Genetically Modified (GM) olive flies: A credible pest management approach?	GeneWatch UK September 2013

UK company Oxitec has developed genetically modified (GM) olive flies, which it is seeking to release in large numbers in olive groves. Oxitec has made an application to make its first experimental releases in netted olive groves in Spain. It has stated it also wishes to release GM olive flies in other countries such as Italy, Greece and Morocco.

This briefing explores the issues raised by the proposed releases.

Oxitec: the company

Oxitec is a UK company producing genetically modified (GM) insects with the aim of creating a global market in GM insects for open release into the environment. Oxitec is a spin-out company from the University of Oxford which manages its investment in the company via Oxford Spin-out Equity Management.[1] Other known major investors include the Boston-based multi-millionaire Landon Clay, Oxford Capital Partners[2], and, since 2012, Asia Pacific Capital[3]. Smaller investors include researchers working in the field.[4] The company has received more than £1.5 million in research funding from UK government sources and the UK Treasury has extended tax relief to investors in an attempt to assist the company grow sufficiently to reach an Initial Public Offering (IPO)[5,6]. UK Trade and Investment (UKTI) has worked with embassies worldwide to seek to help Oxitec to secure markets for its GM insects.[7]

Oxitec has close links to the multinational pesticide and seed company, Syngenta: most of its senior management, including its Chief Executive Hadyn Parry, and two Board members, including the Chair, are ex-Syngenta staff.[8] From March 2009 to June 2011, Oxitec received research funding directly from Syngenta for genetic transformation of *Lepidoptera* (a large order of insects that includes pests such as pink bollworm and diamond back moths[9]).[10] Both Oxitec and Syngenta use a consultancy firm run by Colin Ruscoe, Chair of the British Crop Protection Council,[11] and the same PR agency (The Blue Ball Room)[12], which is run by Parry's wife.

Oxitec products

As well as olive flies, Oxitec is developing other GM agricultural pests, such as fruit flies, bollworms and diamond back moths (cabbage moths), and GM mosquitoes. All the company's GM insects are intended to be released repeatedly in large numbers (multiple 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 lethality' trait, which means that most of the female offspring from these matings do not survive to adulthood to reproduce. This is intended to suppress the numbers of wild insects. 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 calls its patented technology "Release of Insects carrying a Dominant Lethal system" (RIDL).

Oxitec began releases of GM mosquitoes in the Cayman Islands, a British Overseas Territory with no biosafety law, in late 2009, without publishing an environmental risk assessment. The company was criticised by scientists working in the field for its secretive approach and for the poor quality of its risk assessments (which were obtained following the releases via Freedom of Information requests and parliamentary questions, under UK and EU legislation) and lack of peer-reviewed evidence on many biosafety issues.[13,14] The only country currently releasing Oxitec's GM mosquitoes in large-scale experiments is Brazil, following a secret agreement between the UK and Brazilian governments in 2007.[15] Smaller scale experiments involving open releases of GM mosquitoes in the Cayman Islands[16] and Malaysia[17,18] have now stopped. Proposals to release GM mosquitoes in the Florida Keys and Panama are awaiting regulatory assessments. A large number of ethical, scientific and regulatory issues have been raised about these experiments.[19] For example, under the Cartegena Protocol on Biosafety, when exporting GM insect eggs for open release to other countries, Oxitec is supposed to supply a risk assessment that meets EU standards and to copy this to the EU and UK authorities so it can be made publicly available. Oxitec has not followed this process correctly for any of its exports to date and its risk assessments have not met European standards. In addition, Oxitec has repeatedly made unsubstantiated claims of benefit to local people and the press.

The GM mosquitoes currently being released in Brazil differ from Oxitec's GM agricultural pests in that both sexes of the GM mosquitoes are genetically engineered to die at the late larval/pupal stage. For Oxitec's GM agricultural pests, only the female insects are genetically engineered to die at the late larval/pupal stage and males will survive to adulthood (this is known as a "female-killing" approach).[20]

To date the only open release experiments conducted using Oxitec's GM agricultural pests have been in the USA, using Oxitec's GM pink bollworms (a cotton pest), with only the fluorescent trait (not the 'early lethality' trait), and made sterile using radiation. These experiments were halted, partly because of concerns raised by US organic farmers, and they also led to a critical report by the US Department of Agriculture (USDA) Office of Inspector General.[21]

In 2011 in the UK, Oxitec sought to make open releases of GM diamond back moths (*Plutella xylostella*) (a cabbage pest) under "contained use" regulations by claiming that its RIDL technology is equivalent to "biological containment". These proposed releases have not been approved.

