Oxitec's genetically modified moths: summary of concerns

GeneWatch UK

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Summary

The UK-based company Oxitec is seeking to release genetically modified (GM) moths (also known as GE moths) into open fields in New York State. This briefing summarises the concerns about the proposed releases of the moths.

Problems identified with this approach include:

(i) The use of late-acting lethality (rather than sterility) – which means the moths will mate and produce viable offspring, the females of which die mostly at the larval stage. This means that food supplies for humans and animals are likely to become contaminated with dead female GM caterpillars;

(ii) Lack of adequate safety testing to demonstrate that consuming dead GM female caterpillars 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 Diamondback moths in the lab may facilitate the spread of antibiotic resistance via gut bacteria or discharges from the GM insect breeding facility;

(iv) Oxitec's approach is not compatible with conventional or organic controls for other pests. 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 or weeds, where they may survive and breed for multiple generations. Male GM moths 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 moths breed in areas contaminated with the antibiotic tetracycline;

(vi) The use of a strain of diamondback moth which is not indigenous to the area poses further risks, as does the proposed release of non-GM wild diamondback moths, some of which may be expected to disperse and persist in the environment;

(vii) The presence of contamination with dead GM caterpillars 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.

Open releases of GM diamondback moths are premature because of the serious limitations of this technology, lack of information needed to assess the risks, and inadequate regulation.

Although the USDA APHIS permit for the trial includes conditions which require destruction of the crop and spraying of insecticide for 100m around the site, these conditions are insufficient to guarantee that GM diamondback moths, or any wild moths that are released, will not spread to other areas and perhaps into the human food chain. If even a few of the wild moths turn out to be resistant to the insecticide used, or escape spraying, those moths will proliferate. The GM moths may do so too if they develop resistance to the genetic killing mechanism.

Missing safeguards and information include the lack of:

- Adequate safety testing to demonstrate that Oxitec's GM moth larvae are safe for humans, animals and wildlife to eat;
- A published risk assessment which meets European standards: which Oxitec is legally required to provide before exporting GM diamondback moth eggs to the USA for open release into the environment;
- Demonstration from the applicant that the use of tetracyclines in the GM moth breeding facility is compliant with FDA Guidance on the use of antibiotics;
- A detailed risk assessment for the proposed releases of non-GM, wild diamondback moths at the site;
- Clarification of who will be liable for contamination of conventional or organic crops if GM moths spread outside the trial site;
- Adequate public consultation, especially with brassica growers, including organic growers.

Additional steps that should be taken before any open releases of GM insects can be properly considered include:

- Revision of the regulation of GM insects to address the concerns raised by the USDA's own Office of Inspector General, and many others: as part of the current revision of the Coordinated Framework for the Regulation of Biotechnology;
- Implementation of a new approvals process, including a full Environmental Impact Statement, with full public consultation.

Beyond the risks of the current trial, future open releases of Oxitec's GM diamondback moths, perhaps on a commercial scale, are not a credible approach to tackling these pests. Major issues include that:

- these GM insects are not sterile and their dead and surviving larvae will damage and contaminate the crop, making it unlikely to be fit for human consumption;
- this approach is not compatible with either conventional or organic methods of control for other pests, and increases in such pests are therefore likely to result.

1. Proposed releases of GM and non-GM diamondback moths in New York State

Oxitec is working with Cornell University on a plan to release GM diamondback moths (scientific name *Plutella xylostella*) as part of an experiment within the grounds of the Cornell University New York State Agricultural Experiment Station (NYSAES) in Geneva, New York. Up to 1.44 million male GM moths are to be released per year, for three years, in fields planted with brassicas (e.g. broccoli or cabbage) at Research Farm North.[1] Three release sites and three control sites are proposed for the current phase of the experiments, with a total acreage of up to 10 acres per site (60 acres in total). Up to 100,000 GM moths may be released per week for up to the duration of the brassica crop cycle (three to four months each year).

Cornell's researchers have obtained a permit for these experiments from the US Department of Agriculture (USDA)'s Animal and Plant Health Inspection Service (APHIS). The permit expires on 10th November 2017.[2] The purpose of the requested field release is for the applicant to assess the efficacy of GM diamondback moth strain OX4319L-Pxy, in reducing pest populations of non-GM diamondback moths. The intention is that released male GM moths mate with wild female moths, producing offspring which inherit a genetically engineered trait which kills most of the females before adulthood. The aim is to suppress the wild population of these moths by limiting its ability to reproduce.

Cornell's application also states that non-GM moths may be released as part of these experiments: "*If the wild diamondback moth population is not present in sufficient numbers at the trial sites, the experimental field will be artificially infested with male and female moths from a USA-derived wild-type diamondback moth strain currently maintained in the laboratory; dye-marked wild-type moths may also be used in mark-release-recapture experiments to provide a direct comparison with the GE moths*".[3]

Diamondback moths are an agricultural pest[4,5,6,7]: the caterpillars feed on a group of plants called brassicas. Brassicas that are commonly used as food include cabbage, cauliflower, broccoli, Brussels sprouts and canola (also known as oil seed rape). Mustards (including wild mustards) are also eaten by this pest.

The GM moths planned for release are produced by the UK-based company Oxitec, which is now owned by the US company Intrexon.

APHIS published an environmental assessment (EA) of the proposed experiments at NYSAES in 2014 for consultation, and issued a permit in November 2014.[8] Environmental and farmers' groups expressed concerns about the proposed open release experiments and the failure to publish the permit.[9] Their open letter describes the published responses to their concerns in a Finding of No Significant Impact (FONSI) issued by USDA-APHIS[10] as inadequate, and the issuing of the permit as lacking in transparency. Cornell University responded by stating the first year of trials will be contained, although it has not specified the level of containment.[11] Subsequently, the Center for Food Safety (CFS) obtained copies of the permit, application and supporting documents following a Freedom of Information Act (FOIA) request, although a number of these documents are heavily redacted to protect commercial confidentiality. Some information from these documents is cited in this briefing.

2. Oxitec's GM insects

Oxitec is a UK-based company, originally funded by venture capital and UK government grants, with close links to the multinational company Syngenta.[12] In September 2015, Oxitec was acquired by the US-based synthetic biology company Intrexon.[13]

As well as GM diamondback moths, Oxitec is developing other GM agricultural pests, such as fruit flies, 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 diamondback moths and other agricultural pests are known as fsRIDL (female sex-RIDL), a variation of the technology in which only the female offspring are genetically engineered to die.[14]

Although Oxitec frequently describes its insects as “sterile”, this is not the case. The released GM males mate and produce offspring which inherit the late-lethality trait. However, the GM insects’ offspring mainly die at the larval stage in the case of GM mosquitoes, or most of the female (but not the male) offspring die at the larval stage in the case of GM agricultural pests. This means wild female diamondback moths which have mated with the released GM males will lay eggs which inherit the GM “female killing” trait, and GM larvae that develop from these eggs will begin eating the crop before the majority of the female larvae die.

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 have been released in open experiments in the Cayman Islands, Malaysia, Panama and Brazil, but only Brazil has decided to continue with these trials. No country has yet given approval for releases of GM mosquitoes on a commercial scale.[15] Oxitec has previously sought to release GM diamondback moths in the UK[16,17,18,19,20,21,22,23], GM olive flies in Spain[24,25], and GM fruit flies in Brazil, but none of these experiments have 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 current application to release GM moths in New York State, if it goes ahead, would therefore be the first open release anywhere in the world of GM insects with the “female-killing” trait.

