Oxitec's GM fruit flies: issues of concern

GeneWatch UK

February 2018

Mediterranean Fruit Fly (Medfly) is a pest insect which lays its eggs in fruit, causing damage to a wide range of crops.

Following glasshouse experiments, UK-based company Oxitec, which is now owned by the US biotech company Intrexon, has proposed releasing genetically modified (GM) fruit flies in open trials in Western Australia, in an attempt to control this pest.[1] The company will need regulatory approval before doing so. The Department of Agriculture and Food in Western Australia is now in consultation with Australian Government regulatory bodies including the Office of the Gene Technology Regulator for the next phase of testing.[2]

This briefing outlines a number of concerns about Oxitec's GM fruit flies (Medfly). In particular, the GM flies are not sterile, but their female offspring die mostly at the larval stage, while they are inside the fruit. This means that fruit crops will be damaged by the feeding GM larvae and food supplies for humans and animals are likely to become contaminated with dead female GM maggots.

1. Oxitec's GM insects

Oxitec is a UK-based company.[3] In September 2015, Oxitec was acquired by the US-based synthetic biology company Intrexon.[4]

As well as GM fruit flies, Oxitec is developing other GM agricultural insect pests, such as diamondback moths, bollworms and olive flies, and GM mosquitoes. All the company's GM insects are intended to be released repeatedly in large numbers (millions on an experimental scale, or billions if commercialised) into the open to mate with the wild species. The insects are genetically engineered to express a fluorescent trait and a 'late-acting lethality' trait, which means many of the offspring from these matings do not survive to adulthood to reproduce. This is intended to suppress the numbers of wild insects.

Oxitec calls its patented technology "Release of Insects carrying a Dominant Lethal system" (RIDL). Its GM fruit flies and other agricultural pests are known as fsRIDL (female sex-RIDL). These insects use a variation of the trait by which only the female offspring are genetically engineered to die.[5]

Although Oxitec frequently describes its insects as "sterile", this is not the case. The released GM males mate and produce offspring which inherit the genetically engineered late-lethality trait. This means that most of the GM insects' female offspring die at the larval stage in the case of GM agricultural pests (which are female-killing only). Wild female fruit flies which have mated with the released GM males will lay eggs which inherit the GM "female killing" trait inside the fruit. GM larvae (fruit fly maggots) that develop from these eggs will begin eating the fruit crop before the majority of the female larvae die inside the fruit. The male GM larvae which grow inside the fruit are expected to emerge and develop

into adults as normal and to go on to mate with other wild flies, again passing on the female-killing trait.

Oxitec's business plan is dependent on locking its customers in to repeated payments for ongoing releases of its GM insect species with the aim of keeping the target wild species' numbers low.

Oxitec's GM mosquitoes – genetically engineered so that both male and female offspring mostly die at the larval stage - have been released in open experiments in the Cayman Islands, Malaysia, Panama and Brazil. Currently, trials continue only in Brazil and the Cayman Islands. No country has yet given approval for releases of GM mosquitoes on a commercial scale.[6]

Oxitec has previously sought to release GM diamondback moths in the UK[7,8,9,10,11,12,13,14] and the USA[15], GM olive flies in Spain[16,17], and GM fruit flies in Brazil. All these GM agricultural pests are female-killing only. Only one of these open release experiments has taken place, due to concerns about potential impacts on the environment and human health, and the likelihood of contaminating fruit and vegetables with GM insects (discussed further below). The only release was a small scale 'mark release recapture' experiment, using GM diamondback moths, in New York State in 2017.[18] Despite an application to conduct population suppression experiments with these moths, a permit for these was not granted. Earlier, open release experiments were conducted in Arizona in 2007 and 2008, using Oxitec's GM pink bollworms (a cotton pest), with only the fluorescence trait (not the 'late lethality' trait), and made sterile using radiation.[19] Although they used irradiated sterile insects, with only a GM fluorescence trait, the GM bollworm experiments were halted, partly over US organic farmers' concerns about contamination of their crops with genetically modified organisms (GMOs).[20,21] They also led the US Department of Agriculture (USDA) Office of Inspector General to make a highly critical report which argues that USDA APHIS' controls over GM insect research are inadequate and that regulations need to be strengthened.[22]

The proposed application to release GM fruit flies in Western Australia, if it goes ahead, could therefore be the first open release anywhere in the world of GM insects with the "female-killing" trait for the purpose of suppressing a wild insect population.

2. The Mediterranean Fruit Fly (Medfly) and existing methods of control

Medfly (*Ceratitis capitata*), a species of fruit fly which has spread worldwide from its native area in the Mediterranean region is thought to have originated in Africa. It is a highly invasive species which causes extensive damage to many fruit crops, such as mangoes, oranges, peaches, apples, figs and cherries.[23,24] Its spread to Australia, where it is categorised as a major "quarantine pest"[25], is likely to be a result of accidental transportation during trade. It is currently present in Western Australia but has been eradicated from other states.