Oxitec's Brazilian partners Moscamed applied to regulators to release GM Mediterranean fruit flies (Medfly, *Ceratitis capitata*) in Brazil in January 2013.[22] No public risk assessment has been made available for this application and it has yet to be approved.

The current application to release GM Olive Fly in Spain, if approved, would therefore be the first open release anywhere in the world of GM insects with the "female-killing" trait. Oxitec has indicated in its application that it also plans releases of GM olive fly in Italy. Other countries mentioned in Oxitec materials as possible future markets for GM Olive Fly include Greece, Israel and Morocco.

Oxitec's GM olive flies and proposed trial

Oxitec's GM olive flies are described in a scientific paper published by researchers working for the company.[23] The GM olive flies have a female-killing trait: this means male offspring survive to adulthood but females die at the late larval or early pupal stage, in the absence of the antibiotic tetracycline (which is used as an antidote to the genetic killing mechanism, to breed the insects in the lab). The insects are also genetically engineered to be fluorescent when observed under a microscope.

Oxitec's 'conditional lethality trait' is created by genetically engineering the female insects to express a protein called tTA (tetracycline-controlled transactivator). High level expression of tTA kills the insects at the larval stage, although the mechanism for this is not fully understood. Tetracycline (an antibiotic which is used commonly in agriculture and medicine) binds to tTA and prevents it leading to the expression of more tTA, allowing the insects to survive to adulthood. This allows them to be bred in the laboratory by including tetracycline in their feed, which acts as an antidote to the genetic killing mechanism.

Oxitec's current application is for an experimental release of its OX3097D-Bol strain of GM olive flies in an olive growing area approximately 8km from the port at Tarragona, in the Catalonia region of Spain, in six netted plots in a 1000km^2 area, over a an 8 week period.[24] The numbers to be released are not provided in the application, since this will depend on the numbers of wild olive flies. The netting is not expected to contain all the GM olive flies (i.e. it is not a caged trial) and Oxitec expects reptiles, small mammals and birds to still be present at the site. Traps will be deployed before, during and post release both inside and outside the netting to monitor numbers. The stated objectives of the proposed study are to:

- Establish the performance of the GM olive flies when competing with wild males for wild females
- Gather information on the longevity of the GM olive fly in a field environment
- Evaluate different release methods.

Although Oxitec has reported results of caged trials studying the mating of the GM olive fly and impacts on wild olive flies, it has not published any caged trials to study biosafety issues. Oxitec's application also includes no plans to assess biosafety issues, as the company believes its GM insects pose negligible risks.

Olive production

The EU is a global leader in olive production, accounting for almost 70% of total world output, and the main net exporter towards non-producing areas.[25] Spain alone produces 36% of the world's olive oil, and the sector is a major contributor to the economies of Greece, Italy and Portugal, and is also important to Croatia, Cyprus, France and Slovenia. Much smaller quantities are grown in Malta. The greatest concentration of olive oil production is found in two Spanish provinces, Cordoba and Jaen in Andalusia, which account for more than a third of EU output.

Olives have a wide diversity of production ranging from low input traditional plantations to super-intensive modern plantations. Additionally, there is a growing trend for organic plantations managed without chemical input, and subject to the most rigorous production standards. Most production volume goes to olive oil, with the rest eaten as table olives.

Maintaining product value and avoiding 'commoditisation' are regarded as critical to the olive oil value chain and sustainability is regarded as an imperative in Europe.[26]

Olive groves are found throughout the Mediterranean region, including in non-EU countries such as Morocco, Tunisia, Turkey, Syria, Albania, Montenegro, Serbia, Egypt, Jordan, Lebanon, Israel and Palestine. Between them, Mediterranean countries supply some 90% of the global olive oil market.

The USA, Argentina and Peru are the largest producers of olives outside the Mediterranean region.[27]

The olive fly

The main olive pest is the olive fruit fly (*Bactrocera oleae*), also known as olive fly, while other important insect pests are: the olive moth (*Prays oleae*) and the black scale insect (*Saissetia oleae*).[28] All three occur widely on olives in the Mediterranean region, causing significant financial losses. Losses due to olive fly are estimated at 5% of olive production or $800 million a year.[29]

The olive fly has been established as a pest in the Mediterranean basin for more than 2,000 years, but appears to have originated in Africa. It is also present in the Near and Middle East and has now spread to California, probably on olives imported from the Mediterranean. Olive fly has been reported in Central America, and on wild olives in China, but not yet in South America or Australia.