3. Lack of adequate US regulation for the release of GM insects

Since 1986, the US government has regulated genetically modified (GM) organisms (also known as genetically engineered - GE - organisms) under a regulatory framework known as the Coordinated Framework for the Regulation of Biotechnology (Coordinated Framework) (51 FR 23302[26], 57 FR 22984[27]). APHIS regulations at 7 Code of Federal Regulations (CFR) part 340 rely on authority granted by the Plant Protection Act (PPA), as amended (7 United States Code (U.S.C.) 7701–7772), to regulate the introduction (importation, interstate movement, or release into the environment) of certain GM organisms and products. The USDA APHIS permit for the proposed GM diamondback moth experiments was issued under 7 CFR Part 340, which covers the introduction of organisms and products altered or produced through genetic engineering which are plant pests or which there is reason to believe are plant pests.[28]

However, the Coordinated Framework is widely considered to be inadequate for the regulation of open releases of GM insects.

In 2002, the US National Academy of Sciences published a report on GM animals which stated that aquatic organisms and insects present the greatest environmental concerns, because their mobility poses serious containment problems, and because they easily can become feral and compete with indigenous populations.[29] The report expressed concerns about gaps in regulation. The Pew Initiative on Food and Biotechnology published a further report in 2004 on gaps in the regulatory system for GM insects in the USA, and a report of a workshop on the issues.[30,31] A central finding of the Pew report was that there are gaps in the regulatory framework in place to review the many issues raised by the potential introduction of GM insects into wild populations. There is no specific regulation on the release of GM insects, no law that clearly covers all the risks and all of the types of GM insects and no single regulatory body: the U.S. Department of Agriculture (USDA), the Food and Drug Administration (FDA) and the Environmental Protection Agency (EPA) could all play a role.

Nevertheless, USDA APHIS initiated oversight of GM insects by issuing permits first for contained experiments and then open releases of GM bollworms containing a fluorescent marker trait.[32] There were 14 US government-funded field trials over a nine year period, beginning in 2002. The only open release experiments were conducted in Arizona in 2007 and 2008, using Oxitec's GM pink bollworms (a cotton pest), with only the fluorescent trait (not the 'late lethality' trait), and made sterile using radiation.[33]

Although they used irradiated sterile insects, with only a GM fluorescent trait, the GM bollworm experiments were halted, partly because of concerns raised by US organic farmers about contamination of their crops with genetically modified organisms (GMOs).[34,35] They also led to a highly critical report by the US Department of Agriculture (USDA) Office of Inspector General. This report argues that USDA APHIS' controls over GM insect research are inadequate and that regulations need to be strengthened.[36] The report also criticises APHIS' Center for Plant Health Science Technology (CPHST) for spending about $550,000 on developing GM plant pests such as the pink bollworm, the Mediterranean fruit fly, and the Mexican fruit fly (in collaborations with Oxitec) without any formal process for selecting which projects would receive funding. The report's recommendations were accepted by APHIS, requiring it to clarify its role, draft specific GM insect regulations, and make more transparent research funding decisions. However, no attempt to draft specific regulations appears to have been made to date.

The Environmental Impact Statement (EIS) published by APHIS in 2008 which recommended the use of GM insects was also found to be "scientifically deficient" when reviewed by scientists at the Max Planck Institute.[37] They report that the document reverses an earlier more cautious view published by APHIS in 2001, without providing the substantial body of evidence required to back up its assertions. However, this "scientifically deficient" 2008 APHIS report and later reports made under the framework criticised by the USDA Office of Inspector General continue to be cited in the current Environmental Assessment for the proposed release of GM diamondback moths.

The White House announced plans to revise the Coordinated Framework for the Regulation of Biotechnology in July 2015.[38] Rather than proceeding with open releases under the current inadequate regulatory regime, this revision could provide an opportunity to ensure that the concerns which have been raised about the regulation of GM insects are properly addressed.

4. Transboundary movement regulations for genetically modified organisms (GMOs)

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.[39] This Regulation requires that the environmental risk assessment (ERA) provided by the exporter meets the standards of EU rules on risk assessment contained in EU Directive 2001/18/EC[40].

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 the Directive. 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.[41] These include a number of issues, discussed further below, that were not considered in APHIS' Environmental Assessment, such as safety testing of GM larvae that may be swallowed during the releases or enter the food chain via contaminated crops, and the impact of the releases on non-target species, including other pests.

Although the USA is not a Party to the Cartagena Protocol, Oxitec is still required to provide a risk assessment which meets EU standards to the importer, and this should be publicly available under freedom of information laws in both the exporting and importing country. No such risk assessment is currently available.[42]

Oxitec has a poor track record of meeting the transboundary notification requirements when exporting its GM mosquito eggs to other countries. [43,44,45]

The UK Department for the Environment, Food and Rural Affairs (Defra) has admitted that Oxitec breached UK and EU regulations 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.[46] The Department has since been warned about the importance of the regulation by the EU authorities.[47] 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.[48] 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.[49]

In Brazil, the risk assessment included in the documents when GM mosquitoes were exported for open release was produced by Oxitec's partner the University of São Paulo, not by the exporter, and it omits most of the issues required to be covered prior to export under EU law. [50] 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.[51] The summary risk assessment relates to the decision by 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 is included, highlighting the lack of consensus on some issues. The Brazilian Public Health Association, ABRASCO, has also criticised Oxitec's approach.[52]

USDA APHIS has previously authorised the import of GM diamondback moth strains from Oxitec for contained use only. This permit expired on August 14th, 2012. The transboundary notification requirements under the Cartagena Protocol apply only to the first export 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 diamondback moths take place in New York State.

5. Concerns about impacts of the proposed releases on human health, the environment and farming

Oxitec's GM moths 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 (which is used as a kind of 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 special type of microscope.

Oxitec has published several papers in scientific journals which contain information about its GM moths.[53,54,55,56] Some further information is contained in USDA APHIS' Environmental Assessment[57] and in the FOIA documents obtained subsequently by the Center for Food Safety.[58] However, these documents leave many unanswered questions about impacts on health and the environment; as well as issues regarding impacts on farmers' markets for their crops and who will be liable if anything goes wrong. A full Environmental Impact Statement (EIS) was not conducted prior to USDA APHIS' approval of the proposed GM moth trials in New York State.

Use of Oxitec's technology requires wild moths to be vastly outnumbered by the GM male moths, which are repeatedly released in order to seek to suppress the population of wild moths. In caged experiments conducted at Cornell, initial release ratios of 20 GM male moths to one wild male moth, increased to rates of 40 to 1 in the next generation, were predicted to be sufficient for population suppression when used alone, whereas lower rates (starting at 3 to 1 and increased to 5 to 1) were used in combination with GM broccoli which had been genetically modified to be pest resistant.[59] GM males were released into the cages on a daily basis because repeated releases are required to try to ensure each generation of eggs inherits the genetic killing mechanism. Release ratios may need to be higher if experiments are conducted in the open air, where wild moths can move in from the surrounding area. This means millions of GM insects would be repeatedly released at the experimental stage, and billions of GM insects would be repeatedly released if the technology were ever used on a commercial scale. The caged trials took six to ten weeks to suppress the population of wild moths in the cages.

The application also proposes making releases of non-GM (wild-type) diamond back moths, from a laboratory strain. This means releasing known pests into the environment without the genetic killing mechanism. These releases will occur as part of an initial mark-release recapture experiment (described below) and also if the wild diamondback moth population is not present in sufficient numbers at the trial sites, in which case the experimental field will be artificially infested with male and female moths from a USA-derived wild-type diamondback moth strain currently maintained in the laboratory (the ‘Vero Beach’ strain from Florida).[60]

The proposed trial consists of two parallel experiments in the first year[61]:
(i) A mark-release-recapture (MRR) experiment, releasing approximately 10,000 GM diamondback moths (OX4319L-Pxy strain) plus 10,000 ‘Vero Beach’ wildtype diamondback moths on a single day. This experiment will be replicated an unspecified number of times for up to six weeks. Monitoring traps set at different distances and directions from the release point will be changed every two days to study dispersal and longevity.
(ii) A mating-performance test in a diamondback moth infested cabbage field with up to 12 field cages. Between 20 and 200 males, followed by 20 to 200 females will be released into each cage from the two stains (OX4319L and wild). Mating success of each male strain will be studied over 2-3 weeks by collecting larvae from the cabbage leaves, rearing them to pupae in the lab and checking for the fluorescent marker in the GM strain.

