The collection of this information is authorized by the Plant Protection Act of 2000. The information will be used to determine eligibility to receive all types of permits. No permit will be issued until this application has been approved.

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
BIOTECHNOLOGY REGULATORY SERVICE

APPLICATIONS FOR PERMIT OR COURTESY PERMIT UNDER 7 CFR 340

(Genetically Engineered Organisms or Products)

1. NAME, ADDRESS, TELEPHONE, AND EMAIL OF APPLICANT

Name: Dr. Anthony Shelton
Position:
Organization: Cornell University/NYSAES
Organization Unique ID:
Address: 630 W. North St.
(b)(6)
Geneva, NY 14456
County/Province:
Township/Island:
Day Telephone: (b)(6)
FAX:
Alternate:
Email 1: (b)(6)
Email 2:

2. INTRODUCTION TYPE

- [ ] Importation
- [ ] Interstate Movement
- [ ] Interstate Movement and Release
- [x] Release

3. PERMIT TYPE

- [x] Standard Permit
- [ ] Courtesy Permit

4. PURPOSE OF PERMIT

- [ ] Industrial Product
- [ ] Pharmaceutical Product
- [ ] Phytoremediation
- [x] Traditional

5. CONFIDENTIAL BUSINESS INFORMATION VERIFICATION (CBI)

Does this application contain CBI? [ ] Yes [x] No
CBI Justification:
N/A

6. REQUEST TYPE

[x] **New** [ ] **Amendment** [ ] **Renewal**

Amendment/Renewal Description:
Previous Permit Number(s):

7. MEANS OF MOVEMENT

Import by air; releases manually from the ground/vehicles.

8. VARIANCE VERIFICATION

Have you previously applied for variance(s) that you wish to apply to this permit? [ ] Yes [x] No
Variance Number(s):

If so, describe in a brief summary how the variance will be applied:
N/A

9. REGULATED ARTICLE

Scientific Name: Plutella xylostella

Common Name: Diamondback moth

Cultivar and/or Breeding Line:

Any biological material (e.g., culture medium, or host material) accompanying the regulated Article during movement:
Artificial insect diet. This diet will be frozen at -15°C for 12 h prior to import.

Country and locality where the donor organism, recipient organism, and vector or vector agent were collected, developed, and produced:
All final engineering of the transforming constructs was performed at Oxitec Ltd, in the United Kingdom.
The genes used from the donor organisms and the piggyBac-derived portions of the vectors used to build the transforming construct were cloned off-site. The recipient organism is the moth, Plutella xylostella, which is endemic in temperate regions around the world, including the USA. The recipient Plutella xylostella strain for the transformation was a wild-type strain obtained from Syngenta (public limited company), UK, which has been reared in Oxitec insectaries since 2008.

Processes, Procedures, and Safeguards Description:

This is a permit request for 3 years of seasonal releases (April to October) of a female-lethal, genetically marked diamondback moth (maximum 100,000 moths/week), in brassica fields at the Cornell University research station, Geneva NY.
Males of the transgenic moths will be released in cultivated brassica plots and biological parameters of these moths, such as dispersal and persistence, measured using traps, for example baited with synthetic sex pheromone. The moths of the OX4319L-Pxy strain carry a stable, heritable marker the DsRed2 fluorescent protein, viewed by fluorescence microscope or detected by PCR and their female progeny die in the absence of a dietary repressor (tetracycline or suitable analogues supplied in their artificial diet). The male-selecting (female-lethal) penetrance of the strain is >99% (Jin et al. 2013). The marker provides a means of distinguishing released moths from wild moths, and female-lethality is a self-limiting trait in the wild.
All genetically modified moths will be reared in insectaries at Cornell University, Geneva NY. The facilities and their general operation have been inspected and approved through a previous importation permit (12-227-102m). Larval rearing will be conducted in quarantine using the same approved procedures as in this previous permit. Only moths homozygous for the conditional lethal transgene, reared off tetracycline, will be released. Adult moths will be transported in sealed containers, with at least two layers of containment, labeled as follows "CORNELL UNIVERSITY GENETICALLY MODIFIED MOTHS FOR RELEASE AT CORNELL UNIVERSITYS NEW YORK STATE AGRICULTURAL EXPERIMENT STATION - AUTHORIZED PERSONNEL ONLY". Insects will be transported by hand or in a vehicle; for each batch the number of containers and identity of member of staff supervising the release will be recorded.

The transgenic diamondback moths encode no toxic or allergen proteins. The DsRed2 marker protein has been evaluated in a New Protein Consultation by the FDA-CFSAN in the USA for human safety, and they raised no objections to its use in corn plants. This involved an assessment of the amino acid sequence using bioinformatics analyses in accordance with the Guidance provided by Codex (2003), the lability of the protein in simulated gastric fluid (SGF) and an examination of the gene source and history of exposure, as well as the toxicity of the protein using bioinformatics analysis. The amino acid sequence in OX4319L-Pxy is the same as that evaluated in the NPC. It has been further evaluated in an Environmental Assessment (EA) by the USDA (http://www.aphis.usda.gov/brs/aphisdocs/08_33801p_dpra.pdf), which concluded that the corn transformation event that contained the DsRed2 gene was unlikely to become a plant pest risk. Additional EAs on another GE moth, GE pink bollworm, expressing fluorescent genes similar to DsRed2 have also been conducted (http://www.gpo.gov/fdsys/pkg/FR-2006-04-19/html/E6-5878.htm) and concluded that it was unlikely to present any hazard to the environment.
The other protein coding region, tTAV, is regulated by sequences from the sex-determination gene, doublesex, from pink bollworm (Pectinophora gossypiella), that produce different splice variants in males and females: the female transcript comprises coding sequence for the tetracycline-repressible transcription factor, tTAV, which interacts with the upstream tetracycline response element, tetO (or tRE), to form a positive-feedback loop that results in insect lethality prior to adulthood. Under the control of the doublesex sex-alternate splicing, lethality is induced only in females. The tTAV amino acid sequence in OX4319L-Pxy has also been evaluated independently using the bioinformatics analyses provided by Codex (2003) for both potential allergenicity and toxicity. No homologies with known allergens or toxins were determined. This study is available on request.
Tetracycline can be provided to the insect in larval artificial diet to suppress female death and permit colony rearing in the laboratory. Neither piggyBac transposase activity nor any antibiotic resistance is conferred to the transgenic diamondback moth by the introduced genetic material.
This female-specific lethal trait was previously discussed in a USDA Environmental Impact Statement published in October 2008, entitled Use of Genetically Engineered Fruit Fly and Pink Bollworm in APHIS Plant Pest Control Programs, which concluded that the use of genetically engineered fruit flies and pink bollworm in APHIS plant pest control programs were the environmentally preferred alternative (Record of Decision (Federal Register Vol 74 (87) 21314 2009).
Reference:
Jin L, et al. 2013 Engineered female-specific lethality for control of pest lepidoptera. ACS Synthetic Biology, 2:160-166.

10. ARTICLE SUPPLIER AND/OR DEVELOPER

Name	Location	Contact Information
Dr. (b)(6)	Oxitec Ltd 71 Milton Park Abingdon OX144RX United Kingdom **County:** Oxford	**Day Telephone:** 0044-1235-832393 **FAX:** **Email:** (b)(6)

11. PHENOTYPES/GENOTYPE

1) Phenotypic Designation Name: visual marker; repressible lethality

Identifying Line(s): OX4319L-Pxy, OX4319N-Pxy, OX4767A-Pxy

Construct(s): OX4319, OX4767

Mode of Transformation: Direct Injection

Phenotype Description:
A description of the anticipated or actual expression of the altered genetic material in the regulated article and how that expression differs from the expression in the non-modified parental organism.

The introduced genetic material in the diamondback moth comprises three protein coding regions, one for marking the insects and two for inducing death before the insect reaches adulthood (in this instance, females only). The former allows the expression of a DsRed2 fluorescent protein originally derived from a coral (Discosoma sp.). The transgenic diamondback moth with the marker gene fluoresces when excited by illumination of the appropriate wavelength. These fluorescent proteins, which have been used as markers in a wide range of vertebrate and invertebrate species, confer no known competitive advantage or disadvantage to the recipient, and no ecological or other consequences resulting from incorporation of these markers into the transgenic diamondback moth can be envisioned. The non-modified diamondback moth has no fluorescent protein gene; therefore, it does not fluoresce when illuminated under the same light frequency. Neither piggyBac transposase activity nor any antibiotic resistance is conferred to the transgenic diamondback moth by the introduced genetic material.

Phenotype(s)

MG - Visual marker; DsRed2 Fluorescent Protein Expression

Genotype(s)

Screenable Marker
Gene: DsRed2 **from** Discosoma sp. - Screenable marker gene DsRed2 from Discosoma spp - Allows the expression of a fluorescent protein from Discosoma spp. Fluorescent protein of the GFP superfamily (DsRed2) under the control of a hr5ie1 promoter/enhancer sequence, which is from Autographa californica nuclear polyhedrosis virus (AcMNPV). A transgenic diamondback moth with the marker gene will fluoresce when excited by intense illumination of the appropriate wavelength. These fluorescent proteins, which have been used as markers in a wide range of vertebrate and invertebrate species, confer no known competitive advantage or disadvantage to the recipient, and no adverse ecological effect or other consequences resulting from incorporation of these markers into the transgenic diamondback moth are envisioned. Expression of a fluorescent protein will therefore permit released modified moths to be distinguished from unmodified.

Vector Sequence: piggyBac (non-autonomous) **from** piggyBac from Trichoplusia ni (moth) - Transformation Vector from Trichoplusia ni (moth) - Effects germline transformation of diamondback moth from piggyBac from Trichoplusia ni (moth) - 3' 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. In the construct used for transformation of the pink bollworm, the transposase gene of the piggyBac element was irreversibly destroyed by insertion of the transgene. 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 melanogaster 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.

Repressible lethality
Gene: tTAV **from** Escherichia coli (bacterium) and Herpes simplex (virus) - Tetracycline-repressible transcriptional activator from tTAV is a synthetic fusion of the tetR protein from Escherichia coli with VP16 from a type 1 herpes simplex virus. The tTA protein binds to and activates expression from the tetracycline response element (tRE), which includes multiple copies of the specific DNA sequence to which tTA binds (tetO) (Gossen et al., 1994; Gossen & Bujard, 1992). tTAV also binds tetracyclines with high affinity; the tetracycline-bound form of tTAV does not bind DNA. tTAV therefore acts as a tetracycline-regulated switch. In the absence of tetracycline, it will induce expression

from tRE, whereas in the presence of tetracycline it will not. High-level expression of tTAV is thought to be deleterious to cells as it can repress their normal transcription; low-level expression has no known effect other than activation of tRE (Berger, et al., 1990; Damke et al., 1995; Gillespie et al., 1997; Gong et al., 2005; Gossen and Bujard, 1992; Salghetti et al., 2001). tTAV has been used in fungi, plants, mice and Drosophila melanogaster with no known adverse effects. Unmodified Plutella xylostella do not have a tTAV activity.

Regulatory sequence: doublesex genomic region **from** Pink bollworm, Pectinophora gossypiella - Female-specificity is conferred using truncated sex-alternate splicing sequences from the doublesex gene of Pectinophora gossypiella. Sequence encoding tTAV is inserted into this splicing sequence, allowing for the expression of tTAV in a sex-specific manner, resulting in a conditional female-lethal system (Jin et al. 2013).

A full list of construct components is provided in the attached Table of genetic elements.

References:
Berger SL, et al. 1990 Selective inhibition of activated but not basal transcription by the acidic activation domain of VP16: evidence for transcriptional adaptors. Cell 61, 1199-1208.
Damke H, et al. 1995 Tightly regulated and inducible expression of dominant interfering dynamin mutant in stably transformed HeLa cells. Meth Enzymol 257, 209-220.
Gillespie JP, et al. 1997 Biological mediators of insect immunity. Annu Rev Entomol 42, 611-643.
Gong P, et al. 2005 A dominant lethal genetic system for autocidal control of the Mediterranean fruitfly. Nat Biotechnol 23, 453-456.
Gossen M, et al. 1994 Inducible gene expression systems for higher eukaryotic cells. Curr Opin Biotechnol 5, 516-520.
Gossen M, and Bujard H 1992 Tight control of gene expression in mammalian cells by tetracycline- responsive promoters. Proc Natl Acad Sci USA 89, 5547-5551.
Salghetti S, et al. 2001 Regulation of transcriptional activation domain function by ubiquitin. Science 293, 1651-1653.
Jin L, et al. 2013 Engineered female-specific lethality for control of pest Lepidoptera. ACS Synthetic Biology, 2:160-166.

12. INTRODUCTION

Release Site

Location Name & Description	Location Address	Contact(s)
1) Research Farm North	NY **County:** Ontario **Proposed Release Start Date:** 4/1/2014 **Proposed Release End Date:** 3/31/2017 **No. of Releases:** up to 72/year **Quantity:** up to 100,000 moths/wk; 10 acres acres	

Location Unique ID: RFN1097

Location GPS Coordinates: (b)(4)

Release Site History: Managed agricultural, cropping, research. Managed agricultural land around release site.

Critical Habitat Involved?: ___ Yes _X_ No

13. DESIGN PROTOCOLS

Production Design

A detailed description of the purpose for the introduction of the regulated article including detailed description of the proposed experimental and/or production design:

The diamondback moth strains OX4319L-Pxy, OX4319N-Pxy and OX4767A-Pxy show a tetracycline-repressible female-lethal phenotype, which could serve as an insecticide-free means of controlling pest populations of diamondback moth in the field in a species-specific manner. Successful pest control will rely upon strong performance of released males, in terms of female-seeking behavior and mating competitiveness. We will seek to measure relevant performance traits in one or more mark-release-recapture field experiments. These will be followed by pest suppression trials, in which Plutella xylostella-infested fields will be treated with fsRIDL male Plutella xylostella, and the wild populations monitored and compared with those of fields not so treated.

In the mark-release-recapture experiments, we will release up to 20,000 male fsRIDL Plutella xylostella (per release; up to 100,000 males per week) from single or multiple points in experimental fields of up to 10 acres planted with brassicas (e.g. cabbage or broccoli). Traps (e.g. sticky traps baited with synthetic sex pheromone) will be placed in and around the field, up to 1 km from the release point, to recapture the male moths. Traps will be collected at least once per week and the recaptured moths screened for the fluorescence marker. Additional PCR screening will be conducted to validate this visual screening. Trapping will continue until no fsRIDL male moths are recaptured for 2 consecutive weeks. To permit an overlapping series of releases in each experimental field that can be independently monitored on the traps, fsRIDL male moths will be sometimes be additionally marked, for example using different-colored fluorescent powders, which are commonly used in such field experiments with insects (reviewed by Eagler & Jackson 2001 Ann Rev Entomol 46:511-543). Crop sampling, in which a proportion of the in-field plants will be collected and closely examined in the laboratory for Plutella xylostella larvae and pupae (wild-type and transgenic), will be conducted at regular intervals to assess mating success of the fsRIDL males. Each experimental field will be surrounded by an approximately 10m-deep border free of potential host plants. Upon completion of the experiment, the insecticide Coragen (chlorantraniliprole) will be sprayed on the plants and surrounding area to kill remaining diamondback moth larvae.
Data from these preliminary field experiments, indicating the fsRIDL male release rate required to achieve a given overflooding rate (b)(4) dispersal and field longevity, will inform the release st ack moths in a suppression trial (how many fsRIDL males to release, how frequently and from how many points), requested as part of this permit application. All of the described trials will require monitoring of a wild diamondback moth population in up to six experimental fields (up to three treated with fsRIDL male moths, and up to three untreated). These fields, of up to 10 acres in size, will be planted with brassica plants (e.g. cabbage or broccoli). If the wild diamondback moth population is not present in sufficient numbers at the trial sites, the experimental fields 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 GM moths. A proportion of the experimental fields will be subjected to regular releases (b)(4) of fsRIDL male moths, in numbers greater than the estimated recruitm e moths in the environment, to achieve an over-flooding effect of fsRIDL males on the wild male diamondback moth population. For each experiment, fsRIDL male releases will be conducted for up to the duration of a brassica crop cycle (anticipated as 3-4 months). Adult traps (e.g. sticky traps baited with synthetic sex pheromone) will be placed in and around each field to monitor the relative numbers of wild-type and fsRIDL males present, and to assess their dispersal. The populations of wild-type moths in each field, including those receiving no fsRIDL males, will be monitored using the adult traps described and periodic crop sampling. Releases will consist of up to 100,000 fsRIDL male moths per week (depending on the overflooding ratio required) over the treatment fields over the course of these suppression experiments. Trapping will continue after the last releases of fsRIDL male moths, and will continue until at least 2 weeks of zero fsRIDL recaptures. Upon completion of the experiment, the insecticide Coragen (chlorantraniliprole) will be sprayed on the plants and surrounding area (within 100 m radius of treated fields) to kill remaining diamondback moth larvae. Post-experiment pheromone trapping will continue for 2 weeks to monitor field longevity of fsRIDL moths.

Destination or Release Description

A detailed description of the intended destination (including final and all intermediate destinations), uses, and/or distribution of the regulated article (e.g., greenhouses, laboratory, or growth chamber location; field trial location, pilot project location; production, propagation, and manufacture location; proposed sale and distribution location):

All genetically modified male diamondback moth used in the trials will be reared as larvae on non-tetracycline artificial diet. Releases will be conducted from the ground or vehicle on Cornell University New York State Agricultural Research Station. Releases will be conducted (b (b)(4) , depending on experimental requirements. lease sites (up to 1000 m radius from release site) will be monitored with traps (e.g. sticky traps baited with synthetic sex pheromone). Traps will be collected at least weekly to count the number of genetically modified moths and wild moths captured on each trap. Samples in the laboratory will be screened for presence of the DsRed2 fluorescent marker, using fluorescence microscopy, and this will be validated by PCR detection of the DNA construct in

selected samples. Some non-viable insect samples will be sent to Oxitecs labs in the UK for the PCR analysis. Prior to each field release, samples from each cohort of male fsRIDL moths will be screened for the fluorescent marker and sexed. Only male moths will be released; the effect on the crop will therefore likely be negligible: male activity is restricted to finding and mating females, feeding on nectar from flowers, and taking shelter during the day.

Confinement Protocols

A detailed description of the proposed procedures, processes, and safeguards which will be used to prevent escape and dissemination of the regulated article at each of the intended destinations:

Adult genetically modified moths will be transported in sealed containers labeled as follows "CORNELL UNIVERSITY GENETICALLY MODIFIED MOTHS FOR RELEASE AT CORNELL UNIVERSITYS NEW YORK STATE AGRICULTURAL EXPERIMENT STATION - AUTHORIZED PERSONNEL ONLY". Insects will be transported by hand or in a vehicle by authorized personnel.
The conditional lethality expressed by the fsRIDL construct means that female progeny from matings with Oxitec male insects die in the absence of tetracycline, and the trait is therefore unlikely to persist in the environment. Other mitigation measures include the lack of known sexually compatible relatives of Plutella xylostella in the USA; the piggyBac transposable element used for the transformation has no endogenous functioning transposase, rendering it non-autonomous (it cannot mobilize itself); the release area will be monitored extensively with traps to attract and collect Plutella xylostella moths; release fields are no larger than 10 acres; the Plutella xylostella can be sprayed with insecticide at any time in the case of observed adverse events; the genetically engineered Plutella xylostella will be securely managed and contained in production and transport; and all viable insects reared for this trial that are not required for release or additional analysis will be devitalized by freezing.

Final Disposition Method: ☒ Destruction/Devitalization ☐ Other ☐ Storage in Contained Facility

Final Disposition Description: All unused genetically modified eggs, larvae, pupae and moths not released, or not needed in the mass-rearing, will be frozen at a minimum of -15°C ± 5°C for 48 h to destroy all life stages.

14. ATTACHMENTS

Attachments
BRS Importation permit (Exp.9/2013) (10/18/2013 @ 10:05 AM)
Cornell University proposed field release site (10/15/2013 @ 10:51 AM)
OX4319L allele persistence report (10/17/2013 @ 09:29 AM)
OX4319L chlortetracycline sensitivity report (10/17/2013 @ 09:22 AM)
OX4319L construct sequencing report (10/17/2013 @ 09:20 AM)
OX4319L molecular characterisation report (10/17/2013 @ 09:21 AM)
OX4319L population suppression cages report (10/17/2013 @ 09:23 AM)
OX4319L resistance management report (10/17/2013 @ 09:24 AM)
Peer-reviewed publication (Jin et al. 2013) describing development/testing of RIDL DBM (9/26/2013 @ 09:51 AM)
Table of genetic elements, OX4319 and OX4767 (10/15/2013 @ 10:50 AM)
Threatened or endangered species (10/15/2013 @ 10:52 AM)
tTAV expression levels report (10/17/2013 @ 09:21 AM)

15. ADDITIONAL INFORMATION

16. COURTESY JUSTIFICATION

I, *Anthony Shelton*, hereby certify that the information in this application and all attachments is complete and accurate to the best of my knowledge and belief.

I acknowledge this is not an application to move or import select agents, the genes expressing select agents, or the toxins made by the select agents, as described in 9 CFR 121.

I will not introduce the regulated articles described in this application until APHIS has deemed the application complete and has granted the permit. By signing this permit, I agree to comply with any and all state, local, and tribal laws and regulations that may apply to the introduction of the articles described in this applications.

If there are any changes to the information disclosed in this application, I will contact APHIS.

17. SIGNATURE OF RESPONSIBLE PERSON	**18. DATE**
Anthony Shelton	October 24, 2013

The collection of this information is authorized by the Plant Protection Act of 2000. The information will be used to determine eligibility to receive all types of permits. No permit will be issued until this application has been approved.

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
BIOTECHNOLOGY REGULATORY SERVICE

APPLICATIONS FOR PERMIT OR COURTESY PERMIT UNDER 7 CFR 340

(Genetically Engineered Organisms or Products)

1. NAME, ADDRESS, TELEPHONE, AND EMAIL OF APPLICANT

Name: Dr. Anthony Shelton
Position:
Organization: Cornell University/NYSAES
Organization Unique ID:
Address: 630 W. North St.
(b)(6)
Geneva, NY 14456
County/Province:
Township/Island:
Day Telephone: (b)(6)
FAX:
Alternate:
Email 1: (b)(6)
Email 2:

2. INTRODUCTION TYPE

- [ ] Importation
- [ ] Interstate Movement
- [ ] Interstate Movement and Release
- [x] Release

3. PERMIT TYPE

- [x] Standard Permit
- [ ] Courtesy Permit

4. PURPOSE OF PERMIT

- [ ] Industrial Product
- [ ] Pharmaceutical Product
- [ ] Phytoremediation
- [x] Traditional

5. CONFIDENTIAL BUSINESS INFORMATION VERIFICATION (CBI)

Does this application contain CBI? [ ] Yes [x] No
CBI Justification:
N/A

6. REQUEST TYPE

[x] **New** [ ] **Amendment** [ ] **Renewal**

Amendment/Renewal Description:
Previous Permit Number(s):

7. MEANS OF MOVEMENT

Import by air; releases manually from the ground/vehicles.

8. VARIANCE VERIFICATION

Have you previously applied for variance(s) that you wish to apply to this permit? [ ] Yes [x] No
Variance Number(s):

If so, describe in a brief summary how the variance will be applied:
N/A

9. REGULATED ARTICLE

Scientific Name: Plutella xylostella

Common Name: Diamondback moth

Cultivar and/or Breeding Line:

Any biological material (e.g., culture medium, or host material) accompanying the regulated Article during movement:
Artificial insect diet. This diet will be frozen at -15°C for 12 h prior to import.

Country and locality where the donor organism, recipient organism, and vector or vector agent were collected, developed, and produced:
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Processes, Procedures, and Safeguards Description:

WARNING: Any use of ePermits to make materially false, fictitious, or fraudulent statements or representations is subject to civil penalties of up to $250,000 (7 U.S.C. § 7734(b)) or punishable by a fine of not more than $10,000, or imprisonment of not more than 5 years, or both (18 U.S.C. §1001).

This is a permit request for 3 years of seasonal releases (April to October) of a female-lethal, genetically marked diamondback moth (maximum 100,000 moths/week), in brassica fields at the Cornell University research station, Geneva NY.
Males of the transgenic moths will be released in cultivated brassica plots and biological parameters of these moths, such as dispersal and persistence, measured using traps, for example baited with synthetic sex pheromone. The moths of the OX4319L-Pxy strain carry a stable, heritable marker the DsRed2 fluorescent protein, viewed by fluorescence microscope or detected by PCR and their female progeny die in the absence of a dietary repressor (tetracycline or suitable analogues supplied in their artificial diet). The male-selecting (female-lethal) penetrance of the strain is >99% (Jin et al. 2013). The marker provides a means of distinguishing released moths from wild moths, and female-lethality is a self-limiting trait in the wild.
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Name	Location	Contact Information
Dr. (b)(6)	Oxitec Ltd 71 Milton Park Abingdon OX144RX United Kingdom **County:** Oxford	**Day Telephone:** 0044-1235-832393 **FAX:** **Email:** (b)(6)

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1) Phenotypic Designation Name: visual marker; repressible lethality

Identifying Line(s): OX4319L-Pxy, OX4319N-Pxy, OX4767A-Pxy

Construct(s): OX4319, OX4767

Mode of Transformation: Direct Injection

Phenotype Description:
A description of the anticipated or actual expression of the altered genetic material in the regulated article and how that expression differs from the expression in the non-modified parental organism.

The introduced genetic material in the diamondback moth comprises three protein coding regions, one for marking the insects and two for inducing death before the insect reaches adulthood (in this instance, females only). The former allows the expression of a DsRed2 fluorescent protein originally derived from a coral (Discosoma sp.). The transgenic diamondback moth with the marker gene fluoresces when excited by illumination of the appropriate wavelength. These fluorescent proteins, which have been used as markers in a wide range of vertebrate and invertebrate species, confer no known competitive advantage or disadvantage to the recipient, and no ecological or other consequences resulting from incorporation of these markers into the transgenic diamondback moth can be envisioned. The non-modified diamondback moth has no fluorescent protein gene; therefore, it does not fluoresce when illuminated under the same light frequency. Neither piggyBac transposase activity nor any antibiotic resistance is conferred to the transgenic diamondback moth by the introduced genetic material.

Phenotype(s)

MG - Visual marker; DsRed2 Fluorescent Protein Expression

Genotype(s)

Screenable Marker
Gene: DsRed2 **from** Discosoma sp. - Screenable marker gene DsRed2 from Discosoma spp - Allows the expression of a fluorescent protein from Discosoma spp. Fluorescent protein of the GFP superfamily (DsRed2) under the control of a hr5ie1 promoter/enhancer sequence, which is from Autographa californica nuclear polyhedrosis virus (AcMNPV). A transgenic diamondback moth with the marker gene will fluoresce when excited by intense illumination of the appropriate wavelength. These fluorescent proteins, which have been used as markers in a wide range of vertebrate and invertebrate species, confer no known competitive advantage or disadvantage to the recipient, and no adverse ecological effect or other consequences resulting from incorporation of these markers into the transgenic diamondback moth are envisioned. Expression of a fluorescent protein will therefore permit released modified moths to be distinguished from unmodified.

Vector Sequence: piggyBac (non-autonomous) **from** piggyBac from Trichoplusia ni (moth) - Transformation Vector from Trichoplusia ni (moth) - Effects germline transformation of diamondback moth from piggyBac from Trichoplusia ni (moth) - 3' 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. In the construct used for transformation of the pink bollworm, the transposase gene of the piggyBac element was irreversibly destroyed by insertion of the transgene. 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 melanogaster 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.

Repressible lethality
Gene: tTAV **from** Escherichia coli (bacterium) and Herpes simplex (virus) - Tetracycline-repressible transcriptional activator from tTAV is a synthetic fusion of the tetR protein from Escherichia coli with VP16 from a type 1 herpes simplex virus. The tTA protein binds to and activates expression from the tetracycline response element (tRE), which includes multiple copies of the specific DNA sequence to which tTA binds (tetO) (Gossen et al., 1994; Gossen & Bujard, 1992). tTAV also binds tetracyclines with high affinity; the tetracycline-bound form of tTAV does not bind DNA. tTAV therefore acts as a tetracycline-regulated switch. In the absence of tetracycline, it will induce expression

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Regulatory sequence: doublesex genomic region **from** Pink bollworm, Pectinophora gossypiella - Female-specificity is conferred using truncated sex-alternate splicing sequences from the doublesex gene of Pectinophora gossypiella. Sequence encoding tTAV is inserted into this splicing sequence, allowing for the expression of tTAV in a sex-specific manner, resulting in a conditional female-lethal system (Jin et al. 2013).

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12. INTRODUCTION

Release Site

Location Name & Description	Location Address	Contact(s)
1) Research Farm North	NY **County:** Ontario **Proposed Release Start Date:** 4/1/2014 **Proposed Release End Date:** 3/31/2017 **No. of Releases:** up to 72/year **Quantity:** up to 100,000 moths/wk; 10 acres acres	

Location Unique ID: RFN1097

Location GPS Coordinates: (b)(4)

Release Site History: Managed agricultural, cropping, research. Managed agricultural land around release site.

Critical Habitat Involved?: ___ Yes _X_ No

13. DESIGN PROTOCOLS

Production Design

A detailed description of the purpose for the introduction of the regulated article including detailed description of the proposed experimental and/or production design:

The diamondback moth strains OX4319L-Pxy, OX4319N-Pxy and OX4767A-Pxy show a tetracycline-repressible female-lethal phenotype, which could serve as an insecticide-free means

of controlling pest populations of diamondback moth in the field in a species-specific manner. Successful pest control will rely upon strong performance of released males, in terms of female-seeking behavior and mating competitiveness. We will seek to measure relevant performance traits in one or more mark-release-recapture field experiments. These will be followed by pest suppression trials, in which Plutella xylostella-infested fields will be treated with fsRIDL male Plutella xylostella, and the wild populations monitored and compared with those of fields not so treated.