Female olive flies lay their eggs in ripening olives, where the newly hatched larvae (maggots) feed until they either pupate in the olive or exit to pupate on the ground. Damage is caused in a number of ways: when immature fruit are pierced by the females laying eggs this can cause them to drop to the ground; the larvae consume part of the crop; larvae present in the olives prevent them being sold as table olives (although some contamination is considered acceptable in olive oil); and the presence of bacteria, yeasts and molds in the damaged olives increases oil acidity and damages its quality and hence its commercial value.

Olive fly larvae feed only on olives, but adults have a varied diet, including insect honeydews, plant nectar, plant pollens, fruit saps and gums, and nutrient sources such as bird dung, bacteria and yeasts. Larval development is dependent on temperature, with a lower threshold between 7.5^{o}C and 10^{o}C and an upper threshold of 30 to 32^{o}C. In most regions, the olive fly appears best adapted to develop in the autumn, when olives are in the best condition for larval growth, and to enter dormancy in winter. It is unclear whether high summer temperatures, and low humidity, may cause the fruit fly to disperse to cooler sites.[30]

Adult females can lay from 50 to 400 eggs, usually one in each olive.[31] Eggs hatch in 2-3 days, larvae develop in approximately 20 days, and pupae in 8 to 10 days. Adults can live from 2 to 7 months. Olive flies usually stay within a single grove but can travel several miles: the olive fruit fly spread through California at a rate of about 100 miles a year. In Europe, the damage threshold for table olives is 1% and for olive oil is 10%. Numbers of larvae per fruit appears to be low in Europe, but some evidence of competition between larvae has been reported in California, with one to eleven larvae per fruit at some field sites.[32]

The number of generations of olive flies per season varies between two and five in different parts of Europe. In Spain, the olive fly normally has three generations per year from summer to autumn, overwintering underground. Olive fly pupae can overwinter in the soil, and ploughing may be less effective than previously thought at destroying them.[33]

Approaches to olive fly control

Environmental sustainability is regarded as the driver for many of the challenges facing the olive industry today, including pest and disease management.[34]

Organophosphate insecticides (dimethoate and fenthion) have been used for many years to combat the olive fly in (non-organic) olive fly plantations in Europe. However, the environmental damage and treatment costs are regarded as significant, and there are also concerns about the contamination of olive oils with pesticide residues.[35,36] The use of pyrethroid insecticides (e.g. deltamethrin) has increased more recently. Some insecticides used in olive cultivation, e.g. dimethoate, are also blamed for a reduction of insect species, including several that help to control pest species. Varying levels of resistance to organophosphate insecticides has developed in olive fly populations and there is also some resistance to pyrethroids. More recently, the insecticide spinosad (based on a compound found in the bacterial species *S. Spinosa*) has been incorporated into a bait spray (GF-120

Naturalyte, marketed by Dow Agrosciences) for increased efficacy with less active ingredients.[37]

The EC's LIFE programme developed guidelines for the improvement of olive cultivation and processing, which promote eco-friendly cultivation techniques including methods to minimise the application of pesticides.[38] Where pesticides are used, timing applications is likely to be important to minimise use and improve efficacy.[39]

Mass trapping is another approach which can reduce the need for spraying. Organic farmers in Spain use the OLIPE (Olivarera de Pedroches) trap, which can keep olive fly damage below about 10%.[40] The trap is made from a plastic bottle containing yeast bait solution. Pheromone traps (a type of insect trap that uses pheromones to lure insects)[41] have significantly improved trapping rates and can be combined with other approaches such as early picking of olives to reduce fruit damage. Mass trapping alone does not reduce damage sufficiently for high quality olive oil production but can be successfully combined with copper hydroxide sprayings (a widely used fungicide-bactericide approved for both organic and conventional agricultural production).[42]

More recently, particle film technology based on pressure spraying of a protective barrier of kaolin (china clay) has been introduced and shown to significantly reduce the incidence of both olive fly and olive black scale. Kaolin has no negative effects on human health or the environment and will not lead to the development of resistance, however some research has suggested spraying can reduce numbers of other insects, including natural enemies of olive flies, and reduce biological diversity.[43]

In California, olive fly suppression is undertaken using mass traps to attract and kill (either commercially- or home-made); pressure spraying of a barrier film protectant (kaolin clay); and/or an the insecticidal bait spray GF-120 Naturalyte (no other pesticides are approved for use).[44] Olive grove sanitation measures, such as removing fallen fruit, can also reduce infestation.