These experiments will be used to work out the numbers needed for the subsequent open release population suppression experiments “*whilst maintaining the trial within the limits outlined in the release permit application 13-297-102r*”. The application states these limits are: up to 72 releases a year; up to 20,000 males per release; up to 100,000 moths per week; in fields of up to 10 acres planted with brassicas (e.g. cabbage or broccoli); up to 6 experimental fields (3 treated and 3 untreated); releases for up to the duration of the brassica crop cycle (3-4 months).[62]

The proposed trials are focused only on studying the efficacy of the technique, not on resolving any outstanding biosafety issues (as discussed below).

The genetic changes made to the diamondback moths are shown in Table 1. Genetic elements from viruses, bacteria, moths, bollworms, flies and coral have been used to genetically engineer the moths.

Table 1: Summary of Oxitec’s description of the genetic elements in the *Plutella xylostella* strain OX4319L-Pxy[63]

Construct component name	Construct component type	Component function	Donor	Detailed description
piggybac 5’	Vector sequence	Germline transformation	*piggyBac* from *Trichoplusia ni* (cabbage looper moth)	5’ end of *piggyBac*. *piggyBac* is a DNA (deoxyribonucleic acid) transposable element that, only when its ITR (inverted terminal repeats) are intact, is capable of integrating DNA flanking by element-specific DNA into other DNA through mediation of a transposase encoded by an ORF (open reading frame) within the element. Transformation was effected by introducing, with the transforming construct, a helper plasmid that supplied

				transposase activity but was itself unable to transpose into other DNA. This transposition-defective helper plasmid has an ORF encoding *piggyBac* transposase under the control of the *Drosophila melangoster* hsp70 promoter. One of the inverted terminal repeats that flank the wild-type *piggyBac* transposase in *piggyBac* has been removed in the helper plasmid so that the helper plasmid cannot itself integrate even though it encodes for active *piggyBac* transposase.
piggyBac 3'	Vector sequence	Germline transformation	*piggyBac* from *Trichoplusia ni* (moth)	As above.
polyA	Regulatory sequence 3'UTR	Stabilize mRNA	Virus	Regulatory sequence that helps stabilize mRNA
Nls	Nuclear localization sequence	Localises DsRed2 protein into the nuclei of cells	Synthetic	NLS causes DsRed2 protein to accumulate within the nuclei of cells. Allows for spatial patterning of protein expression, which is useful to distinguish fluorescence patterns.
DsRed2	Protein coding sequence	Express DsRed2	*Discosoma Sp* (coral)	This allows the expression of a fluorescent protein. The transgenic DBM with the marker gene fluoresces when excited by illumination of the appropriate wavelength.
Intron	Regulatory sequence for 5' UTR	Requirement for translation	*Drosophilia melanogaster* (common fruit fly)	Stabilizes mRNA and required for translation of mRNA
ie1/Hr5	Enhancer/ Promoter sequence	Control expression of DsRed2	*Autographa californica* nuclear polyhedris virus (AcMNPV)	Promoter from *immediate-early-1* gene and *hr5* enhancer region
tetOx7	Synthetic regulatory sequence	Control of gene expression in a tet-repressible manner	Synthetic	Enhancer region to control gene expression

PBW *dsx* genomic region	Splicing	Gives female specificity	*Pectinophora gossypiella* (pink bollworm)	Contains an alternative splicing intron allowing the sex-specific expression of sequence inserted into the female-specific exon (in this case TAV).
hsp70	promoter sequence	Minimal promoter	*Drosophila melangoster* (fruit fly)	Minimal promoter to enable transcription of gene
tetR	Protein coding region (gene)	Component of tTAV protein	Tn10-specified tetracycline-resistance operon of *E. coli* (bacteria)	In the presence of the antibiotic tetracycline tetR does not bind to its operators located within the promoter region of the operon and allows transcription
tTAV (comprising of tetR and VP16 domains)	Protein coding region (Gene)	Expression tTAV protein		tTAV is a tet-responsive transcriptional factor. It is a fusion of tetR from *E.coli* and the VP16 transcriptional activator from HSV. By combining tetR with the C-terminal domain of VP16 from HSV, known to be essential for the transcription of the immediate early viral genes a hybrid transactivator was generated that stimulates minimal promoters fused to tetracycline operator (tetO) sequences. These promoters are silent in the presence of low concentrations of tetracycline, which prevents the tetracycline-controlled transactivator (tTA) from binding to tetO sequences.
DRo K10	Regulatory sequence 3'UTR	Stabilize mRNA		Regulatory sequence that helps stabilize mRNA
VP16	Protein coding region	Component of tTAV protein	Herpes simplex (virus)	Component of synthetic transcription factor tTA

This is a new technology and its impacts on health and the environment are poorly understood. Some of the concerns are outlined below, but there may be other problems that have not yet been identified.

5.1 Dead GM caterpillars contaminating the environment and food supply

Oxitec's GM insects are not sterile. The adult males 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 caterpillars). Since female diamondback moths lay their eggs on the crop, the caterpillars will begin to eat it before dying on the crop. This means that the crop may face significant damage before any population suppression effect begins (this took six weeks in laboratory experiments) and the crop will become contaminated with large numbers of dead GM caterpillars.

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 most of the female insects at the late larval stage, although the mechanism for this is not fully understood. Cornell plans to release one strain of Oxitec's GM diamondback moths in New York State, known as OX4319L-Pxy.[64] The company reports that 9% of GM females from this strain survived the caterpillar stage to become pupae, in laboratory experiments, with about 1% surviving until adulthood.[65] This means that about 41% of the offspring of the GM moths that are released (most of the female offspring) are expected to die as caterpillars when they are feeding on the crop, and another 8% are expected to die as pupae.

Oxitec often compares its approach to the Sterile Insect Technique (SIT) in which insects are released which have been sterilised by radiation. SIT has been successful in tackling some pests, reducing the wild population because the released males mate with wild females but cannot reproduce. However, one important difference is that Oxitec's GM insects are not sterile: they mate successfully and reproduce, but the female offspring mainly die as larvae (caterpillars).

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. 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*." [66] This same problem applies to Oxitec's diamondback moths.

Oxitec reports that it took six weeks to achieve a considerable reduction in reproductive output of diamondback moths in a caged trial in the UK, and ten weeks to reduce the reproductive output to zero.[67] Open air use will generally be much less effective, meaning that crop damage caused by both wild and GM caterpillars is likely to continue for several months during the releases. In the open air, the wild population is unlikely to become extinct because wild moths will fly in from surrounding crops or be blown in on the wind from further afield. Instead, further releases will need to be made each season to maintain the suppression effect.

Crop damage is not the only problem caused by "late acting lethality": it also means that dead GM caterpillars are likely to contaminate the crop. Oxitec plans to release large numbers of GM males to mate with wild females, with the intention that all the eggs laid by the females inherit the genetically engineered "late-acting lethality trait". Instead of most of these eggs developing into caterpillars that later pupate and become adult moths, most of the female caterpillars are expected to be killed by the inherited genetic killing mechanism before they reach adulthood. Death of most female GM moths at the larval stage will significantly increase the number of larvae dying in the brassica crop (and in wild relative brassica weeds), compared to current conventional or organic farming, since about 41% of the offspring (i.e. most of the females) are expected to die at this stage if the technology works correctly, rather than pupating or reaching adulthood. 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 diamondback moth larvae or brassica crops, or wild relatives of these crops which diamondback moths may also feed on. This will include humans if the crop enters the food chain.