The pest has been eliminated from the eastern states, possibly as a result of competition from the Queensland fruit fly (*Bactrocera tryoni*), combined with control measures in affected orchards.[26] In New South Wales, Medfly was first recorded in 1898 but had disappeared by 1948. In Queensland, it was formerly present in the southeast and first recorded in 1909, but disappeared during the 1930s. In Victoria, Medfly was first recorded in 1909 and had disappeared by the 1940s.[27]

Medfly was first identified in Western Australia in 1897, but early action was not taken to control the pest. A range of techniques have since been used to attempt to control Medfly in Western Australia, including traps, lures and sprays, quarantine controls, and disinfestation of infested fruit.[28] In 2005, researchers studied why Medfly in the Kimberley region of Western Australia is only established at Broome.[29] They found that the eight host plants most important to Medfly survival and abundance in Broome are kumquat, guava, orange jessamine, mango, Barbados cherry, yellow oleander, Pacific almond and blackberry tree. The authors concluded that it is likely that medfly can only maintain populations in areas close to human habitation, and eradication from these areas would lead to eradication from the whole Kimberley region.

Flight by the adult flies and the transport of infested fruits are the major means of movement and dispersal to previously uninfested areas. There is evidence that Medfly can fly at least 20 km.[30] It has been suggested that buffer areas in area-wide integrated pest management of Mediterranean fruit fly should be at least 1.3 km wide, based on monitoring experiments in Spain[31]. Eggs and larvae (maggots) may be transported in fruit; and pupae in contaminated soil.[32]

Adult female Medfly puncture the host fruit with their ovipositor to lay their eggs below its skin. The eggs hatch inside the fruit within 2-4 days (up to 16-18 days in cool weather) and the larvae feed for another 6-11 days (at 13-28°C). Fully grown larvae jump out of the fruit into the soil below, where pupae form in the soil under the host plant and adult Medflies emerge after 6-11 days (at 24-26°C; longer in cool conditions). Adults live for up to 2 months (as measured in field cages, perhaps longer in the wild). Adult activity is reduced or suspended at temperatures around 30°C, when the flies seek out cooler areas. The lower and upper temperatures that permit coordinated movement of adults are within the range of 5.4–6.6°C and 42.4–43.0°C, respectively, but these values may vary.[33] Medfly distribution is therefore highly seasonal.[34]

When detected, it is important to gather and destroy all fallen and infected host fruits and to monitor the population using traps.[35,36]

Until recently, the usual choice of insecticide has been the organophosphate Malathion (also known as Maldison). It is usually combined in a bait spray with a protein which gives off ammonia to attract the flies, so that insecticide can then be applied to just a few selected places in the orchard. In 2015, the World Health Organisation's cancer agency, IARC, categorised malathion as 'probably carcinogenic to humans'[37] and this chemical is currently under reconsideration by the Australian Pesticides and Veterinary Medicines Authority (APVMA)[38].

Insecticide use carries a high risk of pesticide residues on fruit, negative effects on ecosystems, and the development of insect resistance. Malathion has been associated with harmful impacts on beneficial insects, including bees and the natural enemies of pest insects. It is now banned in the European Union (EU) and is being replaced with more environmentally friendly alternatives such as spinosad (an insecticide based on chemical compounds found in the bacterial species *Saccharopolyspora spinose*), again combined with bait. Although Malathion is somewhat more effective, spinosad appears able to provide an effective level of control, with less environmental impact.[39] Spinosad is relatively benign to natural enemies of pests (predators and parasites), however it can harm some species of parasitoids through sub-lethal effects, including loss of reproductive capacity, reduced longevity, etc.[40]

Traps and lures may also be used: mass trapping of females and males using densely-spaced baited traps is being used extensively in the Mediterranean region.[41] The trapping technique is based on placing a high density of traps with an attractant and a toxicant, aiming to capture the highest number of adults. A study in clementine groves in Spain found that 25 traps per hectare provided a valid stand-alone method to protect mid-season varieties, with less than 0.5% of the fruits damaged at harvest.[42] For early-season varieties, a higher density of traps was required (50 traps per hectare), combined with chemical treatments to only the perimeter row of the grove, resulting in less than 2% of fruits damaged at harvest. Attract-and-kill methods reduce costs and can provide effective pest control. For example, a Spanish attract-and-kill device impregnated with deltamethrin (a pyrethroid) and attractants achieved good control of Medfly in early clementine orchards in Spain.[43] Control was as effective as mass trapping, but at lower cost.

One of the most effective control techniques against fruit flies in general is to wrap fruit, either in newspaper, a paper bag, or in the case of long/thin fruits, a polythene sleeve. This is a simple physical barrier to Medfly laying eggs inside the fruit, but it has to be applied before the stage at which the fruit is attacked.[44]

Commercially produced baits and traps, and fruit exclusion bags, are available for use by organic farmers.[45]

An alternative method of control is the sterile insect technique (SIT), which has been used against Medfly in Costa Rica, Italy, Mexico, Nicaragua, Peru, Spain, Tunisia and the USA, with the largest ongoing programme in Mexico (Programa Moscamed). The sterile insect technique (SIT) requires the release of millions of flies which have been sterilised using radiation into the wild population, so that there is a strong likelihood of wild females mating with sterile males.[46] Alternatively, wild females may be sterilised with chemicals: field trials in Spain, in which lufenuron (an insect growth regulator) was mixed with food-based attractants, provide evidence for the effectiveness of this technique.[47] Females fed lufenuron or that have mated with lufenuron-fed males can reduce or prevent egg hatching, producing a continuous reduction in fruit fly populations year after year. In the Spanish study, this technique was as effective as malathion treatment in citrus, and more effective in persimmon, from the second year of treatment.

