that Oxitec is seeking to commercialize. The first expresses a red fluorescent colouring gene. Without a lethal gene, these GE bollworms are additionally irradiated. The red colouring transgene enables identification of SIT irradiated insects in the field,[6] a function that is normally accomplished by use of dyes. Oxitec, however, mentions that "anecdotal field experience" in the United States suggests that dye is insufficient because weakly tinted insects might be mistaken for wild ones.[7] The case for use of GE insects to perform this simple function is weak, as dyes have been successfully used for decades and weakly dyed insects can be subjected to lab analysis. Nevertheless, field trials of the GE bollworm have been conducted in the US.

The second GE pink bollworm expresses a lethal gene, although its practical use seems questionable because the US SIT programme to eradicate pink bollworm, at which the Oxitec insect is aimed, is widely regarded as being on the verge of successful completion.[8]

Mediterranean fruit fly: The Mediterranean fruit fly (*Ceratitis capitata*), sometimes simply called "medfly", is a pest of fruit crops, especially citrus. The fly leaves its eggs under the skin of fruit on the tree. Hatched larvae feed inside the ripening fruit, ruining it. Larvae transported by the fruit trade have repeatedly caused outbreaks of medfly in new areas. It is thus a frequent target of eradication programmes, and the reason for numerous fruit quarantine programmes in many countries.

Mexican fruit fly: The Mexican fruit fly (*Anastrepha ludens*), or "mexfly", is another citrus pest, but of more limited distribution and economic importance than the medfly. Like the medfly, mexfly larvae feed on citrus fruit. Primarily a pest in Mexico and Central America, the mexfly is occasionally found in the United States, especially in south Texas, where periodic appearances in a citrus-growing region on the border with Mexico provoke chemical and SIT eradication efforts. The US has also supported mexfly (as well as medfly) SIT programmes in Mexico and Guatemala, primarily as a means of creating a firewall against incursions into the US.

Olive fruit fly: As its name indicates, the olive fruit fly (*Bactrocera oleae*) is a pest of olives. Formerly restricted to the Eastern Hemisphere, the olive fruit fly has recently become established in the Americas. Available controls include chemicals, traps, resistant olive varieties, biological control and spraying trees with non-toxic repellents. In the past, use of SIT to eradicate the olive fruit fly has been impractical due to difficulties with captive-rearing of the flies, particularly providing them with an appropriate diet. Recent research, however, has started to provide answers to these problems.

Oxitec posits that its GE olive fly offers an improvement over SIT; however, the major advances that are making widespread use of SIT in olive fly control a more real prospect are related to olive fly breeding and rearing, rather than genetic engineering.

Tomato leaf miner: The tomato leaf miner (*Tuta absoluta*) originated in South America and is now spreading elsewhere, including tomato crops in the Mediterranean and Middle East. It can also attack eggplant (brinjal), tobacco, potatoes and other crops. Available controls include chemicals, biological controls and pheromone traps. Oxitec has entered into an agreement with Certis Europe, a subsidiary of Japan's Mitsui Chemical, to develop the GE insect.

Chapter 5

Practical Impediments

There are many practical impediments to commercial use of GE insects in agricultural pest control. While the challenges vary from insect to insect and location to location, the following are among those that are frequently a concern:

Genetic background: Populations of the same species are not necessarily alike, especially if the individuals come from different places, for instance, populations separated by an ocean. This variation from region to region poses a challenge to pest control using GE insects because in many situations, the captive-reared population should be of the same genetic background as the wild one, among other things, in order to avoid the possible introduction of genes that would worsen the pests' impact.

Population differences are relevant for some of the widely disseminated agricultural pests targeted by Oxitec. For instance, pink bollworms in India appear to show greater resistance to Bt toxin genes than their counterparts in the US. In mosquitoes, different strains of the same species may be more or less effective vectors of human disease.

Thus, the particulars of the strain(s) of insect that is genetically engineered can have great impact on its potential use in the field, possibly rendering the GE insect ineffective or even backfiring by introducing traits that invigorate pest populations. With transformation and establishment of new GE insect strains involving substantial scientific and regulatory investments, consistently maintaining appropriate captive-reared strains is not a simple question.