Because of the problem of contamination, USDA APHIS has added a condition to permit for the proposed trial which states [68]: "*THIS IS A CROP DESTRUCT TRIAL. Brassicas will be destroyed at the end of the research field trial. No plants or produce shall be used for food or feed. Upon completion of the experiment, the insecticide shall be sprayed on the plants and the surrounding area within 100m radius of treated fields to kill remaining diamondback moth larvae*".

Although this is an important condition to reduce the likelihood of humans eating crops contaminated with GM caterpillars, safety testing of the GM moths and caterpillars remains extremely important because:

1. Treatment of crops with insecticide within 100m radius is insufficient to prevent contamination of crops further afield (discussed further below);
2. People in the area might swallow adult moths due to the very large numbers of adult males released in a small area;
3. Wildlife will consume adult moths and larvae during the experiment.

In addition, safety must be demonstrated before such technology could ever be put into commercial use. It makes sense to do this before any open releases take place, as the costs and risks of open trials are unnecessary if the moths (especially the larvae) are not safe to eat.

If food does become contaminated, dead GM caterpillars in the crop are likely to be off-putting to consumers and may pose risks to health. Conventionally-grown crops contain very few caterpillars as pesticides usually kill them before they reach this stage, but crops grown using Oxitec's GM insects are likely to have many more caterpillars even than those organically grown crops which are not treated with pesticides (by an order of magnitude or more). This is because a relatively small number of caterpillars would die in the crop naturally, compared to the large numbers that will die as larvae because they contain the genetically engineered "female killing" trait. Further, the crops produced during the GM moth releases are likely to suffer significant damage before the GM female caterpillars die, as well as damage from GM male caterpillars which will mostly survive to adulthood.

Another potential exposure route for humans is through swallowing the adult moths 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.[69] This is because the releases of wild 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, Oxitec provides a commercial reference for toxicity testing of the red fluorescent marker, DsRed2, by Pioneer DuPont.[70] 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*".[71] Other than a bioinformatics report (discussed further below), 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. This mechanism is commonly used by researchers 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 only one feeding study, in which its GM *Ae. aegypti* mosquito larvae were fed to two different species of a type of mosquito that eats other mosquitoes (known as *Toxorhynchites*).[72] No feeding studies have been published for Oxitec's GM diamondback moths or any of its other GM insect pest species, and no feeding trials have been published which study potential impacts on birds, mammals, reptiles or amphibians, such as lizards or frogs.

Oxitec has provided a bioinformatics report by a consultant, Dr Richard E Goodman of the University of Nebraska, which compares the chemical sequences of the tTA (also known as tTAV) and DsRed2 (fluorescent) proteins with known toxins and allergens in databases. The bioinformatics report was made publicly available for the first time in 2015 in response to FOIA requests by the Center for Food Safety[73] and was not published during the 2014 consultation. The released version is poorly copied so that it is difficult to read. The bioinformatics report considers exposure only through mosquito bites and saliva, not through the dietary route, which is more relevant to the GM diamondback moths, although the searches conducted may be relevant to both. Oxitec's submission to the regulators, which summarises this report, notes some similarities between the tTAV protein and known allergens (a 22% match with tropomyosin from *Neptunea polycostata*, a gastropod; and a 27.5% match with a salivary protein from the mosquito *Aedes albopictus*).[74] The full report adds a 23.2% match between DsRed2 and *Daucus carota* (wild carrot or "Queen Anne's-Lace"), a mild allergen. However, these partial matches are not considered further as they are less than 35%. The report also cites two papers which refer to toxicity of DsRed2[75,76], however the author of the bioinformatics report states that he does not believe that these papers demonstrate toxicity. Oxitec reports that the most significant match with tTAV from the toxicity point of view was to the TetR protein of *Escherichia fergusonii*, which was tagged as a toxin because it is a toxic bacterium: however the company and its consultant state that no evidence was found that the protein itself is toxic.

In the scientific literature, there is some evidence that enhanced tTA expression can have adverse effects (loss of neurons affecting cognitive behaviour) in transgenic (GM) mice.[77] Other mice studies have detected adverse effects on the lung.[78,79] The bioinformatics report fails to identify or cite this evidence, instead claiming that these constructs have been inserted in mice and other animals without any adverse impacts. The reported adverse effects are caused by production of tTA in the cells of these mice through genetic engineering, rather than through eating tTA. However, they suggest that more evidence is needed before concerns about safety are dismissed. In the case of the rat feeding trails reported for Green Fluorescent protein (cited above), the authors state: "*Moreover,* *transgenic animals that constitutively express GFP have been reported as being healthy… This may differ from dietary exposure because the GFP in transgenic animals is located intracellularly and toxicity or allergenicity may function differently than when exposed extracellularly. Nevertheless, these data do suggest that GFP is minimally toxic, a conclusion that is supported by this feeding study*". However, for tTA, the opposite is true, as adverse effects have been reported in transgenic mice. These adverse effects should be taken as a warning sign that further testing – including feeding studies - is required.

Considerably more data, based on specific feeding trials in relevant species, is therefore needed to establish that consumption of GM moth 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 diamondback moth eggs to the USA or other countries (as detailed above); (ii) future exports of crops produced using GM moths 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.[80] 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.[81]

An application by Oxitec 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.[82,83] Oxitec re-applied to release GM olive flies in Spain in 2015, without providing any further safety information.[84] This application was rejected.[85]

In 2014, the Brazilian regulator CTNBio approved experimental releases of Oxitec's GM Mediterranean Fruit Fly (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.[86] 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.[87] Contamination of crops with GM moths or flies could have serious impacts on the ability of farmers to sell them, especially on the organic or European markets, as detailed further below.

The evidence provided for the proposed releases of GM diamondback moths falls far short of the data or precautions needed to assess safety of the GM moths, for example to birds and mammals, including humans, which may eat them, especially at the larval stage when they will contaminate food crops. Farm workers and animals might also swallow adult flies due to the very large numbers involved in the releases.

Species which could be affected if consuming the GM moths is harmful include species which are endangered, threatened or of special concern and which may feed on diamondback moths or larvae or on brassicas in New York State: such as the Northern Long-Eared Bat, Grasshopper Sparrow and the New Cottontail Rabbit, which are identified as relevant species in the APHIS EA. Migratory birds also forage for insects in or adjacent to fields containing brassica crops.

5.2 Potential to spread antibiotic resistance in food and the environment

Even if the genetic changes to the moths, including expression of tTA, were demonstrated to be safe for humans, animals and wildlife to consume, concerns would exist about the dead GM larvae in the crop spreading antibiotic resistant bacteria into the environment, as discussed below.

Oxitec uses tetracycline (an antibiotic which is used commonly in agriculture and medicine) as a kind of antidote to the genetic killing mechanism, allowing it to breed its 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. Including tetracycline in their feed allows the female insects to live to adulthood rather than dying at the larval stage.

The use of tetracycline to breed the GM diamondback moths in the lab carries the risk of spreading antibiotic resistance, which could pose a major risk to human and animal health.[88] This is because insect guts are reservoirs for antibiotic resistance genes which can be spread into the environment. [89,90,91] GM insect production in factories exposed to antibiotics could lead to drug resistance in bacteria in their guts so that the insects disseminate antibiotic resistance when released into the environment.[92,93] Oxitec's GM diamondback moths are reared on an artificial diet containing 100µg/mL of tetracycline[94] or chlortetracycline (CTC, another antibiotic in the tetracycline family)[95]. These are both common antibiotics which are being phased out for uses other than treating animal diseases, according to FDA Guidance.[96,97] The Guidance is intended to ensure that antibiotics including tetracycline and chlortetracycline are only available for use in veterinary or human medicine. This is because antibiotic resistance can make antibiotics useless to treat diseases, with serious implications for human and animal health.