Thus, there have been several recent improvements in olive fly control, but there are also new methods under investigation. Biological control of olive flies with natural enemies (parasitoids) has been attempted, with limited success to date, but with the prospect of improvements being made following further research.[45,46] Microbial pesticides, such as strains of bacteria, are also being investigated, as they have low toxicity and high biodegradability, and are regarded as environmentally friendly alternatives to existing pesticides.[47] Insect growth regulators are another potential alternative with low toxicity to humans and reduced risk of harm to beneficial organisms.[48,49]

It is also possible that the classical Sterile Insect Technique (SIT), using irradiated insects, can be improved sufficiently for use in olive flies without the use of GM insects (and especially the risks associated with late-lethality at the larval stage, rather than genuine sterility). SIT involves releasing large numbers of irradiated male insects, which are sterile, to mate with wild pests. Matings between wild females and sterile males can suppress the wild population, and, if releases occur on a large-scale over time, sometimes eliminate it. Difficulties with applying SIT to olive flies include problems with mass-rearing of olive flies in the laboratory. Improving diets for mass-reared flies (including enriching with bacteria, which appears to have a major effect on other types of fruit fly[50]), and other methods to improve quality, are currently under investigation.[51]

Another alternative approach (already being tested for mosquitoes in the field[52]) is the infection of pest species with *Wolbachia*, a type of bacteria that causes sterility in females mated with infected males.[53,54]

Olive cultivars vary in their susceptibility to olive fly infection but there are no current reported attempts to plant resistant varieties, or to plant olives with smaller fruit as hedgerows to better support parasitoids.[55] Agro-ecological approaches such as these may have potential to further reduce infestation.

GM olive flies: a useful role in protecting olives?

> "[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 as approach would be detrimental as it would result in significant damage of larvae to the agricultural produce*." Expert report to the European Food Safety Authority, 2010.[56]

Oxitec describes its GM olive flies as an improvement to the Sterile Insect Technique (SIT) which has been applied successfully to a number of other pest species, but which has so far been unsuccessful for olive flies.

However, in comparison with SIT, a major problem with the use of Oxitec's GM agricultural pests is that, rather than being sterile, the female offspring of the matings between GM males and wild females die mostly at the late larval or pupal stage i.e. towards the end of the larval (maggot) life stage. Because the larval stage is when olive flies damage the fruit, it is unlikely that Oxitec's approach can actually prevent much, if any, of the damage to the crop, even if it successfully suppresses the wild population. The fact that the female offspring of Oxitec's GM insects survive to the late larval or early pupal stage (in the absence of tetracycline) is unintended: if Oxitec's genetic mechanism caused sterility, so that no eggs were laid or no larvae were produced, this would avoid the damage that the GM larvae will cause to the crop.

A second problem is the efficacy of this approach to reducing olive fly populations, and the "release ratio" of GM males to wild males that may be required. In its GM mosquito trials Oxitec has used release ratios of up to 54 to 1 before it observed any reduction in wild populations.[57] This means that GM males may need to vastly outnumber wild males if they are to mate successfully. Such large numbers suggest that Oxitec's GM insects may be no more competitive with wild males than irradiated insects are.

An important 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 sites of damage to insects' DNA, whereas the RIDL system relies on a specific genetic modification. This means that, unlike with irradiated insects, GM insects which survive and breed successfully – i.e. which overcome the genetic 'late-lethality' mechanism – could evolve rapidly during mass production.[58] If this happens, the lethality effect could rapidly disappear as resistance develops in production facilities or in the field. Oxitec has published some computer modelling of how resistance to RIDL might evolve.[59] Another potential mechanism for resistance is that wild females may become unreceptive to mating with released males.[60] This implies that, even if population suppression is effective, resistance could develop relatively rapidly.

Mass breeding of GM insects will also result in loss of fitness over time (due to inbreeding, known as the "colony effect").[61] 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 mosquitoes and new wild mosquitoes would have to be created periodically and introduced to try to increase the fitness of the colony.