In a feasibility study using the Sterile Insect Technique with irradiated flies, Medfly was eradicated in December 1984 from Carnarvon in Western Australia (based on finding no infestation of adults or larvae for a period equivalent to 3 fly generations).[48] Initially, no insecticide was used, but as the program progressed, it was found necessary to use insecticide bait spraying to knock the natural population down to a very low level, to enable successful over flooding with sterile flies.[49] From October 1984 to January 1985, a time period sufficient for at least three fruit fly generations, no flies were caught in the 180 traps situated in and around Carnarvon. However, as there were no quarantine barriers to prevent re-infestation, infested fruit was subsequently brought in from the South West. From 1989-1991, SIT was used successfully to eradicate another fruit fly species - Queensland fruit fly (Q-fly) - from Western Australia at a cost of $8 million. A pilot SIT programme for Medfly subsequently took place in Broome in 2000.[50] Trapping data over the three years of the trial showed that the eight month period of releases suppressed, but did not eradicate, flies.[51] A cost-benefit analysis suggested that eradication of Medfly from Australia would cost around $70 million over a six year period: if the area of horticulture expanded over a 20-year period, then the benefits of eradication could equal or exceed the cost.[52] However, a 'systems approach' is likely to continue to be needed, including baiting and quarantine controls.[53]

More recently, the Western Australia Government earmarked Carnarvon for the release of millions of sterile male Medflies in 2016, using flies sterilised using radiation.[54,55] The four-year pilot Medfly sterilisation programme is part of the Boosting Biosecurity Defences project. SIT will be combined with other control methods such as baiting.[56] This is a pilot project which aims to demonstrate the level of industry and community participation required to be successful.[57] In 2017, fruit fly numbers in Carnarvon were reportedly being monitored and reduced through hygiene measures (such as picking up fallen fruit), in order to lower the Medfly numbers in Carnarvon sufficiently to start a sterilised male program.[58]

Currently, SIT is also being developed further for Q-fly, as part of the SITplus Partnership, led by Western Australia.[59] A new Port Augusta facility will have the capacity to produce 50 million sterile male Q-flies each week, as part of the State Government's $5 million a year program to keep the Queensland fruit fly out of South Australia.[60]

3. Regulation of GMOs, including imports of GM insects

Unlike SIT, which uses irradiated or chemically treated sterile flies, Oxitec's approach involves repeated mass releases of millions of genetically modified (GM) flies into the environment. Open releases of genetically modified organisms (GMOs) are covered by laws and regulations which require an environmental and public health risk assessment, before a licence is issued for experimental or commercial use. The relevant law in Australia is the Gene Technology Act 2000 and consequent regulations and amendments.[61,62]

The Office of the Gene Technology Regulator (OGTR) has been established within the Australian Government Department of Health to provide administrative support to the Gene Technology Regulator in the performance of the functions under the Gene Technology Act 2000.[63] The Regulator has specific responsibility to protect the health and safety of people, and to protect the environment, by identifying risks posed by or as a result of gene technology, and by managing those risks. If an application for open release of GM flies is made, the OGTR will prepare and post a summary of the application on their website. The OGTR will then prepare and post a Risk Assessment for public comment.

The Biosecurity Act 2015 is also relevant, as it relates to pests that may cause harm to plants, including quarantine pests such as Medfly.[64] In addition, the Biological Control Act 1984 provides the legal framework for assessing and authorising biological control activities.[65]

Imports of GMOs are also regulated by the Gene Technology Act and exports from the UK to Australia are covered by European Union (EU) law.

Under European Union (EU) law, Oxitec should provide a publicly available environmental risk assessment which meets European standards before exporting GM insect eggs for open release to foreign countries. This legal requirement arises because Oxitec's GM insect eggs are live genetically modified organisms (LMOs) covered by the Cartagena Protocol on Biosafety to the Convention on Biological Diversity, to which the UK is a Party. The relevant legal requirements for export are implemented in the UK through the European Regulation (EC) 1946/2003 on transboundary movement of genetically modified organisms.[66] This Regulation requires that the environmental risk assessment (ERA), which an exporter provides, meets the standards of EU rules on risk assessment contained in EU Directive 2001/18/EC[67]. This requirement is in addition to the requirement for a risk assessment to be published by the OGTR under Australian law.

For GMOs which are not plants, a list of issues that must be covered by the risk assessment is included in Annex II, D.1 of Directive 2001/18/EC. Guidance published by the European Food Safety Authority (EFSA) outlines the evidence that Oxitec would need to provide for its GM insects to be placed on the EU market ('placing on the market' means making available to third parties, whether in return for payment or free of charge), highlighting the issues that should be considered in the ERA.[68]

Although Australia is not a Party to the Cartagena Protocol, under European law, Oxitec is still required to provide a risk assessment which meets EU standards, to the competent authority of the importing country, and this should be publicly available under freedom of information laws in both the exporting and importing country. No such risk assessment is yet available.[69]

Oxitec has a poor track record of meeting the transboundary notification requirements when exporting its GM mosquito eggs to other countries. [70,71,72]

The UK Department for the Environment, Food and Rural Affairs (Defra) has admitted that Oxitec breached UK and EU regulations, on implementing the Cartagena Protocol on Biosafety, when it failed to provide a risk assessment to the Panamanian authorities prior to exporting GM mosquito eggs to Panama for open release. But Defra says it will not enforce the regulation because Panama did not want the risk assessment.[73] EU authorities have since warned the Department about the importance of the regulation.[74] The Gorgas Institute, which acted as Oxitec's partner for its experiments in Panama, did produce a risk assessment, but this is clearly marked "Uso confinado" (confined use) and does not meet EU or international standards for open release of GM insects.[75] Panama has not supplied any risk assessment documents to the Cartagena Protocol's Biosafety Clearing House. However, Panama has since decided to discontinue experiments with Oxitec's GM mosquitoes.[76]