This issue came to the fore in the UK, where Oxitec has approached regulators seeking approval for field trials of its GE diamondback moth. British regulators responded coolly to Oxitec's approach, noting that the company's genetically engineered moth strain is of North American rather than UK origin, raising the possibility that it could introduce new insecticide resistance or other undesirable traits in UK moth populations.[9]

Assortative mating: Another problem is assortative mating. This is the process, frequently ill-understood, in which insects segregate themselves in selection of mates. For instance, in SIT programmes, wild-type insects may prefer to mate with other wild-type insects, reducing the effectiveness of sterile insect releases. Getting inside the "reproductive minds" of insects to understand factors influencing assortative mating, and what can be done to captive strains to improve their competitiveness, can be a significant scientific challenge. If captive-reared insects are less attractive to their wild counterparts, then the effectiveness of the released insects is reduced. Conventional breeding is used to address this problem with SIT. With GE insects, however, addressing assortative mating problems may be more difficult, from both practical and regulatory perspectives.

Keeping up with Nature: Captive-bred insect strains may need to be kept "up to date" with those in the wild for reasons in addition to mating preferences. Over time, inbred captive insect strains may need new genetic stock from the wild, to restore vigour, confer new traits appearing in wild insects, etc. In SIT programmes this is accomplished through insect breeding. For a genetic engineering company, however, shifting transgenes onto new genetic backgrounds, by new transformations or by conventional breeding, raises biosafety questions and may be subject to a higher burden to prove safety, given the risks associated with genetic engineering.

Scale and cost: SIT efforts are typically government or government-supported programmes. There are good reasons why: Mass rearing and release of sterile insects typically requires large infrastructure. Rearing and handling facilities need to take significant containment precautions to avoid becoming a source of pests themselves. Programmes frequently target large areas under cultivation, requiring regular and systematic distribution of sterile insects over a large physical area, often by aircraft. The attendant practical and legal complexities are significant and do not easily lend themselves to the private sector.

Contamination of organic production: Use of genetically engineered insects in pest control may also present problems for organic agriculture. The use of genetically engineered insects to control pests may not meet organic standards, and given the imprecise dissemination of released insects, an organic farmer may find himself or herself an unwilling participant in a GE pest control programme. Also, egg-laying insects may deposit GE eggs on organic crops, causing direct GE contamination.

Under US organic standards, use of genetic engineering is an "excluded method"; thus, Oxitec's GE insects could not be deliberately used. More likely to worry organic farmers, however, is the potential problem of contamination. If Oxitec's insects were detected in a harvested crop, then it could not be sold as organic.[10] The result is that use of Oxitec's insects would create a burden on organic farmers to prevent contamination by the flying insects, both in order to preserve the value of their crops and because failure to perform due diligence to avoid GE contamination could result in a farmer losing organic certification.

Later-acting lethality: In many insect pest species, the main damage caused to crops is done not by adult populations, but at the larval stages of growth. For instance, olive flies inject their eggs into fruit, and it is the growing larvae, feeding from the inside (and inviting bacterial and other infections), that ruin the olive.

Typically, irradiated insects that are released by SIT programmes are sexually mature but do not successfully reproduce. These insects (or their mates) thus do not deposit viable eggs on crops that they infest, thereby limiting the damage attributable to the insect release.

With Oxitec's approach, however, the lethal genes introduced into GE insects may not take effect in those insects' offspring until the late larval stage. This means that GE eggs are deposited on host plants, those eggs hatch, and the resulting larvae feed on their host, even if the insects are "programmed" to die before reaching sexual maturity. When they die, in cases such as the olive fly, they are likely to die on or inside the host plant. The result is that in many cases, Oxitec's GE insects and their larvae have the potential to cause greater crop damage than irradiated insects of the same species.