In the mark-release-recapture experiments, we will release up to 20,000 male fsRIDL Plutella xylostella (per release; up to 100,000 males per week) from single or multiple points in experimental fields of up to 10 acres planted with brassicas (e.g. cabbage or broccoli). Traps (e.g. sticky traps baited with synthetic sex pheromone) will be placed in and around the field, up to 1 km from the release point, to recapture the male moths. Traps will be collected at least once per week and the recaptured moths screened for the fluorescence marker. Additional PCR screening will be conducted to validate this visual screening. Trapping will continue until no fsRIDL male moths are recaptured for 2 consecutive weeks. To permit an overlapping series of releases in each experimental field that can be independently monitored on the traps, fsRIDL male moths will be sometimes be additionally marked, for example using different-colored fluorescent powders, which are commonly used in such field experiments with insects (reviewed by Hagler & Jackson 2001 Ann Rev Entomol 46:511-543). Crop sampling, in which a proportion of the in-field plants will be collected and closely examined in the laboratory for Plutella xylostella larvae and pupae (wild-type and transgenic), will be conducted at regular intervals to assess mating success of the fsRIDL males. Each experimental field will be surrounded by an approximately 10m-deep border free of potential host plants. Upon completion of the experiment, the insecticide Coragen (chlorantraniliprole) will be sprayed on the plants and surrounding area to kill remaining diamondback moth larvae.
Data from these preliminary field experiments, indicating the fsRIDL male release rate required to achieve a given overflooding rate (e.g. (b)(4) dispersal and field longevity, will inform the release strategy ack moths in a suppression trial (how many fsRIDL males to release, how frequently and from how many points), requested as part of this permit application. All of the described trials will require monitoring of a wild diamondback moth population in up to six experimental fields (up to three treated with fsRIDL male moths, and up to three untreated). These fields, of up to 10 acres in size, will be planted with brassica plants (e.g. cabbage or broccoli). If the wild diamondback moth population is not present in sufficient numbers at the trial sites, the experimental fields 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 GM moths. A proportion of the experimental fields will be subjected to regular releases ((b)(4)) of fsRIDL male moths, in numbers greater than the estimated recruitme le moths in the environment, to achieve an over-flooding effect of fsRIDL males on the wild male diamondback moth population. For each experiment, fsRIDL male releases will be conducted for up to the duration of a brassica crop cycle (anticipated as 3-4 months). Adult traps (e.g. sticky traps baited with synthetic sex pheromone) will be placed in and around each field to monitor the relative numbers of wild-type and fsRIDL males present, and to assess their dispersal. The populations of wild-type moths in each field, including those receiving no fsRIDL males, will be monitored using the adult traps described and periodic crop sampling. Releases will consist of up to 100,000 fsRIDL male moths per week (depending on the overflooding ratio required) over the treatment fields over the course of these suppression experiments. Trapping will continue after the last releases of fsRIDL male moths, and will continue until at least 2 weeks of zero fsRIDL recaptures. Upon completion of the experiment, the insecticide Coragen (chlorantraniliprole) will be sprayed on the plants and surrounding area (within 100 m radius of treated fields) to kill remaining diamondback moth larvae. Post-experiment pheromone trapping will continue for 2 weeks to monitor field longevity of fsRIDL moths.

Destination or Release Description

A detailed description of the intended destination (including final and all intermediate destinations), uses, and/or distribution of the regulated article (e.g., greenhouses, laboratory, or growth chamber location; field trial location, pilot project location; production, propagation, and manufacture location; proposed sale and distribution location):

All genetically modified male diamondback moth used in the trials will be reared as larvae on non-tetracycline artificial diet. Releases will be conducted from the ground or vehicle on Cornell University's New York State Agricultural Research Station. Releases will be conducted (b)(4) (b)(4) depending on experimental requirements.
ase sites (up to 1000 m radius from release site) will be monitored with traps (e.g. sticky traps baited with synthetic sex pheromone). Traps will be collected at least weekly to count the number of genetically modified moths and wild moths captured on each trap. Samples in the laboratory will be screened for presence of the DsRed2 fluorescent marker, using fluorescence microscopy, and this will be validated by PCR detection of the DNA construct in selected samples. Some non-viable insect samples will be sent to Oxitec's labs in the UK for the PCR analysis. Prior to each field release, samples from each cohort of male fsRIDL moths will be screened for the fluorescent marker and sexed. Only male moths will be released; the effect on the crop will therefore likely be negligible: male activity is restricted to finding and mating females, feeding on nectar from flowers, and taking shelter during the day.

Confinement Protocols

A detailed description of the proposed procedures, processes, and safeguards which will be used to prevent escape and dissemination of the regulated article at each of the intended destinations:

Adult genetically modified moths will be transported in sealed containers labeled as follows "CORNELL UNIVERSITY GENETICALLY MODIFIED MOTES FOR RELEASE AT CORNELL UNIVERSITYS NEW YORK STATE AGRICULTURAL EXPERIMENT STATION - AUTHORIZED PERSONNEL ONLY". Insects will be transported by hand or in a vehicle by authorized personnel.
The conditional lethality expressed by the fsRIDL construct means that female progeny from matings with Oxitec male insects die in the absence of tetracycline, and the trait is therefore unlikely to persist in the environment. Other mitigation measures include the lack of known sexually compatible relatives of Plutella xylostella in the USA; the piggyBac transposable element used for the transformation has no endogenous functioning transposase, rendering it non-autonomous (it cannot mobilize itself); the release area will be monitored extensively with traps to attract and collect Plutella xylostella moths; release fields are no larger than 10 acres; the Plutella xylostella can be sprayed with insecticide at any time in the case of observed adverse events; the genetically engineered Plutella xylostella will be securely managed and contained in production and transport; and all viable insects reared for this trial that are not required for release or additional analysis will be devitalized by freezing.

Final Disposition Method: ☒ Destruction/Devitalization ☐ Other ☐ Storage in Contained Facility

Final Disposition Description: All unused genetically modified eggs, larvae, pupae and moths not released, or not needed in the mass-rearing, will be frozen at a minimum of -15°C + 5°C for 48 h to destroy all life stages.

14. ATTACHMENTS

Attachments
BRS Importation permit (Exp.9/2013) (10/18/2013 @ 10:05 AM)
Cornell University proposed field release site (10/15/2013 @ 10:51 AM)
OX4319L allele persistence report (10/17/2013 @ 09:23 AM)
OX4319L chlortetracycline sensitivity report (10/17/2013 @ 09:22 AM)
OX4319L construct sequencing report (10/17/2013 @ 09:20 AM)
OX4319L molecular characterisation report (10/17/2013 @ 09:21 AM)
OX4319L population suppression cages report (10/17/2013 @ 09:23 AM)
OX4319L resistance management report (10/17/2013 @ 09:24 AM)
Peer-reviewed publication (Jin et al. 2013) describing development/testing of RIDL DBM (9/26/2013 @ 09:51 AM)
Table of genetic elements, OX4319 and OX4767 (10/15/2013 @ 10:50 AM)
Threatened or endangered species (10/15/2013 @ 10:52 AM)
tTAV expression levels report (10/17/2013 @ 09:21 AM)

15. ADDITIONAL INFORMATION

16. COURTESY JUSTIFICATION

I, *Anthony Shelton*, hereby certify that the information in this application and all attachments is complete and accurate to the best of my knowledge and belief.

I acknowledge this is not an application to move or import select agents, the genes expressing select agents, or the toxins made by the select agents, as described in 9 CFR 121.

I will not introduce the regulated articles described in this application until APHIS has deemed the application complete and has granted the permit. By signing this permit, I agree to comply with any and all state, local, and tribal laws and regulations that may apply to the introduction of the articles described in this applications.

If there are any changes to the information disclosed in this application, I will contact APHIS.

17. SIGNATURE OF RESPONSIBLE PERSON	**18. DATE**
Anthony Shelton	October 24, 2013

The collection of this information is authorized by the Plant Protection Act of 2000. The information will be used to determine eligibility to receive all types of permits. No permit will be issued until this application has been approved.

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
BIOTECHNOLOGY REGULATORY SERVICE

APPLICATIONS FOR PERMIT OR COURTESY PERMIT UNDER 7 CFR 340

(Genetically Engineered Organisms or Products)

1. NAME, ADDRESS, TELEPHONE, AND EMAIL OF APPLICANT

Name: Dr. Anthony Shelton
Position:
Organization: Cornell University/NYSAES
Organization Unique ID:
Address: 630 W. North St.
(b)(6)
Geneva, NY 14456
County/Province:
Township/Island:
Day Telephone: (b)(6)
FAX:
Alternate:
Email 1: (b)(6)
Email 2:

2. INTRODUCTION TYPE

- [ ] Importation
- [ ] Interstate Movement
- [ ] Interstate Movement and Release
- [x] Release

3. PERMIT TYPE

- [x] Standard Permit
- [ ] Courtesy Permit

4. PURPOSE OF PERMIT

- [ ] Industrial Product
- [ ] Pharmaceutical Product
- [ ] Phytoremediation
- [x] Traditional

5. CONFIDENTIAL BUSINESS INFORMATION VERIFICATION (CBI)

Does this application contain CBI? [ ] Yes [x] No
CBI Justification:
N/A

6. REQUEST TYPE

[x] **New** [ ] **Amendment** [ ] **Renewal**

Amendment/Renewal Description:
Previous Permit Number(s):

7. MEANS OF MOVEMENT

Import by air; releases manually from the ground/vehicles.

8. VARIANCE VERIFICATION

Have you previously applied for variance(s) that you wish to apply to this permit? [ ] Yes [x] No
Variance Number(s):

If so, describe in a brief summary how the variance will be applied:
N/A

9. REGULATED ARTICLE

Scientific Name: Plutella xylostella

Common Name: Diamondback moth

Cultivar and/or Breeding Line:

Any biological material (e.g., culture medium, or host material) accompanying the regulated Article during movement:
Artificial insect diet. This diet will be frozen at -15°C for 12 h prior to import.

Country and locality where the donor organism, recipient organism, and vector or vector agent were collected, developed, and produced:
All final engineering of the transforming constructs was performed at Oxitec Ltd, in the United Kingdom.
The genes used from the donor organisms and the piggyBac-derived portions of the vectors used to build the transforming construct were cloned off-site. The recipient organism is the moth, Plutella xylostella, which is endemic in temperate regions around the world, including the USA. The recipient Plutella xylostella strain for the transformation was a wild-type strain obtained from Syngenta (public limited company), UK, which has been reared in Oxitec insectaries since 2008.

Processes, Procedures, and Safeguards Description:

This is a permit request for 3 years of seasonal releases (April to October) of a female-lethal, genetically marked diamondback moth (maximum 100,000 moths/week), in brassica fields at the Cornell University research station, Geneva NY.
Males of the transgenic moths will be released in cultivated brassica plots and biological parameters of these moths, such as dispersal and persistence, measured using traps, for example baited with synthetic sex pheromone. The moths of the OX4319L-Pxy strain carry a stable, heritable marker the DsRed2 fluorescent protein, viewed by fluorescence microscope or detected by PCR and their female progeny die in the absence of a dietary repressor (tetracycline or suitable analogues supplied in their artificial diet). The male-selecting (female-lethal) penetrance of the strain is >99% (Jin et al. 2013). The marker provides a means of distinguishing released moths from wild moths, and female-lethality is a self-limiting trait in the wild.
All genetically modified moths will be reared in insectaries at Cornell University, Geneva NY. The facilities and their general operation have been inspected and approved through a previous importation permit (12-227-102m). Larval rearing will be conducted in quarantine using the same approved procedures as in this previous permit. Only moths homozygous for the conditional lethal transgene, reared off tetracycline, will be released. Adult moths will be transported in sealed containers, with at least two layers of containment, labeled as follows "CORNELL UNIVERSITY GENETICALLY MODIFIED MOTHS FOR RELEASE AT CORNELL UNIVERSITYS NEW YORK STATE AGRICULTURAL EXPERIMENT STATION - AUTHORIZED PERSONNEL ONLY". Insects will be transported by hand or in a vehicle; for each batch the number of containers and identity of member of staff supervising the release will be recorded.

The transgenic diamondback moths encode no toxic or allergen proteins. The DsRed2 marker protein has been evaluated in a New Protein Consultation by the FDA-CFSAN in the USA for human safety, and they raised no objections to its use in corn plants. This involved an assessment of the amino acid sequence using bioinformatics analyses in accordance with the Guidance provided by Codex (2003), the lability of the protein in simulated gastric fluid (SGF) and an examination of the gene source and history of exposure, as well as the toxicity of the protein using bioinformatics analysis. The amino acid sequence in OX4319L-Pxy is the same as that evaluated in the NPC. It has been further evaluated in an Environmental Assessment (EA) by the USDA (http://www.aphis.usda.gov/brs/aphisdocs/08_33801p_dpra.pdf), which concluded that the corn transformation event that contained the DsRed2 gene was unlikely to become a plant pest risk. Additional EAs on another GE moth, GE pink bollworm, expressing fluorescent genes similar to DsRed2 have also been conducted (http://www.gpo.gov/fdsys/pkg/FR-2006-04-19/html/E6-5878.htm) and concluded that it was unlikely to present any hazard to the environment.
The other protein coding region, tTAV, is regulated by sequences from the sex-determination gene, doublesex, from pink bollworm (Pectinophora gossypiella), that produce different splice variants in males and females: the female transcript comprises coding sequence for the tetracycline-repressible transcription factor, tTAV, which interacts with the upstream tetracycline response element, tetO (or tRE), to form a positive-feedback loop that results in insect lethality prior to adulthood. Under the control of the doublesex sex-alternate splicing, lethality is induced only in females. The tTAV amino acid sequence in OX4319L-Pxy has also been evaluated independently using the bioinformatics analyses provided by Codex (2003) for both potential allergenicity and toxicity. No homologies with known allergens or toxins were determined. This study is available on request.
Tetracycline can be provided to the insect in larval artificial diet to suppress female death and permit colony rearing in the laboratory. Neither piggyBac transposase activity nor any antibiotic resistance is conferred to the transgenic diamondback moth by the introduced genetic material.
This female-specific lethal trait was previously discussed in a USDA Environmental Impact Statement published in October 2008, entitled Use of Genetically Engineered Fruit Fly and Pink Bollworm in APHIS Plant Pest Control Programs, which concluded that the use of genetically engineered fruit flies and pink bollworm in APHIS plant pest control programs were the environmentally preferred alternative (Record of Decision (Federal Register Vol 74 (87) 21314 2009).
Reference:
Jin L, et al. 2013 Engineered female-specific lethality for control of pest lepidoptera. ACS Synthetic Biology, 2:160-166.

10. ARTICLE SUPPLIER AND/OR DEVELOPER

Name	Location	Contact Information
Dr. (b)(6)	Oxitec Ltd 71 Milton Park Abingdon OX144RX United Kingdom **County:** Oxford	**Day Telephone:** 0044-1235-832393 **FAX:** **Email:** (b)(6)

11. PHENOTYPES/GENOTYPE

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A detailed description of the purpose for the introduction of the regulated article including detailed description of the proposed experimental and/or production design:

The diamondback moth strains OX4319L-Pxy, OX4319N-Pxy and OX4767A-Pxy show a tetracycline-repressible female-lethal phenotype, which could serve as an insecticide-free means of controlling pest populations of diamondback moth in the field in a species-specific manner. Successful pest control will rely upon strong performance of released males, in terms of female-seeking behavior and mating competitiveness. We will seek to measure relevant performance traits in one or more mark-release-recapture field experiments. These will be followed by pest suppression trials, in which Plutella xylostella-infested fields will be treated with fsRIDL male Plutella xylostella, and the wild populations monitored and compared with those of fields not so treated.

In the mark-release-recapture experiments, we will release up to 20,000 male fsRIDL Plutella xylostella (per release; up to 100,000 males per week) from single or multiple points in experimental fields of up to 10 acres planted with brassicas (e.g. cabbage or broccoli). Traps (e.g. sticky traps baited with synthetic sex pheromone) will be placed in and around the field, up to 1 km from the release point, to recapture the male moths. Traps will be collected at least once per week and the recaptured moths screened for the fluorescence marker. Additional PCR screening will be conducted to validate this visual screening. Trapping will continue until no fsRIDL male moths are recaptured for 2 consecutive weeks. To permit an overlapping series of releases in each experimental field that can be independently monitored on the traps, fsRIDL male moths will be sometimes be additionally marked, for example using different-colored fluorescent powders, which are commonly used in such field experiments with insects (reviewed by Eagler & Jackson 2001 Ann Rev Entomol 46:511-543). Crop sampling, in which a proportion of the in-field plants will be collected and closely examined in the laboratory for Plutella xylostella larvae and pupae (wild-type and transgenic), will be conducted at regular intervals to assess mating success of the fsRIDL males. Each experimental field will be surrounded by an approximately 10m-deep border free of potential host plants. Upon completion of the experiment, the insecticide Coragen (chlorantraniliprole) will be sprayed on the plants and surrounding area to kill remaining diamondback moth larvae.
Data from these preliminary field experiments, indicating the fsRIDL male release rate required to achieve a given overflooding rate (e.g. (b)(4)), dispersal and field longevity, will inform the release strategy dback moths in a suppression trial (how many fsRIDL males to release, how frequently and from how many points), requested as part of this permit application. All of the described trials will require monitoring of a wild diamondback moth population in up to six experimental fields (up to three treated with fsRIDL male moths, and up to three untreated). These fields, of up to 10 acres in size, will be planted with brassica plants (e.g. cabbage or broccoli). If the wild diamondback moth population is not present in sufficient numbers at the trial sites, the experimental fields 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 GM moths. A proportion of the experimental fields will be subjected to regular releases (b)(4)) of fsRIDL male moths, in numbers greater than the estimated recruitm le moths in the environment, to achieve an over-flooding effect of fsRIDL males on the wild male diamondback moth population. For each experiment, fsRIDL male releases will be conducted for up to the duration of a brassica crop cycle (anticipated as 3-4 months). Adult traps (e.g. sticky traps baited with synthetic sex pheromone) will be placed in and around each field to monitor the relative numbers of wild-type and fsRIDL males present, and to assess their dispersal. The populations of wild-type moths in each field, including those receiving no fsRIDL males, will be monitored using the adult traps described and periodic crop sampling. Releases will consist of up to 100,000 fsRIDL male moths per week (depending on the overflooding ratio required) over the treatment fields over the course of these suppression experiments. Trapping will continue after the last releases of fsRIDL male moths, and will continue until at least 2 weeks of zero fsRIDL recaptures. Upon completion of the experiment, the insecticide Coragen (chlorantraniliprole) will be sprayed on the plants and surrounding area (within 100 m radius of treated fields) to kill remaining diamondback moth larvae. Post-experiment pheromone trapping will continue for 2 weeks to monitor field longevity of fsRIDL moths.

Destination or Release Description

A detailed description of the intended destination (including final and all intermediate destinations), uses, and/or distribution of the regulated article (e.g., greenhouses, laboratory, or growth chamber location; field trial location, pilot project location; production, propagation, and manufacture location; proposed sale and distribution location):

All genetically modified male diamondback moth used in the trials will be reared as larvae on non-tetracycline artificial diet. Releases will be conducted from the ground or vehicle on Cornell University New York State Agricultural Research Station. Releases will be conducted (b)(4) (b)(4) , depending on experimental requirements.
lease sites (up to 1000 m radius from release site) will be monitored with traps (e.g. sticky traps baited with synthetic sex pheromone). Traps will be collected at least weekly to count the number of genetically modified moths and wild moths captured on each trap. Samples in the laboratory will be screened for presence of the DsRed2 fluorescent marker, using fluorescence microscopy, and this will be validated by PCR detection of the DNA construct in

selected samples. Some non-viable insect samples will be sent to Oxitecs labs in the UK for the PCR analysis. Prior to each field release, samples from each cohort of male fsRIDL moths will be screened for the fluorescent marker and sexed. Only male moths will be released; the effect on the crop will therefore likely be negligible: male activity is restricted to finding and mating females, feeding on nectar from flowers, and taking shelter during the day.

Confinement Protocols

A detailed description of the proposed procedures, processes, and safeguards which will be used to prevent escape and dissemination of the regulated article at each of the intended destinations:

Adult genetically modified moths will be transported in sealed containers labeled as follows "CORNELL UNIVERSITY GENETICALLY MODIFIED MOTHS FOR RELEASE AT CORNELL UNIVERSITYS NEW YORK STATE AGRICULTURAL EXPERIMENT STATION - AUTHORIZED PERSONNEL ONLY". Insects will be transported by hand or in a vehicle by authorized personnel.
The conditional lethality expressed by the fsRIDL construct means that female progeny from matings with Oxitec male insects die in the absence of tetracycline, and the trait is therefore unlikely to persist in the environment. Other mitigation measures include the lack of known sexually compatible relatives of Plutella xylostella in the USA; the piggyBac transposable element used for the transformation has no endogenous functioning transposase, rendering it non-autonomous (it cannot mobilize itself); the release area will be monitored extensively with traps to attract and collect Plutella xylostella moths; release fields are no larger than 10 acres; the Plutella xylostella can be sprayed with insecticide at any time in the case of observed adverse events; the genetically engineered Plutella xylostella will be securely managed and contained in production and transport; and all viable insects reared for this trial that are not required for release or additional analysis will be devitalized by freezing.

Final Disposition Method: ☒ Destruction/Devitalization ☐ Other ☐ Storage in Contained Facility

Final Disposition Description: All unused genetically modified eggs, larvae, pupae and moths not released, or not needed in the mass-rearing, will be frozen at a minimum of -15°C ± 5°C for 48 h to destroy all life stages.

14. ATTACHMENTS

Attachments
BRS Importation permit (Exp.9/2013) (10/18/2013 @ 10:05 AM)
Cornell University proposed field release site (10/15/2013 @ 10:51 AM)
OX4319L allele persistence report (10/17/2013 @ 09:29 AM)
OX4319L chlortetracycline sensitivity report (10/17/2013 @ 09:22 AM)
OX4319L construct sequencing report (10/17/2013 @ 09:20 AM)
OX4319L molecular characterisation report (10/17/2013 @ 09:21 AM)
OX4319L population suppression cages report (10/17/2013 @ 09:23 AM)
OX4319L resistance management report (10/17/2013 @ 09:24 AM)
Peer-reviewed publication (Jin et al. 2013) describing development/testing of RIDL DBM (9/26/2013 @ 09:51 AM)
Table of genetic elements, OX4319 and OX4767 (10/15/2013 @ 10:50 AM)
Threatened or endangered species (10/15/2013 @ 10:52 AM)
tTAV expression levels report (10/17/2013 @ 09:21 AM)

15. ADDITIONAL INFORMATION

16. COURTESY JUSTIFICATION

I, *Anthony Shelton*, hereby certify that the information in this application and all attachments is complete and accurate to the best of my knowledge and belief.

I acknowledge this is not an application to move or import select agents, the genes expressing select agents, or the toxins made by the select agents, as described in 9 CFR 121.

I will not introduce the regulated articles described in this application until APHIS has deemed the application complete and has granted the permit. By signing this permit, I agree to comply with any and all state, local, and tribal laws and regulations that may apply to the introduction of the articles described in this applications.

If there are any changes to the information disclosed in this application, I will contact APHIS.

17. SIGNATURE OF RESPONSIBLE PERSON	**18. DATE**
Anthony Shelton	October 24, 2013

United States Department of Agriculture
Animal and Plant Health Inspection Service
Biotechnology Regulatory Services
4700 River Road, Unit 147
Riverdale, Maryland
20737-1236

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
BIOTECHNOLOGY REGULATORY SERVICE

PERMIT UNDER 7 CFR 340

(Genetically Engineered Organisms or Products)

This permit was generated electronically via the ePermits system

Enclosed is the BRS Permit Application

PERMITTEE NAME:	Dr. Anthony Shelton
ORGANIZATION:	Cornell University/NYSAES
ADDRESS:	630 W. North St. (b)(6) Geneva, NY 14456
PHONE:	(b)(6)
FAX:	
RELEASE:	NY

PERMIT NUMBER	13-297-102r
DATE ISSUED:	November 10, 2014
EFFECTIVE:	November 10, 2014
EXPIRES:	**November 10, 2017**
INTRODUCTION TYPE	Release
PERMIT TYPE	Standard
PURPOSE OF PERMIT	Tracitional

Under the conditions specified, this permit authorizes the following:

Regulated Article: Plutella xylostella

THIS PERMIT HAS BEEN APPROVED ELECTRONICALLY BY THE FOLLOWING BRS OFFICIAL VIA EPERMITS

SIGNATURE OF BRS OFFICIAL	DATE
John Turner	November 10, 2014

The collection of this information is authorized by the Plant Protection Act of 2000. The information will be used to determine eligibility to receive all types of permits. No permit will be issued until this application has been approved.

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
BIOTECHNOLOGY REGULATORY SERVICE

APPLICATIONS FOR PERMIT OR COURTESY PERMIT UNDER 7 CFR 340

(Genetically Engineered Organisms or Products)

1. NAME, ADDRESS, TELEPHONE, AND EMAIL OF APPLICANT

Name: Dr. Anthony Shelton
Position:
Organization: Cornell University/NYSAES
Organization Unique ID:
Address: 630 W. North St.
(b)(6)
Geneva, NY (b)(6)
County/Province:
Township/Island:
(b)(6)
Day Telephone:
FAX:
Alternate:
Email 1: (b)(6)
Email 2:

2. INTRODUCTION TYPE

- [ ] Importation
- [ ] Interstate Movement
- [ ] Interstate Movement and Release
- [x] Release

3. PERMIT TYPE

- [x] Standard Permit
- [ ] Courtesy Permit

4. PURPOSE OF PERMIT

- [ ] Industrial Product
- [ ] Pharmaceutical Product
- [ ] Phytoremediation
- [x] Traditional

5. CONFIDENTIAL BUSINESS INFORMATION VERIFICATION (CBI)

Does this application contain CBI? [ ] Yes [x] No
CBI Justification:
N/A

6. REQUEST TYPE

[x] **New** [ ] **Amendment** [ ] **Renewal**

Amendment/Renewal Description:
Previous Permit Number(s):

7. MEANS OF MOVEMENT

Import by air; releases manually from the ground/vehicles.

8. VARIANCE VERIFICATION

Have you previously applied for variance(s) that you wish to apply to this permit? [ ] Yes [x] No
Variance Number(s):

If so, describe in a brief summary how the variance will be applied:
N/A

9. REGULATED ARTICLE

Scientific Name: Plutella xylostella

Common Name: Diamondback moth

Cultivar and/or Breeding Line:

Any biological material (e.g., culture medium, or host material) accompanying the regulated Article during movement:
Artificial insect diet. This diet will be frozen at -15°C for 12 h prior to import.

Country and locality where the donor organism, recipient organism, and vector or vector agent were collected, developed, and produced:
All final engineering of the transforming constructs was performed at Oxitec Ltd, in the United Kingdom.
The genes used from the donor organisms and the piggyBac-derived portions of the vectors used to build the transforming construct were cloned off-site. The recipient organism is the moth, Plutella xylostella, which is endemic in temperate regions around the world, including the USA. The recipient Plutella xylostella strain for the transformation was a wild-type strain obtained from Syngenta (public limited company), UK, which has been reared in Oxitec insectaries since 2008.

Processes, Procedures, and Safeguards Description:

WARNING: Any use of ePermits to make materially false, fictitious, or fraudulent statements or representations is subject to civil penalties of up to $250,000 (7 U.S.C. § 7734(b)) or punishable by a fine of not more than $10,000, or imprisonment of not more than 5 years, or both (18 U.S.C. §1001).

This is a permit request for 3 years of seasonal releases (April to October) of a female-lethal, genetically marked diamondback moth (maximum 100,000 moths/week), in brassica fields at the Cornell University research station, Geneva NY.
Males of the transgenic moths will be released in cultivated brassica plots and biological parameters of these moths, such as dispersal and persistence, measured using traps, for example baited with synthetic sex pheromone. The moths of the OX4319L-Pxy strain carry a stable, heritable marker the DsRed2 fluorescent protein, viewed by fluorescence microscope or detected by PCR and their female progeny die in the absence of a dietary repressor (tetracycline or suitable analogues supplied in their artificial diet). The male-selecting (female-lethal) penetrance of the strain is >99% (Jin et al. 2013). The marker provides a means of distinguishing released moths from wild moths, and female-lethality is a self-limiting trait in the wild.
All genetically modified moths will be reared in insectaries at Cornell University, Geneva NY. The facilities and their general operation have been inspected and approved through a previous importation permit (12-227-102m). Larval rearing will be conducted in quarantine using the same approved procedures as in this previous permit. Only moths homozygous for the conditional lethal transgene, reared off tetracycline, will be released. Adult moths will be transported in sealed containers, with at least two layers of containment, labeled as follows "CORNELL UNIVERSITY GENETICALLY MODIFIED MOTHS FOR RELEASE AT CORNELL UNIVERSITYS NEW YORK STATE AGRICULTURAL EXPERIMENT STATION - AUTHORIZED PERSONNEL ONLY". Insects will be transported by hand or in a vehicle; for each batch the number of containers and identity of member of staff supervising the release will be recorded.

The transgenic diamondback moths encode no toxic or allergen proteins. The DsRed2 marker protein has been evaluated in a New Protein Consultation by the FDA-CFSAN in the USA for human safety, and they raised no objections to its use in corn plants. This involved an assessment of the amino acid sequence using bioinformatics analyses in accordance with the Guidance provided by Codex (2003), the lability of the protein in simulated gastric fluid (SGF) and an examination of the gene source and history of exposure, as well as the toxicity of the protein using bioinformatics analysis. The amino acid sequence in OX4319L-Pxy is the same as that evaluated in the NPC. It has been further evaluated in an Environmental Assessment (EA) by the USDA (http://www.aphis.usda.gov/brs/aphisdocs/08_33801p_dpra.pdf), which concluded that the corn transformation event that contained the DsRed2 gene was unlikely to become a plant pest risk. Additional EAs on another GE moth, GE pink bollworm, expressing fluorescent genes similar to DsRed2 have also been conducted (http://www.gpo.gov/fdsys/pkg/FR-2006-04-19/html/E6-5878.htm) and concluded that it was unlikely to present any hazard to the environment.
The other protein coding region, tTAV, is regulated by sequences from the sex-determination gene, doublesex, from pink bollworm (Pectinophora gossypiella), that produce different splice variants in males and females: the female transcript comprises coding sequence for the tetracycline-repressible transcription factor, tTAV, which interacts with the upstream tetracycline response element, tetO (or tRE), to form a positive-feedback loop that results in insect lethality prior to adulthood. Under the control of the doublesex sex-alternate splicing, lethality is induced only in females. The tTAV amino acid sequence in OX4319L-Pxy has also been evaluated independently using the bioinformatics analyses provided by Codex (2003) for both potential allergenicity and toxicity. No homologies with known allergens or toxins were determined. This study is available on request.
Tetracycline can be provided to the insect in larval artificial diet to suppress female death and permit colony rearing in the laboratory. Neither piggyBac transposase activity nor any antibiotic resistance is conferred to the transgenic diamondback moth by the introduced genetic material.
This female-specific lethal trait was previously discussed in a USDA Environmental Impact Statement published in October 2008, entitled Use of Genetically Engineered Fruit Fly and Pink Bollworm in APHIS Plant Pest Control Programs, which concluded that the use of genetically engineered fruit flies and pink bollworm in APHIS plant pest control programs were the environmentally preferred alternative (Record of Decision (Federal Register Vol 74 (87) 21314 2009).
Reference:
Jin L, et al. 2013 Engineered female-specific lethality for control of pest lepidoptera. ACS Synthetic Biology, 2:160-166.

10. ARTICLE SUPPLIER AND/OR DEVELOPER

Name	Location	Contact Information
Dr. (b)(6)	Oxitec Ltd 71 Milton Park Abingdon OX144RX United Kingdom **County:** Oxford	**Day Telephone:** 0044-1235-832393 **FAX:** **Email:** (b)(6)

11. PHENOTYPES/GENOTYPE

1) Phenotypic Designation Name: visual marker; repressible lethality

Identifying Line(s): OX4319L-Pxy, OX4319N-Pxy, OX4767A-Pxy

Construct(s): OX4319, OX4767

Mode of Transformation: Direct Injection

Phenotype Description:
A description of the anticipated or actual expression of the altered genetic material in the regulated article and how that expression differs from the expression in the non-modified parental organism.