Immigration and emigration of olive flies may contribute to the levels of olive fly in spring and summer[62] and this will complicate the effects of any population suppression programme, since olive flies from neighbouring orchards may take advantage of the reduction in competition if a fall in populations is achieved. Again, this will limit the likely success of the proposed approach.

Another potentially major problem is the difficulty of using a species-specific approach in the presence of multiple pests. Although olive fly is currently the main pest of olives, this may not be the case if Oxitec's GM olive flies are successful in reducing numbers. This is because olive flies compete with other pests for resources. If the population of olive flies were successfully suppressed (even temporarily), the olive moth (*Prays oleae*) and the black scale insect (*Saissetia oleae*) might increase in numbers.

This situation could be regarded as analogous to problems with GM insect-resistant crops (Bt crops) which have developed in China and Brazil. In China, secondary pests which are not affected by the Bt toxins in its GM cotton crop have become a major problem.[63,64,65] In Brazil, the Agricultural Ministry recently issued a warning about a massive explosion in corn ear worm (*Helicoverpa armigera*) in areas growing Bt maize.[66] These examples show how reductions in competition or natural enemies can lead to an explosion in another type of pest.

In addition, combining spraying (including of organic-approved substances) with GM insect releases may be difficult since any spraying which affects Oxitec's GM insects could reduce the potential effectiveness of the releases further.

GM olive flies in the food chain

"*Olive oil must be labeled clearly to inform consumers of its intrinsic values and place of origin, whilst also providing a means of distinguishing it from poorer quality oils and/or imitations. Transparency should be facilitated by the appropriate instruments. It is only by being familiar with a product that you can appreciate it, be willing to pay the price for it and understand that it is good value for money*". Benedetto Orlandi, President of the Copa (Committee of Professional Agricultural Organisations) and Cogeca (General Confederation of Agricultural Cooperatives in the EU)Working Party on Olive Oil and Table Oils, 2010.[67]

Because the female offspring of Oxitec's GM olive flies mostly die at the late larval/pupal stage, this means that many GM olive fly larvae (maggots) could die inside the olives where the female has laid her eggs. If Oxitec's GM olive flies are used in commercial production, many dead GM olive fly maggots, as well as some live ones, are likely to enter the food chain. It is questionable whether this will be acceptable to consumers or the industry. If not, experimental releases represent an unnecessary risk and a waste of money. If experiments do go ahead, it will be important to take steps to prevent any olives or olive oil produced during experimental releases of GM olive flies from entering the food chain.

In its scientific paper on GM olive flies, Oxitec argues that GM insects entering the food chain could be excluded from the scope of EU regulations in a similar way to foods produced by fermentation with GM micro-organisms where the GM organism is not present in the final product, and that any GM insects in the food chain should be treated as "technically unavoidable", to avoid triggering labelling requirements for GM food. However, because Oxitec's GM olive flies are not sterile but produce female offspring which are genetically programmed to die at the late larval/early pupal stage, it is clear that very large numbers of dead GM maggots could end up in the food chain in a manner which cannot be regarded as unavoidable or accidental. In addition, there could be some surviving female GM maggots, or live male ones (which are expected to survive to adulthood). Under EU legislation, food containing GMOs is regulated and should be labelled and traceable, as well as subject to a food safety assessment.

However, there remains considerable uncertainty about how regulators will handle the issue of GM insects in the food chain. EFSA's Guidance on the Environmental Risk Assessment of GM animals[68] states: "*This Guidance Document considers primarily effects of GM animals on human health through routes of exposure other than ingestion or intake... However, 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...*". However, the EFSA Guidance Document on the risk assessment of food and feed from GM animals explicitly excludes consideration of GM insects in the food chain, on the grounds that insects are not normally consumed as food. The failure of EFSA to consult on the subject of GM insects in the food chain is currently the subject of a complaint from GeneWatch UK to the European Ombudsman.

Further, there has been no consultation with growers, retailers or consumers about traceability and labelling requirements for the use of GM olive flies in olive production; nor have guidelines been developed to address the problems of co-existence with organic or conventional producers who do not want GM olive flies in contact with their produce. This is particularly important because releases of fluorescent GM bollworms were halted in the USA partly due to concerns that they were incompatible with organic certification. Unless these issues are resolved, there could be serious negative impacts on the olive trade associated with the use of GM olive flies.