Apart from the practical impediments highlighted above, there are also serious concerns about the potential environmental and health risks related to releasing GE insects into the environment. While it is not within the scope of this paper to discuss the biosafety issues, some examples of potential environmental risks could include unintended effects on biological diversity such as new or more vigorous pests; harm or loss of other species and disruption of ecological communities and ecosystem processes; vertical and horizontal gene transfer and the consequences thereof; persistence of the transgene in the ecosystem; and evolutionary responses which may have adverse consequences.

Chapter 6

Relationship with Other Companies

Oxitec presents itself as an independent British company, but it appears to have strong staff and management ties with Swiss agrochemical giant Syngenta.

Two of the four seats on Oxitec's Board of Directors are held by former Syngenta managers. (The other two are held by representatives of private equity firms that have invested in the company.)[11]

Three out of five members of Oxitec's management team are also Syngenta alumni. CEO Hadyn Parry, Regulatory Affairs Manager Camilla Beech and Director of Business Development Glen Slade were all at Syngenta prior to Oxitec.

Syngenta has directly funded research on GE insects by Luke Alphey, Oxitec's Director.[12] There are also links through former employees: Oxitec's director of business development from 2006 to 2010 came to the company from Syngenta, where she worked in mergers and acquisitions.[13]

The companies further share the same small public relations consulting company, which provides editing and communications services for both Oxitec and Syngenta's management office in Switzerland.[14]

Despite the numerous connections, no public explanation of the relationship between the companies has been made.

Oxitec also has ties with other spinoff companies, which have arisen from six of its patent applications related to methods to detect specific DNA mutations in biological samples (see box).

Oxitec's Extended Family

Key Oxitec staff and investors have formed spinoff companies in human health named 360 Genomics and Genefirst Ltd, both based in Oxford, UK. Both companies' focus is on DNA diagnostics, and both appear to use patent applications filed by Oxitec between 2008 and 2011.

Formed in 2007 with venture capital backing, 360 Genomics currently sells DNA tests for human cancers; however, according to the company's website, these do not have regulatory approval and are thus limited to research use. The company calls its technology "polymerase chain displacement reaction", and it claims the technology is an improvement over classic polymerase chain reaction (PCR). 360 Genomics is led by Fu Guoliang, an Oxitec researcher. Hadyn Parry, the CEO of Oxitec, sits on its board. Nearly five years after the company's founding, however, 360 Genomics is not growing, and a promised alliance with a major pharmaceutical company does not appear to have taken place.

Genefirst Ltd is a more recent Oxitec spinoff, founded in 2011. Directed by Fu and Oxitec's scientific director Luke Alphey, Genefirst has a similar focus as 360 Genomics, promising new DNA-based diagnostics. It also appears to rely on intellectual property applications filed by Oxitec. The UK-based Genefirst appears related to a Chinese company with almost the same name, Genefirst Technology, that was founded by Fu in 2009.

Chapter 7

Conclusion

Oxitec's ambition appears to go well beyond GE mosquitoes, extending into use of a variety of species of GE insects to control pest populations for globally important crops. With its publicly ambiguous but apparently close relationship with Syngenta, should the company achieve regulatory approval(s) for its GE agricultural pests, it may be able to depend upon the larger company's marketing experience and reach – senior staff were formerly Syngenta employees and it already shares communications strategies.

Yet Oxitec must also overcome serious hurdles and has a built-in dependence on sterile insect technique. While the company attempts to play this dependence as an advantage – muddying distinctions between SIT and genetic engineering – the comparatively advanced, proven and well-developed SIT techniques, and their continuing evolution, are also strong arguments against undertaking the risks associated with field release of genetically engineered agricultural pests.

For example, Oxitec's pink bollworm that expresses a red fluorescence gene is promoted by the company as facilitating identification of captive-reared insects on the field. Yet the same function has been accomplished with dyes for many years, as part of a programme (in the US) that has proven so effective that the pest is on the verge of eradication and the SIT programme, as a consequence, on the cusp of being terminated. Are genetically engineered insects really necessary to serve a function already accomplished by dyes and other markers in a notably successful programme?