The introduced genetic material in the diamondback moth comprises three protein coding regions, one for marking the insects and two for inducing death before the insect reaches adulthood (in this instance, females only). The former allows the expression of a DsRed2 fluorescent protein originally derived from a coral (Discosoma sp.). The transgenic diamondback moth with the marker gene fluoresces when excited by illumination of the appropriate wavelength. These fluorescent proteins, which have been used as markers in a wide range of vertebrate and invertebrate species, confer no known competitive advantage or disadvantage to the recipient, and no ecological or other consequences resulting from incorporation of these markers into the transgenic diamondback moth can be envisioned. The non-modified diamondback moth has no fluorescent protein gene; therefore, it does not fluoresce when illuminated under the same light frequency. Neither piggyBac transposase activity nor any antibiotic resistance is conferred to the transgenic diamondback moth by the introduced genetic material.

Phenotype(s)

MG - Visual marker; DsRed2 Fluorescent Protein Expression

Genotype(s)

Screenable Marker
Gene: DsRed2 **from** Discosoma sp. - Screenable marker gene DsRed2 from Discosoma spp - Allows the expression of a fluorescent protein from Discosoma spp. Fluorescent protein of the GFP superfamily (DsRed2) under the control of a hr5ie1 promoter/enhancer sequence, which is from Autographa californica nuclear polyhedrosis virus (AcMNPV). A transgenic diamondback moth with the marker gene will fluoresce when excited by intense illumination of the appropriate wavelength. These fluorescent proteins, which have been used as markers in a wide range of vertebrate and invertebrate species, confer no known competitive advantage or disadvantage to the recipient, and no adverse ecological effect or other consequences resulting from incorporation of these markers into the transgenic diamondback moth are envisioned. Expression of a fluorescent protein will therefore permit released modified moths to be distinguished from unmodified.

Vector Sequence: piggyBac (non-autonomous) **from** piggyBac from Trichoplusia ni (moth) - Transformation Vector from Trichoplusia ni (moth) - Effects germline transformation of diamondback moth from piggyBac from Trichoplusia ni (moth) - 3' 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. In the construct used for transformation of the pink bollworm, the transposase gene of the piggyBac element was irreversibly destroyed by insertion of the transgene. 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 melanogaster 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.

Repressible lethality
Gene: tTAV **from** Escherichia coli (bacterium) and Herpes simplex (virus) - Tetracycline-repressible transcriptional activator from tTAV is a synthetic fusion of the tetR protein from Escherichia coli with VP16 from a type 1 herpes simplex virus. The tTA protein binds to and activates expression from the tetracycline response element (tRE), which includes multiple copies of the specific DNA sequence to which tTA binds (tetO) (Gossen et al., 1994; Gossen & Bujard, 1992). tTAV also binds tetracyclines with high affinity; the tetracycline-bound form of tTAV does not bind DNA. tTAV therefore acts as a tetracycline-regulated switch. In the absence of tetracycline, it will induce expression

from tRE, whereas in the presence of tetracycline it will not. High-level expression of tTAV is thought to be deleterious to cells as it can repress their normal transcription; low-level expression has no known effect other than activation of tRE (Berger, et al., 1990; Damke et al., 1995; Gillespie et al., 1997; Gong et al., 2005; Gossen and Bujard, 1992; Salghetti et al., 2001). tTAV has been used in fungi, plants, mice and Drosophila melanogaster with no known adverse effects. Unmodified Plutella xylostella do not have a tTAV activity.

Regulatory sequence: doublesex genomic region **from** Pink bollworm, Pectinophora gossypiella - Female-specificity is conferred using truncated sex-alternate splicing sequences from the doublesex gene of Pectinophora gossypiella. Sequence encoding tTAV is inserted into this splicing sequence, allowing for the expression of tTAV in a sex-specific manner, resulting in a conditional female-lethal system (Jin et al. 2013).

A full list of construct components is provided in the attached Table of genetic elements.

References:
Berger SL, et al. 1990 Selective inhibition of activated but not basal transcription by the acidic activation domain of VP16: evidence for transcriptional adaptors. Cell 61, 1199-1208.
Damke H, et al. 1995 Tightly regulated and inducible expression of dominant interfering dynamin mutant in stably transformed HeLa cells. Meth Enzymol 257, 209-220.
Gillespie JP, et al. 1997 Biological mediators of insect immunity. Annu Rev Entomol 42, 611-643.
Gong P, et al. 2005 A dominant lethal genetic system for autocidal control of the Mediterranean fruitfly. Nat Biotechnol 23, 453-456.
Gossen M, et al. 1994 Inducible gene expression systems for higher eukaryotic cells. Curr Opin Biotechnol 5, 516-520.
Gossen M, and Bujard H 1992 Tight control of gene expression in mammalian cells by tetracycline- responsive promoters. Proc Natl Acad Sci USA 89, 5547-5551.
Salghetti S, et al. 2001 Regulation of transcriptional activation domain function by ubiquitin. Science 293, 1651-1653.
Jin L, et al. 2013 Engineered female-specific lethality for control of pest Lepidoptera. ACS Synthetic Biology, 2:160-166.

12. INTRODUCTION

Release Site

Location Name & Description	Location Address	Contact(s)
1) Research Farm North	NY **County:** Ontario **Proposed Release Start Date:** 4/1/2014 **Proposed Release End Date:** 3/31/2017 **No. of Releases:** up to 72/year **Quantity:** up to 100,000 moths/wk; 10 acres acres	

Location Unique ID: RFN1097

Location GPS Coordinates: (b)(4)

Release Site History: Managed agricultural, cropping, research. Managed agricultural land around release site.

Critical Habitat Involved?: ___ Yes _X_ No

13. DESIGN PROTOCOLS

Production Design

A detailed description of the purpose for the introduction of the regulated article including detailed description of the proposed experimental and/or production design:

The diamondback moth strains OX4319L-Pxy, OX4319N-Pxy and OX4767A-Pxy show a tetracycline-repressible female-lethal phenotype, which could serve as an insecticide-free means

of controlling pest populations of diamondback moth in the field in a species-specific manner. Successful pest control will rely upon strong performance of released males, in terms of female-seeking behavior and mating competitiveness. We will seek to measure relevant performance traits in one or more mark-release-recapture field experiments. These will be followed by pest suppression trials, in which Plutella xylostella-infested fields will be treated with fsRIDL male Plutella xylostella, and the wild populations monitored and compared with those of fields not so treated.

In the mark-release-recapture experiments, we will release up to 20,000 male fsRIDL Plutella xylostella (per release; up to 100,000 males per week) from single or multiple points in experimental fields of up to 10 acres planted with brassicas (e.g. cabbage or broccoli). Traps (e.g. sticky traps baited with synthetic sex pheromone) will be placed in and around the field, up to 1 km from the release point, to recapture the male moths. Traps will be collected at least once per week and the recaptured moths screened for the fluorescence marker. Additional PCR screening will be conducted to validate this visual screening. Trapping will continue until no fsRIDL male moths are recaptured for 2 consecutive weeks. To permit an overlapping series of releases in each experimental field that can be independently monitored on the traps, fsRIDL male moths will be sometimes be additionally marked, for example using different-colored fluorescent powders, which are commonly used in such field experiments with insects (reviewed by Hagler & Jackson 2001 Ann Rev Entomol 46:511-543). Crop sampling, in which a proportion of the in-field plants will be collected and closely examined in the laboratory for Plutella xylostella larvae and pupae (wild-type and transgenic), will be conducted at regular intervals to assess mating success of the fsRIDL males. Each experimental field will be surrounded by an approximately 10m-deep border free of potential host plants. Upon completion of the experiment, the insecticide Coragen (chlorantraniliprole) will be sprayed on the plants and surrounding area to kill remaining diamondback moth larvae.
Data from these preliminary field experiments, indicating the fsRIDL male release rate required to achieve a given overflooding rate ((b)(4)), dispersal and field longevity, will inform the release str dback moths in a suppression trial (how many fsRIDL males to release, how frequently and from how many points), requested as part of this permit application. All of the described trials will require monitoring of a wild diamondback moth population in up to six experimental fields (up to three treated with fsRIDL male moths, and up to three untreated). These fields, of up to 10 acres in size, will be planted with brassica plants (e.g. cabbage or broccoli). If the wild diamondback moth population is not present in sufficient numbers at the trial sites, the experimental fields 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 GM moths. A proportion of the experimental fields will be subjected to regular releases (b)(4)) of fsRIDL male moths, in numbers greater than the estimated recruitm le moths in the environment, to achieve an over-flooding effect of fsRIDL males on the wild male diamondback moth population. For each experiment, fsRIDL male releases will be conducted for up to the duration of a brassica crop cycle (anticipated as 3-4 months). Adult traps (e.g. sticky traps baited with synthetic sex pheromone) will be placed in and around each field to monitor the relative numbers of wild-type and fsRIDL males present, and to assess their dispersal. The populations of wild-type moths in each field, including those receiving no fsRIDL males, will be monitored using the adult traps described and periodic crop sampling. Releases will consist of up to 100,000 fsRIDL male moths per week (depending on the overflooding ratio required) over the treatment fields over the course of these suppression experiments. Trapping will continue after the last releases of fsRIDL male moths, and will continue until at least 2 weeks of zero fsRIDL recaptures. Upon completion of the experiment, the insecticide Coragen (chlorantraniliprole) will be sprayed on the plants and surrounding area (within 100 m radius of treated fields) to kill remaining diamondback moth larvae. Post-experiment pheromone trapping will continue for 2 weeks to monitor field longevity of fsRIDL moths.

Destination or Release Description

A detailed description of the intended destination (including final and all intermediate destinations), uses, and/or distribution of the regulated article (e.g., greenhouses, laboratory, or growth chamber location; field trial location, pilot project location; production, propagation, and manufacture location; proposed sale and distribution location):

All genetically modified male diamondback moth used in the trials will be reared as larvae on non-tetracycline artificial diet. Releases will be conducted from the ground or vehicle on Cornell University's New York State Agricultural Research Station. Releases will be conducted (b)(4) , depending on experimental requirements. lease sites (up to 1000 m radius from release site) will be monitored with traps (e.g. sticky traps baited with synthetic sex pheromone). Traps will be collected at least weekly to count the number of genetically modified moths and wild moths captured on each trap. Samples in the laboratory will be screened for presence of the DsRed2 fluorescent marker, using fluorescence microscopy, and this will be validated by PCR detection of the DNA construct in selected samples. Some non-viable insect samples will be sent to Oxitec's labs in the UK for the PCR analysis. Prior to each field release, samples from each cohort of male fsRIDL moths will be screened for the fluorescent marker and sexed. Only male moths will be released; the effect on the crop will therefore likely be negligible: male activity is restricted to finding and mating females, feeding on nectar from flowers, and taking shelter during the day.

Confinement Protocols

A detailed description of the proposed procedures, processes, and safeguards which will be used to prevent escape and dissemination of the regulated article at each of the intended destinations:

Adult genetically modified moths will be transported in sealed containers labeled as follows "CORNELL UNIVERSITY GENETICALLY MODIFIED MOTES FOR RELEASE AT CORNELL UNIVERSITYS NEW YORK STATE AGRICULTURAL EXPERIMENT STATION - AUTHORIZED PERSONNEL ONLY". Insects will be transported by hand or in a vehicle by authorized personnel.
The conditional lethality expressed by the fsRIDL construct means that female progeny from matings with Oxitec male insects die in the absence of tetracycline, and the trait is therefore unlikely to persist in the environment. Other mitigation measures include the lack of known sexually compatible relatives of Plutella xylostella in the USA; the piggyBac transposable element used for the transformation has no endogenous functioning transposase, rendering it non-autonomous (it cannot mobilize itself); the release area will be monitored extensively with traps to attract and collect Plutella xylostella moths; release fields are no larger than 10 acres; the Plutella xylostella can be sprayed with insecticide at any time in the case of observed adverse events; the genetically engineered Plutella xylostella will be securely managed and contained in production and transport; and all viable insects reared for this trial that are not required for release or additional analysis will be devitalized by freezing.

Final Disposition Method: ☒ Destruction/Devitalization ☐ Other ☐ Storage in Contained Facility

Final Disposition Description: All unused genetically modified eggs, larvae, pupae and moths not released, or not needed in the mass-rearing, will be frozen at a minimum of -15°C + 5°C for 48 h to destroy all life stages.

14. ATTACHMENTS

Attachments
BRS Importation permit (Exp.9/2013) (10/18/2013 @ 10:05 AM)
Cornell University proposed field release site (10/15/2013 @ 10:51 AM)
OX4319L allele persistence report (10/17/2013 @ 09:23 AM)
OX4319L chlortetracycline sensitivity report (10/17/2013 @ 09:22 AM)
OX4319L construct sequencing report (10/17/2013 @ 09:20 AM)
OX4319L molecular characterisation report (10/17/2013 @ 09:21 AM)
OX4319L population suppression cages report (10/17/2013 @ 09:23 AM)
OX4319L resistance management report (10/17/2013 @ 09:24 AM)
Peer-reviewed publication (Jin et al. 2013) describing development/testing of RIDL DBM (9/26/2013 @ 09:51 AM)
Table of genetic elements, OX4319 and OX4767 (10/15/2013 @ 10:50 AM)
Threatened or endangered species (10/15/2013 @ 10:52 AM)
tTAV expression levels report (10/17/2013 @ 09:21 AM)

15. ADDITIONAL INFORMATION

16. COURTESY JUSTIFICATION

I, *Anthony Shelton*, hereby certify that the information in this application and all attachments is complete and accurate to the best of my knowledge and belief.

I acknowledge this is not an application to move or import select agents, the genes expressing select agents, or the toxins made by the select agents, as described in 9 CFR 121.

I will not introduce the regulated articles described in this application until APHIS has deemed the application complete and has granted the permit. By signing this permit, I agree to comply with any and all state, local, and tribal laws and regulations that may apply to the introduction of the articles described in this applications.

If there are any changes to the information disclosed in this application, I will contact APHIS.

17. SIGNATURE OF RESPONSIBLE PERSON	**18. DATE**
Anthony Shelton	October 24, 2013

SUPPLEMENTAL PERMIT CONDITIONS

For Release of Plutella xylostella

(1) All persons working with the genetically-engineered (GE) diamondback moth must be informed of these permit conditions. Anyone working with these insects must agree to and sign/ initial these conditions before beginning work. These signed conditions do not need to be submitted to USDA/ APHIS but must be readily accessible in the event of an inspection and presented upon request.

Note: these conditions may be copied and stored electronically for electronic signature and initialing provided that the permit number, authorized organisms and life stages, release locations, and authorization statement all appear on the document with the permit number. The residency condition does not need to be signed. Signing these conditions only indicates that the person working under this permit has read them; the permit holder is the sole responsible party under this permit.

(2) Modifications to the containment of these organisms must be approved prior to making changes by applying for an amendment in ePermits.

(3) This permit does not authorize movement or use of plant pathogens listed in the Public Health Security and Bioterrorism Preparedness and Response Act of 2002. If any organism listed as a Select Agent is identified from materials associated with this research, the permit holder is required to notify APHIS, Agricultural Select Agent Program (ASAP) immediately by phone at 301-851-3300, and within seven (7) days submit APHIS/CDC Form 4 (Report of Identification of a Select Agent or Toxin in a Clinical or Diagnostic Laboratory) to APHIS, ASAP; 4700 River Rd, Unit 2, Riverdale, MD 20737 (see instructions at: http://www.aphis.usda.gov/programs/ag_selectagent/index.shtml). Failure to comply with this requirement is a violation of the Agricultural Bioterrorism Protection Act of 2002.

(4) If organisms that are not authorized in this permit are received, the permit holder must take all prudent measures to contain the organism(s) and notify the BRS Compliance Staff by contacting a compliance officer immediately (that is, within one business day) by calling 301-851-3935 or by e-mail to BRSCompliance@aphis.usda.gov. The permit holder must immediately notify the permit unit of the destruction of regulated organisms received under this permit, as above. Similarly, the permit holder must immediately notify the permit unit if facilities are destroyed or decommissioned for any reason.

(5) The permit holder must maintain an official permanent work assignment at the address identified on this permit. If the permit holder ceases assignment/affiliation at the address identified on this permit, or personnel circumstances change in any way, then a compliance officer must be notified at the BRS Compliance Staff immediately (that is, within one business day) by either (a) email to BRSCompliance@aphis.usda.gov, (b) verbal communication at 301-851-3935, or (c) conventional mail to BRS Compliance Staff, 4700 River Road, Riverdale, Unit 91, MD 20737. The permit holder must destroy all regulated organisms prior to departure unless the permit holder either (a) requests cancellation of this permit and complies with all permit-specific termination conditions, (b) applies for and receives a permit to move the organisms to a new facility, or (c) relinquishes control of the regulated organisms to a qualified individual who obtained a permit for the continued use of these regulated organisms prior to this permit holder's departure.

(6) All laboratories and growth chambers where this genetically engineered insect is employed will be locked with limited access by authorized personnel. In each area where a regulated genetically engineered organism is used, at least one sign must be posted stating that a regulated genetically engineered organism is being used.

(7) Upon completion of research all engineered insects (except those retained for future research), will be disposed of by freezing at -20 for 24 hours/or autoclaving.

(8) There is to be no further distribution of these genetically engineered insects under this permit without prior approval from State (intrastate movement) and Federal regulatory officials.

(9) THIS AUTHORIZATION IS VALID FOR THE RELEASE INTO THE ENVIRONMENT OF THIS GENETICALLY ENGINEERED INSECT ONLY IN THE AREAS DESCRIBED IN THE APPLICATION.

(10) THIS IS A CROP DESTRUCT TRIAL. Brassicas will be destroyed at the end of the research field trial. No plants of produce shall be used for food or feed. Upon completion of the experiment, the insecticide shall be sprayed on the plants and surrounding area within a 100 m radius of treated fields to kill remaining diamondback moth larvae.

(11) Without prior notice and during reasonable hours authorized Plant Protection and Quarantine and State regulatory officials shall be allowed to inspect the conditions under which this genetically engineered insect is being kept.

(12) Reporting an Unauthorized or Accidental Release

a. According to the regulation in 7 CFR § 340.4(f)(10)(i), APHIS shall be notified orally immediately upon discovery and notified in writing within 24 hours in the event of any accidental or unauthorized release of the regulated article.

- For immediate verbal notification, contact APHIS BRS Compliance Staff at (301) 851-3935 and ask to speak to a Compliance and Inspection staff member. Leave a verbal report on voicemail if the phone is not answered by a Compliance Officer.
- In addition, in the event of an emergency in which you need to speak immediately to APHIS personnel regarding the situation, you may call:

The APHIS/BRS Regional Biotechnologist assigned in the region where the field test occurs:
For Western Region, contact the Western Region Biotechnologist at (970) 494-7513
or e-mail: BRSWRBT@aphis.usda.gov
For Eastern Region, contact the Eastern Region Biotechnologist at (919) 855-7622 or e-mail: BRSERBT@aphis.usda.gov Or

The APHIS State Plant Health Director for the state where the unauthorized release occurred. The list of APHIS State Plant Health Directors is available at:
http://www.aphis.usda.gov/services/report_pest_disease/report_pest_disease.shtml.
or
http://pest.ceris.purdue.edu/stateselect.html

b. Written notification should be sent by one of the following means:

By e-mail:
BRSCompliance@aphis.usda.gov

By mail:
Biotechnology Regulatory Services (BRS)

Permit Number: 13-297-102r

THIS PERMIT HAS BEEN APPROVED ELECTRONICALLY BY THE FOLLOWING BRS OFFICIAL VIA EPERMITS	
SIGNATURE OF BRS OFFICIAL	**DATE**
John Turner	November 10, 2014

Regulatory Operations Program
USDA/APHIS
4700 River Rd. Unit 91
Riverdale, MD 20737

Additional instructions for reporting compliance incidents may be found at http://www.aphis.usda.gov/biotechnology/compliance_incident.shtml

(13) No person shall move a regulated article interstate unless the number of the limited permit appears on the outside of the shipping container.

THIS PERMIT HAS BEEN APPROVED ELECTRONICALLY BY THE FOLLOWING BRS OFFICIAL VIA EPERMITS	
SIGNATURE OF BRS OFFICIAL	**DATE**
John Turner	November 10, 2014

United States Department of Agriculture
Animal and Plant Health Inspection Service
Biotechnology Regulatory Services
4700 River Road, Unit 147
Riverdale, Maryland
20737-1236

Standard Permit Conditions for the Introduction of a Regulated Article

(7 CFR 340.4 (f))

Permit Conditions: A person who is issued a permit and his/her employees or agents shall comply with the following conditions , and any supplemental conditions which shall be listed on the permit, as deemed by the Deputy Administrator to be necessary to prevent the dissemination and establishment of plant pests:

(1) The regulated article shall be maintained and disposed of (when necessary) in a manner so as to prevent the dissemination and establishment of plant pests.

(2) All packaging material, shipping containers, and any other material accompanying the regulated article shall be treated or disposed of in such a manner as to prevent the dissemination and establishment of plant pests.

(3) The regulated article shall be kept separate from other organisms, except as specifically allowed in the permit.

(4) The regulated article shall be maintained only in areas and premises specified in the permit.

(5) An inspector shall be allowed access, during regular business hours, to the place where the regulated article is located and to any records relating to the introduction of a regulated article.

(6) The regulated article shall, when possible, be kept identified with a label showing the name of the regulated article, and the date of importation.

(7) The regulated article shall be subject to the application of measures determined by the Administrator to be necessary to prevent the accidental or unauthorized release of the regulated article.

(8) The regulated article shall be subject to the application of remedial measures (including disposal) determined by the administrator to be necessary to prevent the spread of plant pests.

(9) A person who has been issued a permit shall submit to APHIS a field test report within 6 months after the termination of the field test. A field test report shall include the APHIS reference number, methods of observation, resulting data, and analysis regarding all deleterious effects on plants, nontarget organisms, or the environment.

(10) APHIS shall be notified within the time periods and manner specified below, in the event of the following occurrences:

(i) Orally notified immediately upon discovery and notify in writing within 24 hours in the event of any accidental or unauthorized release of the regulated article;

(ii) In writing as soon as possible but not later than within 5 working days if the regulated article or associated host organism is found to have characteristics substantially different from those listed in the application for a permit or suffers any unusual occurrence (excessive mortality or morbidity, or unanticipated effect on non-target organisms).

(11) A permittee or his/her agent and any person who seeks to import a regulated article into the United States shall:

(i) Import or offer the regulated article for entry only through any USDA plant inspection station listed in 7 CFR 319.37-14;

(ii)Notify APHIS promptly upon arrival of any regulated article at a port of entry, of its arrival by such means as a manifest, customs entry document, commercial invoice, waybill, a broker's document, or a notice form provided for such purpose; and

(iii) Mark and identify the regulated article in accordance with 7 CFR 340.7.

Rev. January 1, 2010

Permit Number: 13-297-102r

THIS PERMIT HAS BEEN APPROVED ELECTRONICALLY BY THE FOLLOWING BRS OFFICIAL VIA EPERMITS	
SIGNATURE OF BRS OFFICIAL	**DATE**
John Turner	November 10, 2014

Any regulated article introduced not in compliance with the requirements of 7 CFR 340 or any standard or supplemental permit conditions, shall be subject to the immediate application of such remedial measures or safeguards as an inspector determines necessary, to prevent the introduction of such plant pests. Any regulated article introduced not in compliance with the requirements of 7 Code of Federal Regulation Part 340 or any standard or supplemental permit conditions, shall be subject to the immediate application of such remedial measures or safeguards as an inspector determines necessary, to prevent the introduction of such plant pests. The responsible party may be subject to fines or penalties as authorized by the Plant Protection Act (7 U.S.C. 7701-7772).

Permit Number: 13-297-102r

THIS PERMIT HAS BEEN APPROVED ELECTRONICALLY BY THE FOLLOWING BRS OFFICIAL VIA EPERMITS	
SIGNATURE OF BRS OFFICIAL	**DATE**
John Turner	November 10, 2014

APPENDIX I

Please see OX4319L-Pxy population suppression cage report (10/07/2013) attached to the release permit application for details on the previous caged trial carried out using the OX4319L-Pxy strain.

APPENDIX II

Importation permit 12-227-102m

USDA United States Department of Agriculture
APHIS Animal and Plant Health Inspection Service
Biotechnology Regulatory Services
4700 River Road, Unit 147
Riverdale, Maryland
20737-1236

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
BIOTECHNOLOGY REGULATORY SERVICE

PERMIT UNDER 7 CFR 340

(Genetically Engineered Organisms or Products)

This permit was generated electronically via the ePermits system

Enclosed is the BRS Permit Application

PERMITTEE NAME:	Prof. Anthony Shelton
ORGANIZATION:	Cornell/NYSAES
ADDRESS:	614 W. North St. Geneva, NY 14456
PHONE:	(b)(6)
FAX:	315-787-(b)(6)
DESTINATION:	NY

PERMIT NUMBER	12-227-102m
DATE ISSUED:	September 12, 2012
EFFECTIVE:	September 15, 2012
EXPIRES:	**September 15, 2013**
INTRODUCTION TYPE	Importation
PERMIT TYPE	Standard
PURPOSE OF PERMIT	Tracitional

Under the conditions specified, this permit authorizes the following:

Regulated Article: Plutella xylostella

THIS PERMIT HAS BEEN APPROVED ELECTRONICALLY BY THE FOLLOWING BRS OFFICIAL VIA EPERMITS

SIGNATURE OF BRS OFFICIAL	DATE
Steven M. Bennett	September 12, 2012

The collection of this information is authorized by the Plant Protection Act of 2000. The information will be used to determine eligibility to receive all types of permits. No permit will be issued until this application has been approved.

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
BIOTECHNOLOGY REGULATORY SERVICE

APPLICATIONS FOR PERMIT OR COURTESY PERMIT UNDER 7 CFR 340

(Genetically Engineered Organisms or Products)

1. NAME, ADDRESS, TELEPHONE, AND EMAIL OF APPLICANT

Name: Prof. Anthony Shelton
Position: Professor of Entomology
Organization: Cornell/NYSAES
Organization Unique ID:
Address: 614 W. North St.
Geneva, NY 14456
County/Province:
Township/Island:
Day Telephone: (b)(6)
FAX: 315-787-(b)(6)
Alternate: 315-729-(b)(6)
Email 1: (b)(6)
Email 2:

2. INTRODUCTION TYPE

- [x] Importation
- [ ] Interstate Movement
- [ ] Interstate Movement and Release
- [ ] Release

3. PERMIT TYPE

- [x] Standard Permit
- [ ] Courtesy Permit

4. PURPOSE OF PERMIT

- [ ] Industrial Product
- [ ] Pharmaceutical Product
- [ ] Phytoremediation
- [x] Traditional

5. CONFIDENTIAL BUSINESS INFORMATION VERIFICATION (CBI)

Does this application contain CBI? [ ] Yes [x] No
CBI Justification:
N/A

6. REQUEST TYPE

[x] **New** [ ] **Amendment** [ ] **Renewal** [ ] **Variance** [ ] **Amendment, Renewal and/or Variance**

Amendment/Renewal Description:
Previous Permit Number(s):

7. MEANS OF MOVEMENT

Express carrier or in baggage or carry-on luggage

8. VARIANCE VERIFICATION

Have you previously applied for variance(s) that you wish to apply to this permit? [ ] Yes [x] No
Variance Number(s):

If so, describe in a brief summary how the variance will be applied:
N/A

9. REGULATED ARTICLE

Scientific Name: Plutella xylostella

Common Name: Diamondback moth

Cultivar and/or Breeding Line:
Phenotypic designation name: fsRIDL

Any biological material (e.g., culture medium, or host material) accompanying the regulated Article during movement:
Artificial insect diet

Country and locality where the donor organism, recipient organism, and vector or vector agent were collected, developed, and produced:
All final engineering of the transforming constructs was performed at Oxitec Ltd, in the United Kingdom.
The genes used form the donor organisms and the piggyBac-derived portions of the vectors used to build the transforming construct were cloned off-site. The recipient organism is the moth, Plutella xylostella, which is endemic in temperate regions around the world, including the USA.
The recipient Plutella xylostella strain for the transformation was a wild-type strain obtained from Syngenta plc, UK, which has been reared in Oxitec insectaries since 2008.

Processes, Procedures, and Safeguards Description:

10. ARTICLE SUPPLIER AND/OR DEVELOPER

Name	Location	Contact Information
(b) (6)	Oxitec Ltd 71 Milton Park Oxford OX14 4RX United Kingdom **County:** Oxfordshire	**Day Telephone:** +44 1235 832393 **FAX:** +44 1235 861138 **Email:** (b) (6))

11. PHENOTYPES/GENOTYPE

1) Phenotypic Designation Name: fsRIDL

Identifying Line(s):

Construct(s): fsRIDL

Mode of Transformation: Direct injection

Phenotype Description:
A description of the anticipated or actual expression of the altered genetic material in the regulated article and how that expression differs from the expression in the non-modified parental organism.

Genetically modified Plutella xylostella were produced using the transposable element piggyBac, isolated from the cabbage looper moth, Trichiplusia ni. These transposons insert in single or multiple copies in the moth genome. Ie1/Hr5 specific promoters derived from Autographa californica nuclear polyhedrosis virus (AcMNPV) are used to drive the expression of the fluorescent proteins DsRed2, which enables easy visualization of the transgenes.

Phenotype(s)

MG - Pigment composition altered

OC - Sterile

Genotype(s)

Sterile
Gene: Screenable marker Gene **from** Discosoma spp - Allows the expression of a fluorescent protein from Discosoma spp. this allows the expression of a fluorescent protein of the GFP superfamily (DsRed2) under the control of a hr5ie1 promoter/enhancer sequence, which is from Autographa californica nuclear polyhedrosis virus (AcMNPV). A transgenic diamondback moth with the marker gene will fluoresce when excited by illumination of the appropriate wavelength. These fluorescent proteins, which have been used as markers in a wide range of vertebrate and invertebrate species, confer no known competitive advantage or disadvantage to the recipient, and no adverse ecological effect or other consequences resulting from incorporation of these markers into the transgenic diamondback moth are envisioned. The unmodified moths are not strongly fluorescent. Expression of a fluorescent protein will therefore permit all other modified moths to be distinguished from unmodified.

Enhancer: Enhancer **from** Escherichia coli - Tetracycline-repressible transcriptional activator from tTAV is a synthetic fusion of the tetR protein from Escherichia coli with VP16 from a type 1 herpes simplex virus the tTA protein binds to and activates expression from the tetracycline response element (tRE), which includes multiple copies of the specific DNA sequence to which tTA binds (tetO) (Gossen et al., 1994; Gossen & Bujard, 1992). tTAV also binds tetracyclines with high affinity; the tetracycline-bound form of tTAV does not bind DNA. tTAV therefore acts as a tetracycline-regulated switch. In the absence of tetracycline it will induce expression from tRE, whereas in the presence of tetracycline in will not. High-level expression of tTAV is thought to be deleterious to cells as it can repress their normal transcription; low-level expression has no known effect other than activation of tRE (Berger, et al., 1990; Damke et al., 1995; Gillespie et al., 1997; Gong et al., 2005; Gossen and Bujard, 1992; Salghetti et al., 2001). tTAV has been used in fungi, plants, mice and D. melanogaster with no known adverse effects. Unmodified Plutella xylostella do not have a tTAV activity.