The risk assessment included in the documents when GM mosquitoes were first exported for open release in Brazil was produced by Oxitec's then research partner the University of São Paulo, not by the exporter. It omits most of the issues required to be covered prior to export under EU law. [77] This is also in breach of UK and EU legal requirements. Brazil supplied risk assessment documents to the Cartagena Protocol's Biosafety Clearing House only in August 2014, more than three years after starting open release experiments.[78] The summary risk assessment relates to the decision of Brazil's biosafety regulator CTNBio to approve commercial releases, although commercial releases have yet to be approved by Brazil's health surveillance authority, ANVISA. A brief dissenting opinion from two of the experts on the committee[79] is included, highlighting the lack of consensus on some issues.

The transboundary notification requirements under the Cartagena Protocol apply only to the first export to a country for open release, not to exports for contained use.

The requirement for the exporter to provide a risk assessment which meets EU standards may be important in determining liability if anything goes wrong, because the onus is on the company to provide information which is complete and correct. Thus, it is important that this risk assessment is publicly available before any open releases of GM fruit flies take place in Western Australia.

4. Oxitec's GM Medfly

Oxitec's GM flies have a female-killing trait: this means male offspring survive to adulthood but most of the female offspring die at the late larval or early pupal stage, in the absence of the antibiotic tetracycline (used as a kind of antidote to the genetic killing mechanism, to breed the insects in the lab). In contrast, the males survive to adulthood, as if they were normal flies, but are able to pass the GM killing trait to their female offspring. The insects are also genetically engineered to be fluorescent when observed under a special type of microscope.

The development and testing of Oxitec's GM Medfly is described in a number of scientific journal papers.[80,81,82] The strain that has been selected for further testing is called OX3864A and is described in more detail in a 2014 paper, which reports a number of experiments conducted in cages at the universities of Oxford and Crete.[83]

More recently, Oxitec scientists and Western Australia's Department of Agriculture and Food have conducted further experiments in glasshouses at a laboratory in Western Australia.[84] The project website states that the mating performance of the Oxitec males was comparable with sterile males irradiated at low levels. However, no results have yet been published in a peer-reviewed journal. This means there is no public information about many important issues, such as the contamination of fruit with GM fruit fly larvae and the damage that they cause to it.

Although Oxitec has referred in press releases to additional experiments in Morocco[85], no published information on these experiments appears to be available.

Oxitec argues that its GM flies can improve on the performance of radiation-sterilised flies used in SIT. The performance of SIT has limitations due to the effects of mass-rearing and the effects of radiation, which reduces the fitness of the male flies. However, Oxitec's flies show limited gains in fitness compared to irradiated flies[86] and will have a similar level of difficulty in mass rearing. Mass breeding of mosquitoes results in loss of fitness over time (due to inbreeding, known as the "colony effect").[87] Loss of fitness means that fewer males will mate with wild females and effectiveness will be reduced. In the use of irradiated SIT, new wild insects can be added to the colony prior to irradiation in order to increase the fitness. With RIDL, new back-crosses between the parent line of GM flies and new wild flies would have to be created periodically and introduced to increase the fitness of the colony: adding to complexity and costs.

Another key difference between the Sterile Insect Technique (SIT) using irradiated insects and the release of genetically modified (GM) insects is that radiation-induced sterility involves multiple chromosome breaks in the insects' DNA, whereas the RIDL system relies on a specific genetic modification. Radiation-induced sterility therefore has built-in redundancy that is not provided by molecular genetic approaches.[88] A number of authors have speculated that any genetic or molecular event that allows the GM mosquitoes to survive and breed successfully could therefore be rapidly selected for during mass production.[89] If this happens, the conditional lethality effect could rapidly disappear as resistance develops in production facilities or in the field. A related problem is the concern that contamination of the breeding facility with wild flies could also lead to mass production of flies without the killing mechanism: this reportedly happened in a previous Oxitec experiment with GM mosquitoes in caged trials in Mexico.[90]

Perhaps most importantly, Oxitec's GM insects are not sterile: the female-killing approach results in most of the females dying at the larval stage. In the case of GM fruit flies, many will die as maggots when they are still inside the fruit and after causing significant damage to it.

In addition, Oxitec's GM insects are bred using the common antibiotic tetracycline in their feed, which acts as a chemical switch to turn off the killing mechanism and allow egg-laying females to be produced. The use of tetracycline in this way raises two concerns: whether the insects or waste from the insect factory will spread antibiotic resistant bacteria into the environment; and whether the insects will encounter sufficient tetracycline in the environment after they are released to turn off the killing mechanism.

These issues and concerns are discussed further below.

4.1 GM maggots in fruit crops

Oxitec's GM flies are not sterile. Unlike irradiated flies, which do not normally produce offspring, the adult GM male flies mate with wild females which lay eggs and reproduce. However, the female offspring are genetically programmed to die, mainly at the larval stage (i.e. as maggots), or as pupae. Since fruit flies lay their eggs in the crop, the maggots will still be able to eat it before the female maggots begin to die inside the fruit. Some maggots may leave the fruit and die as pupae on the ground beneath the trees, but many will die inside the fruit. This means that the crop may suffer significant damage before any population suppression effect begins and the crop will become contaminated with large numbers of dead GM maggots.