In its application, Oxitec provides a reference for toxicity testing of the red fluorescent marker, DsRed2, but 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 is not fully understood and no safety data appears to be available. There is some evidence that enhanced tTA expression can have adverse effects (loss of neurons affecting cognitive behaviour) in transgenic mice.[69]

GM olive flies in the environment

In its application, Oxitec has stated that the potential environmental impacts are negligible given the conditions of the trial and that no ecosystem monitoring is necessary. However, the application contains no evidence to substantiate this claim.

Oxitec states that standard olive fly traps, such as McPhail traps or Yellow sticky traps, will be set up within the release sites and outside the netting to monitor the olive fly population. However, a recent study concluded that yellow sticky panel traps give a poor representation of the olive fruit fly population density of olive groves and found that McPhail traps captured only 0.5% of the flies present within a radius of 20m in the studied field.[70] Oxitec also states that the GM olive flies can be detected by the fluorescent marker DsRed2. However, a study of the market by the company using its GM bollworms found that the fluorescent trait began to disappear in this species over a matter of days after they were caught in traps, especially in hot weather.[71] There are no proposals regarding the monitoring of impacts on other species, including monitoring for possible increases in the major non-target pests (the olive moth and the black scale insect). It is therefore difficult to have much confidence in Oxitec's monitoring plans or the ability of their experiments to detect potential adverse effects.

Before the implications of releasing GM olive flies are even considered, it is first important to consider the proposed olive fly strain. According to Oxitec's application, the parent lines of the GM olive flies originated in Greece and have been outcrossed into additional strains from the Mediterranean basin. Their journal paper explains that this involved outcrossing a Greek laboratory strain (Democritus) for five generations with the Agrov wild-type strain from Israel. Although olive flies are native to Spain, Oxitec has developed a non-native strain and this has implications for the proposed releases. In the UK, Oxitec has been prevented from releasing a GM Diamond Back Moth because of concerns about the use of a North American background strain, which is subject to controls under plant pest control regulations.[72] Releasing non-native strains can be problematic if the strain is resistant to insecticides or in any way more fit to survive or to damage olives than native Spanish strains. Oxitec has not provided any justification in its application for its proposal to release a non-native strain of olive fly, or the results of any safety tests (for example, of insecticide resistance).

Concerns about GM olive flies in the environment include:

(1) The extent to which GM olive flies can disperse and breed;
(2) Direct adverse impacts (e.g. toxicity) on non-target species;
(3) Impacts on ecosystems – including predators, prey and competitor species - of the population suppression approach, especially the risk of an increase in other types of pest.

There is currently a limited understanding of the factors that affect olive fly populations and its natural enemies and the complex interactions between the various components in olive orchard agro-ecosystems.[73]

Since Oxitec uses a female-killing approach for its GM agricultural pests, GM males, which survive for multiple generations, will become widely dispersed in the environment unless they are physically contained. The proposed olive fly experiments are significantly different from the current experiments in Brazil with GM mosquitoes, where both male and female offspring are programmed to die. In addition, some GM female olive flies are likely to survive. Although Oxitec reports in its paper that the killing mechanism is 100% effective for its female GM olive flies, it does not report the number of flies tested. With the large numbers needed for open releases it is almost inevitable that some female offspring survive to adulthood. If female GM olive flies encounter sufficient levels of the antibiotic tetracycline in the environment, this could increase their survival rates. Survival rates of Oxitec's GM mosquitoes are up to 18% in the presence of cat food (which contains industrially farmed meat containing the antibiotic), compared to 3-4% on fish food, which is assumed to be uncontaminated[74]. The tetracycline class of antibiotics is one of the most commonly used in human and veterinary medicine[75] and is detectable in foodstuffs such as meats[76], milk[77], farmed fish[78] and honey[79]; in animal slurry[80]; and in human sewage[81].

Because of problems with dispersal and survival (especially the expected survival of all male offspring), it is likely to be impossible to ensure all GM olive flies are removed from the environment if any harmful effects are identified after their release. GM olive flies (males, or surviving females) may leave the site through flying, or adults or larvae may be transported on people or materials leaving the site. Olive flies have been transported worldwide on untreated olive fruits, leading, for example, to their spread from the Mediterranean to California. Some surviving GM olive fly maggots will be transported inside olives, if the fruits from the proposed trial are not destroyed. Because some newly hatched larvae exit the olive to pupate in the soil, it is also likely to be difficult to clear the site of all GM insects following the trial.