Gene: Female-specificity **from** Escherichia coli - tTAV is inserted into genomic regions containing specific sequences enabling the alternative splicing allowing for the expression of tTAV in a sex-specific manner, resulting in a female-lethal system.

12. INTRODUCTION

Point of Origin

Location Name & Description	Location Address	Contact(s)
1) Oxitec Ltd	United Kingdom	

Destination

Location Name & Description	Location Address	Contact(s)
1) Cornell University/NYSAES - Barton Lab	(b)(4) Geneva, NY 14456 **County:** Ontario **Proposed Start Date:** 9/14/2012 **Proposed End Date:** 9/13/2013 **Quantity:** 5000 Individual Adult, eggs, larvae or pupae **Inspected by BRS or PPQ?** Yes **Previous Permit No.:** 63937	

13. DESIGN PROTOCOLS

Production Design

A detailed description of the purpose for the introduction of the regulated article including detailed description of the proposed experimental and/or production design:

N/A

Destination or Release Description

A detailed description of the intended destination (including final and all intermediate destinations), uses, and/or distribution of the regulated article (e.g., greenhouses, laboratory, or growth chamber location; field trial location, pilot project location; production, propagation, and manufacture location; proposed sale and distribution location):

Shipment of no more than 5,000 Plutella xylostella eggs/larvae/pupae from the United Kingdom to Cornell University will involve packaging the moths into shatter-resistant plastic containers with a further two layers of containment, which will be closed and sealed with tape. The box will be labelled as to its contents, origin, destination and contact telephone numbers.
Upon arrival at the insectaries at Cornell University the shipment of moths will be opened and maintained inside the Barton Lab (b)(4) .
At this time there are no plans f eld trials or sale of these insects. The received insects will be used to populate a laboratory colony that will be used for research purposes within the laboratory and trials in fully contained glasshouses with restricted access.

Confinement Protocols

A detailed description of the proposed procedures, processes, and safeguards which will be used to prevent escape and dissemination of the regulated article at each of the intended destinations:

Transgenic diamondback moths at Cornell University wi l be housed within Barton Lab in room (b)(4)) that are BSL2 facilities. Each walk-in

(b)(4)

netting are hung and insects and plants are placed into cages. The mesh of the cages does not permit adults or larvae to move through them. Access into the cages is through sleeves sew into the cages.
Even if there are escapes from the cages and through the greenhouse, it has been well documented that diamondback moth is incapable of surviving the winter in our area. Diamondback moth colonizes cabbage in western New York from the shipment of infested transplants from southern areas or greenhouses, or migrates here on weather patterns.
Diamondback moth in the rearing room and glasshouses:
Diamondback moths will be reared according to our established protocols (http://web.entomology.cornell.edu/shelton/diamondback-moth/)
In the laboratory, moths will be reared at 25oC with a natural photoperiod. Eggs are laid on aluminium foil that has been dipped into cabbage juice and larvae and pupae are kept in plastic rearing containers. Pupae are transferred to cages for subsequent egg collection. In caged glasshouse experiments, diamondback moth with be reared on cabbage or broccoli throughout their life-cycle, with some samples moved to the rearing room to be reared as described above. All

adults are kept in sturdy screened, tightly closed cages within the laboratory or glasshouse. All surplus eggs, larvae, pupae or adults, and associated food material, are killed by freezing at -20oC for at least 12 hours.
Shipment of diamondback moth from the United Kingdom to Cornell University will involve placing the moths into shatter-resistant plastic containers with a further two layers of containment, which will be closed and sealed with tape. The box will be labelled as to its contents, origin, destination and contact telephone numbers. That sealed container will be shipped via TNT or another designated carrier or occasionally carried by a traveller.
Final disposition description:
Transgenic diamondback moths will be reared until such time as experiments pertaining to the specific transgenes under investigation are finished. At such time all remaining eggs, larvae, pupae and adults will be frozen at -20oC for 12 hours.

Final Disposition Method: ☒ Destruction/Devitalization ☐ Other ☐ Storage in Contained Facility

Final Disposition Description: Transgenic diamondback moths will be reared until such time as experiments pertaining to the specific transgenes under investigation are finished. At such time all remaining eggs, larvae, pupae and adults will be frozen at -20oC for 12 hours.

14. ATTACHMENTS

15. ADDITIONAL INFORMATION

Insects will be shipped in a shatter-resistant plastic containers with a further two layers of containment, which will be closed and sealed with tape. The box will be labelled as to its contents, origin, destination and contact telephone numbers.

16. COURTESY JUSTIFICATION

I, *Anthony Shelton*, hereby certify that the information in this application and all attachments is complete and accurate to the best of my knowledge and belief.

I acknowledge this is not an application to move or import select agents, the genes expressing select agents, or the toxins made by the select agents, as described in 9 CFR 121.

I will not introduce the regulated articles described in this application until APHIS has deemed the application complete and has granted the permit. By signing this permit, I agree to comply with any and all state, local, and tribal laws and regulations that may apply to the introduction of the articles described in this applications.

If there are any changes to the information disclosed in this application, I will contact APHIS.

17. SIGNATURE OF RESPONSIBLE PERSON	**18. DATE**
Anthony Shelton	August 14, 2012

SUPPLEMENTAL PERMIT CONDITIONS

For Movement of Plutella xylostella

(1) The Plutella xylostella eggs, larvae, or pupae are to be shipped in containers as specified in 7 CFR Part 340.8(4), for insects, mites, and related organisms or as stated in the permit application.

(2) This authorization is strictly for rearing and research in a controlled laboratory environment.

(3) All laboratories and growth chambers where this genetically engineered insect is employed will be locked with limited access by authorized personnel. In each area where a regulated genetically engineered organism is used, at least one sign must be posted on the door or wall stating that a regulated genetically engineered organism is being used.

(4) Upon completion of research all engineered insects (except those retained for future research), will be disposed of by freezing at -20 for 24 hours/or autoclaving for a minimum of 20 minutes.

(5) This authorization for movement under permit, is valid for execution, for a period of 1 year.

(6) There is to be no further distribution of these genetically engineered insects under this permit without prior approval from State (intrastate movement) and Federal regulatory officials (interstate movement).

(7) THIS AUTHORIZATION IS NOT VALID FOR THE RELEASE INTO THE ENVIRONMENT OF THIS GENETICALLY ENGINEERED INSECT.

All necessary precautions must be taken to prevent escape of these genetically engineered insects.

(8) Without prior notice and during reasonable hours authorized Plant Protection and Quarantine and State regulatory officials shall be allowed to inspect the conditions under which this genetically engineered insect is being kept.

(9) Reporting an Unauthorized or Accidental Release

1. According to the regulation in 7 CFR § 340.4(f)(10)(i), APHIS shall be notified orally immediately upon discovery and notified in writing within 24 hours in the event of any accidental or unauthorized release of the regulated article.

- For immediate verbal notification, contact APHIS BRS Compliance Staff at (301) 851-3935 and ask to speak to a Compliance and Inspection staff member. Leave a verbal report on voicemail if the phone is not answered by a Compliance Officer.
- In addition, in the event of an emergency in which you need to speak immediately to APHIS personnel regarding the situation, you may call:

The APHIS/BRS Regional Biotechnologist assigned in the region where the field test occurs:
For Western Region, contact the Western Region Biotechnologist at (970) 494-7513
or e-mail: BRSWRBT@aphis.usda.gov
For Eastern Region, contact the Eastern Region Biotechnologist at (919) 855-7622 or e-mail: BRSERBT@aphis.usda.gov

Or

The APHIS State Plant Health Director for the state where the unauthorized release occurred. The list of APHIS State Plant Health Directors is available at:
http://www.aphis.usda.gov/services/report_pest_disease/report_pest_disease.shtml
or
http://pest.ceris.purdue.edu/stateselect.html

2. Written notification should be sent by one of the following means:

By e-mail:
BRSCompliance@aphis.usda.gov

By mail:
Biotechnology Regulatory Services (BRS)
Regulatory Operations Program
USDA/APHIS
4700 River Rd. Unit 91
Riverdale, MD 20737

3. Additional instructions for reporting compliance incidents may be found at http://www.aphis.usda.gov/biotechnology/compliance_incident.shtml

(10) No person shall move a regulated article interstate unless the number of the limited permit appears on the outside of the shipping container.

THIS PERMIT HAS BEEN APPROVED ELECTRONICALLY BY THE FOLLOWING BRS OFFICIAL VIA EPERMITS	
SIGNATURE OF BRS OFFICIAL	**DATE**
Steven M. Bennett	September 12, 2012

United States Department of Agriculture
Animal and Plant Health Inspection Service
Biotechnology Regulatory Services
4700 River Road, Unit 147
Riverdale, Maryland
20737-1236

Standard Permit Conditions for the Introduction of a Regulated Article

(7 CFR 340.4 (f))

Permit Conditions: A person who is issued a permit and his/her employees or agents shall comply with the following conditions , and any supplemental conditions which shall be listed on the permit, as deemed by the Deputy Administrator to be necessary to prevent the dissemination and establishment of plant pests:

(1) The regulated article shall be maintained and disposed of (when necessary) in a manner so as to prevent the dissemination and establishment of plant pests.

(2) All packaging material, shipping containers, and any other material accompanying the regulated article shall be treated or disposed of in such a manner as to prevent the dissemination and establishment of plant pests.

(3) The regulated article shall be kept separate from other organisms, except as specifically allowed in the permit.

(4) The regulated article shall be maintained only in areas and premises specified in the permit.

(5) An inspector shall be allowed access, during regular business hours, to the place where the regulated article is located and to any records relating to the introduction of a regulated article.

(6) The regulated article shall, when possible, be kept identified with a label showing the name of the regulated article, and the date of importation.

(7) The regulated article shall be subject to the application of measures determined by the Administrator to be necessary to prevent the accidental or unauthorized release of the regulated article.

(8) The regulated article shall be subject to the application of remedial measures (including disposal) determined by the administrator to be necessary to prevent the spread of plant pests.

(9) A person who has been issued a permit shall submit to APHIS a field test report within 6 months after the termination of the field test. A field test report shall include the APHIS reference number, methods of observation, resulting data, and analysis regarding all deleterious effects on plants, nontarget organisms, or the environment.

(10) APHIS shall be notified within the time periods and manner specified below, in the event of the following occurrences:

(i) Orally notified immediately upon discovery and notify in writing within 24 hours in the event of any accidental or unauthorized release of the regulated article;

(ii) In writing as soon as possible but not later than within 5 working days if the regulated article or associated host organism is found to have characteristics substantially different from those listed in the application for a permit or suffers any unusual occurrence (excessive mortality or morbidity, or unanticipated effect on non-target organisms).

(11) A permittee or his/her agent and any person who seeks to import a regulated article into the United States shall:

(i) Import or offer the regulated article for entry only through any USDA plant inspection station listed in 7 CFR 319.37-14;

(ii)Notify APHIS promptly upon arrival of any regulated article at a port of entry, of its arrival by such means as a manifest, customs entry document, commercial invoice, waybill, a broker's document, or a notice form provided for such purpose; and

(iii) Mark and identify the regulated article in accordance with 7 CFR 340.7.

Rev. January 1, 2010

Permit Number: 12-227-102m

THIS PERMIT HAS BEEN APPROVED ELECTRONICALLY BY THE FOLLOWING BRS OFFICIAL VIA EPERMITS	
SIGNATURE OF BRS OFFICIAL	**DATE**
Steven M. Bennett	September 12, 2012

Any regulated article introduced not in compliance with the requirements of 7 CFR 340 or any standard or supplemental permit conditions, shall be subject to the immediate application of such remedial measures or safeguards as an inspector determines necessary, to prevent the introduction of such plant pests. Any regulated article introduced not in compliance with the requirements of 7 Code of Federal Regulation Part 340 or any standard or supplemental permit conditions, shall be subject to the immediate application of such remedial measures or safeguards as an inspector determines necessary, to prevent the introduction of such plant pests. The responsible party may be subject to fines or penalties as authorized by the Plant Protection Act (7 U.S.C. 7701-7772).

Permit Number: 12-227-102m

THIS PERMIT HAS BEEN APPROVED ELECTRONICALLY BY THE FOLLOWING BRS OFFICIAL VIA EPERMITS	
SIGNATURE OF BRS OFFICIAL	**DATE**
Steven M. Bennett	September 12, 2012

APPENDIX III

Field trial plan as detailed in the release permit application 13-297-102r together with supplementary trial design information.

The collection of this information is authorized by the Plant Protection Act of 2000. The information will be used to determine eligibility to receive all types of permits. No permit will be issued until this application has been approved.

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
BIOTECHNOLOGY REGULATORY SERVICE

APPLICATIONS FOR PERMIT OR COURTESY PERMIT UNDER 7 CFR 340

(Genetically Engineered Organisms or Products)

1. NAME, ADDRESS, TELEPHONE, AND EMAIL OF APPLICANT

Name: Dr. Anthony Shelton
Position:
Organization: Cornell University/NYSAES
Organization Unique ID:
Address: 630 W. North St.
(b)(6)
Geneva, NY 14456
County/Province:
Township/Island:
Day Telephone: (b)(6)
FAX:
Alternate:
Email 1:
Email 2:

2. INTRODUCTION TYPE

- [ ] Importation
- [ ] Interstate Movement
- [ ] Interstate Movement and Release
- [x] Release

3. PERMIT TYPE

- [x] Standard Permit
- [ ] Courtesy Permit

4. PURPOSE OF PERMIT

- [ ] Industrial Product
- [ ] Pharmaceutical Product
- [ ] Phytoremediation
- [x] Traditional

5. CONFIDENTIAL BUSINESS INFORMATION VERIFICATION (CBI)

Does this application contain CBI? ☐ Yes ☒ No
CBI Justification:
N/A

6. REQUEST TYPE

☒ New ☐ Amendment ☐ Renewal

Amendment/Renewal Description:
Previous Permit Number(s):

7. MEANS OF MOVEMENT

Import by air; releases manually from the ground/vehicles.

8. VARIANCE VERIFICATION

Have you previously applied for variance(s) that you wish to apply to this permit? ☐ Yes ☒ No
Variance Number(s):

If so, describe in a brief summary how the variance will be applied:
N/A

9. REGULATED ARTICLE

Scientific Name: Plutella xylostella

Common Name: Diamondback moth

Cultivar and/or Breeding Line:

Any biological material (e.g., culture medium, or host material) accompanying the regulated Article during movement:
Artificial insect diet. This diet will be frozen at -15°C for 12 h prior to import.

Country and locality where the donor organism, recipient organism, and vector or vector agent were collected, developed, and produced:
All final engineering of the transforming constructs was performed at Oxitec Ltd, in the United Kingdom.
The genes used from the donor organisms and the piggyBac derived portions of the vectors used to build the transforming construct were cloned off-site. The recipient organism is the moth, Plutella xylostella, which is endemic in temperate regions around the world, including the USA. The recipient Plutella xylostella strain for the transformation was a wild type strain obtained from Syngenta (public limited company), UK, which has been reared in Oxitec insectaries since 2008.

Processes, Procedures, and Safeguards Description:

WARNING: Any use of ePermits to make materially false, fictitious, or fraudulent statements or representations is subject to civil penalties of up to $250,000 (7 U.S.C. § 7734(b)) or punishable by a fine of not more than $10,000, or imprisonment of not more than 5 years, or both (18 U.S.C. §1001).

This is a permit request for 3 years of seasonal releases (April to October) of a female-lethal, genetically marked diamondback moth (maximum 100,000 moths/week), in brassica fields at the Cornell University research station, Geneva NY.
Males of the transgenic moths will be released in cultivated brassica plots and biological parameters of these moths, such as dispersal and persistence, measured using traps, for example baited with synthetic sex pheromone. The moths of the OX4319L-Pxy strain carry a stable, heritable marker the DsRed2 fluorescent protein, viewed by fluorescence microscope or detected by PCR and their female progeny die in the absence of a dietary repressor (tetracycline or suitable analogues supplied in their artificial diet). The male-selecting (female-lethal) penetrance of the strain is >99% (Jin et al. 2013). The marker provides a means of distinguishing released moths from wild moths, and female lethality is a self limiting trait in the wild.
All genetically modified moths will be reared in insectaries at Cornell University, Geneva NY. The facilities and their general operation have been inspected and approved through a previous importation permit (12 227 102m). Larval rearing will be conducted in quarantine using the same approved procedures as in this previous permit. Only moths homozygous for the conditional lethal transgene, reared off tetracycline, will be released. Adult moths will be transported in sealed containers, with at least two layers of containment, labeled as follows "CORNELL UNIVERSITY GENETICALLY MODIFIED MOTHS FOR RELEASE AT CORNELL UNIVERSITYS NEW YORK STATE AGRICULTURAL EXPERIMENT STATION - AUTHORIZED PERSONNEL ONLY". Insects will be transported by hand or in a vehicle; for each batch the number of containers and identity of member of staff supervising the release will be recorded.

The transgenic diamondback moths encode no toxic or allergen proteins. The DsRed2 marker protein has been evaluated in a New Protein Consultation by the FDA-CFSAN in the USA for human safety, and they raised no objections to its use in corn plants. This involved an assessment of the amino acid sequence using bioinformatics analyses in accordance with the Guidance provided by Codex (2003), the lability of the protein in simulated gastric fluid (SGF) and an examination of the gene source and history of exposure, as well as the toxicity of the protein using bioinformatics analysis. The amino acid sequence in OX4319L Pxy is the same as that evaluated in the NPC. It has been further evaluated in an Environmental Assessment (EA) by the USDA (http://www.aphis.usda.gov/brs/aphisdocs/08_33801p_dpra.pdf), which concluded that the corn transformation event that contained the DsRed2 gene was unlikely to become a plant pest risk. Additional EAs on another GE moth, GE pink bollworm, expressing fluorescent genes similar to DsRed2 have also been conducted (http://www.gpo.gov/fdsys/pkg/FR-2006-04-19/html/E6-5878.htm) and concluded that it was unlikely to present any hazard to the environment.
The other protein coding region, tTAV, is regulated by sequences from the sex-determination gene, doublesex, from pink bollworm (Pectinophora gossypiella), that produce different splice variants in males and females: The female transcript comprises coding sequence for the tetracycline repressible transcription factor, tTAV, which interacts with the upstream tetracycline response element, tetO (or tRE), to form a positive-feedback loop that results in insect lethality prior to adulthood. Under the control of the doublesex sex alternate splicing, lethality is induced only in females. The tTAV amino acid sequence in OX4319L-Pxy has also been evaluated independently using the bioinformatics analyses provided by Codex (2003) for both potential allergenicity and toxicity. No homologies with known allergens or toxins were determined. This study is available on request.
Tetracycline can be provided to the insect in larval artificial diet to suppress female death and permit colony rearing in the laboratory. Neither piggyBac transposase activity nor any antibiotic resistance is conferred to the transgenic diamondback moth by the introduced genetic material.
This female-specific lethal trait was previously discussed in a USDA Environmental Impact Statement published in October 2008, entitled Use of Genetically Engineered Fruit Fly and Pink Bollworm in APHIS Plant Pest Control Programs, which concluded that the use of genetically engineered fruit flies and pink bollworm in APHIS plant pest control programs were the environmentally preferred alternative (Record of Decision (Federal Register Vol 74 (87) 21314 2009).
References:
Jin L, et al. 2013 Engineered female-specific lethality for control of pest Lepidoptera. ACS Synthetic Biology, 2:160-166.

10. ARTICLE SUPPLIER AND/OR DEVELOPER

Name	Location	Contact Information
(b) (6)	Oxitec Ltd 71 Milton Park Abingdon OX144RX United Kingdom **County:** Oxford	**Day Telephone:** 0044-1235-832393 **FAX:** **Email:** (b) (6)

11. PHENOTYPES/GENOTYPE

1. **Phenotypic Designation Name:** visual marker; repressible lethality

Identifying Line(s): OX4319L-Pxy, OX4319N-Pxy, OX4767A-Pxy

Construct(s): OX4319, OX4767

Mode of Transformation: Direct Injection

Phenotype Description:
A description of the anticipated or actual expression of the altered genetic material in the regulated article and how that expression differs from the expression in the non-modified parental organism.

The introduced genetic material in the diamondback moth comprises three protein coding regions, one for marking the insects and two for inducing death before the insect reaches adulthood (in this instance, females only). The former allows the expression of a DsRed2 fluorescent protein originally derived from a coral (Discosoma sp.). The transgenic diamondback moth with the marker gene fluoresces when excited by illumination of the appropriate wavelength. These fluorescent proteins, which have been used as markers in a wide range of vertebrate and invertebrate species, confer no known competitive advantage or disadvantage to the recipient, and no ecological or other consequences resulting from incorporation of these markers into the transgenic diamondback moth can be envisioned. The non-modified diamondback moth has no fluorescent protein gene; therefore, it does not fluoresce when illuminated under the same light frequency. Neither piggyBac transposase activity nor any antibiotic resistance is conferred to the transgenic diamondback moth by the introduced genetic material.

Phenotype(s)

MG - Visual marker; DsRed2 Fluorescent Protein Expression

Genotype(s)

Screenable Marker
Gene: DsRed2 **from** Discosoma sp. Screenable marker gene DsRed2 from Discosoma spp
Allows the expression of a fluorescent protein from Discosoma spp. Fluorescent protein of the GFP superfamily (DsRed2) under the control of a hr5ie1 promoter/enhancer sequence, which is from Autographa californica nuclear polyhedrosis virus (AcMNPV). A transgenic diamondback moth with the marker gene will fluoresce when excited by intense illumination of the appropriate wavelength. These fluorescent proteins, which have been used as markers in a wide range of vertebrate and invertebrate species, confer no known competitive advantage or disadvantage to the recipient, and no adverse ecological effect or other consequences resulting from incorporation of these markers into the transgenic diamondback moth are envisioned. Expression of a fluorescent protein will therefore permit released modified moths to be distinguished from unmodified.

Vector Sequence: piggyBac (non autonomous) **from** piggyBac from Trichoplusia ni (moth) - Transformation Vector from Trichoplusia ni (moth) - Effects germline transformation of diamondback moth from piggyBac from Trichoplusia ni (moth) - 3' 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. In the construct used for transformation of the pink bollworm, the transposase gene of the piggyBac element was irreversibly destroyed by insertion of the transgene. 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 melanogaster 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.

Repressible Lethality
Gene: tTAV **from** Escherichia coli (bacterium) and Herpes simplex (virus)
Tetracycline-repressible transcriptional activator from tTAV is a synthetic fusion of the tetR protein from Escherichia coli with VP16 from a type 1 herpes simplex virus. The tTA protein binds to and activates expression from the tetracycline response element (TRE), which includes multiple copies of the specific DNA sequence to which tTA binds (tetO) (Gossen et al., 1994; Gossen & Bujard, 1992). tTAV also binds tetracyclines with high affinity; the tetracycline bound form of tTAV does not bind DNA. tTAV therefore acts as a tetracycline-regulated switch. In the absence of tetracycline, it will induce expression

from tRE, whereas in the presence of tetracycline it will not. High-level expression of tTAV is thought to be deleterious to cells as it can repress their normal transcription; low-level expression has no known effect other than activation of tRE (Berger, et al., 1990; Damke et al., 1995; Gillespie et al., 1997; Gong et al., 2005; Gossen and Bujard, 1992; Salghetti et al., 2001). tTAV has been used in fungi, plants, mice and Drosophila melanogaster with no known adverse effects. Unmodified Plutella xylostella do not have a tTAV activity.

Regulatory sequence: doublesex genomic region **from** Pink bollworm, Pectinophora gossypiella - Female-specificity is conferred using truncated sex-alternate splicing sequences from the doublesex gene of Pectinophora gossypiella. Sequence encoding tTAV is inserted into this splicing sequence, allowing for the expression of tTAV in a sex-specific manner, resulting in a conditional female-lethal system (Jin et al. 2013).

A full list of construct components is provided in the attached Table of genetic elements.

References:
Berger SL, et al. 1990 Selective inhibition of activated but not basal transcription by the acidic activation domain of VP16: evidence for transcriptional adaptors. Cell 61, 1199-1208.
Damke H, et al. 1995 Tightly regulated and inducible expression of dominant interfering dynamin mutant in stably transformed BeLa cells. Meth Enzymol 257, 209 220.
Gillespie JP, et al. 1997 Biological mediators of insect immunity. Annu Rev Entomol 42, 611-643.
Gong P, et al. 2005 A dominant lethal genetic system for autocidal control of the Mediterranean fruitfly. Nat Biotechnol 23, 453-456.
Gossen M, et al. 1994 Inducible gene expression systems for higher eukaryotic cells. Curr Opin Biotechnol 5, 516 520.
Gossen M, and Bujard H 1992 Tight control of gene expression in mammalian cells by tetracycline- responsive promoters. Proc Natl Acad Sci USA 89, 5547-5551.
Salghetti S, et al. 2001 Regulation of transcriptional activation domain function by ubiquitin. Science 293, 1651-1653.
Jin L, et al. 2013 Engineered female-specific lethality for control of pest Lepidoptera. ACS Synthetic Biology, 2:160 166.

12. INTRODUCTION

Release Site

Location Name / Description	Location Address	Parcel(s)
1) Research Farm North	N? **County:** Ontario **Proposed Release Start Date:** 4/1/2014 **Proposed Release End Date:** 3/31/2017 **No. of Releases:** up to 72/year **Quantity:** up to 100,000 moths/wk; 10 acres acres	

Location Unique ID: REN_007

Location GPS Coordinates: (b)(4)

Release Site History: Managed agricultural, cropping, research. Managed agricultural land around release site.

Critical Habitat Involved?: [redacted] Yes [redacted] No

13. DESIGN PROTOCOLS

Production Design

A detailed description of the purpose for the introduction of the regulated article including detailed description of the proposed experimental and/or production design:

The diamondback moth strains GX4319L Pxy, GX4319N Pxy and GX4767A Pxy show a tetracycline repressible female lethal phenotype, which could serve as an insecticide free means of controlling pest populations of diamondback moth in the field in a species specific manner. Successful pest control will rely upon strong performance of released males, in terms of female seeking behavior and mating competitiveness. We will seek to measure relevant performance traits in one or more mark release recapture field experiments. These will be followed by pest suppression trials, in which Plutella xylostella infested fields will be treated with fsRIDL male Plutella xylostella, and the wild populations monitored and compared with those of fields not so treated.

In the mark release recapture experiments, we will release up to 20,000 male fsRIDL Plutella xylostella (per release; up to 100,000 males per week) from single or multiple points in experimental fields of up to 10 acres planted with brassicas (e.g. cabbage or broccoli). Traps (e.g. sticky traps baited with synthetic sex pheromone) will be placed in and around the field, up to 1 km from the release point, to recapture the male moths. Traps will be collected at least once per week and the recaptured moths screened for the fluorescence marker. Additional PCR screening will be conducted to validate this visual screening. Trapping will continue until no fsRIDL male moths are recaptured for 2 consecutive weeks. To permit an overlapping series of releases in each experimental field that can be independently monitored on the traps, fsRIDL male moths will be sometimes be additionally marked, for example using different colored fluorescent powders, which are commonly used in such field experiments with insects (reviewed by Eagler & Jackson 2001 Ann Rev Entomol 46:511 543). Crop sampling, in which a proportion of the in field plants will be collected and closely examined in the laboratory for Plutella xylostella larvae and pupae (wild type and transgenic), will be conducted at regular intervals to assess mating success of the fsRIDL males. Each experimental field will be surrounded by an approximately 10m deep border free of potential host plants. Upon completion of the experiment, the insecticide Coragen (chlorantraniliprole) will be sprayed on the plants and surrounding area to kill remaining diamondback moth larvae.
Data from these preliminary field experiments, indicating the fsRIDL male release rate required to achieve a given overflooding rate (e.g. (b)(4) dispersal and field longevity, will inform the release strategy of fsRIDL male diamondback moths in a suppression trial (how many fsRIDL males to release, how frequently and from how many points), requested as part of this permit application. All of the described trials will require monitoring of a wild diamondback moth population in up to six experimental fields (up to three treated with fsRIDL male moths, and up to three untreated). These fields, of up to 10 acres in size, will be planted with brassica plants (e.g. cabbage or broccoli). If the wild diamondback moth population is not present in sufficient numbers at the trial sites, the experimental fields 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. A proportion of the experimental fields will be subjected to regular releases ((b)(4)) of fsRIDL male moths, in numbers greater than the estimated recruitment rate of wild type male moths in the environment, to achieve an over flooding effect of fsRIDL males on the wild male diamondback moth population. For each experiment, fsRIDL male releases will be conducted for up to the duration of a brassica crop cycle (anticipated as 3 4 months). Adult traps (e.g. sticky traps baited with synthetic sex pheromone) will be placed in and around each field to monitor the relative numbers of wild type and fsRIDL males present, and to assess their dispersal. The populations of wild type moths in each field, including those receiving no fsRIDL males, will be monitored using the adult traps described and periodic crop sampling. Releases will consist of up to 100,000 fsRIDL male moths per week (depending on the overflooding ratio required) over the treatment fields over the course of these suppression experiments. Trapping will continue after the last releases of fsRIDL male moths, and will continue until at least 2 weeks of zero fsRIDL recaptures. Upon completion of the experiment, the insecticide Coragen (chlorantraniliprole) will be sprayed on the plants and surrounding area (within 100 m radius of treated fields) to kill remaining diamondback moth larvae. Post experiment pheromone trapping will continue for 2 weeks to monitor field longevity of fsRIDL moths.

Destination or Release Description

A detailed description of the intended destination (including final and all intermediate destinations), uses, and/or distribution of the regulated article (e.g., greenhouses, laboratory, or growth chamber location; field trial location, pilot project location; production, propagation, and manufacture location; proposed sale and distribution location):

All genetically modified male diamondback moth used in the trials will be reared as larvae on non-tetracycline artificial diet. Releases will be conducted from the ground or vehicle on Cornell University's New York State Agricultural Research Station. Releases will be conducted ™4: (b)(4) , depending on experimental requirements.
The area around the release sites (up to 1000 m radius from release site) will be monitored with traps (e.g. sticky traps baited with synthetic sex pheromone). Traps will be collected at least weekly to count the number of genetically modified moths and wild moths captured on each trap. Samples in the laboratory will be screened for presence of the DsRed2 fluorescent marker, using fluorescence microscopy, and this will be validated by PCR detection of the DNA construct in

selected samples. Some non-viable insect samples will be sent to Oxitec labs in the UK for the PCR analysis. Prior to each field release, samples from each cohort of male fsRIDL moths will be screened for the fluorescent marker and sexed. Only male moths will be released; the effect on the crop will therefore likely be negligible: male activity is restricted to finding and mating females, feeding on nectar from flowers, and taking shelter during the day.

Confinement Protocols

A detailed description of the proposed procedures, processes, and safeguards which will be used to prevent escape and dissemination of the regulated article at each of the intended destinations:

Adult genetically modified moths will be transported in sealed containers labeled as follows "CORNELL UNIVERSITY GENETICALLY MODIFIED MOTHS FOR RELEASE AT CORNELL UNIVERSITYS NEW YORK STATE AGRICULTURAL EXPERIMENT STATION - AUTHORIZED PERSONNEL ONLY". Insects will be transported by hand or in a vehicle by authorized personnel.
The conditional lethality expressed by the fsRIDL construct means that female progeny from matings with Oxitec male insects die in the absence of tetracycline, and the trait is therefore unlikely to persist in the environment. Other mitigation measures include the lack of known sexually compatible relatives of Plutella xylostella in the USA; the piggyBac transposable element used for the transformation has no endogenous functioning transposase, rendering it non-autonomous (it cannot mobilize itself); the release area will be monitored extensively with traps to attract and collect Plutella xylostella moths; release fields are no larger than 10 acres; the Plutella xylostella can be sprayed with insecticide at any time in the case of observed adverse events; the genetically engineered Plutella xylostella will be securely managed and contained in production and transport; and all viable insects reared for this trial that are not required for release or additional analysis will be devitalized by freezing.