As experts advising the European Food Safety Authority have noted: "[Late lethality*] implies that the offspring of the mating between the released arthropods and the wild population carry the transgene and survive beyond the embryo stage…For fruit flies such an approach would be detrimental as it would result in significant damage of larvae to the agricultural produce*." [91]

Oxitec reports that it took eight weeks to begin population suppression following the release of GM flies in cages, and fourteen weeks to reduce the reproductive output to zero.[92] Open air use will generally be much less effective, meaning that crop damage caused by both wild and GM maggots is likely to continue for several months during the releases. In the open air, the wild population is unlikely to become extinct because wild flies will fly in from surrounding habitats and reproduce. Thus, further releases will need to be made each season to maintain the suppression effect.

Death of most female GM flies at the late larval or pupal stage will significantly increase the number of larvae dying in the fruit, compared to current conventional or organic fruit production, where most of the flies would be expected to emerge from the fruit as fully grown larvae. The dead larvae will contain the DsRed2 (fluorescent) and tTA (late lethality) genetically engineered traits. They will be consumed by all species which normally consume the fruit or other nearby produce which Medfly eat. This will include humans if the crop enters the food chain. Dead GM maggots in contaminated fruit are likely to be off-putting to consumers and may pose risks to health, which will need to be carefully assessed.

Another potential exposure route for humans is through swallowing the flies during the releases. Journalists have reported that in Brazil "…*it's impossible to talk during the liberation sessions without accidentally swallowing a few*…" of Oxitec's adult GM mosquitoes due to the very large numbers released to try to swamp the wild population.[93] This is

because the releases of GM males must swamp the wild males by an order of magnitude or more to have any effect on the wild population.

In its application to release GM moths in New York State (since withdrawn but later resubmitted), Oxitec provides a commercial reference for toxicity testing of the red fluorescent marker, DsRed2, by Pioneer DuPont.[94] Oxitec also cites a 26-day feeding study in rats, using GM oil seed rape (canola) genetically modified to express green (not red) fluorescent protein (GFP), which concludes: "*These data indicate that GFP is a low allergenicity risk and provide preliminary indications that GFP is not likely to represent a health risk*".[95] Other than a bioinformatics report, Oxitec provides no evidence regarding the safety of the RIDL genetic mechanism and the high level expression of tTA that kills the insects at the larval stage. The mechanism of action of this killing mechanism is not fully understood and very limited safety data is available. The tetracycline transactivator (tTA) protein is created by fusing one protein, TetR (tetracycline repressor), found in *Escherichia coli* bacteria, with the activation domain of another protein, VP16, found in the Herpes Simplex Virus. Researchers commonly use this mechanism to switch on and off different genetic traits, for example in transgenic (GM) mice used in medical research, but it is not normally present in the human food chain. Oxitec has published one feeding study, in which GM *Ae. aegypti* mosquito larvae were fed to two different species of a type of mosquito that eats other mosquitoes (known as *Toxorhynchites*).[96] More recently, Oxitec published a feeding study on the impact of GM olive flies on one parasitoid (a wasp) and two predators (a spider and a beetle), reporting no adverse effects.[97] No feeding studies have been published for Oxitec's GM fruit flies and no feeding trials have been published which study potential impacts on birds, mammals, reptiles or amphibians, such as lizards or frogs.

Considerably more data, based on specific feeding trials in relevant species, is therefore needed to establish that consumption of GM fruit fly adults or larvae is not harmful to humans, farm animals, pets or wildlife.

European Union (EU) standards are relevant here because: (i) Oxitec is required by EU law to provide a risk assessment which meets EU standards before exporting its GM fruit fly eggs to Australia or other countries (as detailed above); (ii) future exports of crops produced using GM flies to the EU, and perhaps to other countries, will be required to meet these standards. EU Guidance on risk assessment of GM insects published by the European Food Safety Authority (EFSA) requires applicants to assess the effects of toxins or allergens associated with the GM insect animals such as birds, mammals, reptiles and amphibians.[98] It also states (page 8): "…*applicants should also assess the likelihood of oral exposure of humans to GM animals or their products which are not intended for food or feed uses. If such exposure is likely and ingestion or intake will occur at levels which could potentially place humans at risk, then applicants should apply the assessment procedures described in the EFSA Guidance Document on the risk assessment of food and feed from GM animals and on animal health and welfare aspects*". To meet the requirements of the cited Guidance on risk assessment of food and feed, it is likely that repeated dose toxicity studies using laboratory animals would be required.[99]

Oxitec's application to release GM olive flies in Spain, genetically engineered with the same female-killing trait, was withdrawn in 2013, following a request for further information from the regulator, including toxicity testing using feeding trials in relevant species.[100,101] Oxitec re-applied to release GM olive flies in Spain in 2015, without providing the necessary safety information.[102] This application was rejected.[103]

In 2014, the Brazilian regulator CTNBio approved experimental releases of Oxitec's GM Medfly. However, the company has yet to make the transboundary notification for export of GM Medfly required by European Union law, which requires a risk assessment which meets EU standards to be reviewed and accepted by the importer, as described above.[104] The European Commission has notified Brazil that export of fruit contaminated with GM Medfly to the EU would be illegal under EU law and sought further information about the steps that will be taken to ensure such exports do not happen.[105] Contamination of crops with GM flies could have serious impacts on whether farmers can sell them, especially on the organic or European markets.