Cornell's application to USDA APHIS states that all GM diamondback moths used in the trials will be reared as larvae on a non-tetracycline diet. This is consistent with the report of the contained trials undertaken at Cornell to date. [98] 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. Oxitec can do this with its GM agricultural pests (but not its GM mosquitoes) because the males survive and are not killed by the genetic killing mechanism (i.e. the technology is "female killing" only). 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.[99,100] There are very limited studies of these effects in moths but there is some evidence that *Lepidoptera* (the group of insects which includes diamondback moths) can transmit their immune status, influenced by their midgut microbiota, to future generations.[101] If antibiotic resistant bacteria can spread from one generation of moths to the next, they will end up in the environment and subsequently in the food chain when the GM moths are released and reproduce.

No figures are available on the quantity of tetracycline that might be discharged from a laboratory or factory producing GM diamondback moths. However, some information is available about tetracycline use when breeding Oxitec's GM mosquitoes. Oxitec produces about 250 GM mosquito larvae in one breeding tray, creating 220 adults or 110 adult males at an 88% survival rate. Each tray contains one litre of tetracycline water (at 30 µg/ml concentration) and this water may require replacement once a week during the 7 to 10 day development of the eggs to pupae.[102] This means that to produce 2 million male GM mosquitoes a week (the current target for experiments in Brazil) Oxitec requires about 18 to 36 thousand litres of tetracycline water, which then requires disposal. Any tetracycline contaminated water released from the laboratory or GM insect-production factory could lead bacteria in the receiving environment to develop antibiotic resistance, which might spread into bacteria which cause diseases.

No public information is available about the proposed use of tetracyclines to feed GM diamondback moths at Cornell, or the proposed method of disposal. It is unclear how the proposed use of tetracycline for a non-veterinary purpose can be regarded as consistent with FDA Guidance on this issue. The USDA did not mention these concerns in its EA or FONSI, which indicates it likely it did not consider this potential human health effect at all.

5.3 Likely increases in other pests

If Oxitec's releases of GM moths successfully reduce the wild population, even temporarily, other pests are likely to move in and start eating the crop. This is because Oxitec uses a "single species approach" which aims to remove only one species from the ecosystem. However, improving management of one pest can increase outbreaks of another. Other pests compete with diamondback moths for resources, so if the moths are reduced or removed, numbers of other pests are likely to increase. These risks have not been considered in the Environmental Assessment for the proposed experiments at Cornell. In contrast, they would be required to be considered in a risk assessment that met EU standards.

There are numerous other brassica pest species which compete with diamondback moths to eat such crops. They include: cabbage root maggot (*Delia radicum*); flea beetle (*Phyllotreta striolata* and *P. cruciferae*); imported cabbage worm (*Pieris rapae*), also known as the small cabbage white; cabbage looper (*Trichoplusia ni*); cabbage and green peach aphids (*Brevicoryne brassicae* and *Myzus persicae*); onion thrip (*Thips tabaci*); and Swede midge (*Contarinia nasturii*).

Imported cabbageworm, cabbage looper and thrips, as well as diamondback moths, all cause perennial pest problems in New York State and the Swede midge is also recognised as a potentially serious pest.[103] Thus a strategy which only tackles one of these pests, potentially making the crop more available to other pests by reducing competition, may not be effective in limiting damage to the crop. In New York State, the imported cabbageworm is the most common of the three *Lepidoptera* (moths and butterflies) which eat the leaves of plants and which may contaminate the marketable portion of brassica crops by either their presence or their faecal matter.[104] It overwinters locally throughout the Northeast, so it is generally a pest every year. In contrast, the diamondback moth and cabbage looper are commonly carried north on weather fronts from southern overwintering sites. Because this migration does not occur every year, populations are highly variable.

The vast majority of (non-organic) Brassica crops are treated with insecticides prior to any diamondback moth infestation.[105] However, these insecticide applications have usually been dictated by the presence of early season pests such as root maggots (*Delia* species) and flea beetles (*Phyllotreta* species), rather than by diamondback moths.[106] The imported cabbageworm is often controlled using the bacterial insecticide Bt (*Bacillus thuringiensis*) which may also be used to control diamondback moths.[107,108]

Thus, Oxitec's approach is likely to conflict with conventional methods of control for other major pests. Unless alternative management practices are used for all pests (discussed further below), insecticide applications are likely to continue in order to deal with these other types of pests. In such a situation, Oxitec's approach will cost extra money for no benefit, because continued insecticide spraying is likely to also kill the GM diamondback moths, so they cannot contribute to suppression of wild relatives. Alternatively, if insecticide applications cease, and no alternatives are implemented, other pests are likely to become a significant problem in the crop.

The risk of increases in other pests is analogous to problems with GM insect-resistant crops (Bt crops) which have developed in China, Brazil and India. In China, secondary pests which are not affected by the Bt toxins in its GM cotton crop have become a major problem.[109,110,111] In Brazil, the Agricultural Ministry has issued warnings about massive explosions in corn ear worm (*Helicoverpa armigera*) in areas growing Bt maize.[112,113] More recently, a major outbreak of whitefly has been reported in GM cotton in India.[114] These examples show how reductions in competition can lead to an explosion in another type of pest.

5.4 GM insects spreading where they cannot be recalled

A major difference between Oxitec's GM mosquitoes and its GM agricultural pests, such as the GM diamondback moths, is that the GM trait in the agricultural pests is "female killing" only, whereas the both the male and female offspring of the GM mosquitoes die. This means that the male GM moths 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 to most GM males surviving, some female GM moths will survive to adulthood. Cornell researchers plan to release Oxitec's GM diamondback moth OX4319L-Pxy in the proposed experiments in New York State. For this strain, female survival rates to adulthood in the absence of chlortetracycline (CTC, one of the tetracycline family of antibiotics) is reportedly 1%, relative to the wild moths.[115] Because very large numbers of GM moths are expected to reproduce in the environment, the numbers of female survivors could be very high even if survival rates are only 1%. Cornell's permit allows the release of 1.44 million males a year. As a very rough estimate, if all the released GM males mated successfully once, this would lead to about 216 million eggs laid in the crop containing the GM trait (on average a female diamondback moth lays 150 eggs[116]), leading to up to a million surviving female offspring.

In addition, contamination with tetracycline and related antibiotics is widespread in the environment and could lead to significantly increased survival rates. Oxitec has reported female survival rates at different chlortetracycline (CTC) concentrations for the OX4319L-Pxy strain of GM diamondback moth (the numbers tested are not reported).[117] In these tests, no GM females survived to adulthood at CTC concentrations up to 0.01 μg/mL, while at or above 10 μg/mL CTC the female survival rate was similar to that of males. At concentrations of 0.1 μg/mL and 1 μg/mL female survival to adulthood was around 15% and 55% respectively, relative to wild moths.

The tetracyclines are a family of antibiotics any one of which can increase the GM female moth's survival rates. Because of their use in treating animal diseases, tetracyclines commonly contaminate animal manure. Oxytetracycline can be found at concentrations above 500 μg/g in animal manure and doxycycline at up to 78.5 μg/g dry weight in broiler manure.[118,119] 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.[120] These concentrations are likely to be more than enough to inactivate the killing mechanism in the female GM moths if the larvae come into direct contact with contaminated manure. Although diamondback moths do not normally lay their eggs in direct contact with manure, they might change their behaviour if this benefits their survival. Behavioural adaption beneficial for survival could be selected for in the field, leading to females seeking contaminated areas in which to lay their eggs. There is evidence of behavioural resistance developing in a SIT programme using irradiated flies, when females became unreceptive to mating with irradiated males. [121]

Tetracycline levels in industrially farmed animals may also be sufficient to increase GM female moth 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[122] but later admitted to an 18% survival rate of larvae fed on cat food in a published paper.[123] 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.[124] In some circumstances 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.[125]

Resistance to the genetic killing mechanism can also develop through evolution during mass production, when any mutations which arrive by chance to allow the insects to survive and breed will be selected for so they become common in the population (something which can't happen with the traditional Sterile Insect Technique).[126,127,128] Such resistance is another mechanism which could allow more GM female moths to survive and breed.