Final Disposition Method: [X] Destruction/Devitalization [ ] Other [ ] Storage in Contained Facility

Final Disposition Description: All unused genetically modified eggs, larvae, pupae and moths not released, or not needed in the mass rearing, will be frozen at a minimum of -15°C ± 5°C for 48 h to destroy all life stages.

14. ATTACHMENTS

Attachments

- [redacted] (Exp.9/2013) (10/18/2013 @ 10:05 AM)
- Cornell University proposed field release site (10/15/2013 @ 10:51 AM)
- OX4319L allele persistence report (10/17/2013 @ 09:24 AM)
- OX4319L chlortetracycline sensitivity report (10/17/2013 @ 09:22 AM)
- OX4319L construct sequencing report (10/17/2013 @ 09:26 AM)
- OX4319L molecular characterisation report (10/17/2013 @ 09:21 AM)
- OX4319L population suppression cages report (10/17/2013 @ 09:23 AM)
- OX4319L resistance management report (10/17/2013 @ 09:24 AM)
- Peer-reviewed publication (Jin et al. 2013) describing development/testing of RIDL DBM (9/26/2013 @ 09:51 AM)
- Table of genetic elements, OX4319 and OX4767 (10/15/2013 @ 10:56 AM)
- Threatened or endangered species (10/15/2013 @ 10:52 AM)
- tTAV expression levels report (10/17/2013 @ 09:21 AM)

15. ADDITIONAL INFORMATION

16. COURTESY JUSTIFICATION

I, *Anthony Shelton*, hereby certify that the information in this application and all attachments is complete and accurate to the best of my knowledge and belief.

I acknowledge this is not an application to move or import select agents, the genes expressing select agents, or the toxins made by the select agents, as described in 9 CFR 121.

I will not introduce the regulated articles described in this application until APHIS has deemed the application complete and has granted the permit. By signing this permit, I agree to comply with any and all state, local, and tribal laws and regulations that may apply to the introduction of the articles described in this applications.

If there are any changes to the information disclosed in this application, I will contact APHIS.

17. SIGNATURE OF RESPONSIBLE PERSON	18. DATE
Anthony Shelton	October 24, 2013

WARNING: Any use of ePermits to make materially false, fictitious, or fraudulent statements or representations is subject to civil penalties of up to $250,000 (7 U.S.C. § 7734(b)) or punishable by a fine of not more than $10,000, or imprisonment of not more than 5 years, or both (18 U.S.C. §1001).

Further details on the initial experiments proposed as part of the GE diamondback moth (*Plutella xylostella*) field release

Laboratory performance tests carried out at Oxitec Ltd (UK) have identified OX4319L-Pxy as the strain with the most potential for strong performance in the field; therefore, this is the strain that has been reviewed in the ERA and is proposed for use in the open field releases. In light of this amendment to the proposed release protocol and adaptation to the initial releases within the protocol, this amendment has been added.

As part of the initial data collection, two trials will be undertaken in parallel in the first year:

1. A mark-release-recapture (MRR) experiment, approximately 10,000 males each of two strains - OX4319L-Pxy and its non-transgenic counterpart strain 'Vero Beach' – will be co-released on a single day from a single central point in a brassica field (e.g. cabbage), and monitoring traps set at different distances and directions from this point will be changed every 2 days to study dispersal and longevity. Replicates of the releases will be carried out for up to 6 weeks. If releases are expected to result in overlapping groups of moths occurring concurrently in the field (e.g. if release 1 and release 2 are undertaken <1 week apart), consecutive releases will be marked with a different fluorescent powder or other artificial marking method.

2. A mating performance test in a DBM infested cabbage field with up to 12 field cages. Into each cage, we will release 'X' males from two strains - OX4319L-Pxy and wild - and later release 'X' females (X = 20-200). For the next 2-3 weeks, we will collect progeny (either by replacing the cabbages themselves, using sentinel plants, or taking sample leaves from the cabbages), and rear insects in the laboratory to pupation. Sexing these pupae and screening for fluorescence will provide information on the mating success of each male strain.

Combining these two experiments in the first year of the trial will provide information on the performance of the OX4319L-Pxy strain in field settings. Results from this first year of experiments will be used to design experiments in years 2 and 3 in greater detail, while maintaining the trial within the limits outlined in the release permit application 13-297-102r.

Appendix IV

A review of tetracycline in the environment

Agricultural pest species have been controlled with a broad range of pesticides, however the rapid generation time of many species combined with the high selection pressures imposed by monocultures and widespread use of single insecticides has led to the failure of a number of common insecticides.

Particularly in agricultural settings that has been a significant drive to reduce the amount of pesticides used as exampled in the EU where integrated pest management has been enforced as policy in an attempt to reduce the sales and use of pesticides, this has resulted in a drive towards the use of novel pest control strategies (Hillocks, 2012).

A long-standing effective pest control strategy is the sterile-insect-technique (SIT) this involves control of the pest population through releases of irradiated males (Dyck, 2005). These males will mate with the wild females and due to the damage incurred by the dose of radiation the resulting offspring from the cross will not be viable. Whilst this technique has been used since the 1950s for control of Pink Bollworm and New World Screw worm amongst other species, the reduction in fitness of the insect can compromise the cost effectiveness of the program. In other cases the dose of radiation required to sterilize the insect can so greatly impair the fitness of the male that the technique is impractical.

Release of male pest insects which are homozygous for a repressible dominant lethality trait acts as a novel variant of the SIT (Ant et al., 2011; Gong et al., 2005; Jin et al., 2013; Wilke et al., 2009); the released males pass the lethal genetic trait onto all offspring which will prevent the larval development of all females absence of the repressor. Since the majority of agricultural pest damage is caused by ovipositioning and feeding of larval stages; a male only release is preferable to reduce biting incidence and crop damage. The repressible nature of this lethality trait enables the insects to be developed in the mass rearing facilities and absence of the repressor in the environment enables the insects to be used for effective pest control.

Oxitec's novel control is dependent on the absence of tetracycline from the release environment. A review of peer-reviewed studies published in the scientific literature has been carried out in order to assess a range of potential release sites for the presence of tetracycline. Exposure routes have been assessed in order to establish sites and areas that might need to be considered to develop a comprehensive risk.

The levels of tetracycline required to repress the lethality trait is known in the insectaries in order to enable mass rearing of the strains however it is important to establish a more detailed tetracycline response curve for each species to indicate the ability of the strains to survive in a range of tetracycline concentrations. Furthermore for each species levels of tetracycline in their respective receiving environments will need to be evaluated by a thorough literature review before release of the control strain.

Exposure routes

As it is only the juvenile or larval stages of the insects which require tetracycline there is no requirement that the adult stages take up tetracycline to survive. Therefore the habitat of the adult life stages is not considered.

Diamondback moths are general pests of Brassicas and will develop in the leaves of the crop. Adult females lay between 1-8 eggs in groups on the underside of the leaf. 1st instar larvae develop in the plant mesophyll emerging in subsequent instars and feeding on the leaf surface. Larvae then form a loose mesh cocoon to pupate in. The larval stages require tetracycline in order to survive therefore tetracycline concentrations of sufficient concentration need to be present in the leaf on which the insect is eating.

Tetracyclines

Tetracyclines are a family of antibiotics with a closely related molecular structure these include a number of analogues of tetracycline such as; chlortetracycline, oxytetracycline, doxycycline, minocycline, methacycline, demethylchlortetracycline (Grassi, 1993). The development of novel tetracycline analogues has been necessary where antibiotic resistance has arisen and improvements in the water solubility of the analogues have extended their application range. The uses of many of the analogues of tetracycline are summarized in Annex 1.

The ability of these analogues to repress the lethality trait used in Oxitec insects can be inferred through a literature review of the tTA system in other organisms (Orth et al., 1999) in particular, doxycycline has been frequently used to induce repression of the tet-system particularly when the tet-system is used for conditional gene therapy in mice (Robertson et al., 2002). The tetracycline analogues, tetracycline hydrochloride and chlortetracycline are routinely used in the insectaries at Oxitec to repress the lethality trait which enables mass rearing of the strains; these analogues of tetracycline are commonly in veterinary or prophylaxis uses whilst novel analogues of tetracycline such as doxycline are solely used in human therapeutic applications.

In an agricultural setting the most likely sources of tetracycline are from application of manure contaminated with tetracyclines as a result of prophylactic or veterinary applications. Tetracyclines used against plant pathogens was explored and appears to be only applicable to a small number of specific tree species therefore has not been included in this review focussing on diamondback moths.

Incorrect disposal of human therapeutic tetracyclines could result in a potential source of antibiotic contamination in landfill sites or could result in antibiotics in waste water systems.

In order for the lethality trait to be repressed the larval stage of the strain will require exposure to tetracycline. The levels of tetracycline for the developed strains are well documented and provide a baseline for predicting the amount of tetracycline which will be required in future developments.

Levels of tetracycline required to repress the lethality trait in Diamondback moth

Immature stages of the diamondback moth are a known pest of most brassica crop plants. Within the insectaries at Oxitec the lethality trait is repressed by addition of 200mg/L of chlorotetracycline (CTC) to their artificial diet. Manure is often spread on agricultural land as a fertiliser; some manure is known to contain tetracycline potentially in high levels which could accumulate in crop plants.

A dose response curve has been carried out for the OX4319L-Pxy strain to determine the minimum level of CTC required in their diet to repress the lethality trait.

The survival of heterozygous diamondback moths which were reared on different concentrations of CTC in the larval diet indicate that concentrations of 0.01µg/ml CTC are insufficient to enable the full repression of the lethality trait as no females survive to pupation. At concentrations of 0.1 µg/ml CTC in the larval diet over 20% of the female heterozygotes are capable of survival to pupation. Survival to adulthood is much lower at this (0.1 µg/ml) concentration suggesting that despite survival to pupation the females might still be expressing tTAV.

The expression of tTAV has been assessed in different life stages of male and female diamondback moths both on and off diets containing 100 µg/ml CTC. Expression of the tTAV is clearly not repressed in female larvae reared on diets not containing CTC.

Tetracycline in the environment

Tetracycline is a commonly used antibiotic within livestock rearing particularly as the tetracycline analogues chlorotetracycline and oxytetracycline. In the EU prophylactic use of antibiotics has been banned since 2006[13] due to the risk of antibiotic resistance development, which led to a subsequent increase in the amount of therapeutic applications of antimicrobials. (Burow et al., 2014) Antibiotics such as tetracyclines are still used in large quantities within farming (2011). In Canada, Korea and the US, tetracycline amongst other antibiotics, continue to be used as growth promoters in poultry, pigs and cattle (Kim et al., 2011).

The absorption rate of the antibiotics within the animals is known to be small, with up to 72 % of the antibiotic being excreted in faeces and urine within 2 days of antibiotic application. (Kim et al., 2011) The resulting manure is commonly composted for up to four months prior to application as a fertiliser in agricultural land. In theory, the presence of high concentrations of tetracyclines (e.g. tetracycline, chlortetracycline and oxytetracycline) in the soil or manure could lead to levels of these chemicals accumulating in plant foliage posing a threat to human health. Furthermore should the level of the tetracycline bioaccumulate in plant tissues to sufficient levels, the lethality trait in the pest control insect could be repressed.

Tetracyclines are the most commonly used antibiotic in pig farming (Brambilla et al., 2007) the majority of these antibiotics end up in manure in their bioactive form (Kim et al., 2011). Pig manure is often applied to agricultural land as a fertiliser however good farming standards require that the manure is well rotted or composted prior to application. Throughout the composting step the manure can reach high temperatures, particularly in the presence of an organic material such as sawdust which initiates efficient composting. Commercial composts can be composed of 30-50% animal manures with the remaining contents consisting of organic matter (including sawdust)(Kim et al., 2012). It is thought that the high temperatures reached during composting result in breakdown of the tetracyclines by up to 96%, another study has also shown a 90% reduction in veterinary antibiotics in manures when proper composting practises were used. (Kim et al., 2011) The presence of antibiotic resistance genes in livestock manures from different pig farms has shown that tetracycline resistance genes are present in all sections of the waste water treatment plants (Cheng et al., 2013). Tetracyclines readily to

[13] http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2003:268:0029:0043:EN:PDF)

bind to other substances, the chelation of tetracylines out of waste water systems is well documented and in slurry pits it has been shown that oxytetracylines transfer to solid particles. The rate with which tetracyclines bind to solid particles in soil is dependent on the pH, organic matter content and the presence of metals. (Brambilla et al., 2007)

Tetracycline is also sensitive to light and has a short half-life in the environment. Tetracycline is known to be rapidly degraded by ultra-violet radiation (Bautiz and Nogueira, 2006), in the presence of iron or other metal catalysts (Reyes et al., 2006), with total deactivation obtained in 70 minutes. The use of tetracycline in the environment was reviewed by Sarmah et al. (2006) and again tetracycline was found to have rapid degradation (with the bulk of degradation taking place on day 1) and a short half-life in the environment (15-30 days in water and up to 9 days in animal manure). It is likely that the complex nature of the environmental conditions, daily rain intensity, temperature, solar radiation, soil type and size and micro-flora will have an impact on the degradation times, most likely decreasing the half-life compared to those in controlled laboratory conditions. Removal of antibiotics using graphene oxide functionalized magnetic particles has further indicated how tetracycline removal in waste water treatment plants can be improved to decouple the removal of tetracyclines from pH and temperature. (Lin et al., 2013)

Despite the rapid breakdown of tetracycline in the environment the presence of veterinary antibiotics as a soil contaminant has been shown to result in bioaccumulation of antibiotics in crop plants. Antibiotics are thought to be taken up by plants through water transport and passive absorption. (Hu et al., 2010)

An investigation into the contamination of crops with veterinary antibiotics looked at the amount of antibiotic in lettuce and tomato after application of antibiotic treated swine slurry which contained 22.9mg/L chlorotetracycline (Seo et al., 2010). Post-harvest CTC concentrations in the tomato were, on average, 0.7ng/g (fresh weight). It seems that tomato plants may be inefficient at taking up CTC, as concentrations in lettuce (mean, 3.4 ng/g) in the same experiment were nearly five times that seen in tomatoes. This variation in CTC uptake efficiency of different plants was also shown by Kumar et al. in a similar experiment (Kumar et al., 2005), which showed CTC levels in corn and onion generally double that in cabbage.

Another study has investigated the ability of *Zea mays* to absorb tetracycline. An uncontaminated field was subjected to pig slurries contaminated with 15 mg/L of oxytetracycline and 5 mg/L of chlorotetracycline. Tetracyclines analyses on soils and on field plants (roots, stalks, and leaves) did not determine the appreciable presence of tetracyclines in field settings. Residues of 1-50ng/g of oxytetracycline was detected in the roots of *Zea mays* grown in pots contaminated with oxytetracycline at 62.5-1000ng/g of dry soil. (Migliore et al., 2010)

Altogether these studies indicate that whilst the concentration of tetracycline is very low in crops plants grown in tetracycline contaminated soils there is a significant variation in levels of tetracycline detected in different plant species. Therefore prior to the use of RIDL strains evaluation of the crop plants tetracycline uptake will be needed together with the amount quantity of tetracycline needed to repress the lethality of the RIDL trait.

Direct application of antibiotics to crop plants is permitted as a treatment for bacterial infections. Similar to the pesticide residue limits that are established to minimize exposure of consumers, established guidelines for acceptable daily intake of veterinary antibiotics have been established by the Joint FAO/WHO Expert Committee of Food Additives and Contaminants. Tolerance limits for

antibiotic levels in the USA have been established by the Environmental Protection Agency for residues of oxytetracycline in or on peach and pear crops (Maia et al., 2009). In Brazil, tetracyclines are permitted for use on tomato, potato, beans, cucumber, coffee, peach, plum, passion fruit and pepper. The maximum residue level is 0.25mg/kg for all commodities except for plum where the MRL is 0.7 mg/kg. The presence of tetracycline in a tomato crop following direct application of oxytetracycline showed that the 7 days pre-harvest interval is sufficient to reduce the level of tetracycline present in the crop to below the MRL. (No detectable levels of oxytetracycline were present after 4 days). As the tetracycline breaks down rapidly on the surface of the plant it would be anticipated that any RIDL larvae would not have sufficient levels of tetracycline throughout its development to suppress the lethality trait resulting in female insects reaching maturity.

(2011). Antibiotics in Animal Farming, Public health and animal welfare. In Compassion in World Farming

Alphey, L. (2002). Re-engineering the sterile insect technique. Insect biochemistry and molecular biology *32*, 1243-1247.

Alphey, L., Nimmo, D., O'Connell, S., and Alphey, N. (2008). Insect population suppression using engineered insects. In Transgenesis and the Management of Vector-Borne Disease, S. Aksoy, ed. (Austin, Texas, USA: Landes Bioscience and Springer), pp. 93-103.

Alphey, N., Coleman, P.G., Donnelly, C.A., and Alphey, L. (2007). Managing insecticide resistance by mass release of engineered insects. Journal of Economic Entomology *100*, 1642-1649.

Ant, T., Koukidou, M., and Alphey, L. (2011). Genetic engineering of the olive fruit fly, Bactrocera oleae, for use in the sterile insect technique (SIT). 5th Meeting of the IOBC/WPRS WG Integrated Protection of Olive Crops Bulletin.

Bakri, A., Mehta, K., and Lance, D.R. (2005). Sterilizing Insects with Ionizing Radiation In Sterile Insect Technique Principles and Practice in Area-Wide Integrated Pest Management, V.A. Dyck, J. Hendrichs, and A.S. Robinson, eds. (The Netherlands: Springer), pp. 233-268.

Baur, M.E., Kaya, H.K., Tabashnik, B.E., and Chilcutt, C.F. (1998). Suppression of diamondback moth (Lepidoptera: Plutellidae) with an entomopathogenic nematode (Rhabditida: Steinernematidae) and Bacillus thuringiensis Berliner. J Econ Entomol *91*, 1089-1095.

Bautiz, I.R., and Nogueira, R.F.P. (2006). Degradation of tetracycline by photo-Fenton process - Solar irradiation and matrix effects. J Photochemistry and Photobiology A: Chemistry *187*, 33-39.

Bevis, B.J., and Glick, B.S. (2002). Rapidly maturing variants of the Discosoma red fluorescent protein (DsRed). Nature Biotechnology *20*, 83-87.

Blum, S., Basedow, T., and Becker, N. (1997). Culicidae (Diptera) in the diet of predatory stages of anurans (Amphibia) in humid biotopes of the Rhine Valley in Germany. Journal of Vector Ecology *22*, 23-29.

Brambilla, G., Patrizii, M., De Filippis, S.P., Bonazzi, G., Mantovi, P., Barchi, D., and Migliore, L. (2007). Oxytetracycline as environmental contaminant in arable lands. Analytica chimica acta *586*, 326-329.

Brown, B.A., Wallace, R.J., and Onvi, G. (1996). Activities of the glycylcyclines N,N-dimethylglycylamido-minocycline and N,N-dimethylglycylamido-6-demethyl-6-deoxytetracycline against Nocardia spp. and tetracycline-resistant isolates of rapidly growing mycobacteria. Antimicrob Agents Chemother *40*, 874-878.

Burow, E., Simoneit, C., Tenhagen, B.A., and Kasbohrer, A. (2014). Oral antimicrobials increase antimicrobial resistance in porcine E. coli - A systematic review. Prev Vet Med *113*, 364-375.

Charleston, D., and Kfir, R. (2000). The possibility of using Indian mustard, *Brassica juncea*, as a trap crop for the diamondback moth, *Plutella xylostella*, in South Africa. . Crop Protection *19*, 455-460.

Cheng, W., Chen, H., Su, C., and Yan, S. (2013). Abundance and persistance of antibiotic resistance genes in livestock farms: A comprehensive investigation in eastern China. . Environmental International *61*, 1-7.

Chopra, I., and Roberts, M. (2001). Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. Microbiology and molecular biology reviews : MMBR *65*, 232-260 ; second page, table of contents.

CLONTECHniques (2001). Living Colors DsRed2 Improved Red Fluorescent Protein for Use in Living Cells (www.clontech.com).

Codex, A.C. (2003). Appendix III, Guidelines for the conduct of food safety assessment of foods derived from recombinant-DNA plants and Appendix IV, Annex on the assessment of possible allergenicity (Rome: Food and Agriculture Organisation (FAO)), pp. 47-60.

Dyck, V., ed. (2005). Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management (Springer).

EFSA (2009). Statement of EFSA on the consolidated presentation of the joint Scientific Opinion of the GMO and BIOHAZ Panels on the "Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants" and the Scientific Opinion of the GMO Panel on "Consequences of the Opinion on the Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants on Previous EFSA Assessments of Individual GM Plants". . The EFSA Journal *1108*, 1-8.

EFSA (2012). Guidance on the environmental risk assessment of genetically modified animals, E.F.S. Authority, ed. (EFSA).

Elwakil, W., and Mossler, M. (2013). Crop Profile for Cabbage in Florida. (University of Florida).

EPA (1993). Hydroxytetracycline Monohydrochloride and Oxytetracycline Calcium (United States Environmental Protection Agency: United States Environmental Protection Agency).

Fu, G., Condon, K.C., Epton, M.J., Gong, P., Condon, G.C., Morrison, N.I., Dafa'alla, T.H., and Alphey, L. (2007). Female-specific insect lethality engineered using alternative splicing. Nature Biotechnology *25*, 353-357.

Gianessi, L. (2009). The Benefits of Insecticides Use: Cabbage. In CropLife Foundation (Crop Protection Research Institute).

Golizadeh, A.L.I., Kamali, K., Fathipour, Y., and Abbasipour, H. (2007). Temperature-dependent development of diamondback moth, Plutella xylostella (Lepidoptera: Plutellidae) on two brassicaceous host plants. Insect Science *14*, 309-316.

Gong, P., Epton, M., Fu, G., Scaife, S., Hiscox, A., Condon, K., Condon, G., Morrison, N., Kelly, D., and Dafa'alla, T. (2005). A dominant lethal genetic system for autocidal control of the Mediterranean fruitfly. Nat Biotech *23*, 453 - 456.

Goodman, R.E. (2013). Bioinformatics analysis for risks of allergenicity and toxicity of proteins encoded by the two genes introduced into genetically engineered mosquitos *(Aedes aegypti)*, strain OX513A for production of sterile males to reduce vector transmission of important human diseases. (Univeristy of Nebraska).

Grassi, G.G. (1993). Tetracyclines- extending the atypical spectrum. International Journal of Antimicrobial Agents *3*.

Heuer, H., and Smalla, K. (2007). Horizontal Gene Transfer Between Bacteria Environ Biosafety Res *6*, 3-13.

Hillocks, R.J. (2012). Farming with fewer pesticides: EU pesticide review and resulting challenges for UK agriculture. Crop Protection *31*, 85-93.

Hopkinson, R.F., and Soroka, J.J. (2010). Air trajectory model applied to an in-depth diagnosis of potential diamondback moth infestations on the Canadian Prairies. Agricultural and Forest Meteorology *150*, 1-11.

Hu, X., Zhou, Q., and Luo, Y. (2010). Occurrence and source analysis of typical veterinary antibiotics in manure, soil, vegetables and groundwater from organic vegetable bases, northern China. Environ Pollut *158*, 2992-2998.

Ip, T.M., and Wan, D.C.C. (2004). A novel orange fluorescent protein from the Cnidarian tube anemone *Cerianthus sp.* and its multicolored fluorescent mutants. . Luminescence *19*, 129-192.

Jin, L., Walker, A.S., Fu, G., Harvey-Samuel, T., Dafa'alla, T., Miles, A., Marubbi, T., Granville, D., Humphrey-Jones, N., O'Connell, S., *et al.* (2013). Engineered Female-Specific Lethality for Control of Pest Lepidoptera. ACS Synthetic Biology *2*, 160-166.

Kim, K.-R., Owens, G., Kwon, S.-I., So, K.-H., Lee, D.-B., and Ok, Y.S. (2011). Occurrence and Environmental Fate of Veterinary Antibiotics in the Terrestrial Environment. Water, Air, & Soil Pollution *214*, 163-174.

Kim, K.-R., Owens, G., Ok, Y.S., Park, W.-K., Lee, D.B., and Kwon, S.-I. (2012). Decline in extractable antibiotics in manure-based composts during composting. Waste management *32*, 110-116.

Knodel, J., and Ganchiarachchi, M. (2008). Diamondback Moth in Canola; Biological and Integrated Pest Management.

Kumar, K., Gupta, S.C., Baidoo, S.K., Chander, Y., and Rosen, C.J. (2005). Antibiotic uptake by plants from soil fertilized with animal manure. Journal of environmental quality *34*, 2082-2085.

Lee, S.-T., Chu, Y.-I., and Talekar, N.S. (1995). The Mating Behaviour of Diamondback Moth (Lepidoptera: Plutellidae). . Chinese J Entomol *15*, 81-89.

Lee, S., Lee, D.-W., and Boo, K.S. (2005). Sex Pheronmone Compostion of the Diaondback Moth, Plutella xylostella (L.) in Korea. . J Asia-Pacific Entomol *8*, 243-248.

Leggat, P.A. (2009). Safety and Efficacy of Doxycycline Clinical Medicine: Therapeutics *1*, 1069-1072.

Lin, H., McGrath, J., Wang, P., and Lee, T. (2007). Cellular toxicity induced by SRF-mediated transcriptional squelching. Toxicol Sci *96*, 83-91.

Lin, Y., Xu, S., and Li, J. (2013). Fast and highly efficient tetracyclines removal from environmental waters by graphene oxide functionalized magnetic particles. Chemical Engineering Journal *225*, 679-685.

Lukyanov, K.A., Fradkov, A.F., Gurskaya, N.G., Matz, M.V., Labas, Y.A., Savitsky, A.P., Markelov, M.L., Zaraisky, A.G., Zhao, X., Fang, Y., *et al.* (2000). Natural animal coloration can Be determined by a nonfluorescent green fluorescent protein homolog. J Biol Chem *275*, 25879-25882.

Maia, P.P., da Silva, E.C., Rath, S., and Reyes, F.G.R. (2009). Residue content of oxytetracycline applied on tomatoes grown in open field and greenhouse. Food Control *20*, 11-16.

Matz, M., Fradkov, A., Labas, Y., Savitsky, A., Zaraisky, A., Markelov, M., and Lukyanov, S. (1999). Fluorescent proteins from nonbioluminesent Anthozoa sp. Nature Biotechnology *17*, 969-973.

McInnis, D., Lance, D.R., and Jackson, C.G. (1996). Behavioural resistance to the sterile insect release technique by the Mediterranean fruit fly (Diptera: Tephritidae) in Hawaii. . Ann entomol Soc Am *89*, 739-744.

McLaughlin, J.R., Mitchell, E.R., and Kirsch, P. (1994). Mating Disruption of Diamondback Moth (Lepidoptera: Plutellidae) in Cabbage: Reduction of Mating and Suppression of Larval Populations. Journal of Economic Entomology *87*, 1198-1204.

Migliore, L., Godeas, F., De Filippis, S.P., Mantovi, P., Barchi, D., Testa, C., Rubattu, N., and Brambilla, G. (2010). Hormetic effect(s) of tetracyclines as environmental contaminant on Zea mays. Environ Pollut *158*, 129-134.

Nguyen, C., Bahar, M.H., Baker, G., and Andrew, N.R. (2014). Thermal Tolerance Limits of Diamondback Moth in Ramping and Plunging Assays. PLoS ONE *9*, e87535.

Oreenaiza, N., Donald, W., Wong, H.M., Teoh, G.N., Mohamed, K.A., Halim, N.A., Winskill, P., Hadi, A.A., Muhammad, Z.S., Lacroix, R., *et al.* (2013). Oral Ingestion of Transgenic RIDL Ae. aegypti Larvae Has No Negative Effect on Two Predator Toxorhynchites Species. PLoS ONE *8*, e58805.

Orth, P., Schnappinger, D., Sum, P.-E., Ellestad, G.A., Hillen, W., Saenger, W., and Hinrichs, W. (1999). Crystal structure of the tet repressor in complex with a novel tetracycline, 9-(*N,N*-dimethylglycylamido)-6-demethyl-6-deoxy-tetracycline. J Mol Biol *285*, 455-461.

Pavely, C., and Fedorova, M. (2006). Early food safety evaluation for a red fluorescent protein: DsRed2, C. Pavely, ed. (Pioneer Hi-Bred International, Inc: Pioneer, A DuPont Company).

Phuc, H.K., Andreasen, M.H., Burton, R.S., Vass, C., Epton, M.J., Pape, G., Fu, G., Condon, K.C., Scaife, S., Donnelly, C.A., *et al.* (2007). Late-acting dominant lethal genetic systems and mosquito control. BMC Biol *5*, 11.

Reyes, C., Fernadez, J., Freer, J., Mondaca, M., Zaror, C., Malato, S., and Mansilla, H.D. (2006). Degradation and inactivation of tetracycline by TiO2 photocatalysis. J Photochemistry and Photobiology A: Chemistry *184*.

Richards, H.A., Han, C.-T., Hopkins, R.G., Failla, M.L., Ward, W.W., and Stewart, C.N.J. (2003). Safety Assessment of Recombinant Green Fluorescent Protein Orally Administered to Weaned Rats. The Journal of Nutrition *133*, 1909-1912.

Robertson, A., Perea, J., Tolmachova, T., Thomas, P.K., and Huxley, C. (2002). Effects of mouse strain, postion of integration and tetracyline analogue on the tetracycline conditional system in transgenic mice. . Gene *282*, 65-74.

Sambrook, J., and Russell, D. (2001). Molecular Cloning: A Laboratory Manual; third edition (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press).

Sarfraz, M., Dosdall, L.M., and Keddie, B.A. (2006). Diamondback moth–host plant interactions: Implications for pest management. Crop Protection *25*, 625-639.

Sarmah, A., Meyer, M.T., and Boxall, A. (2006). A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics(VA's) in the environment. Chemosphere *65*, 725-759.

Schroeder, P.C., Shelton, A.M., Ferguson, C.S., Hoffmann, M.P., and Petzoldt, C.H. (2000). Application of synthetic sex pheromone for management of diamondback moth, Plutella xylostella, in cabbage. Entomologia Experimentalis et Applicata *94*, 243-248.

Seo, Y., B., C., Kang, C., Jeong, B., and Jung, Y.-S. (2010). Antibiotic Uptake by Plants from Soil Applied with Antibiotic-Treated Animal Manure. Korean J Soil Sci Fert *43*.

Shagin, D.A., Barsova, E.V., Yanushevich, Y.G., Fradkov, A.F., Lukyanov, K.A., Labas, Y.A., Semenova, T.N., Ugalde, J.A., Meyers, A., Nunez, J.M., *et al.* (2004). GFP-like proteins as ubiquitous metazoan superfamily: evolution of functional features and structural complexity. Molecular biology and evolution *21*, 841-850.