If contamination of fruit with GM maggots does occur, failure to conduct human safety tests prior to conducting open release experiments could damage markets far more widely than in the local area of the trial. There would be implications for international as well as domestic markets (including organic markets), since most overseas markets have regulatory approvals processes without which products containing GM insects will not be accepted, as described above. In Australia and New Zealand, foods containing genetically modified organisms (GMOs) must also be labelled.[106] This is also the case in many other countries, such as Thailand, Indonesia, China, South Korea and Japan.[107] In recent years, free trade agreements secured with China, Japan and South Korea have seen an increase in the exports of Australian fruit and nuts, with almonds performing particularly well.[108] Therefore, the implications for Asian export markets of potential contamination with GM fruit fly maggots needs to be very carefully considered.

The Department of Agriculture and Food, Western Australia (DAFWA) seeks to attract investment on the basis of Western Australia's image as "clean and green". Open releases of GM flies might tarnish this image and reputational damage to fruit growers might also occur (due to potential contamination of their crop with GM fruit fly maggots), leading to lost market opportunities.

The crops that might be affected include many types of fruit and some vegetables. Worldwide, Medfly has been recorded from over 260 plant species including fruits, vegetables and nuts. In Western Australia, thin-skinned stone fruit (apricots, nectarines, peaches), mangoes, persimmon, apples, pears and mandarins are particularly susceptible.[109] Medfly will attack all citrus except some lemons and can breed in over-ripe or damaged fruit of less susceptible crops including tomato, eggplant and capsicum. When populations are very high, less preferred hosts such as olives may also be attacked.

Apples, pears and nashis are the main pome fruits grown in Western Australia.[110] Most fruit is sold on local markets with some interstate and overseas sales. About 80% of total strawberry exports from Australia, come from Western Australia.[111] The main destination countries for exports are Singapore, the UAE, Kuwait, Thailand and Malaysia.[112] Strawberries are reported in the scientific literature as a host for Medfly, however a study in Western Australia has reported preliminary findings that support the idea that strawberries are rarely a host for Medfly in the field.[113] The Western Australian citrus industry primarily supplies the local market with oranges, mandarins, grapefruit, lemons and limes.[114] Mangoes can be harvested from September through to April in Western Australia due to the wide range of climates.[115]

Although contamination could affect all farmers, including conventional farmers, there are particular concerns about organic crops because the use of genetic engineering and genetically modified organisms (GMOs) is prohibited in organic standards. A 2016 paper outlines how organic markets could be threatened by contamination with GM insects.[116]

Economic impact on organic farmers could be significant: they could be required to increase a buffer zone, thereby decreasing the acreage on which they are able to grow profitable organic crops, and they could be subject to loss of markets if genetically modified organisms are found on their crops.

For comparison, in some cases contamination with GM crops has caused major (multi-million dollar) damage to markets for conventional or organic crops and foods.[117,118,119,120] Before any open releases of GM pests take place, it is therefore important to have clarity about who will be liable if they contaminate other crops outside the experimental area.

4.2 Potential to spread antibiotic resistance in food and the environment

Antibiotic resistance in some pathogenic microbes is a serious global problem as it can lead to some serious bacterial diseases in humans and/or animals becoming difficult or impossible to treat with antibiotics. In response, Australia has developed a National Antimicrobial Resistance Strategy.[121]

Oxitec uses tetracycline (an antibiotic used commonly in agriculture and medicine) as a kind of antidote to the genetic killing mechanism, allowing it to breed insects in the laboratory or insect factory, prior to making a release of GM males. Tetracycline binds to tTA and prevents it leading to the expression of more tTA so that the genetic killing mechanism does not work until required. Including tetracycline in their feed enables the female insects to live to adulthood rather than dying at the larval stage.

Oxitec's GM fruit flies are reared on an artificial diet containing 100µg/mL of the antibiotic tetracycline.[122] This extensive use of antibiotics in the rearing process is not required when using traditional SIT.

The use of tetracycline to breed the GM fruit flies in the lab carries the risk of spreading antibiotic resistance, which could pose a major risk to human and animal health as tetracyclines are used to treat several diseases including urinary tract infections, acne, gonorrhea, chlamydia, etc.[123] This is because insect guts can be reservoirs for antibiotic resistance genes which can be spread into the environment.[124,125,126] GM insect production in factories exposed to antibiotics could lead to drug resistant bacteria in their guts so that the insects disseminate antibiotic resistance when released into the environment.[127,128] Any female agricultural pests which are inadvertently released with antibiotic resistant bacteria in their guts could transfer antibiotic resistance directly into fruit because female fruit flies pierce the fruit to lay their eggs inside.

With Oxitec's GM agricultural pests (but not its GM mosquitoes) the males survive and are not killed by the genetic killing mechanism (i.e. the technology is "female killing" only). Therefore, it is not necessary to release GM males fed on tetracycline, because the next generation, from eggs produced by females which were bred on tetracycline, can be used instead. However, the parents of the released males must be fed on tetracycline for the females to survive to adulthood and be able to lay eggs. This could still allow the spread of antibiotic resistant bacteria because many bacteria in insects pass from the eggs to the next generation.[129,130] If antibiotic resistant bacteria can spread from one generation of fruit flies to the next, they will end up in the environment and perhaps consequently in the food chain when the GM flies are released and reproduce.