Therefore, most GM males survive and some GM females will 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 moths spread in the environment.

In the FOIA documents, Oxitec has provided a report which shows that the genetic killing trait is selected out of a wild diamondback moth population over time, because it makes the insects unfit to survive.[129] However, the results are dependent on the low survival rate of GM females, which may change over time if resistance develops or they breed in tetracycline-contaminated areas.

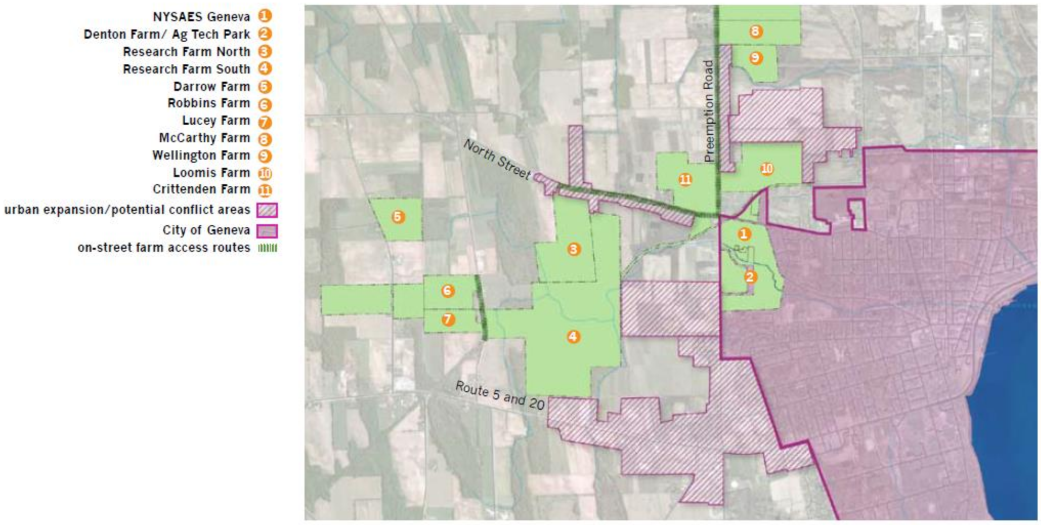
At the conclusion of each proposed open release experiment, the release sites will be treated with the EPA-registered insecticide, Coragen (chlorantraniliprole) to kill any remaining moths. Post-experiment monitoring of diamondback moths with the traps will continue for two weeks after the conclusion of each experiment. The intention to clear all brassica crops and weeds for 10m around the site, followed by spraying over 100m around the site is stated on page 10 of Oxitec's report (appended to the USDA APHIS

Environmental Assessment). Spraying for 100m around the site is a condition of the USDA APHIS permit (as cited above).

APHIS' Environmental Assessment states (page 11) that the proposed release sites are generally surrounded by other agricultural fields, planted to row/vegetables crops, orchards, and vineyards. According to the EA (page 36), within-field and adjacent-field plant communities are anticipated to be similar within the action area, in that there will be a mixture of cultivated crops and weeds of those cultivated crops. Domesticated crops that may be found within the action area include fruits and field crops, including a variety of domesticated cruciferous crops, such as cabbage or broccoli. According to the EA, weeds in the area will include up to 50 species of non-domesticated cruciferous plants which may act as hosts for diamondback moth larvae.

A plan and aerial photograph of the proposed release site at Research Farm North are shown in Figures 1 and 2.

Figure 1: Map of NYSAES showing the location of Research Farm North[130].

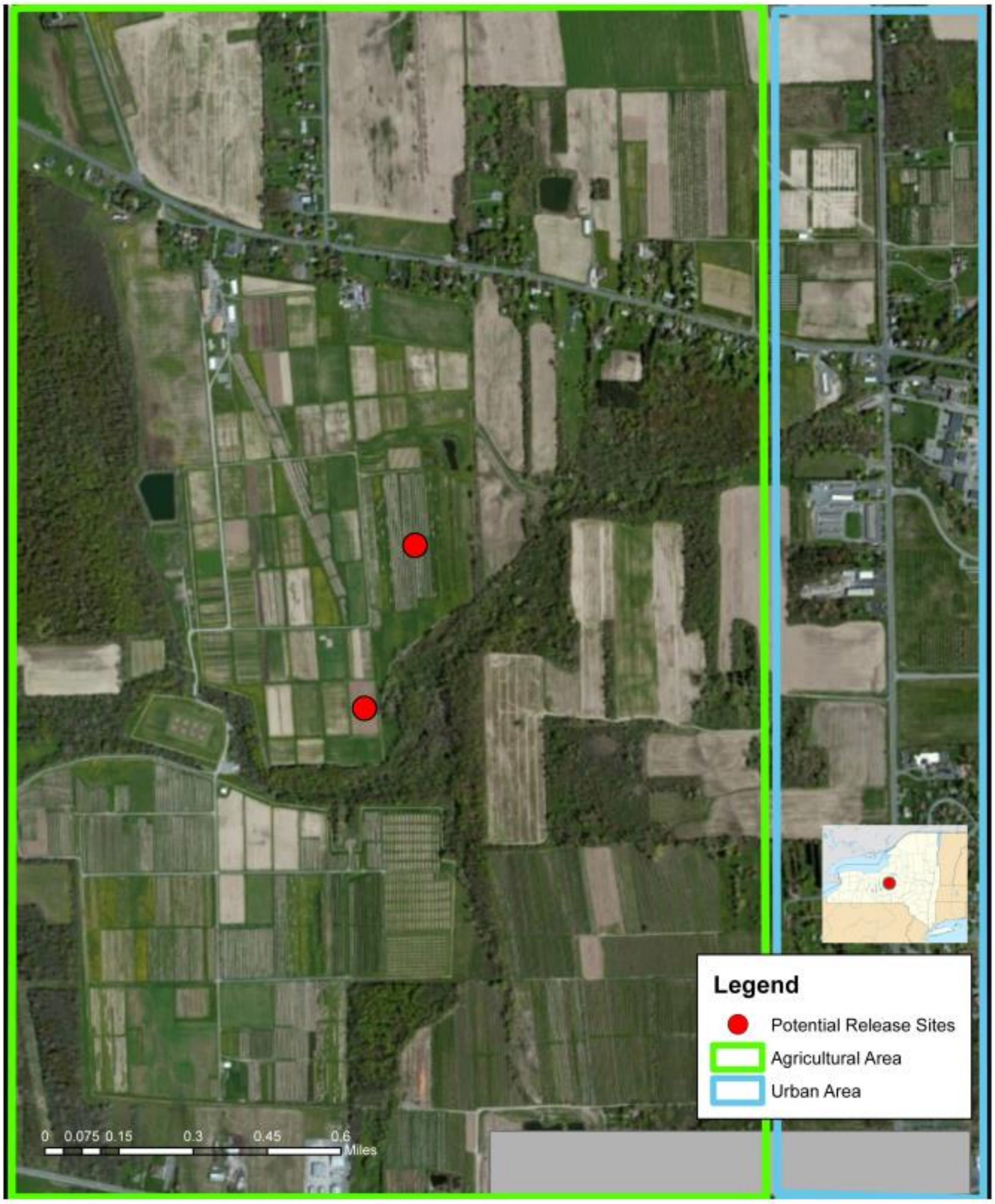


Transport and sale of brassica produce is one mechanism through which this pest has been transported worldwide and from the south to north of the United States.[131] Other mechanisms for spread include: independent flight of adult moths to and from wild relatives; dispersal through the movement of humans, animals and birds; and dispersal assisted by the wind, including mass migration.