Shelton, A. (2001). Regional outbreaks of diamondback moth due to movement of contaminated plants and favourable climatic conditions. Paper presented at: Proceedings of the 4th International Workshop (Melbourne, Australia).

Simmons, G.S., McKemey, A.R., Morrison, N.I., O'Connell, S., Tabashnik, B.E., Claus, J., Fu, G., Tang, G., Sledge, M., Walker, A.S., *et al.* (2011). Field performance of a genetically engineered strain of pink bollworm. PLoS ONE *6*, e24110.

Someya, Y., Yamaguchi, A., and Sawai, T. (1995). A novel glycylcycline, 9-(N,N-dimethylglycylamido)-6-demethyl-6-deoxytetracycline, is neither transported nor recognized by the transposon Tn10-encoded metal-tetracycline/H+antiporter. Antimicrob Agents Chemother *39*, 247-249.

Talekar, N.S., and Shelton, A.M. (1993). Biology, Ecology, and the Management of the Diamondback Moth. Annu Rev Entomol *38*, 275-301.

USDA (2001). Availablity of Environmental Assessment and Finding of No Significant Impact for a Confined Field Test of Genetically Engineered Pink Bollworm M.a.R.P. United States Department of Agriculture, Animal and PLant Health Inspection Service, ed.

Vandenberg, J.D., Shelton, A.M., Wilsey, W.T., and Ramos, M. (1998). Assessment of *Beauveria bassiana* Sprays for Control of Diamondback Moth (Lepidoptera: Plutellidae) on Crucifers. Biological and Microbial Control *91*, 624-630.

Vreysen, M.J. (2005). Monitoring sterile and wild insects in area-wide integrated pest management programmes. In Sterile Insect Technique Principles and practice in area-wide integrated pest management, V.A. Dyck, J. Hendrichs, and A.S. Robinson, eds. (Springer, the Netherlands: Springer), pp. 325-361.

Walters, M., Morrison, N.I., Claus, J., Tang, G., Phillips, C.E., Young, R., Zink, R.T., and Alphey, L. (2012). Field longevity of a fluorescent protein marker in an engineered strain of the pink bollworm, Pectinophora gossypiella (Saunders). PLoS ONE *7*, e38547.

Wilke, A.B.B., Nimmo, D.D., St John, O., Kojin, B.B., Capurro, M.L., and Marrelli, M.T. (2009). Mini-review: Genetic enhancements to the sterile insect technique to control mosquito populations Asian Pacific Journal of Molecular Biology and Biotechnology *17*, 65-74.

Zalucki, M.P., Shabbir, A., Silva, R., Adamson, D., Shu-Sheng, L., and Furlong, M.J. (2012). Estimating the Economic Cost of One of the World's Major Insect Pests, Plutella xylostella (Lepidoptera: Plutellidae): Just How Long Is a Piece of String? Journal of Economic Entomology *105*, 1115-1129.

Annex One. Table 1 Analogues of Tetracycline

Analogues of tetracycline	**Synonyms of tetracyclines**	**Trade names**	**Use of the analogue**	**References**
Chlortetracycline		Aureomycin	Veterinary use Oral therapeutic use	(Chopra and Roberts, 2001) (http://www.octagon-services.co.uk/articles/chlortetracycline.htm)
Doxycycline		*Vibramycin*	Anti-protozoan (used as an anti-malarial), anti-bacterial and anti-helminthic.	(Leggat, 2009)
Oxytetracycline	Hydroxytetracycline	Terramycin	Veterinary and human therapeutic use (therapeutic use in fish Coyne et al. 1997) Oral and parenteral therapeutic use Use in plant bacteria control Oral and parenteral therapeutic use	(Chopra and Roberts, 2001)
6-Methylene-5-hydroxytetracycline	Methacycline	Rondomycin	Treatment of respiratory tract diseases	(Chopra and Roberts, 2001) xPharm: The Comprehensive Pharmacology Reference, 2007, Pages 1-5
6-Deoxy-5-hydroxytetracycline	Doxycycline	Vibramycin	Treatment of respiratory tract diseases	(Chopra and Roberts, 2001)
7-Dimethylamino-6-demethyl-6-deoxytetracycline	Minocycline	Minocin	Human therapeutic use	(Chopra and Roberts, 2001)
Glycylcyclines (a new form of tetracycline)				
9-(N,N-Dimethylglycylamido)-6-demethyl-6-deoxytetracycline			Broad spectrum antibiotic	(Someya et al., 1995)
9-(N,N-Dimethylglycylamido)-minocycline			*Selected species of Nocardia and rapidly growing mycobacteria*	(Brown et al., 1996)
9-(*t*-Butylglycylamido)-minocycline (Also known as Tertiary-butylglycylamidominocycline)		Tigilcycline	Selected human pathogens	(Chopra and Roberts, 2001)

6-Demethyl-7-chlortetracycline (Also known as demethylchlortetracycline)		Declomycin	Oral therapeutic use	(Chopra and Roberts, 2001)
2-N-Pyrrolidinomethyltetracycline (Also known as Rolitetracycline)		Reverin	Oral therapeutic use	(Chopra and Roberts, 2001)
2-N-Lysinomethyltetracycline (also known as Lymecycline)		Tetralysal	Oral and parenteral therapeutic use. Enhanced oral absorption (Human therapeutic use particularly acne)	(Chopra and Roberts, 2001)
N-Methylol-7-chlortetracycline (Also known as Clomocycline)		Megaclor	Oral therapeutic use	(Chopra and Roberts, 2001)
Hydroxytetracycline monohydrochloride			Registered as pesticides; Application tree injection additive to paints	(EPA, 1993)
Oxytetracycline calcium			Registered as pesticides; Application wettable powder, foliar application using ground or aircraft equipment It is also used in veterinary medicine	(EPA, 1993)

At present in human therapeutic use tetracyclines have applications in the treatment of a number of sexually transmitted diseases and treatment of respiratory tract infections (although this use has been in decline). Acne, rosacea and dental infections have also been treated with tetracyclines.

In veterinary medicine tetracyclines persist in treating, *Chlamydia psittaci* an infection of birds and Anaplasmosis, a ruminant tick borne infection. Tetracyclines are also used as plant protection products where they are effective against Fire blight which infects a number of fruit trees.

Table 2. Half-lives of tetracycline analogues

	Half-lives (hours)
Tetracyclines	
Chlortetracycline	5-6
Oxytetracycline	8-9.5
Tetracycline	8-10
Rolitetracycline	7-8
Second-generation tetracyclines	
Demethylchlortetracycline	10-13

Appendix V

Please see OX4319L-Pxy resistance management report (10/07/2013) attached to the release permit application for details on the investigation into the predicted synergistic resistance management benefit of combined use of fsRIDL diamondback moth and transgenic *Bt* broccoli.

Appendix VII

Please see OX4319L-Pxy molecular characterization report (10/07/2013) attached to the release permit application for details on characterization of the transgene insertion in the fsRIDL strain of diamondback moth OX4319L-Pxy.

Appendix VII

A review of all threatened and endangered species which could potentially be present at the release site.

Threatened or endangered species present at the release site

A search was carried out on the IUCN red list of threatened species (http://www.iucnredlist.org/search; accessed 12th August 2013) according to the following search criteria:

Show taxa:
Species
Search by taxonomy:
ANIMALIA
Search by location:
New York
(Native)
Search by systems:
Terrestrial
Match any habitat:
1. Forest
2. Savanna
3. Shrubland
4. Grassland
5. Wetlands (inland)
6. Rocky areas (eg. inland cliffs, mountain peaks)
7. Caves and Subterranean Habitats (non-aquatic)
8. Desert
14. Artificial/Terrestrial
16. Introduced vegetation
17. Other
18. Unknown

Match any threat:
1. Residential & commercial development
2. Agriculture & aquaculture
3. Energy production & mining
4. Transportation & service corridors
5. Biological resource use
6. Human intrusions & disturbance
7. Natural system modifications
8. Invasive & other problematic species & genes
9. Pollution
10. Geological events
11. Climate change & severe weather
12. Other options

Search by assessment:
Categories: CR, EN, VU, DD

This search found only seven species from which only one species, the New Cottontail Rabbit (*Sylvilagus transitionalis*), whilst this is not an aquatic species and has the potential for habitat overlap with the diamondback moth this species this species is a herbivore which is unlikely to directly interact with the released moth.

Further searches on the New York States Department for Environment (http://www.dec.ny.gov/animals/7494.html; accessed 12th August 2013) indicate that there are a number of endangered and threatened animals in the state which are not listed on the IUCN red list. Evaluation of these species for animals which might have a habitat which overlaps with the agricultural pest, diamondback moth, has been carried out and is presented in Table 1.

Overall there are a number of birds which could be present around abandoned agricultural land or nearby open grasslands however occurrence of any special concern bird species in a large highly managed farmland is unlikely. Insects form the diet of many small mammals, reptiles and birds however there is no one species which is reliant on the diamond back moth as a diet source. None of the species listed on the IUCN red list and New York States Department of Environment were reliant on any one species as a food source therefore the impact that this release of diamondback moths would have on the endangered, threatened or special concern animal populations is negligible.

Table 1. Species which could interact with the released Diamondback moths and are present in New York State and are Endangered, threatened or are of special concern.

Common name (*latin name*)	**Distribution**	**Threat**	**New York Status**	**Habitat overlap with diamondback moth**
Loggerhead Shrike (*Lanius ludovicianus*)	Most of Northern America from South Canada to South Mexico.	Threats to this species are unclear however it has been suggested that abandonment of farms and orchards have removed breeding sites. Roadkills and pesticide contamination could also be factors.	Endangered	Feed on beetles, grasshopper sand small rodents therefore it is unlikely that this species will have a direct interaction with the diamondback moth however this species is found in agricultural land.
Vesper Sparrow (*Pooecetes gramineus*)	Open grassy areas in North America	This species requires bare ground as breeding territory, abandonment of farms and regrown of forest areas threaten this species.	Special concern	This species has a diet consisting of insects and seeds. In New York this species is commonly found in the Erie-Ontario Plain and the central Appalachians and is not anticipated to be present in currently managed farmland.

Grasshopper Sparrow (*Ammodramus savannarum*)	Common throughout much of the United States and Southern Canada.	Threats include mowing of grasslands, use of pesticides and loss of grassland by plant succession.	Special concern	This species breeding in meadows, pastures, hayfields and croplands. There could be a habitat overlap between the diamondback moths and this species however interactions are likely to be limited as this is a widespread species and the proposed trial is small.
Golden-Winged Warbler (*Vermivora chrysoptera*)	Breeds throughout north central and north-eastern United States	Maintenance of early successional fields is required to preserve this species.	Special concern	This species breeds in early successional habitats therefore it could be present on any abandoned farmlands near to the release site. This is limited potential for habitat overlap and interaction with this species.

Appendix VIII

Bioinformatics analysis for risks of allergenicity and toxicity of proteins encoded by the two genes introduced into genetically engineered mosquitoes (*Aedes aegypti*), strain OX513A for production of sterile males to reduce vector transmission of important human diseases.

Study Title

Bioinformatics analysis for risks of allergenicity and toxicity of proteins encoded by the two gene genetically engineered mosquitos (*Aedes aegypti*), strain OXS13A for production of sterile males transmission of important human diseases

Authors

Richard E. Goodman

Study Completed On

5 September, 2013

Performing Laboratory

Food Allergy Research and Resource Program
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Laboratory Project ID

Study Number: REG Oxitec OXS13A

000075

Summary:
Genetically modified (GM) *Aedes aegypti* mosquitoes were developed by Oxitec Limited by the insertion
contiguous DNA segment comprising two genes to produce male mosquitoes that carry a lethal dominant
a promoter that allows successful reproduction only in cultured conditions in the presence of tetracycline.
the fluorescent red protein (DsRed2) from an Anthozoan species (corals and sea anemones) that has been
selection marker in a number of plant transformation events (Jach et al., 2001; Dietrich and Maiss, 2002;
Mirabella et al., 2004; Stuitje et al., 2003). The second gene regulates the reproductive development of th
production of a protein with a tetracycline-repressible transcriptional activator fused to and controlling a s
simplex virus VP16 protein. The second gene is a dominant lethal trait as the large majority of males and
trait die before functional adulthood. The engineered male mosquitoes are released into the environment t
females, but production of the next generation of progeny fails to develop to adulthood (Gossen and Bujar
2005; Phuc et al, 2007; Kongmee et al., 2010, Fu et al., 2010).

Regulatory agencies in countries where the genetically modified mosquitoes might be released will have t
human safety issues that might be presented by the GM mosquitoes. Although the safety assessment proc
modified organisms (GMOs) is normally applied in consideration of the safety of the organism for food us
majority of GMO's currently seen by regulators, in this case regulators may consider risks to humans that
mosquito proteins through bites by female GM mosquitoes (males do not bite). Potential exposure routes
exposure to female mosquitoes during mosquito rearing; incomplete sex separation leading to release of s
female OX513A mosquitoes together with the males; incomplete penetrance of the lethal trait leading to p
functional heterozygous female adult OX513A mosquitoes among the offspring of the released homozygo
and wild females. The exposure would be expected to occur through bites and saliva, not through dietary
humans). The primary risk of severe reactions would be assumed to be from the transfer of a protein that
allergic reactions in allergic individuals rather than sensitization de novo. Thus a bioinformatics evaluatic
expressed proteins are not allergens and are not highly identical to allergens as described here, was used a
evaluation step to minimize potential risks for humans. Finally, an evaluation was performed to consider
properties that would be considered toxic in the context of human exposure to a mosquito bite. The bioin
performed and reported here did not uncover any concerns of potential allergenicity, allergenic cross-react
toxicity that would demonstrate a need for further testing regarding safety. The conclusion of the bioinfor
and the evidence of expression patterns demonstrated that the DsRed2 and tTAV proteins do not present a
toxicity to humans.

1.0 Introduction

Oxitec Limited, UK has developed genetically modified *Aedes aegypti* mosquitoes by inserting DNA enco transgenic strain OX513A, using the LA513 transposon - also known as OX513 (Phuc et al., 2007). One l (Matz et al., 1999; Jach et al., 2001; Dietrich and Maiss, 2002; Wenck et al., 2003; Stuitje et al., 2003; Mi Phuc et al., 2007) was used as a marker protein for efficient selection of recombinant mosquitoes. The sec recombinant tetracycline repressible transcriptional activator protein (tTAV) described by Phuc et al. (200 produce homozygous male mosquitoes carrying a dominant lethal gene that prevents the successful develo from wild-type females mating with the GM males into adults Phuc et al , 2007).. Since this species is a ve Fever and Yellow Fever as well as other arboviral diseases, there is an urgent need to reduce the reproduct mosquito as one mechanism to reduce human disease. Laboratory as well as field trials are currently in pr effectiveness of this system (Bargielowski et al., 2011; Lacroix et al., 2012, Harris et al, 2011, Harris et al,

The human safety component of GM organisms (GMOs) normally focuses on the safety of food produced (Codex, 2003) and mosquitoes are not consumed by humans. However, the female mosquitoes must feed mammal hosts (usually humans in the case of *Aedes aegypti*) in order to provide nutritional requirements f Proteins in the saliva of mosquitoes are known to cause allergic reactions in humans. In addition, there is e protein to be introduced through a mosquito bite.

While it would be important to consider whether one or both of the newly introduced proteins (DsRed2 or mosquito might be present in saliva of GM mosquitoes, the primary evaluation should be whether the prot allergens or toxins, or whether they are nearly identical to any known allergen or toxic protein. That is wh requested a bioinformatics analysis of the two expressed proteins. In 2011 a bioinformatics study was perf on tTAV regarding potential similarities to allergens and toxins (Goodman, 2011). The current report pro new updated search that includes the DsRed2 marker protein and tTAV compared to all compiled allergen databases. This report describes the sequences, the datasets, the methods and the results of the bioinforma DsRed2 and tTAV proteins, using the amino acid sequence information provided by Oxitec as the query s

2.0 Purpose

The purpose of this study is to perform an evaluation of the potential allergenicity and toxicity of the DsRe
proteins that are encoded by the genes introduced in OX513A mosquitoes (*Aedes aegypti*) based on publis
the source of the genes and bioinformatics (sequence comparisons) of proteins with known allergens and t
to guide decisions regarding whether additional safety tests would be needed for evaluating these proteins
of allergy or toxicity if there is any human exposure through the bite of the female mosquitoes.

3.0 Methods

3.1 Scientific literature search strategies. The PubMed database (http://www.ncbi.nlm.nih.gov/pubme
U.S. National Library of Medicine was used as the primary data source for scientific literature on alle
The primary question is whether the source of the gene is a common cause of allergy or toxicity. The
publication, date and abstracts) from searches were saved to files for review. All publication abstract
reviewed and any likely relevant publications suggesting adverse health risks were investigated furth
journal articles.

3.1.1 Search for allergenicity. Search terms "gene source" AND "allergen" as well as "gene source"
were used on 10 August, 2013.

3.1.2 Search for toxicity. Search terms "gene source" AND "toxin" as well as "gene source" AND
on 10 August, 2013.

3.2 Amino acid sequences of query proteins. The mosquito transfection clone construct used to deve
described in Phuc et al., 2007.

3.2.1 DsRed2. The DsRed2 amino acid sequence from OX513A was supplied by Oxitec Limited (T
identical to the protein expressed by the transient expression vector pX-DR, GI:237652127 (C
except for an additional three amino acids at the N-terminus that was added in constructing th
transposon (personal communication, C. Beech, 17 May, 2013).

3.2.2 tTAV. The tTAV amino acid sequence from OX513A was supplied from Oxitec Limited (Tat
identical to GI: 60542785, Accession AJ865387, from Gong et al., 2005 and Phuc et al., 2007

Table 1 Amino acid sequences of the novel OXS13A transgenic event mosquito proteins.

Protein (in OXS13A) Common name of nearest source organism *Latin name*	**Protein name** Nearest published sequence GI: Protein length (aa) Percent ID to Oxitec [native publication]	**Protein sequence for OXS13A proteins (supplied by**
DsRed2 Coral *Discosoma sp.*	**DsRed** GI:55976617 225 amino acids 97% (219/225 aa) [Matz et al., 1999]	1 MARMASSENV ITEEMRYKVR MEGTVNGHEF EIEGEGEGRP 51 VTKGGPLPFA WDILSPQFQY GSKVYVKHPA DIPDYKKLSF 101 NFSCGGVATV TQDSSLQDGC FIYKVKFIGV NFPSDGPVMQ 151 KRLYPRDGVL KGEIKKALKL KDGGHYLVEF KSIYMAKKPV 201 KLDITSHNED YTIVEQYERT EGRHSTGL
rTAV GI: 60542785 Synthetic construct from two proteins Bacterial tetracycline repressor *Escherichia coli*	**Tetracycline repressor protein** (3-208 aa) GI:48618887 3 **Transactivating tegument protein** (211-338 aa)	1 MGSRLDKSKV INSALELINE VGIEGLTTRK LAQKLCVEQP 51 ALLDATAIEM LDREHTHFCP LEGRSWQDEL RNNAKSFRCA 101 HLGTRPTEKQ YETLENQLAF LCQQGFSLEN ALYALSAVGH 151 EHQVAKEERE TPTTDSMPPL LRQAIELFDH QGAEPAFLFG 201 QLKCESGSGP AYSAARTKNN YGSTIEGLLD LPDDAPEEA 251 PAGHTRRLST APPTDVSLGD ELHLDGEDVA MAHADALDDF 301 FGPGFTPHDS APYGALDMAD FEFEQMFTDA LGIDEYGG

3.3 Sequence database search strategies.

The AllergenOnline version 13 (http://www.allergenonline.org/) and the NCBI Entrez Protein (http://www.ncbi.nlm.nih.gov/BLAST/) databases were used as the protein amino acid data source comparisons for allergens and toxins (31,601,460 sequences on 14 August, 2013). The AllergenO updated in 12 February 2013 and is maintained by the Food Allergy Research and Resource Progr of Nebraska. Protein entries in the Entrez search and retrieval system is compiled and maintained

National Institutes of Health (U.S.A.). The database is potentially updated or modified daily, and th sequence searches by BLASTP is relevant to the dataset used in the BLASTP searches. BLASTP ar unique computer algorithms that provide similar local alignments and results if the appropriate scor criteria are used.

3.3.1 FASTA3 overall search of AllergenOnline. The potential sequential and inferred structura DSRed2 and tTAV proteins were evaluated using version 13 of AllergenOnline.org.

3.3.2 FASTA3 of AllergenOnline by 80 aa segments. This short segment search is based on the Codex (2003). The rationale is that this might help in identifying structural motifs, much shorter the which might contain a conformational IgE binding epitope. It should also help to identify potentiall proteins that are not true homologues of an allergen that have significant local identities that might immunological target for IgE antibodies in those with allergies to the matched allergen. A match of allergen will suggest further testing for possible cross-reactivity.

3.3.3 BLASTP of NCBI Entrez with "allergen" as keyword limit. The BLASTP is available on website (http://www.ncbi.nlm.nih.gov/BLAST/). The current version is BLASTP 2.2.28+ (28 July, search was used comparing the DSRed2 and tTAV query sequences against the entire Entrez Protein limit option selected to query entries for "allergen", to align only with proteins identified as allergen this BLASTP search is to ensure that a significant match with a newly discovered allergenic sequen been entered into AllergenOnline is not overlooked. Evaluation of the *E* value, the length of the alig percent identity of any identified match is necessary to judge the significance of any alignment usin

3.3.4 BLASTP of NCBI Entrez without keyword limit. The purpose of this BLASTP search is t DSRed2 and tTAV proteins to all known protein sequences to evaluate whether there are other simi other organisms that might provide information of safe exposure to homologues of this protein.

3.3.5 BLASTP of NCBI Entrez with "toxin" as keyword limit. The purpose of this BLASTP s matches to known toxic proteins (toxins) and if alignments share significant identities, to determine would require further testing. The sequences of the DSRed2 and tTAV proteins were compared to t database using "toxin" as a keyword search limit.

080000

4.0 Results and Discussion. The summary results for the PubMed search using the various protein source and the amino acid sequences of the DsRed2 and tTAV proteins, are presented here.

4.1 PubMed Searches. The PubMed scientific literature database was searched for evidence that the Dsl proteins are linked to allergy or toxicity. The search demonstrated that there is no published evidence that t allergens or toxins for mammals and no evidence that implies there might be an association with allergenic important consideration of the safety assessment related to potential toxicity, is an understanding of the me the newly expressed protein. If the protein is an enzyme, potential biological impacts of any new metaboli considered. If the inserted DNA or new protein is a transcription or translational regulator, potential target effects in source organisms or other host organisms should be considered. Identical or nearly identical gen OX513A mosquitoes have been have been inserted in transgenic animals, plants or arthropods by many sci proteins have been expressed within cells of various tissues of the hosts, without adverse impacts (e.g., for al., 2013; for tetracycline repressible transactivator protein see Gong et al., 2005). Since these proteins hav directly within the cells of diverse eukaryotic species without obvious toxic effects, it is highly unlikely the exogenous exposure by any route (ingestion, inhalation or injection) would have adverse biological impact mammalian health.

4.1.1 Allergenicity.

The terms "*Discosoma*" AND "allergen" as well as "*Discosoma*" AND "allergy" were used for evidence of allergy from the source organism of the DsRed2 protein, *Discosoma sp.* No ref when "allergen" was used. Two references were listed when "allergy" was used (Tetrina et al. 2008), but in both cases the studies used the DsRed protein as a fluorescent label to study disea there was no causal relationship with allergy.

Literature searches to evaluate the potential allergenicity (and toxicity) of the source for the somewhat complex. The source of the gene/protein for the tetracycline repressor protein (amin TAV) part of the protein is the Tn10 plasmid in *E. coli* (Gossen and Bujard, 1992; Altschmeid is produced as a fusion protein with the *Herpes simplex* virus protein 16 C by the design of the ((Gong et al., 2005, Phuc et al., 2007).

The *E. coli* bacterium has used as a cloning and expression host for many allergens and tox some strains of *E. coli* are known to produce toxins. Therefore it was expected that simple sear

AND "allergen" or "toxin" would find many irrelevant publications. A search of the terms "*Es*
"allergen" in PubMed returned 665 publication references. Since it is not possible to efficiently
publications for evidence of allergenicity, an additional term, "tetracycline" was used to refine t
tTAV is a tetracycline repressible transactivator. Only one publication was found then, Nishiha
describing the design of a transgenic bacterium that included regulatory expression of a cedar p
2 in *E. coli*. The results suggest that there are no natural allergens in *E. coli*. In order to ensure
the third term was not too restrictive, an additional strategy was used. Since, the sequence of th
to, or nearly identical to tetracycline repressible transactivator proteins that have been cloned fr
bacteria including *Salmonella sp.*, *Shigella sp.*, *Acinetobacter sp.*, and of the taxonomic family
a search was performed using "*Acinetobacter*" AND "allergen" as alternative search terms. F
were identified. Jadhav et al. (2013) found *Acinetobacter sp.* associated with isolates of nosoc
number of patients in hospitals, along with many other microbes, without any connection to all
(2011) published data from a study demonstrating that an intentional lung infection with *Acinet*
reversed the Th2 response of eosinophilia and "allergic response" to ovalbumin in a mouse moc
(2007) tested skin prick tests and IgE responses against a number of gram negative and gram po
commonly found in organic dust, using subjects who work in a poultry hatchery in Poland. The
detail proteins or specific data, only a trend that more workers showed positive precipitin reacti
(typically an IgG antibody complex reaction, indicating Th1 response) to *Escherichia coli* and *A*
baumannii along with other fungi and bacteria than control subjects. Valerio et al. (2005) demo
16S ribosomal DNA, that a few bacterial species were present in cultures and culture medium o
house dust mite (*Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*), including
however the prevalence was low and no proteins were identified or relationship to allergenicity
source of endotoxin in the allergenic mite cultures. Dutkiewicz et al. (2002) measured IgE level
number of bacterial species using sera from workers in a potato processing facility to evaluate p
work-related asthma. Although *Acinetobacter calcoaceticus* was mentioned, there was no refer
important target for IgE, whereas some other bacteria were implicated. Interestingly there is litt
literature for microbial proteins causing allergic reactions, so the importance of the few reports
precipitin antibody binding reactions to bacterial proteins is not obvious (RE Goodman, person
search with "Acinetobacter" AND "allergy" listed 48 references. An attempt to focus on the pr
by inclusion of a third term "tetracycline repressor protein", and did not identify any publicatior
the entire list of 48 publications demonstrated that most reported identification of *Acinetobacter*
microbes in a study group, often from an institute of allergy, or in some cases (e.g. Renz et al., 2

model was used to demonstrate that an infection with *Acinetobacter spp.* may suppress allergi
research points to the conclusion that exposure to *Acinetobacter spp.* protects individuals agai
due to the lipopolysaccharide content that skews reactions toward a Th1 response (Debarry et

The terms "Herpes" AND "VP16" AND "Allergy" were used to evaluate the source of the
portion of tTAV, with nine articles found. However all nine were only listed as they are from
National Institute of Allergy and Infectious Diseases. They did not demonstrate a relationship
simplex VP16 and allergy. A search with "Herpes" AND "VP16" AND "allergen" yielded no

Thus, a search of the literature for publications linking the source organisms, *Discosoma s*
surrogate source (*Acinetobacter sp.*), and *Herpes simplex* did not uncover evidence that would
or the proteins used in OX513A as likely allergens.

4.1.2 Toxicity. A search of PubMed using the taxonomic organism names for the taxonomic source
tTAV along with the terms "toxin" or "toxicity" AND the protein ("DsRed2" or "tTAV") wer

For "Discosoma" AND "toxin", no references were found. For "Discosoma" AND "toxic
were found. One by Long et al. (2005) presented information on a new, mutated form of the r
from *Discosoma sp.*, and suggested that the DsRed2 protein might be toxic if expressed in tra
referring to an earlier paper by Hadjantonakis and Papaioannou (2004). However, after a care
paper by Hadjantonakis and Papaioannou, I believe that their study did not demonstrate toxici
showed a failure to maintain highly expressing red-fluorescent stem cell lines, which might be
effects or a number of other technical issues. The second paper found in this search was by M
which they indicate using DsRed2 as a marker in transgenic mice did not demonstrate any to
transgenic mice. A further search with "DsRed2" found 217 publications and rapid review di
Instead the publications demonstrate that the DsRed2 protein can be expressed in various cell
and transfected mouse cells that have been used for various physiological or toxicological stu
toxicity. For example, a study by Ryu et al. (2013) reported successful transformation and use
with the DsRed2 reporter in a chimeric situation with maintenance of polyclonal tissues havin
EGFP transformed or DsRed2 transformed mice, with no reported toxicity. These were gener
lines of transgenic mice, one with DsRed2 and one with EGFP. The mice were surviving and
Nordin et al. (2013) demonstrated that the OX513A mosquito larvae, which do express both t

proteins can be consumed by two species of fly larvae (*Toxorhynchites spp.*) without apparent t
literature search, there does not appear to be published evidence that the DsRed2 protein is toxi
insects, plants or mammals.

The tTAV protein is a fusion of two proteins. The tetracycline repressible transactivator pro
The second of the fusion protein is from the *Herpes* simplex virus. The first search with "Esche
"toxin" returned over 26,890 publications. Adding the term "tetracycline" reduced that to 229 f
large number. Scanning titles of the 229 reveals that most are related to specific virulence factor
disease in livestock. Addition of the term "transactivator" removed all but one of the 229 publi
(1997) described tetracycline regulated expression of the diphtheria toxin A gene in human glio
element. The toxicity was related to the toxicity of the toxin A gene. These results demonstrate
specific bacterium is associated with toxic responses, there is no obvious indication that the tetr
protein is associated with toxicity.

As in the search for allergens, an additional search was performed using the *Actinetobacter s*
since the proteins are 99% identical and the species are from the same family of bacteria. The i
verify whether other researchers who study this species might have uncovered toxicity associate
A search with "Actinetobacter" AND "toxin" found 210 publications. There is ample evidence t
Actinetobacter sp. is an opportunistic pathogen and that it does produce toxins such as lipopolys
However, limiting the search by including AND "tetracycline" reduced the publication list to tw
antibiotic resistance (Wieczorek et al., 2008 and Loeffelholz et al., 1987). Addition of "transact
list to zero. Finally, "Acinetobacter" AND "tetracycline repressor protein" identified only one
Thompson et al. (2007), which did not identify toxicity associated with the protein. This search
evidence of any toxicity associated with the tetracycline repressor protein of *Actinetobacter sp.*

The VP16 protein of *Herpes simplex* virus is a transcriptional regulator that functions by bir
DNA sequences (TAATGARAT consensus sequence) present in virus genes that are up-regulat
of viral infection (Simmen et al. 1997). It functions within the cell and is not expected to be tak
cells or be active in cells that do not express the protein. In order to consider possible toxicity t
AND "toxicity" as well as "Herpes" AND "toxin" were search in PubMed, returning 1381 refer
references respectively. Adding "VP16" as a search term reduced the number to 7 and 6 referer
The publications were searched for evidence of toxicity related to VP16 and no direct evidence

studies generally relate to attempts to use *Herpes simplex* virus as a transfection vector for effi
mice for mechanistic studies or possibly to humans to treat disease. The vectors have caused t
number of publications are identified in this search. The conclusion of reading those publicati
relevant was negative, that is, no direct toxicity associated with the VP16 protein.