Medfly is a potential vector of human pathogens, such as *E. coli*, to fruits, since it is able to transfer bacteria from animal excreta (one typical feeding site) directly into the fruits where females lay their eggs.[131] Species of gut bacteria in the Medfly have been characterised.[132] Some major components of the Medfly's gut bacterial community are vertically transmitted from the female parent to its offspring, some within the egg and others on the surface of the egg.[133] During oviposition bacteria are transferred to the fruit and establish and proliferate within it, causing its decay and perpetuating the Medfly-associated bacterial community. An initial, egg-borne, diverse community of bacteria expands throughout the fly's life cycle.[134] Thus, it is likely that antibiotic resistance genes can spread from the guts of released GM insects through at least two mechanisms: horizontal gene transfer to bacteria such as *E. Coli* encountered during feeding on excreta and spread through the environment, including onto fruit; or parental transmission via wild female mates or any accidentally released GM females and thence into the fruit and the larvae which grow inside it.

Any tetracycline contaminated water released from the laboratory or GM insect-production factory could also lead to bacteria in the receiving environment developing antibiotic resistance, which might spread into pathogenic bacteria.

Tetracyclines are generally classed under Schedule 4 of the Australian 'Poisons Standard' as Prescription Only Medicine, or Prescription Animal Remedy Substances. These are substances the use or supply of which should be by or on the order of persons permitted by State or Territory legislation to prescribe (i.e. doctors or veterinarians) and should be available from a pharmacist on prescription.[135] Some exceptions are allowed under Schedule 5 i.e. tetracyclines (including chlortetracycline and oxytetracycline) may also be used in preparations: a) for topical application to animals for ocular use only; or b) containing 40 per cent or less of chlortetracycline, when packed and labelled for the treatment of ornamental caged birds or ornamental fish only. However, the current classifications do not appear to permit the use of tetracyclines in the rearing of GM insects.

4.3 Potential increases in competitor species

Medfly is known to interact with other species of fruit fly.[136]

The Queensland fruit fly (*Bactrocera tryoni*), also known as Q-fly, is native to Australia, and also causes extensive damage to fruit crops. Although Q-fly and Medfly co-existed in areas of New South Wales and Victoria for some time, they do not now generally co-exist in Australia, likely because of the differences in egg-laying habits, competition by larvae in fruit and differences in host range.[137] Early research suggested Q-fly distribution is limited to eastern states.[138] A supposedly different species of fruit fly *Bactrocera aquilonis*, is found in northern Western Australia. However, it has since been found to be almost genetically identical to Q-fly, so it may not be a distinct species and should also be treated as a pest.[139,140] Although Q-fly had previously been eradicated from Western Australia (in 1990), two populations were sampled in Derby and Broome in this 2010 study: the latter appeared to originate from the Northern Territory. The existence of competition between Medfly and Q-fly raises the possibility that population suppression of Medfly – if it is successful - could lead to expansion in the territory of the fruit fly *Bactrocera aquilonis* (which is closely related or perhaps identical to Q-fly), due to reduced competition for resources. If so, damage to fruit (at least in northern areas) may simply be continued by another species, or a new Q-fly eradication programme might need to be introduced.

This possibility is supported by evidence that Medfly appears to be outcompeted by other species in some areas where it has been introduced, including Hawaii, Mauritius and Zimbabwe.[141]

This risk also applies to conventional SIT, but not to other methods of control which may be effective against both species.

4.4 GM insects spreading to where they cannot be recalled

A major difference between Oxitec's GM mosquitoes and its GM agricultural pests, such as the GM fruit flies, is that the GM trait in the agricultural pests is "female killing" only, whereas both the male and female offspring of the GM mosquitoes die. This means that the male GM fruit flies are not genetically programmed to die and are likely to survive for many generations, increasing the risk that they will spread widely in the environment.

In addition, contamination with tetracycline and related antibiotics is widespread in the environment and could lead to significantly increased survival rates. For its other GM insects, Oxitec reports low survival rates of females to adulthood, which increase significantly when the larvae feed on tetracycline. For its GM Medfly, Oxitec reports zero female survival to adulthood in the absence of tetracycline, and survival rates equivalent to wild flies when fed on 100 µg/ml of tetracycline.[142] However, no dose response curve is provided to show what percentage of flies might survive if they were to encounter lower levels of tetracycline in the environment.

The tetracyclines are a family of antibiotics any one of which can increase the GM female Medfly's survival rates. Because of their use in treating animal diseases[143], tetracyclines commonly contaminate animal manure. Oxytetracycline can be found in some environments at concentrations above 500 µg/g in animal manure and doxycycline at up to 78.5 µg/g dry weight in broiler manure.[144,145] A global review reports lower but still relevant concentrations of tetracyclines of up to 0.88 µg/g in pig manure, 11.9 µg/g in poultry manure and 0.208 µg/g in cattle manure.[146] These concentrations are likely to be more than enough to at least partially inactivate the killing mechanism in the female GM flies if the larvae or pupae (which emerge in the soil below the trees) come into direct contact with contaminated manure.