The permit conditions which require spraying within 100m of the trial site and destruction of the crop may not be sufficient to prevent contamination of neighbouring crops. Further, contamination will become a much bigger issue if Oxitec's technology is ever used on a commercial scale.

The APHIS EA accepts (page 45) that "*cruciferous crops planted on adjacent fields may experience some herbivory damage from the larval offspring of a GE diamondback moth male and a non-GE diamondback moth female*", however it fails to consider the implications of contamination of neighbouring crops with GM (also known as "GE") diamondback moth larvae, particularly in relation to the lack of safety testing (discussed above) and the potential loss of markets (discussed further below).

Figure 2: Aerial photograph of the potential release sites, reproduced from Figure 2 of the APHIS EA. The action area consists of six sites (3 release sites and 3 control sites). The upper red dot represents five release sites that are directly adjacent to one another, while the lower red dot represents a single release site.



Diamondback moth movement within crops involves a series of short flights within the crop canopy: in experiments in Australia, 95% of released diamondback moths remained within

40 to 106m of the release point, 99% remained within 63 to 117m, and 99.9% remained within 113 to 300m of the release point.[132] If these estimates are applicable to the release site, this means that about 1% of the released moths may move outside the area which is to be sprayed following the trial (which covers only up to 100m). Thus, for the proposed annual release of up to 1.44 million males some 14,400 GM males might fly outside the 100m area to be sprayed, under non-migratory conditions.

There can be significant movement between the crop and neighbouring flowering vegetation, and a small proportion of individuals (less than 1%) may move greater distances from healthy crops to colonize neighbouring crop patches. The authors of the Australian study also cite unpublished data that small percentages of diamondback moths can move between host patches of up to 150m separation. In addition, they state they cannot rule out some moths engaging in long-distance dispersal or migration. When applying population control strategies, such as mating disruption, the authors recommend a minimal separation distance of 3km between target and non-target populations of moths, based on a safety factor of 10 applied to the 300m distance from their release point that 99.9% of moths stayed within in their study. This is far larger than the 100m area to be sprayed following the proposed study of GM diamondback moths, according to the conditions in the USDA APHIS permit.

An earlier study of diamondback moth flight paths in Japan found a mean flight distance of 615m in summer and 286m in autumn for moths captured outside the release field.[133] Although the majority of released moths, which were caught in the release field, were not included in these calculations, this research also demonstrates that released diamondback moths can fly much further than the 100m spraying distance required for the proposed trial in New York State.

Longer-range movement of DBM is achieved through active migration, which explains its global distribution. Migration enables diamondback moths to move from areas that allow year-round persistence into areas that are only seasonally suitable for growth and development so that regions where diamondback moths cannot survive low winter temperatures can be invaded annually from regions where they can overwinter.[134] Ecological studies suggest that the diamondback moth is an actively dispersing species and when environmental conditions dictate, such as when host plants degrade, the species moves large distances to colonise particular regions when climatic conditions are favourable for migration.[135] The authors of the Australian study cited above note that the dispersal pattern from harvested crops is likely to be quite different from that in healthy host crops and that, depending on the location of the closest host patches, moths from harvested crops may have to travel hundreds of meters or even kilometres.

Thus, there may be movement of released diamondback moths (GM or wild moths) from the crop to neighbouring crops or flowering vegetation, and movement might occur over much longer distances if the moths migrate. As well as brassica crops, such as cabbage and broccoli, there are numerous weedy brassicas which may provide viable habitat for diamondback moths, including *Sinapis arvensis* (wild mustard or charlock), *Raphanus raphanistrum* (wild radish), *Brassica rapus* (wild or bird rape), and *Hirschfeldia incana* (hoary mustard).[136]

If migration occurs, there may even be potential for diamondback moths released in New York State to contaminate the Canadian canola crop[137] as well as brassica production. In Ontario, diamondback moths generally arrive from the South although they can sometimes also overwinter.[138]

Although the main mechanism for crop damage in northern climates is re-infestation via long-distance dispersal by the wind, small numbers may overwinter in cold climates, potentially allowing survival of the GM trait. In Canada, Alberta's Department of Agriculture and Rural Development reports that overwintering diamondback moths were found in central Alberta in the early 1990s i.e. considerably further north than the proposed trial site.[139,140] Adults have also recently been found in spring emergence traps in Saskatchewan and have been collected (in small numbers) very early in spring in Manitoba. The average temperature in January (the coldest month) in Geneva, New York, where the proposed experiments are sited, is -8.9^{o}C[141], compared to a lower lethal temperature where 25% survived of -15.2^{o}C in laboratory tests.[142] This does not provide confidence in claims made in the Environmental Assessment that GM diamondback moths cannot overwinter at the release site, particularly if there is unintentional survival of females due to failure of the killing mechanism.

5.5 Release of non-native strains and wild-type moths

In the UK, Oxitec was prevented from releasing its GM diamondback moth partly because of concerns about the use of a North American background strain, which is subject to controls under plant pest control regulations.[143] Using a non-native strain can introduce undesirable traits which might not be present in a local strain of pest e.g. the introduced strain of pest could do more crop damage, be more invasive, or be resistant to treatment with some insecticides.

The strain of diamondback moth used by Oxitec is not indigenous to New York State but originates in Vero Beach, Florida, USA. [144] According to the Oxitec document appended to the USDA-APHIS Environmental Assessment, this strain has been tested for susceptibility to the insecticide *Bt* and is unlikely to have developed resistance to other insecticides as it is a laboratory strain (page 16). However, no tests of resistance to other insecticides have been reported, nor has data been provided on other properties, such as invasiveness.

Caged experiments at Cornell have shown how releasing GM diamondback moths to mate with wild ones may slow the development of resistance in wild Bt-resistant strains of moth.[145] Whilst this could be a beneficial impact, it highlights how other traits in a non-native strain of moth could also spread into the wild moth population through mating.

The application (page 5) also proposes releases of non-GM (wild-type) diamond back moths, from a laboratory strain. These releases would be: (i) as part of the initial mark-release-recapture experiment; and (ii) to infest the trial sites if insufficient numbers of wild moths are present to undertake the population suppression trial. This means releasing known pests into the environment without the genetic killing mechanism. These wild-type moths are also likely to spread outside the release site and beyond the 100m spray area, as detailed above. The USDA APHIS has provided no risk assessment for such a proposed release, which was discovered only through the FOIA request cited above.

5.6 Other possible effects of the releases on other species

The proposed releases will significantly increase the number of adult diamondback moths in the short-term (by an order of magnitude or more), due to the need to swamp each wild male with many GM ones. If the experiment is successful, the number of dead caterpillars in the crop will also increase, as the wild population is replaced by the GM one. In the longer term, if population suppression is successful, the number of moths should fall due to the lower survival rate of the GM moths compared to the wild ones. In practice, wild male moths might move away, in response to the releases, and cause an increase in pests in neighbouring fields. Wild moths could also move back into the site as the local population falls and competition is reduced. Other competitor pest species might also increase if the number of diamondback moths does fall. In the following season, re-infestation is likely and repeated releases of GM male moths may take place again. The changing cycle of releases implies significant changes to the ecosystem, including short-term increases in adult moths and larvae for predator species, followed by temporary falls in numbers if population suppression is successful. As well as possible direct adverse effects of consuming GM adult moths or larvae (considered above), significant fluctuations in the availability of the moths as food might cause some problems for some species.

Horizontal gene transfer (HGT) is defined as any process in which an organism incorporates genetic material from another organism into its genome without being the offspring of that organism. HGT is common in viruses and bacteria but is much rarer in plants and other animals.[146] Oxitec has introduced genetic modifications to its GM insects using something called the *piggyBac* transposon: this is a small piece of DNA that inserts itself into another place in the genome. Some scientists have expressed concerns that the *piggyBac* transposon could move from a GM insect to a virus and then from the virus to another insect.[147,148,149] Although such changes are likely to be rare, mass rearing of GM insects prior to release could provide an opportunity for this to happen.[150] Oxitec's GM insects contain a complex system of genetic elements from other species (see Table 1) and it is unclear what would happen if these were transferred to other organisms.