The deliberate release of Genetically Modified Organisms (GMOs), including GM insects, into the environment in the EU is governed by Directive 2001/18/EC. The proposed release is an experimental (not commercial) release, and the decision on approval will therefore be taken at a national or regional level in Catalonia or Spain. Nevertheless, surviving GM insects could be transported further afield and cross-boundary issues will have to be considered, necessitating extensive consultation.

The recent guidance published by the European Food Safety Authority (EFSA) outlines the type of evidence that Oxitec would need to provide for a commercial-scale release.[82] For GMOs other than plants Directive 2001/18/EC requires information on the following issues (Annex II, D.1):
1. Likelihood of the GMO to become persistent and invasive in natural habitats under the conditions of the proposed release(s).
2. Any selective advantage or disadvantage conferred to the GMO and the likelihood of this becoming realised under the conditions of the proposed release(s).
3. Potential for gene transfer to other species under conditions of the proposed release of the GMO and any selective advantage or disadvantage conferred to those species.
4. Potential immediate and/or delayed environmental impact of the direct and indirect interactions between the GMO and target organisms (if applicable).
5. Potential immediate and/or delayed environmental impact of the direct and indirect interactions between the GMO with non-target organisms, including impact on population levels of competitors, prey, hosts, symbionts, predators, parasites and pathogens.
6. Possible immediate and/or delayed effects on human health resulting from potential direct and indirect interactions of the GMO and persons working with, coming into contact with or in the vicinity of the GMO release(s).
7. Possible immediate and/or delayed effects on animal health and consequences for the feed/food chain resulting from consumption of the GMO and any product derived from it, if it is intended to be used as animal feed.
8. Possible immediate and/or delayed effects on biogeochemical processes resulting from potential direct and indirect interactions of the GMO and target and non-target organisms in the vicinity of the GMO release(s).
9. Possible immediate and/or delayed, direct and indirect environmental impacts of the specific techniques used for the management of the GMO where these are different from those used for non-GMOs.

Directive 2001/18/EC requires that the introduction of GMOs into the environment should be carried out according to the “step by step” principle. This means that the containment of GMOs is reduced and the scale of release increased gradually, step by step, but only if evaluation of the earlier steps in terms of protection of human health and the environment indicates that the next step can be taken. It is therefore important to note that although Oxitec has conducted some caged trials on the efficacy of its product (i.e. on mating fitness) it has provided no evidence from laboratory studies or cages trials regarding biosafety issues, such as:

- Toxicity testing of consumption of GM olive flies (at various life stages) on other species (including humans);
- Potential interactions between large-scale releases of GM olive flies and other species (e.g. competition effects with other pests);
- Impacts of environmental contamination with tetracycline on GM larval survival rates.

Nor has Oxitec collected or provided any data on:

- The baseline ecosystem at the site, including typical fluctuations in olive fly and the main competitor pest species;
- Other species present at the site.

Nor has Oxitec attempted to provide validated computer models of possible impacts on complex ecosystems of successful or unsuccessful suppression of the olive fly population in the target olive grove (including, for example, a potential influx of olive flies from other areas, or increases in non-target pests).

Oxitec's application is therefore seriously premature, because important information needed to make a decision on potential environmental impacts is almost entirely missing from the application.

Conclusions

The fact that Oxitec's GM olive fly female offspring die at the larval stage means olive fruit damage is still likely to be significant and olives are likely to be contaminated with large numbers of dead GM olive fly larvae. It is difficult to justify the experimental trials of GM olive flies proposed by Oxitec in Spain and elsewhere because this is unlikely to be acceptable to either growers or consumers. In addition, effectiveness is likely to be limited, and risks include the spread of GM olive flies beyond the trial site, and a possible surge in other types of pest.

Open releases of Oxitec's GM olive flies are also premature due to:

- The company's failure to observe the 'step-by-step' approach and to provide any evidence from laboratory or caged trials, ecosystem monitoring, or modelling, that health or environmental risks have been properly considered;
- Unresolved regulatory issues regarding GM insects in the food chain.

Commercial use of Oxitec's technology would raise significant concerns about potential cross-border environmental impacts and loss of markets for olive growers who do not want to use GM. Research has already delivered recent significant improvements in the sustainability of olive fly control, and many other alternatives are being investigated for the future.

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