The combined search information failed to identify any evidence that the proteins fused to
tTAV protein or the DsRed2 proteins have any known toxicity.

4.2 Sequence comparison of the DsRed2 and tTAV proteins in OX513A to allergens. The amino aci
DsRed2 protein and the tTAV protein (Table 1) were compared to known allergens using both a full-leng
search and a sliding window of 80 comparisons against AllergenOnline.org, version 13. Additionally, a B
performed against the NCBI database using keyword search limits of "allergen" and "toxin".

4.2.1 Full length FASTA3 vs. AllergenOnline. Results of the full length FASTA3 searches of the l
AllergenOnline version 13 did not identify any significant alignment with an allergen. Scoring results
protein showing alignments with E scores less than 1 are shown in Table 3 and demonstrate no signifi
allergen. The low-level alignment with various sequences of the same carrot PR-10 protein are insigni
identities (%) are markedly below the level that is likely to indicate cross-reactivity (< 50% identity, A
also below the 35% identity level suggested by Codex (2003) as a match that may possibly be cross-re
no scientific basis for assuming the DsRed2 protein is sufficiently similar to any allergen to suspect cr
is no rationale for performing serum IgE tests based on overall alignment, the most predictive bioinfor

Table 2. Overall FASTA3 search of AllergenOnline.org database with the DsRed2 protein (225 a
Only proteins identified with matches to allergens or putative allergens in AllergenOnline.org version 1
score smaller than 1 are shown. None of the results were significant using the criteria of >35% identity
alignments of at least 80 amino acids.

Sequence GI #	Organism	Description	Length aa	E score	% Identity	aa A l
3023/9155	*Daucus carota* carrot	Pathogenesis Related protein (Bet v 1 like)	154	0.23	23.2	
302379157	*Daucus carota* carrot	Pathogenesis Related protein (Bet v 1 like)	154	0.36	23.2	
302379159	*Daucus carota* carrot	Pathogenesis Related protein (Bet v 1 like)	154	0.36	23.2	
19912791	*Daucus carota* carrot	Pathogenesis Related protein (Bet v 1 like)	154	0.42	23.2	
302379151	*Daucus carota* carrot	Pathogenesis Related protein (Bet v 1 like)	154	0.42	23.2	
302379153	*Daucus carota* carrot	Pathogenesis Related protein (Bet v 1 like)	154	0.58	23.2	

Similarly, the tTAV protein amino acid sequence does not show any significant FASTA alig
any known allergen in the AllergenOnline.org database (Table 3). The only alignment was to tr
of the sea snail or whelk (Neptunea polycostata) and at only 22.1% identity with an E score of 0
is considered irrelevant for potential cross-reactivity.

Table 3. Overall FASTA3 search of AllergenOnline.org database with the tTAV protein (338 amino

Only proteins identified with matches to allergens or putative allergens in AllergenOnline.org version 13, score smaller than 1 are shown. None of the results were significant using the criteria of >35% identity o alignments of at least 80 amino acids. In addition to testing the complete 338 aa tTAV protein, the 208 aa repressor protein and the VP16 127 aa proteins were tested independently and only the N-terminal 208 aa the intact tTAV aligned with tropomyosin of sea snail. The complete tTAV alignment is shown here.

Sequence GI #	Organism	Description	Length aa	E score	% Identity	aa Alig len
219806590	*Neptunea polycostata* Sea snail (whelk)	Tropomyosin	284	0.053	22.1	18

480000

4.2.2 Sliding 80-amino acid window FASTA3 vs. AllergenOnline.org database. Results of the comp DsRed2 and tTAV protein sequences were tested against all of the sequences in Allergenonline.org versio comparisons did not identify any possible match of > 35% identity with any known allergen in the databas cross-reactions for allergic individuals is very low, and the data indicate there is no reason to perform seru there is not a target allergen to suspect cross-reactivity. Tables 5 and 6 give an indication of the results obt and tTAV proteins respectively.

Table 4. Scanning 80-mer Sliding Window Search Results for DsRed2 protein

80mer Sliding Window Search Results

Database	AllergenOnline Database v13 (February 12, 2013)
Input Query	>DsRed2 MASSENVIKEFMRFKVRMEGTVNGHEFEIEGEGRPYEGRQTVKLKVTKGG LPFSWDILSPQFQYGSKVYVKHPADIPDYKKLSFPEGFKWERVMNFEDGGVVTVTQDSS LQDGEFIYKVKLRGTNFPSDGPVMQKKTMGWEASSERMYPEDGALKGEIKQRLKLKDGGH YDAEVKTTYKAKKPVQLPGAYNVNIKLDITSHNEDYTIVEQYERAEGRHSTG
Length	225
Number of 80 mers	149
Number of Sequences with hits	0

No Matches of Greater than 35% Identity Found

AllergenOnline Database v13 (February 12, 2013)

Table 5. Scanning 80-mer Sliding Window Search Results for tTAV protein of OX513A

80mer Sliding Window Search Results

Database	AllergenOnline Database v13 (February 12, 2013)
Input Query	>query MPSRLDKSKVINSALELLNEVGIEGLTTRKLAQKLGVEQPTLYWHVKNKRALL LDRHHTHFCPLEGESWQDFLRNNAKSFRCALLSHRDGAKVHLGTRPTEKQYE LCQQGFSLENALYALSAVGHFTLGCVLEDQEHQVAKEERETPTTDSMPPLLRQ QGAEPAFLFGLELIICGLEKQLKCESGSGPAYSRARTKNNYGSTIEGLLDLP DLADSRLSFLEAGHIRRLSTAPPTDYSLGDELHLDGEDVAMAHADALDDFDL PGPGFTPHDSAPYGALDMADFEFEQMFTDALGIDEYGG
Length	338
Number of 80 mers	259
Number of Sequences with hits	0

No Matches of Greater than 35% Identity Found

AllergenOnline Database v13 (February 12, 2013)

4.2.3 Eight amino acid match. Because some countries still require a search for any exact match o contiguous amino acids between the GM protein and any known allergen, that comparison was perfc AllergenOnline.org database, version 13. Both the DsRed2 and the tTAV full length sequences wer AllergenOnline.org search query box and tested. The results of these searches were negative.

4.2.4 BLASTP of NCBI Entrez using "allergen". The full-length amino acid sequences of the Ds proteins were compared to sequences in NCBI-Entrez, which were designated as "allergen" in the N August, 2013.

000089

The top two aligned matches to DsRed2 have (Tables 6) have significantly small *E* scores, sugg
evolutionary homology. However, the identity matches are low (25% in a 212 amino acid alignment
pollen allergen Cry j 1 fused to a green fluorescent protein and 24% in a 212 amino acid alignment
Bla g 1 fused to a green fluorescent protein). The low identity match is not considered a likely indic
reactivity (Alberse, 2000). But importantly, the two matched sequences are synthetic constructs th
fluorescent marker protein that was originally derived from *Aequorea victoria* (GI:634009) describe
The alignments of DsRed2 to those two synthetic constructs were only in the region of the green flu
which is not known to cause allergies. The other alignments of DsRed2 were not significant as judg
score values (>0.001) and low identity matches (25% to 57%) with very short-partial protein alignm
proteins would not be considered homologues of the DsRed2.

Table 6. BLASTP of NCBI Entrez with DsRed2 using the keyword "allergen". The scoring alignment
10 are shown for this DsRed2 protein vs. all proteins labeled with the keyword "allergen" in the NCBI Entrez
2013, using BLASTP. The sequence identities are low and / or the length of alignments are very short, indicti
and that the overall structure is unlikely to be similar. Thus even if the NCBI sequence is a proven allergen, t
likelihood of cross-reactivity by the DsRed2 protein.

Sequence GI#	Organism	Description	Length aa	*E* score	
223005744	*Synthetic construct* Cryptomera japonica AND GFP origin *Aequorea victoria*	Synthetic construct of T cell epitopes of the Cry j 1 *Cryptomera japonica)* and green fluorescent protein	412	1e-16	
529482053	*Synthetic construct* *Blatella germanica* AND GFP origin *Aequorea*	Synthetic construct of cockroach allergen Bla g 1 and green fluorescent protein	416	1e-14	

	victoria			
116333554	*Lactobacillus brevis* bacteria	Hypothetical protein LVIS_0955	321	0.34
156370878	*Nematostella vectensis* Sea anemone	Predicted protein sea anemone MD-2 like protein	299	3.1
493609361	*Oscillochloris trichoides* bacteria	Allergen V5/Tpx-1 family protein	495	4.0
403416894	*Fibroporia radiculosa* Brown rot fungus	Predicted protein MD-2 like protein	524	4.1

The tTAV protein only showed one very minor alignment by BLASTP limited by "allergen" (T alignment is insignificant and does not represent an indication of possible cross-reactivity as descrit (2000) and Goodman et al. (2008).

Table 7. BLASTP of NCBI Entrez with tTAVusing the keyword "allergen". The only identified scoring al
scores below 10 is shown for this tTAV. The sequence identities are low and / or the length of alignments are v
unlikely homology and that the overall structure is unlikely to be similar. Thus even if the NCBI sequence is a f
is very little likelihood of cross-reactivity by the tTAV protein.

Sequence GI#	Organism	Description	Length aa	E score	%
493199135	*Treponema vincentii* Spirochette	Hypothetical protein, SCP like protein	221	1.7	

4.2.5 BLASTP of NCBI without keyword limit. The full-length of the DsRed2 and tTAV proteins
all sequences in NCBI-Entrez database on 14 August, 2013. The DsRed2 protein has 100 alignments
identity, mostly with synthetic constructs of transfection vectors as it is a marker gene/protein. Simil
alignments of near-100% identity with synthetic constructs with partial alignments to tetracycline rep
with *Herpes simplex* 1 VP16. Because so many entries in NCBI show synthetic constructs that resear
transfecting various organisms, it is difficult to trace out the origin of the proteins. The original litera
necessary to evaluate the origins (see the Introduction and Section 4.1 for references).

4.3 BLASTP of NCBI Entrez with "toxin". The full-length sequences of the DsRed2 and tTAV protei
sequences in NCBI-Entrez, which were designated as "toxin" in the NCBI database on 14 August, 2013.
proteins with E scores smaller than 10 are shown for each of the two proteins (Tables 8– 9).

DsRed2. The best scoring alignment with DsRed2 was to synthetic constructs. For the best aligned pro
alignment of 98% identity is to a 223 amino acid portion of the synthetic protein construct that is from a
(DsRed1) that is in the Green Fluorescent protein family, with a secondary poor alignment of 24% identi
acids to the green fluorescent protein (Liu et al. 2011). The second best aligned sequence was a similar c
authors (Liu et al., 2011), using slightly different order and sequences. The construct was used to transfor
physiological function. There is no evidence that the red fluorescent portion of the protein (with nearly 1(
DsRed2) is toxic. The third sequence with the next best alignments is a similar synthetic construct for te
using a botulinum toxin substrate, by a different group, but with two green fluorescent proteins rather tha
red (Itoh et al., 2002). The fourth protein sequence with an alignment is another cloning construct with a
protein and with a botulinum toxin in the same construct (Band et al. 2010). The fifth protein, phytoene
the toxic bacterium Corynebacterium ulcerans is the highest scoring protein that is not a fluorescent mark
et al., 2012). However, the alignment is poor and the sequence is merely one of the sequences discovered
sequencing of the bacterium. The protein sequence (GI:397655072) was then compared to all of NCBI b
turns out to be one of the highly conserved enzymes related to phytoene desaturases, that are rather ubiqu
evidence was found that this protein is toxic and furthermore, the sequence alignment to DsRed2 is weak
protein is a transcription regulator from *Clostridium botulinum*, a toxic organism. A search of PubMed
publications that describe toxicity associated with the transcriptional regulators of *C. botulinum*. In genet
regulators are only functional if they are expressed inside the cell of the organism containing the gene tha
found no evidence that this protein could be taken in by the cells of other, non-bacterial organisms and ca
changes.

Table 8. BLASTP of NCBI Entrez "toxin" with DsRed2 from OX513A. The best scoring alignments to putativ
NCBI Entrez database on 14 August, 2013, were identified by BLASTP with the full-length sequence of the DsRed2
OX513A.

Sequence GI#	Organism	Description	Length aa	*E* score	%
172054575	Synthetic construct Primary alignment	EGFP-Pak1-Rac1-dsRed1-CAAX fusion with Rac related to a botulinum toxin substrate Alignment only to red fluorescent protein peptide	798	2e-145	
172054575	Synthetic construct Secondary alignment	EGFP-Pak1-Rac1-dsRed1-CAAX fusion with Rac related to a botulinum toxin substrate Alignment only to green fluorescent protein peptide	798	1e-14	
167965513	Synthetic construct Primary alignment	dsRed1/Pak1/Rac1/ECFP fusion protein Alignment to red fluorescent protein	775	1e-144	
167965513	Synthetic construct Secondary alignment	dsRed1/Pak1/Rac1/ECFP fusion protein Alignment to green fluorescent protein	775	4e-14	
230959331	Synthetic construct Primary alignment	Raichu-1011x, rac and cdc42 Alignment to green fluorescent protein	763	1e-15	
230959331	Synthetic construct Secondary alignment	dsRed1/Pak1/Rac1/ECFP fusion protein Alignment to green fluorescent protein	763	2e-14	
259490938	Synthetic construct	deltaLC-GFP-BoNT/A rev	1230	2e-15	
397655072	Corynebacterium ulcerans bacteria	Phytoene dehydrogenase	544	0.17	
182674319	Corynebacterium botulinum bacteria	Transcriptional regulator AraC family	395	8.4	

tTAV. The best scoring alignment with tTAV was to a tetracycline repressor protein TetR from *Escherichia fergu* alignment is very significant, however the bacteria is considered a source of toxicity. But the TetR protein has vei TetR proteins of many species of bacteria. It is also a regulatory protein expressed in the bacteria, which acts on t to suppress or promote expression of bacterial proteins. It is not known to be taken up by cells of other organisms effects. The next alignment is almost identical to the first and from another species of the genus. In fact it is 100% (196 aa) segment of the protein of tTAV. Running a BLAST comparison of these two proteins demonstrates very with many cloned gene/proteins as the activity is useful for tetracycline dependent gene regulation, as used in OX. a few alignments of much lower length and identity, all beginning near the N-terminus of the tTAV protein. Howe very poor alignment to a segment of tTAV beginning at amino acid 224, but that was also a transcriptional regulat found of homology of the tTAV protein to a true toxin.

Table 9. BLASTP of NCBI Entrez "toxin" with tTAV protein from OX513A. The best scoring alignments to in the NCBI Entrez database on 14 August, 2013, were identified by BLASTP with the full-length sequence of the OX513A. There were 93 alignments with *E* scores less than 10 using this sequence and the term "toxin" by BLAS cases it is clear that the sequence is "identified" as a toxin due to the toxicity of the source organism (from a numt Importantly, only a few sequences had high identity matches over any extensive length of sequence. Those were

Sequence GI#	Organism	Description	Length aa	*E* score
218561676	*Escherichia fergusonii* bacteria	Tetracycline repressor protein TetR	208	3e-150
388377844	*Escherichia coli* bacteria	Tetracycline repressor protein TetR	197	4e-142
394430501	*Escherichia coli* bacteria	Tetracycline repressor protein TetR	141	1e-81
190903672	*Escherichia coli* bacteria	Tetracycline repressor protein class A from transposon 1721	219	2e-65
388363196	*Escherichia coli* bacteria	Tetracycline repressor protein from E. coli strain o111:H8, TetR C	225	2e-64

310286451	*Escherichia coli* bacteria	Tetracycline repressor protein Class A from E. coli	217	1e-63
397654352	*Corynebacterium ulcerans* bacteria	TetR family transcriptional regulator	203	1e-12
300850578	*Enterococcus faecalis* bacteria	TetR family transcriptional regulator	220	2e-11
292642929	*Enterococcus faecalis* bacteria	TetR family transcriptional regulator	222	2e-08
429514288	*Enterococcus faecalis* bacteria	TetR/AcrR family transcriptional regulator	189	1e-5

4.4 Bioinformatics summary for the DsRed2 and tTAV proteins of OX513A. Although the results o the sources of the genes transferred into OX513A were challenging due to the some extensive annotation or toxicity associated with the source organisms, careful evaluation of the abstracts and publications as w did not identify publications with sufficient evidence to suspect the DsRed2 or tTAV proteins represent t toxicity.

None of the results from the bioinformatics searches of the DsRed2 or tTAV protein amino acid s these proteins represent a risk of allergy or toxicity that is greater than a typical dietary protein. There we either protein to known allergens with more than 50% identity over the full-length. There were no matche over 80 or more amino acid segments compared to known or putative allergens. There were no identical contiguous amino acid segments. These highly conservative comparisons did not identify sequence simil suggest the proteins are allergens or are sufficiently similar to an allergen to cause cross-reactions. They matches to toxins to suggest they may be toxic.

5.0 Conclusions

No convincing evidence was found to suggest that the DsRed2 protein or the rTAV protein expressed in the mosquitos represent risks of allergy or toxicity to humans (or other mammals). Based on the guidelines of Alimentarius Commission (2003 and 2009), and on common practices for evaluation of potential risks of al from GMO (plants, animals or microbes), there is no reason to perform additional tests to evaluate potentia toxicity for these proteins. Although the guidelines are intended primarily evaluating potential food safety potential risks from genetically engineered organisms, the same safety evaluation process is scientifically s for evaluating other potential routes of exposure, namely via airway (inhalation of insect body parts) or thro (e.g. mosquito saliva). There is no evidence that these proteins pose any risk of eliciting allergic or toxic re

6.0 References

Aalberse RC. (2000). Structural biology of allergens. *J Allergy Clin Immunol* 106:228-238.

Altschmied L, Baumeister R, Pfleiderer K and Hillen W. (1988). A threonine to alanine exchange at position 40 of recognition of the sixth base pair of *tet* operator from GC to AT. EMBO J 7(12):4011-4017.

Band PA, Blais S, Neubert TA, Cardozo TJ, Ichtchenko K. (2010). Recombinant derivatives of botulinum neurotox trafficking studies and neuronal delivery. *Protein Expr Purif* 71(1):62-73.

Bargielowski I, Nimmo D, Alphey L, Koella JC. (2011). Comparison of life history characteristics of the genetical and a wild type strain of *Aedes aegypti*. *PLOS One* 6(6): e20699. doi:10.1371/journal.pone.0020699.

Baur I PW, Oberer DM, Breakefield XO, Reeves SA. (1997). Regulated expression of the diphtheria toxin A gene i using prokaryotic transcriptional control elements. J Neurosurg 87(1):89-95.

Chen S, Songkumarn P, Liu J, Want GL. (2009). A versatile zero background T-vector system for gene cloning an *Plant Physiol* 150(3):1111-1121.

Codex Alimentarius Commission. (2003). Alinorm 03/34: Joint FAO/WHO Food Standard Programme, Codex Ali Commission, Twenty-Fifth Session, Rome, Italy 30 June-5 July, 2003. Appendix III, Guideline for the conduct

assessment of foods derived from recombinant-DNA plants and Appendix IV, Annex on the assessment of possi
47-60.

Debarry J, Hanuszkiewicz A, Stein K, Holst O, Heine H. (2010). The allergy –protective properties of *Acinetobacte*
imparted by its lipopolysaccharide. Allergy 65:690-697.

Dietrich C, Maiss E. (2002). Red fluorescent protein DsRed from *Discosoma sp.* as a reporter protein in hi
Biotechniques 32(2):286-293.

Dutkiewicz J, Skorska C, Krysinska-Traczyk E, Cholewa G, Sitkowska J, Mianowski J, Gora A. (2002). F
of potato processing workers to work-related microbial allergens. 9(2):237-242.

Fu G, Lees RS, Nimmo D, Aw D, Jin L, Gray P, Berendonk TU, White-Cooper H, Scaife S, Phuc HK, Ma
Jasinskiene N, James AA, Alphey L. (2010). Female-specific flightless phenotype for mosquito control
USA 107(10):4550-4554.

Gong P, Epton MJ, Fu G, Scaife S, Hiscox A, Condon KC, Condon GC, Morrison NI, Kelly DW, Dafa'all
Alphey L. (2005). A dominant lethal genetic system for autocidal control of the Mediterranean fruitfly. .
23(4):453-456.

Goodman RE. (2008). Performing IgE serum testing due to bioinformatics matches in the allergenicity assessment
Chem Toxicol 46:S24-S34

Goodman RE. (2011). Bioinformatics evaluation of transgenic protein tTAV from mosquito (2 August, 2011, unpu

Goodman RE, Vieths S, Sampson HA, Hill D, Ebisawa M, Taylor SL, van Ree R. (2008). Allergenicity assessmen
modified crops—what makes sense? *Nat Biotechnol* 26(1):73-81.

Gossen M, Bujard H. (1992). Tight control of gene expression in mammalian cells by tetracycline-responsive prom
Sci USA 89:5547-5551.

Jach G, Binot E, Frings S, Luxa K, Schell J. (2001). Use of red fluorescent protein from *Discosoma sp.* (ds
plant gene expression. *Plant Journal* 28(4):483-491.

Jadhav S, Sahasrabudhe T, Kalley V, Gandham N. (2013). The microbial colonization profile of respirator significance of the role of disinfection: a blinded study. J Clin Deagn Res 7(6):1021-1026.

Hadjantonakis AK, Papaioannou VE. (2004). Dynamic in vivo imaging and cell tracking using a histone fusion in mice. BMC Biotechnol 4:33 doi:10.1186/1472-6750-4-33.

Itoh RE, Kurokawa K, Ohba Y, Yoshizaki H, Mochizuki N and Matsuda M. (2002). Activation of rac and by fluorescent resonance energy transfer-based single-molecule probes in the membrane of living cells. 22(18):6582-6591.

Lacroix R, McKemey AR, Raduan N, Kwee Wee L, Ilong Ming W, Guat Ney T, Rahidah AAS, Salman S, Nordin O, Ilanum ATN, Angamuthu C, Marlina Mansor S, Lees RS, Naish N, Scaife S, Gray P, Labbe D, Alphey L, Vasan SS, Lim LH, Wasi AN, Murad S. (2012). Open field release of genetically engineer *Aedes aegypti* in Malaysia. PLOS One 7(8): e42771. Doi:10.1371/journal.pone.0042771.

Liu R, Ren D, Liu Y, Deng Y, Sun B, Zhang Q, Guo X. (2011). Biosensors of DsRed as FRET partner wi quantitatively imaging induced activation of Rac, Cdc42 in living cells. *Mol Imaging Biol* 13(3):424-43

Loeffelholz MJ, Rana F, Modrzakowski MC, Blazyk J. (1987). Effect of plasmid RP1 on phase changes in membranes and lipopolysaccharide from *Acinetobacter calcoaceticus*: a Fourier transform infrared study 26(21):6644-6648.

Long JZ, Lacoan CS, Hadjantonakis AK. (2005). Genetic and spectrally distinct in vivo imaging: embryoi mice with widespread expression of a monomeric red fluorescent protein. BMC Biotechnol 5:20 doi:1(20.

Matz MV, Fradkov AF, Labas YA, Savitsky AP, Zaraisky AG, Markelov ML, Lukyanov SA. (1999). Fluo from nonbioluminescent Anthozoa species. Nat Biotechnol 17(10):969-973.

Mirabella R, Franken C, van der Krogt GNM, Bisseling T, Geurts R. (2004). Use of the fluorescent timer reporter to monitor dynamics of gene activity in plants. *Plant Physiol* 135:1879-1887.

Murata K, Imai M, Nakanishi S, Watanabe D, Pastan I, Kobayashi K, Nihira T, Mochizuki H, Yamada S, I
M. (2011). Compensation of depleted neuronal subsets by new neurons in a local area of the adult olfac
Neurosci. 31(29):10540-10557.

Nishihara K, Kanemori M, Kitagawa M, Yanagi H, Yura T. (1998). Chaperone coexpression plasmids: dif
synergistic roles of DnaK-DnaJ-GrpE and GroEL-GroES in assisting folding of an allergen of Japanese
in *Escherichia coli*. Appl. Environ Microbiol 64(5):1694-1699.

Nordin O, Donald W, Ming WH, Ney TG, Mohamed KA, Halim NA, Winskill P, Hadi AA, Muhammad Z
S, McKemey AR, Beech C, Shahnaz M, Alphey L, Nimmo DD, Nazni WA, Lee HL. (2013). Oral inge
RIDL *Ae. aegypti* larvae has no negative effect on two predator Toxorhynchites species. PLOS One 8(2
10.1371/journal pone.0058805.

Phuc HK, Andreasen MH, Burton RS, Vass C, Epton MJ, Pape G, Fu G, Condon KC, Scaife S, Donnelly
White-Cooper H, Alphey L. (2007). Late-acting dominant lethal genetic systems and mosquito control.

Qiu H, Kuolee R, Harris G, Zhou H, Miller H, Patel GB, Chen W. (2011). *Acinetobacter baumannii* infec
eosinophilia and lung pathology in a mouse model of allergic asthma. PLOS One 6(7):e22004. Doi
10.1371/journal.pone.0022004.

Renz H, Conrad M, Brand S, Teich R, Garn H, Pfefferle PI. (2011). Allergic diseases, gene-environment i
66 (Suppl 95):10-12.

Ryu JY, Siswanto A, Harimoto K, Tagawa YI. (2013). Chimeric analysis of EGFP and DsRed2 transgeni
polyclonal maintenance of pancreatic acini. *Transgenic Res* 22:549-556.

Sekizuka T, Yamamoto A, Komiya T, Kenri T, Takeuchi F, Shibayama K, Takahashi M, Kuroda M, Iwak
Corynebacterium ulcerans 0102 carries the gene encoding diphtheria toxin on a prophage different fror
NCTC 12129. *BMC Microbiol* 12(1):72.

Simmen KA, Newell A, Robinson M, Mills JS, Canning G, Handa R, Parkes K, Borkakoti N, Jupp R. (19
interactions in the *Herpes simplex* virus type 1 VP16-induced complex: VP16 peptide inhibition and m
host cell factor requirements. J Virol 71(5):3886-3894.

Skorska C, Mackiewicz B, Golec M, Cholewa G, Chmielowiec-Korzeniowska A, Dutkiewicz J. (2007). E
exposure to organic dust in workers of a modern hatchery. Ann Agric Environ Med 14(2):341-345.

Stuije AR, Verbree EC, van der Linden KH, Mietkiewska EM, Nap JP, Kneppers TJA. (2003). Seed-exp
proteins as versatile tools for easy (co)transformation and high-throughput functional genomics in *Ara*
Biotechnol J 1:301-309.

Tawfik VL, Regan MR, Haenggeli C, LaCroix-Fralish ML, Nutile-McMenemy N, Perez N, Rothstein JD,
Propentofylline-induced astrocyte modulation leads to alternations in glial glutamate promoter activati
nerve transection. *Neuroscience* 152(4):1086-1092.

Tetrina NL, Levenson EA, Ehrenfeld E. (2010). Viable polioviruses that encode 2A proteins with fluore
Virol 84(3):1477-1488.

Thompson SA, Maani EV, Lindell AH, King CJ, McArthur V. (2007). Novel tetracycline resistance dete
an environmental strain of *Serratia marcescens*. Appl Env Microbiol 73(7):2199-2206.

Tsien RY. (1998). The green fluorescent protein. *Annu Rev Biochem* 67:509-544.

Valerio CR, Murray P, Arlian LG, Slater JE. (2005). Bacterial 16S rhosomal DNA in house dust mite cu
Immunol 116(6):1296-1300.

Wenck A, Pugieux C, Turner M, Dunn M, Stacy C, Tiozzo A, Dunder E, van Grinsven E, Khan R, Sigar
Reed J, Drayton P, Oliver D, Trafford H, Legris G, Rushton H, Tayab S, Launis K, Chang Y-F, Chen J
(2003). Reef-coral proteins as visual, non-destructive reporters for plant transformation. *Plant Cell Re*

Wieczorek P, Sacha P, Hauschild T, Zorawski M, Krawczyk M, Tryniszewska E. (2008). Multidrug resist
baumannii-the role of AdeABC (RND family) efflux pump in resistance to antibiotics. Folia Histochem
46(3):257-267.

7.0 Appendix 1, AllergenOnline Database, version 13, February, 2013 (see attached PDF).

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ANIMAL AND PLANT HEALTH INSPECTION SERVICE
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(Genetically Engineered Organisms or Products)

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RELEASE:	NY

PERMIT NUMBER	13-297-102r
DATE ISSUED:	November 10, 2014
EFFECTIVE:	November 10, 2014
EXPIRES:	**November 10, 2017**
INTRODUCTION TYPE	Release
PERMIT TYPE	Standard
PURPOSE OF PERMIT	Tracitional

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Regulated Article: Plutella xylostella

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BIOTECHNOLOGY REGULATORY SERVICE

APPLICATIONS FOR PERMIT OR COURTESY PERMIT UNDER 7 CFR 340

(Genetically Engineered Organisms or Products)

1. NAME, ADDRESS, TELEPHONE, AND EMAIL OF APPLICANT

Name: Dr. Anthony Shelton
Position:
Organization: Cornell University/NYSAES
Organization Unique ID:
Address: 630 W. North St.
(b)(6)
Geneva, NY (b)(6)
County/Province:
Township/Island:
(b)(6)
Day Telephone:
FAX:
Alternate:
Email 1: (b)(6)
Email 2:

2. INTRODUCTION TYPE

- [ ] Importation
- [ ] Interstate Movement
- [ ] Interstate Movement and Release
- [x] Release

3. PERMIT TYPE

- [x] Standard Permit
- [ ] Courtesy Permit

4. PURPOSE OF PERMIT

- [ ] Industrial Product
- [ ] Pharmaceutical Product
- [ ] Phytoremediation
- [x] Traditional

5. CONFIDENTIAL BUSINESS INFORMATION VERIFICATION (CBI)

Does this application contain CBI? [ ] Yes [x] No
CBI Justification:
N/A

6. REQUEST TYPE

[x] **New** [ ] **Amendment** [ ] **Renewal**

Amendment/Renewal Description:
Previous Permit Number(s):

7. MEANS OF MOVEMENT

Import by air; releases manually from the ground/vehicles.

8. VARIANCE VERIFICATION

Have you previously applied for variance(s) that you wish to apply to this permit? [ ] Yes [x] No
Variance Number(s):

If so, describe in a brief summary how the variance will be applied:
N/A

9. REGULATED ARTICLE

Scientific Name: Plutella xylostella

Common Name: Diamondback moth

Cultivar and/or Breeding Line:

Any biological material (e.g., culture medium, or host material) accompanying the regulated Article during movement:
Artificial insect diet. This diet will be frozen at -15°C for 12 h prior to import.

Country and locality where the donor organism, recipient organism, and vector or vector agent were collected, developed, and produced:
All final engineering of the transforming constructs was performed at Oxitec Ltd, in the United Kingdom.
The genes used from the donor organisms and the piggyBac-derived portions of the vectors used to build the transforming construct were cloned off-site. The recipient organism is the moth, Plutella xylostella, which is endemic in temperate regions around the world, including the USA. The recipient Plutella xylostella strain for the transformation was a wild-type strain obtained from Syngenta (public limited company), UK, which has been reared in Oxitec insectaries since 2008.