5.7 Liability and contamination

According to Cornell University, with more than 12,000 acres grown annually, New York ranks in the top three states nationally for both fresh market and kraut cabbage.[151] Fresh cabbage is sold in retail and wholesale markets and is used for coleslaw, egg rolls and other products. Broccoli is grown on an estimated 800 acres throughout New York State.[152] Multiple plantings are typically grown along with other Cole crops such as cauliflower, cabbage and kale on small-scale diversified vegetable farms. Broccoli is predominantly grown from transplants set in April and May for a spring crop and in late June through August for a fall crop.

Open releases of GM and wild diamondback moths raise concerns that these may spread into neighbouring crops, either directly or after a period of survival and spread through wild relatives of these crops. Both GM and wild moths could damage such crops. In addition, the spread of GM diamondback moths could lead to contamination of crops with large numbers of dead GM caterpillars, as described above.

If crop contamination with GM caterpillars 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 (including the EU) have a regulatory approvals process without which products containing GM insects will not be accepted on the market, as described above. In the EU, foods containing genetically modified organisms (GMOs) must also be labelled.[153] Further, there may be cross-border issues with Canada if GM diamondback moths spread across the border (see discussion of off-site dissemination above), with implications for the canola industry as well.

Although contamination could affect all farmers, including conventional farmers, there are particular concerns about organic crops because the use of genetic engineering, or genetically modified organisms (GMOs), is prohibited in organic products. To meet the USDA organic regulations, farmers and processors must show they aren't using GMOs and that they are protecting their products from contact with prohibited substances, such as GMOs, from farm to table.[154,155] However, unlike many pesticides, there aren't specific tolerance levels in the USDA organic regulations for GMOs. As such, National Organic Program policy states that trace amounts of GMOs don't automatically mean the farm is in violation of the USDA organic regulations. In these cases, the certifying agent will investigate how the inadvertent presence occurred and recommend how it can be better prevented in the future: for example, by requiring a larger buffer zone. As described above, spraying for 100m around the release site, as the USDA permit requires, is unlikely to be sufficient to guarantee that there is no contamination. 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 GM crops have caused major (multi-million dollar) damage to markets for conventional or organic crops and foods.[156,157,158,159] 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.

6. Alternatives

The diamondback moth is one of several secondary crop pests that have dramatically increased in importance owing to the overuse of broad-spectrum insecticides since their introduction in the late 1940s.[160] Intensive insecticide use in high-value Brassica crop production has led to increased selection pressure for resistance, particularly in the tropics and subtropics.

However, many alternative approaches to diamondback moth control have been developed and used successfully including: crop rotation; intercropping and (with somewhat variable results) trap cropping (using collards or Indian mustard around fields to attract the pests).[161] Mating disruption, using the female pheromone of the diamondback moth, may also contribute to integrated pest management. In addition, biological control using natural enemies can be successful and is an active topic of research.

Development of an agroecosystem management approach includes management of individual fields to minimize pest damage through careful use of available control measures, as well as broader regional measures.[162] In the Bajío region of Mexico, the general approach to managing individual fields since 1988 has been based on field sampling and action thresholds that permit relatively large populations early in the development of the crop but call for strict control as harvest approaches. By 2007, 3-4 insecticide applications (significantly fewer than pre-1988) were generally sufficient for insect control in each field and natural control of diamondback moth, particularly parasitism, remained high during the early phases of crop development. There has been a shift in insecticide use over time: a gradual shift from synthetic insecticides to Bt took place for many growers from 1992 to 1995, combined with biological control, cultural controls, and host-free periods (periodic moratoria on planting cruciferous crops, known as the “veda”). After 2000, three new effective insecticides became available: spinosad, indoxacarb, and emamectin-benzoate, although these produced new challenges in maintaining important non-chemical controls.

The greater potential for improving control of diamondback moth appears to be changing the agroecosystem itself at larger spatial and temporal scales, perhaps by production and processing of additional crops in the region i.e. reducing reliance on monoculture.

Cornell has produced a guide for organic production of broccoli, cauliflower, cabbage and Brussels sprouts, which includes Integrated Pest Management (IPM) techniques such as identifying and assessing pest populations, keeping accurate pest history records, selecting the proper site, and preventing pest outbreaks through use of crop rotation, resistant varieties and biological controls.[163]
Recommendations for diamondback moth are:

- Scout weekly (examine the underside of leaves on plants) and be aware for thresholds for use of organic insecticides. Spinosad, Bt and neem products are approved for organic production and rotation of use (applying one class to each insect generation) will help to avoid resistance development in the pests;
- Do not plant near previously infested fields;
- Consider using trap cropping with yellow rocket or collards, although this has shown variable results;
- Consider isolating the crop through growing tall barriers of a non-host crop such as sorghum Sudan or sweet corn: this can reduce infestations from these low flying pests in small, isolated plantings;
- Inspect transplants to prevent DBM from being introduced;
- Consider floating row covers which can provide a barrier between the crop and pest, but note these can be expensive and may reduce crop yields;
- Consider using natural enemies, particularly *Diadegma insulare*, which can reduce DBM populations by more than 80%;
- Limit weeds in the brassica family, such as wild mustard, yellow rocket, and shepherds purse, which serve as alternate hosts for DBM and can contribute to a quick buildup of populations;
- Plough down crop residues after harvest to destroy existing eggs and larvae which could build populations over the season.

As noted above, in the North East USA, diamondback moth is regarded as a secondary pest which is not present in significant numbers every year. It is therefore important to use integrated pest management which also targets other species. In addition, Oxitec's approach is not compatible with organic farming. The likely presence of large numbers of dead GM caterpillars in the crop, as discussed above, would not be compatible with organic production systems and could put organic certification at risk.

7. Conclusions and recommendations

Open releases of GM diamondback moths are premature because of the serious limitations of this technology, lack of information needed to assess the risks, and inadequate regulation.

Although the USDA APHIS permit for the proposed trial includes conditions which require destruction of the crop and spraying of insecticide for 100m around the site, these conditions are insufficient to guarantee that GM diamondback moths, or any wild moths that are released, will not spread to other areas and perhaps into the human food chain.

Missing safeguards and information include the lack of:

- Adequate safety testing to demonstrate that Oxitec's GM moth larvae are safe for humans, animals and wildlife to eat;

- A published risk assessment which meets European standards: which Oxitec is legally required to provide before exporting GM diamondback moth eggs to the USA for open release into the environment;
- Demonstration from the applicant that the use of tetracyclines in the GM moth breeding facility is compliant with FDA Guidance on the use of antibiotics;
- A detailed risk assessment for the proposed releases of non-GM, wild diamondback moths at the site;
- Clarification of who will be liable for contamination of conventional or organic crops if GM moths spread outside the trial site;
- Adequate public consultation, especially with brassica growers, including organic growers.

Additional steps that should be taken before any open releases of GM insects can be properly considered include:

- Revision of the regulation of GM insects to address the concerns raised by the USDA's own Office of Inspector General, and many others, about gaps in regulation: as part of the current revision of the Coordinated Framework for the Regulation of Biotechnology;
- Implementation of a new approvals process, including a full Environmental Impact Statement, with full public consultation.

Beyond the risks of the current trial, future open releases of Oxitec's GM diamondback moths, perhaps on a commercial scale, are not a credible approach to tackling these pests. Major issues include that:

- these GM insects are not sterile and their dead and surviving larvae will damage and contaminate the crop, making it unlikely to be fit for human consumption;
- this approach is not compatible with either conventional or organic methods of control for other pests, and increases in such pests are therefore likely to result.

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