In some countries, fruit trees could be another source of exposure because oxytetracycline is sometimes used in fruit production to treat bacterial diseases of plants, especially fire blight in pear and apple and bacterial spot in peach and nectarine.[147] However, fire blight is currently eradicated from Australia and strict quarantine measures are in place.[148]

Tetracycline levels in industrially farmed animals may also be sufficient to increase GM female fruit fly survival. When Oxitec's GM mosquitoes were fed cat food containing industrially farmed chicken, which probably contained the antibiotic tetracycline, their survival rate increased to 15-18%. Oxitec originally hid this information[149] but later admitted to an 18% survival rate of larvae fed on cat food in a published paper.[150] In one study, levels of tetracycline from beef carcasses at a slaughterhouse in Iran were 131.0 µg/kg in meat, 254.9 µg/kg in liver and 409.1 µg/kg in kidney.[151]

Resistance to the genetic killing mechanism can also develop through evolution during mass production. Mutations that arise by chance which allow the insects to survive and breed will be selected for, so they become more common in the population (something which can't happen with the traditional Sterile Insect Technique).[152,153,154] Such resistance is another mechanism which could allow more GM female flies to survive and breed. In addition,

behavioural adaptation, beneficial for survival, could be selected for in the field. There is evidence of behavioural resistance developing in a SIT programme using irradiated flies, when females became unreceptive to mating with irradiated males.[155]

Therefore, most GM males survive and some GM females may survive as well, perhaps in increasing numbers as they develop resistance or find sources of tetracyclines in the environment. There is a risk that these GM flies will spread in the environment, either by flying or via dispersal of contaminated fruit or soil, as described above.

4.5 Release of non-native strains

Traditional Sterile Insect Technique (SIT) programmes typically use one of two laboratory strains of Medfly, known as Vienna 7 and Vienna 8, developed using genetic material from flies of Middle Eastern origin.[156] Oxitec used a different strain, called TOLIMAN, as the wild type background for its GM flies. TOLIMAN is a wild type strain originating from Guatemala, which has been maintained at Oxitec's UK labs since 2004.

Introgression is the transfer of genetic information from one strain or species to another as a result of inter-breeding. Oxitec claims to have tested the insecticide susceptibility of the strains to minimize the risk that release might introgress insecticide resistance into wild populations (Supplementary Table 3: insecticides tested were α-cypermethrin, Spinosad, Dimethoate and Thiacloprid).[157] However, the release of non-native strains risks introducing new traits into wild populations: this might include resistance to insecticides which were not included in these tests, or others traits such as invasiveness.

5. Costs

In 2011, Oxitec published a paper claiming that its GM mosquito technology is cost effective at preventing dengue.[158] The computer modelling in this paper was conducted before any experimental results were published and is therefore out of date. In practice, Oxitec has struggled to demonstrate that its GM mosquito technology is effective or cost-effective.[159] However, this 2011 paper does contain some information on the costs of constructing and operating facilities to produce sterile insects using irradiation (the sterile insect technique, SIT). Costs are given in US dollars at 2008 prices. The cost of construction of a SIT facility varies considerably from $50,000 to $26 million. The cost of production of sterile insects is given as a mean of $813 per million insects (range $172 to $1,639 per million insects). In Brazil, cost of production of irradiated SIT Medflies ranges from $400 to $700 for every million flies released and experts have questioned whether Oxitec's GM flies could be produced any cheaper.[160] Apart from the cost of production of the flies, Oxitec expects to make a profit from its patented RIDL system. It therefore seems unlikely that costs would be any lower than a conventional SIT programme, even if the technology was effective and did not encounter any of the problems outlined above. However, because Oxitec's technology will leave GM maggots in the fruit, the risk of lost markets and the costs that would incur will be considerably higher than with other methods of control.

Conclusions

Oxitec, which is now owned by the US biotech company Intrexon, has proposed releasing genetically modified (GM) fruit flies in open trials in Western Australia, in an attempt to control this pest. However, Oxitec's technology is not a credible approach to pest control.

Problems identified with this approach include:

(i) The use of late-acting lethality (rather than sterility) – which means the flies will mate and produce viable offspring, the females of which die mostly at the larval stage. This means that fruit crops will be damaged by the feeding GM larvae and food supplies for humans and animals are likely to become contaminated with dead female GM maggots;
(ii) Lack of adequate safety testing to demonstrate that consuming dead GM female maggots in crops will be safe for humans, birds or animals, including threatened species. Adult insects could also be swallowed during mass releases;
(iii) In addition, the use of tetracycline to breed the GM flies in the lab may facilitate the spread of antibiotic resistance via gut bacteria or discharges from the GM insect breeding facility;
(iv) Impacts of the single-species approach on other pests may include increases in the numbers of such pests or establishment in new areas: this may include invasive pests;
(v) The use of a female-killing approach, in which only the female GM larvae die, is likely to lead to the dispersal of GM males to neighbouring crops, where they may survive and breed for multiple generations. Male GM flies may spread over significant distances in the longer term, via migration, or if contaminated crops enter the food chain. Surviving females may also be dispersed and the numbers of female survivors may increase as resistance develops or if the GM flies breed in areas contaminated with the antibiotic tetracycline;
(vi) The use of a strain of Medfly which is not indigenous to the area poses further risks, as new undesirable traits might be introduced into the wild Medfly population;
(vii) The presence of contamination with dead GM maggots in a crop is not compatible with organic production systems and could put organic certification at risk. Contamination would also likely damage markets for both organic and conventional crops, including export markets, many of which require safety testing and labelling of GMOs. It is unclear who would be liable for the loss of markets in the event of such contamination.

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