Similarly, Oxitec puts forth a GE olive fly as a potential improvement in olive fly SIT programmes. Yet the major advances in olive fly SIT in recent years are improvements in insect diet and rearing techniques – items unrelated to genetic engineering. And a key “advantage” of GE olive fly alleged by Oxitec (greater mating compatibility with wild types) is being addressed by other researchers without the use of genetic engineering.

While through its intellectual property claims the company has positioned itself to potentially reap great benefits from commercial use of GE agricultural pests, when assessed on their merits, and weighed against non-GE options, Oxitec’s GE bugs should ultimately be viewed as uncompelling offerings.

Endnotes

1 The World Intellectual Property Organization's online database (PatentScope) contains only limited information on patent application status outside of a small number of countries. The information available in PatentScope is presented in the table, but is not complete. Patents may have been applied for and/or granted in other jurisdictions.

2 In some species at some radiation doses, insects will successfully reproduce, but the progeny will typically be sterile.

3 Gong, P et al. (2005). A dominant lethal genetic system for autocidal control of the Mediterranean fruit fly. *Nature Biotechnology* 23(4), 453-456. Also: Simmons, GS et al. (2011). Field Performance of a Genetically Engineered Strain of Pink Bollworm. *PLoS ONE* 6(9): e24110. doi:10.1371/journal.pone.0024110

4 A trap crop, planted around or alongside the primary crop, offers the pest an alternative place to lay eggs. For diamondback moths, scientists are evaluating *Brassicaceae* trap crops on which the moth prefers to lay its eggs, but upon which the eggs typically do not survive.

5 Pink bollworm should not be confused with *Helicoverpa zea*, *Helicoverpa armigera* and other species also referred to as "bollworms" and that also attack cotton (among other plants).

6 SIT programmes use traps to identify new infestations and to monitor populations of released insects.

7 Simmons, GS et al. (2011). Field Performance of a Genetically Engineered Strain of Pink Bollworm. *PLoS ONE* 6(9): e24110. doi:10.1371/journal.pone.0024110

8 See, for example, Blake, C and H Cline (2010). Final curtain for pink bollworm. *Southwest Farm Press*, 19 August. URL: http://southwestfarmpress.com/cotton/final-curtain-pink-bollworm

9 Letter from the UK Health and Safety Executive to Oxitec, 11 December 2011. Obtained by GeneWatch UK under the UK Freedom of Information Act.

10 Sligh, Michael. Rural Advancement Foundation International, personal communication, 5 September 2012.

11 Oxitec (2012). Our Team (web page). URL: http://www.oxitec.com/who-we-are/our-team/ (accessed 25 October 2012).

12 EFSA (2012). Declaration of Interests Database – Annual Declaration of Interests for Luke Alphey. URL: http://ess.efsa.europa.eu/doi/doiweb/wg/263347

13 Rectory Farmhouse Ltd (2012). Ann Kramer (web page). URL: http://www.rectory-farmhouse.com/our-team/ann-kramer/ (accessed 29 August 2012).

14 The Blue Ball Room (2012). Clients (web page). URL: http://www.theblueballroom.com/clients.php (accessed 29 August 2012).

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No. 16 Buzz or Bust for Genetically Engineered Insects? *by Edward Hammond* (32 pages)

Venturing into a potentially lucrative new field, British company Oxitec – better known for its efforts to commercialize genetically engineered (GE) mosquitoes – is now seeking to use similar technologies in the commercial application of GE insects in agricultural pest control. The firm claims that reproductive defects genetically engineered into agricultural insect pests can help suppress pest populations. Oxitec has claimed broad patents related to the specific techniques used in the GE insects, which cover a wide range of insects and even other types of animals.

However, as this paper reveals, this technology suffers from several practical shortcomings which could not only curtail its effectiveness but also exacerbate the very pest problem it aims to address. In addition, serious concerns have been raised over the potential environmental and health risks related to the release of GE insects into the wild.

All these considerations must prompt a rethink of the use of GE insects, especially in light of successful advances made by non-GE approaches to agricultural pest control.

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