Processes, Procedures, and Safeguards Description:

WARNING: Any use of ePermits to make materially false, fictitious, or fraudulent statements or representations is subject to civil penalties of up to $250,000 (7 U.S.C. § 7734(b)) or punishable by a fine of not more than $10,000, or imprisonment of not more than 5 years, or both (18 U.S.C. §1001).

This is a permit request for 3 years of seasonal releases (April to October) of a female-lethal, genetically marked diamondback moth (maximum 100,000 moths/week), in brassica fields at the Cornell University research station, Geneva NY.
Males of the transgenic moths will be released in cultivated brassica plots and biological parameters of these moths, such as dispersal and persistence, measured using traps, for example baited with synthetic sex pheromone. The moths of the OX4319L-Pxy strain carry a stable, heritable marker the DsRed2 fluorescent protein, viewed by fluorescence microscope or detected by PCR and their female progeny die in the absence of a dietary repressor (tetracycline or suitable analogues supplied in their artificial diet). The male-selecting (female-lethal) penetrance of the strain is >99% (Jin et al. 2013). The marker provides a means of distinguishing released moths from wild moths, and female-lethality is a self-limiting trait in the wild.
All genetically modified moths will be reared in insectaries at Cornell University, Geneva NY. The facilities and their general operation have been inspected and approved through a previous importation permit (12-227-102m). Larval rearing will be conducted in quarantine using the same approved procedures as in this previous permit. Only moths homozygous for the conditional lethal transgene, reared off tetracycline, will be released. Adult moths will be transported in sealed containers, with at least two layers of containment, labeled as follows "CORNELL UNIVERSITY GENETICALLY MODIFIED MOTHS FOR RELEASE AT CORNELL UNIVERSITYS NEW YORK STATE AGRICULTURAL EXPERIMENT STATION - AUTHORIZED PERSONNEL ONLY". Insects will be transported by hand or in a vehicle; for each batch the number of containers and identity of member of staff supervising the release will be recorded.

The transgenic diamondback moths encode no toxic or allergen proteins. The DsRed2 marker protein has been evaluated in a New Protein Consultation by the FDA-CFSAN in the USA for human safety, and they raised no objections to its use in corn plants. This involved an assessment of the amino acid sequence using bioinformatics analyses in accordance with the Guidance provided by Codex (2003), the lability of the protein in simulated gastric fluid (SGF) and an examination of the gene source and history of exposure, as well as the toxicity of the protein using bioinformatics analysis. The amino acid sequence in OX4319L-Pxy is the same as that evaluated in the NPC. It has been further evaluated in an Environmental Assessment (EA) by the USDA (http://www.aphis.usda.gov/brs/aphisdocs/08_33801p_dpra.pdf), which concluded that the corn transformation event that contained the DsRed2 gene was unlikely to become a plant pest risk. Additional EAs on another GE moth, GE pink bollworm, expressing fluorescent genes similar to DsRed2 have also been conducted (http://www.gpo.gov/fdsys/pkg/FR-2006-04-19/html/E6-5878.htm) and concluded that it was unlikely to present any hazard to the environment.
The other protein coding region, tTAV, is regulated by sequences from the sex-determination gene, doublesex, from pink bollworm (Pectinophora gossypiella), that produce different splice variants in males and females: the female transcript comprises coding sequence for the tetracycline-repressible transcription factor, tTAV, which interacts with the upstream tetracycline response element, tetO (or tRE), to form a positive-feedback loop that results in insect lethality prior to adulthood. Under the control of the doublesex sex-alternate splicing, lethality is induced only in females. The tTAV amino acid sequence in OX4319L-Pxy has also been evaluated independently using the bioinformatics analyses provided by Codex (2003) for both potential allergenicity and toxicity. No homologies with known allergens or toxins were determined. This study is available on request.
Tetracycline can be provided to the insect in larval artificial diet to suppress female death and permit colony rearing in the laboratory. Neither piggyBac transposase activity nor any antibiotic resistance is conferred to the transgenic diamondback moth by the introduced genetic material.
This female-specific lethal trait was previously discussed in a USDA Environmental Impact Statement published in October 2008, entitled Use of Genetically Engineered Fruit Fly and Pink Bollworm in APHIS Plant Pest Control Programs, which concluded that the use of genetically engineered fruit flies and pink bollworm in APHIS plant pest control programs were the environmentally preferred alternative (Record of Decision (Federal Register Vol 74 (87) 21314 2009).
Reference:
Jin L, et al. 2013 Engineered female-specific lethality for control of pest lepidoptera. ACS Synthetic Biology, 2:160-166.

10. ARTICLE SUPPLIER AND/OR DEVELOPER

Name	Location	Contact Information
Dr. (b)(6)	Oxitec Ltd 71 Milton Park Abingdon OX144RX United Kingdom **County:** Oxford	**Day Telephone:** 0044-1235-832393 **FAX:** **Email:** (b)(6)

11. PHENOTYPES/GENOTYPE

1) Phenotypic Designation Name: visual marker; repressible lethality

Identifying Line(s): OX4319L-Pxy, OX4319N-Pxy, OX4767A-Pxy

Construct(s): OX4319, OX4767

Mode of Transformation: Direct Injection

Phenotype Description:
A description of the anticipated or actual expression of the altered genetic material in the regulated article and how that expression differs from the expression in the non-modified parental organism.

The introduced genetic material in the diamondback moth comprises three protein coding regions, one for marking the insects and two for inducing death before the insect reaches adulthood (in this instance, females only). The former allows the expression of a DsRed2 fluorescent protein originally derived from a coral (Discosoma sp.). The transgenic diamondback moth with the marker gene fluoresces when excited by illumination of the appropriate wavelength. These fluorescent proteins, which have been used as markers in a wide range of vertebrate and invertebrate species, confer no known competitive advantage or disadvantage to the recipient, and no ecological or other consequences resulting from incorporation of these markers into the transgenic diamondback moth can be envisioned. The non-modified diamondback moth has no fluorescent protein gene; therefore, it does not fluoresce when illuminated under the same light frequency. Neither piggyBac transposase activity nor any antibiotic resistance is conferred to the transgenic diamondback moth by the introduced genetic material.

Phenotype(s)

MG - Visual marker; DsRed2 Fluorescent Protein Expression

Genotype(s)

Screenable Marker
Gene: DsRed2 **from** Discosoma sp. - Screenable marker gene DsRed2 from Discosoma spp - Allows the expression of a fluorescent protein from Discosoma spp. Fluorescent protein of the GFP superfamily (DsRed2) under the control of a hr5ie1 promoter/enhancer sequence, which is from Autographa californica nuclear polyhedrosis virus (AcMNPV). A transgenic diamondback moth with the marker gene will fluoresce when excited by intense illumination of the appropriate wavelength. These fluorescent proteins, which have been used as markers in a wide range of vertebrate and invertebrate species, confer no known competitive advantage or disadvantage to the recipient, and no adverse ecological effect or other consequences resulting from incorporation of these markers into the transgenic diamondback moth are envisioned. Expression of a fluorescent protein will therefore permit released modified moths to be distinguished from unmodified.

Vector Sequence: piggyBac (non-autonomous) **from** piggyBac from Trichoplusia ni (moth) - Transformation Vector from Trichoplusia ni (moth) - Effects germline transformation of diamondback moth from piggyBac from Trichoplusia ni (moth) - 3' 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. In the construct used for transformation of the pink bollworm, the transposase gene of the piggyBac element was irreversibly destroyed by insertion of the transgene. 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 melanogaster 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.

Repressible lethality
Gene: tTAV **from** Escherichia coli (bacterium) and Herpes simplex (virus) - Tetracycline-repressible transcriptional activator from tTAV is a synthetic fusion of the tetR protein from Escherichia coli with VP16 from a type 1 herpes simplex virus. The tTA protein binds to and activates expression from the tetracycline response element (tRE), which includes multiple copies of the specific DNA sequence to which tTA binds (tetO) (Gossen et al., 1994; Gossen & Bujard, 1992). tTAV also binds tetracyclines with high affinity; the tetracycline-bound form of tTAV does not bind DNA. tTAV therefore acts as a tetracycline-regulated switch. In the absence of tetracycline, it will induce expression

from tRE, whereas in the presence of tetracycline it will not. High-level expression of tTAV is thought to be deleterious to cells as it can repress their normal transcription; low-level expression has no known effect other than activation of tRE (Berger, et al., 1990; Damke et al., 1995; Gillespie et al., 1997; Gong et al., 2005; Gossen and Bujard, 1992; Salghetti et al., 2001). tTAV has been used in fungi, plants, mice and Drosophila melanogaster with no known adverse effects. Unmodified Plutella xylostella do not have a tTAV activity.

Regulatory sequence: doublesex genomic region **from** Pink bollworm, Pectinophora gossypiella - Female-specificity is conferred using truncated sex-alternate splicing sequences from the doublesex gene of Pectinophora gossypiella. Sequence encoding tTAV is inserted into this splicing sequence, allowing for the expression of tTAV in a sex-specific manner, resulting in a conditional female-lethal system (Jin et al. 2013).

A full list of construct components is provided in the attached Table of genetic elements.

References:

Berger SL, et al. 1990 Selective inhibition of activated but not basal transcription by the acidic activation domain of VP16: evidence for transcriptional adaptors. Cell 61, 1199-1208.
Damke H, et al. 1995 Tightly regulated and inducible expression of dominant interfering dynamin mutant in stably transformed HeLa cells. Meth Enzymol 257, 209-220.
Gillespie JP, et al. 1997 Biological mediators of insect immunity. Annu Rev Entomol 42, 611-643.
Gong P, et al. 2005 A dominant lethal genetic system for autocidal control of the Mediterranean fruitfly. Nat Biotechnol 23, 453-456.
Gossen M, et al. 1994 Inducible gene expression systems for higher eukaryotic cells. Curr Opin Biotechnol 5, 516-520.
Gossen M, and Bujard H 1992 Tight control of gene expression in mammalian cells by tetracycline- responsive promoters. Proc Natl Acad Sci USA 89, 5547-5551.
Salghetti S, et al. 2001 Regulation of transcriptional activation domain function by ubiquitin. Science 293, 1651-1653.
Jin L, et al. 2013 Engineered female-specific lethality for control of pest Lepidoptera. ACS Synthetic Biology, 2:160-166.

12. INTRODUCTION

Release Site

Location Name & Description	Location Address	Contact(s)
1) Research Farm North	NY **County:** Ontario **Proposed Release Start Date:** 4/1/2014 **Proposed Release End Date:** 3/31/2017 **No. of Releases:** up to 72/year **Quantity:** up to 100,000 moths/wk; 10 acres acres	

Location Unique ID: RFN1097

Location GPS Coordinates: (b)(4)

Release Site History: Managed agricultural, cropping, research. Managed agricultural land around release site.

Critical Habitat Involved?: ___ Yes _X_ No

13. DESIGN PROTOCOLS

Production Design

A detailed description of the purpose for the introduction of the regulated article including detailed description of the proposed experimental and/or production design:

The diamondback moth strains OX4319L-Pxy, OX4319N-Pxy and OX4767A-Pxy show a tetracycline-repressible female-lethal phenotype, which could serve as an insecticide-free means

of controlling pest populations of diamondback moth in the field in a species-specific manner. Successful pest control will rely upon strong performance of released males, in terms of female-seeking behavior and mating competitiveness. We will seek to measure relevant performance traits in one or more mark-release-recapture field experiments. These will be followed by pest suppression trials, in which Plutella xylostella-infested fields will be treated with fsRIDL male Plutella xylostella, and the wild populations monitored and compared with those of fields not so treated.

In the mark-release-recapture experiments, we will release up to 20,000 male fsRIDL Plutella xylostella (per release; up to 100,000 males per week) from single or multiple points in experimental fields of up to 10 acres planted with brassicas (e.g. cabbage or broccoli). Traps (e.g. sticky traps baited with synthetic sex pheromone) will be placed in and around the field, up to 1 km from the release point, to recapture the male moths. Traps will be collected at least once per week and the recaptured moths screened for the fluorescence marker. Additional PCR screening will be conducted to validate this visual screening. Trapping will continue until no fsRIDL male moths are recaptured for 2 consecutive weeks. To permit an overlapping series of releases in each experimental field that can be independently monitored on the traps, fsRIDL male moths will be sometimes be additionally marked, for example using different-colored fluorescent powders, which are commonly used in such field experiments with insects (reviewed by Hagler & Jackson 2001 Ann Rev Entomol 46:511-543). Crop sampling, in which a proportion of the in-field plants will be collected and closely examined in the laboratory for Plutella xylostella larvae and pupae (wild-type and transgenic), will be conducted at regular intervals to assess mating success of the fsRIDL males. Each experimental field will be surrounded by an approximately 10m-deep border free of potential host plants. Upon completion of the experiment, the insecticide Coragen (chlorantraniliprole) will be sprayed on the plants and surrounding area to kill remaining diamondback moth larvae.
Data from these preliminary field experiments, indicating the fsRIDL male release rate required to achieve a given (b)(4)), dispersal and field longevity, will inf dback moths in a suppression trial (how many fsRIDL males to release, how frequently and from how many points), requested as part of this permit application. All of the described trials will require monitoring of a wild diamondback moth population in up to six experimental fields (up to three treated with fsRIDL male moths, and up to three untreated). These fields, of up to 10 acres in size, will be planted with brassica plants (e.g. cabbage or broccoli). If the wild diamondback moth population is not present in sufficient numbers at the trial sites, the experimental fields 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 GM moths. A proportion of the experimental fields will be subjected to regular releases (b)(4)) of fsRIDL male moths, in numbers greater than the estimated recruitm le moths in the environment, to achieve an over-flooding effect of fsRIDL males on the wild male diamondback moth population. For each experiment, fsRIDL male releases will be conducted for up to the duration of a brassica crop cycle (anticipated as 3-4 months). Adult traps (e.g. sticky traps baited with synthetic sex pheromone) will be placed in and around each field to monitor the relative numbers of wild-type and fsRIDL males present, and to assess their dispersal. The populations of wild-type moths in each field, including those receiving no fsRIDL males, will be monitored using the adult traps described and periodic crop sampling. Releases will consist of up to 100,000 fsRIDL male moths per week (depending on the overflooding ratio required) over the treatment fields over the course of these suppression experiments. Trapping will continue after the last releases of fsRIDL male moths, and will continue until at least 2 weeks of zero fsRIDL recaptures. Upon completion of the experiment, the insecticide Coragen (chlorantraniliprole) will be sprayed on the plants and surrounding area (within 100 m radius of treated fields) to kill remaining diamondback moth larvae. Post-experiment pheromone trapping will continue for 2 weeks to monitor field longevity of fsRIDL moths.

Destination or Release Description

A detailed description of the intended destination (including final and all intermediate destinations), uses, and/or distribution of the regulated article (e.g., greenhouses, laboratory, or growth chamber location; field trial location, pilot project location; production, propagation, and manufacture location; proposed sale and distribution location):

All genetically modified male diamondback moth used in the trials will be reared as larvae on non-tetracycline artificial diet. Releases will be conducted from the ground or vehicle on Cornell University's New York State Agricultural Research Station. Releases will be conducted up
(b) (4) depending on experimental requirements.
The area around the release sites (up to 1000 m radius from release site) will be monitored with traps (e.g. sticky traps baited with synthetic sex pheromone). Traps will be collected at least weekly to count the number of genetically modified moths and wild moths captured on each trap. Samples in the laboratory will be screened for presence of the DsRed2 fluorescent marker, using fluorescence microscopy, and this will be validated by PCR detection of the DNA construct in selected samples. Some non-viable insect samples will be sent to Oxitec's labs in the UK for the PCR analysis. Prior to each field release, samples from each cohort of male fsRIDL moths will be screened for the fluorescent marker and sexed. Only male moths will be released; the effect on the crop will therefore likely be negligible: male activity is restricted to finding and mating females, feeding on nectar from flowers, and taking shelter during the day.

Confinement Protocols

A detailed description of the proposed procedures, processes, and safeguards which will be used to prevent escape and dissemination of the regulated article at each of the intended destinations:

Adult genetically modified moths will be transported in sealed containers labeled as follows "CORNELL UNIVERSITY GENETICALLY MODIFIED MOTES FOR RELEASE AT CORNELL UNIVERSITYS NEW YORK STATE AGRICULTURAL EXPERIMENT STATION - AUTHORIZED PERSONNEL ONLY". Insects will be transported by hand or in a vehicle by authorized personnel.
The conditional lethality expressed by the fsRIDL construct means that female progeny from matings with Oxitec male insects die in the absence of tetracycline, and the trait is therefore unlikely to persist in the environment. Other mitigation measures include the lack of known sexually compatible relatives of Plutella xylostella in the USA; the piggyBac transposable element used for the transformation has no endogenous functioning transposase, rendering it non-autonomous (it cannot mobilize itself); the release area will be monitored extensively with traps to attract and collect Plutella xylostella moths; release fields are no larger than 10 acres; the Plutella xylostella can be sprayed with insecticide at any time in the case of observed adverse events; the genetically engineered Plutella xylostella will be securely managed and contained in production and transport; and all viable insects reared for this trial that are not required for release or additional analysis will be devitalized by freezing.

Final Disposition Method: ☒ Destruction/Devitalization ☐ Other ☐ Storage in Contained Facility

Final Disposition Description: All unused genetically modified eggs, larvae, pupae and moths not released, or not needed in the mass-rearing, will be frozen at a minimum of -15°C + 5°C for 48 h to destroy all life stages.

14. ATTACHMENTS

Attachments
BRS Importation permit (Exp.9/2013) (10/18/2013 @ 10:05 AM)
Cornell University proposed field release site (10/15/2013 @ 10:51 AM)
OX4319L allele persistence report (10/17/2013 @ 09:23 AM)
OX4319L chlortetracycline sensitivity report (10/17/2013 @ 09:22 AM)
OX4319L construct sequencing report (10/17/2013 @ 09:20 AM)
OX4319L molecular characterisation report (10/17/2013 @ 09:21 AM)
OX4319L population suppression cages report (10/17/2013 @ 09:23 AM)
OX4319L resistance management report (10/17/2013 @ 09:24 AM)
Peer-reviewed publication (Jin et al. 2013) describing development/testing of RIDL DBM (9/26/2013 @ 09:51 AM)
Table of genetic elements, OX4319 and OX4767 (10/15/2013 @ 10:50 AM)
Threatened or endangered species (10/15/2013 @ 10:52 AM)
tTAV expression levels report (10/17/2013 @ 09:21 AM)

15. ADDITIONAL INFORMATION

16. COURTESY JUSTIFICATION

I, *Anthony Shelton*, hereby certify that the information in this application and all attachments is complete and accurate to the best of my knowledge and belief.

I acknowledge this is not an application to move or import select agents, the genes expressing select agents, or the toxins made by the select agents, as described in 9 CFR 121.

I will not introduce the regulated articles described in this application until APHIS has deemed the application complete and has granted the permit. By signing this permit, I agree to comply with any and all state, local, and tribal laws and regulations that may apply to the introduction of the articles described in this applications.

If there are any changes to the information disclosed in this application, I will contact APHIS.

17. SIGNATURE OF RESPONSIBLE PERSON	**18. DATE**
Anthony Shelton	October 24, 2013

SUPPLEMENTAL PERMIT CONDITIONS

For Release of Plutella xylostella

(1) All persons working with the genetically-engineered (GE) diamondback moth must be informed of these permit conditions. Anyone working with these insects must agree to and sign/ initial these conditions before beginning work. These signed conditions do not need to be submitted to USDA/ APHIS but must be readily accessible in the event of an inspection and presented upon request.

Note: these conditions may be copied and stored electronically for electronic signature and initialing provided that the permit number, authorized organisms and life stages, release locations, and authorization statement all appear on the document with the permit number. The residency condition does not need to be signed. Signing these conditions only indicates that the person working under this permit has read them; the permit holder is the sole responsible party under this permit.

(2) Modifications to the containment of these organisms must be approved prior to making changes by applying for an amendment in ePermits.

(3) This permit does not authorize movement or use of plant pathogens listed in the Public Health Security and Bioterrorism Preparedness and Response Act of 2002. If any organism listed as a Select Agent is identified from materials associated with this research, the permit holder is required to notify APHIS, Agricultural Select Agent Program (ASAP) immediately by phone at 301-851-3300, and within seven (7) days submit APHIS/CDC Form 4 (Report of Identification of a Select Agent or Toxin in a Clinical or Diagnostic Laboratory) to APHIS, ASAP; 4700 River Rd, Unit 2, Riverdale, MD 20737 (see instructions at: http://www.aphis.usda.gov/programs/ag_selectagent/index.shtml). Failure to comply with this requirement is a violation of the Agricultural Bioterrorism Protection Act of 2002.

(4) If organisms that are not authorized in this permit are received, the permit holder must take all prudent measures to contain the organism(s) and notify the BRS Compliance Staff by contacting a compliance officer immediately (that is, within one business day) by calling 301-851-3935 or by e-mail to BRSCompliance@aphis.usda.gov. The permit holder must immediately notify the permit unit of the destruction of regulated organisms received under this permit, as above. Similarly, the permit holder must immediately notify the permit unit if facilities are destroyed or decommissioned for any reason.

(5) The permit holder must maintain an official permanent work assignment at the address identified on this permit. If the permit holder ceases assignment/affiliation at the address identified on this permit, or personnel circumstances change in any way, then a compliance officer must be notified at the BRS Compliance Staff immediately (that is, within one business day) by either (a) email to BRSCompliance@aphis.usda.gov, (b) verbal communication at 301-851-3935, or (c) conventional mail to BRS Compliance Staff, 4700 River Road, Riverdale, Unit 91, MD 20737. The permit holder must destroy all regulated organisms prior to departure unless the permit holder either (a) requests cancellation of this permit and complies with all permit-specific termination conditions, (b) applies for and receives a permit to move the organisms to a new facility, or (c) relinquishes control of the regulated organisms to a qualified individual who obtained a permit for the continued use of these regulated organisms prior to this permit holder's departure.

(6) All laboratories and growth chambers where this genetically engineered insect is employed will be locked with limited access by authorized personnel. In each area where a regulated genetically engineered organism is used, at least one sign must be posted stating that a regulated genetically engineered organism is being used.

(7) Upon completion of research all engineered insects (except those retained for future research), will be disposed of by freezing at -20 for 24 hours/or autoclaving.

(8) There is to be no further distribution of these genetically engineered insects under this permit without prior approval from State (intrastate movement) and Federal regulatory officials.

(9) THIS AUTHORIZATION IS VALID FOR THE RELEASE INTO THE ENVIRONMENT OF THIS GENETICALLY ENGINEERED INSECT ONLY IN THE AREAS DESCRIBED IN THE APPLICATION.

(10) THIS IS A CROP DESTRUCT TRIAL. Brassicas will be destroyed at the end of the research field trial. No plants of produce shall be used for food or feed. Upon completion of the experiment, the insecticide shall be sprayed on the plants and surrounding area within a 100 m radius of treated fields to kill remaining diamondback moth larvae.

(11) Without prior notice and during reasonable hours authorized Plant Protection and Quarantine and State regulatory officials shall be allowed to inspect the conditions under which this genetically engineered insect is being kept.

(12) Reporting an Unauthorized or Accidental Release

a. According to the regulation in 7 CFR § 340.4(f)(10)(i), APHIS shall be notified orally immediately upon discovery and notified in writing within 24 hours in the event of any accidental or unauthorized release of the regulated article.

- For immediate verbal notification, contact APHIS BRS Compliance Staff at (301) 851-3935 and ask to speak to a Compliance and Inspection staff member. Leave a verbal report on voicemail if the phone is not answered by a Compliance Officer.
- In addition, in the event of an emergency in which you need to speak immediately to APHIS personnel regarding the situation, you may call:

The APHIS/BRS Regional Biotechnologist assigned in the region where the field test occurs:
For Western Region, contact the Western Region Biotechnologist at (970) 494-7513
or e-mail: BRSWRBT@aphis.usda.gov
For Eastern Region, contact the Eastern Region Biotechnologist at (919) 855-7622 or e-mail: BRSERBT@aphis.usda.gov Or

The APHIS State Plant Health Director for the state where the unauthorized release occurred. The list of APHIS State Plant Health Directors is available at:
http://www.aphis.usda.gov/services/report_pest_disease/report_pest_disease.shtml.
or
http://pest.ceris.purdue.edu/stateselect.html

b. Written notification should be sent by one of the following means:

By e-mail:
BRSCompliance@aphis.usda.gov

By mail:
Biotechnology Regulatory Services (BRS)

Permit Number: 13-297-102r

THIS PERMIT HAS BEEN APPROVED ELECTRONICALLY BY THE FOLLOWING BRS OFFICIAL VIA EPERMITS	
SIGNATURE OF BRS OFFICIAL	**DATE**
John Turner	November 10, 2014

Regulatory Operations Program
USDA/APHIS
4700 River Rd. Unit 91
Riverdale, MD 20737

Additional instructions for reporting compliance incidents may be found at http://www.aphis.usda.gov/biotechnology/compliance_incident.shtml

(13) No person shall move a regulated article interstate unless the number of the limited permit appears on the outside of the shipping container.

THIS PERMIT HAS BEEN APPROVED ELECTRONICALLY BY THE FOLLOWING BRS OFFICIAL VIA EPERMITS	
SIGNATURE OF BRS OFFICIAL	**DATE**
John Turner	November 10, 2014

United States Department of Agriculture
Animal and Plant Health Inspection Service
Biotechnology Regulatory Services
4700 River Road, Unit 147
Riverdale, Maryland
20737-1236

Standard Permit Conditions for the Introduction of a Regulated Article

(7 CFR 340.4 (f))

Permit Conditions: A person who is issued a permit and his/her employees or agents shall comply with the following conditions , and any supplemental conditions which shall be listed on the permit, as deemed by the Deputy Administrator to be necessary to prevent the dissemination and establishment of plant pests:

(1) The regulated article shall be maintained and disposed of (when necessary) in a manner so as to prevent the dissemination and establishment of plant pests.

(2) All packaging material, shipping containers, and any other material accompanying the regulated article shall be treated or disposed of in such a manner as to prevent the dissemination and establishment of plant pests.

(3) The regulated article shall be kept separate from other organisms, except as specifically allowed in the permit.

(4) The regulated article shall be maintained only in areas and premises specified in the permit.

(5) An inspector shall be allowed access, during regular business hours, to the place where the regulated article is located and to any records relating to the introduction of a regulated article.

(6) The regulated article shall, when possible, be kept identified with a label showing the name of the regulated article, and the date of importation.

(7) The regulated article shall be subject to the application of measures determined by the Administrator to be necessary to prevent the accidental or unauthorized release of the regulated article.

(8) The regulated article shall be subject to the application of remedial measures (including disposal) determined by the administrator to be necessary to prevent the spread of plant pests.

(9) A person who has been issued a permit shall submit to APHIS a field test report within 6 months after the termination of the field test. A field test report shall include the APHIS reference number, methods of observation, resulting data, and analysis regarding all deleterious effects on plants, nontarget organisms, or the environment.

(10) APHIS shall be notified within the time periods and manner specified below, in the event of the following occurrences:

(i) Orally notified immediately upon discovery and notify in writing within 24 hours in the event of any accidental or unauthorized release of the regulated article;

(ii) In writing as soon as possible but not later than within 5 working days if the regulated article or associated host organism is found to have characteristics substantially different from those listed in the application for a permit or suffers any unusual occurrence (excessive mortality or morbidity, or unanticipated effect on non-target organisms).

(11) A permittee or his/her agent and any person who seeks to import a regulated article into the United States shall:

(i) Import or offer the regulated article for entry only through any USDA plant inspection station listed in 7 CFR 319.37-14;

(ii)Notify APHIS promptly upon arrival of any regulated article at a port of entry, of its arrival by such means as a manifest, customs entry document, commercial invoice, waybill, a broker's document, or a notice form provided for such purpose; and

(iii) Mark and identify the regulated article in accordance with 7 CFR 340.7.

Rev. January 1, 2010

Permit Number: 13-297-102r

THIS PERMIT HAS BEEN APPROVED ELECTRONICALLY BY THE FOLLOWING BRS OFFICIAL VIA EPERMITS	
SIGNATURE OF BRS OFFICIAL	**DATE**
John Turner	November 10, 2014

United States Department of Agriculture
Animal and Plant Health Inspection Service
Biotechnology Regulatory Services
4700 River Road, Unit 147
Riverdale, Maryland
20737-1236

Any regulated article introduced not in compliance with the requirements of 7 CFR 340 or any standard or supplemental permit conditions, shall be subject to the immediate application of such remedial measures or safeguards as an inspector determines necessary, to prevent the introduction of such plant pests. Any regulated article introduced not in compliance with the requirements of 7 Code of Federal Regulation Part 340 or any standard or supplemental permit conditions, shall be subject to the immediate application of such remedial measures or safeguards as an inspector determines necessary, to prevent the introduction of such plant pests. The responsible party may be subject to fines or penalties as authorized by the Plant Protection Act (7 U.S.C. 7701-7772).

Permit Number: 13-297-102r

THIS PERMIT HAS BEEN APPROVED ELECTRONICALLY BY THE FOLLOWING BRS OFFICIAL VIA EPERMITS	
SIGNATURE OF BRS OFFICIAL	**DATE**
John Turner	November 10, 2014

Assignment Sheet

Folder

Application Form: BRS Permit
Application Number: 13-297-102r
Applicant: Shelton, Anthony
Organization: Cornell University/NYSAES

Role	Staff	Description
Biotechnologist (Permit):	Beetham, Patricia ▼	
ERAD1:	Beetham, Patricia ▼	NEPA Decision Summary, TES, Permit denial review
ERAD2:	Beetham, Patricia ▼	Management Review of State Package
ERAD3:	Turner, John ▼	Final ERAD BRS Permit Review
Program Specialist:	Diggs, Kimberly ▼	
RegOps:	Turner, John ▼	

Save Changes

Assignment Log

Anita Drummond	Denise McRae	Program Specialist	Anita Drummond	10/24/13 10:33 AM
Denise McRae	Steven Bennett	Program Specialist	Denise McRae	10/24/13 11:03 AM
Steven Bennett	Kimberly Diggs	Program Specialist	Steven Bennett	10/24/13 02:42 PM
Thomas Nesbitt	Patricia Beetham	Biotechnologist (Permit)	Thomas Nesbitt	10/28/13 02:48 PM
Patricia Beetham	Carlos Blanco	Biotechnologist (Permit)	Thomas Nesbitt	12/02/13 07:44 AM
Thomas Nesbitt	Margaret Jones	ERAD1	Margaret Jones	10/21/14 05:35 AM
Thomas Nesbitt	Margaret Jones	ERAD3	Margaret Jones	10/21/14 05:35 AM
Thomas Nesbitt	Margaret Jones	ERAD2	Margaret Jones	10/21/14 05:35 AM
Margaret Jones	Patricia Beetham	ERAD2	Margaret Jones	10/24/14 02:31 PM
Margaret Jones	John Turner	ERAD3	Margaret Jones	10/24/14 02:31 PM
Steven Bennett	Monica Galli	RegOps	Margaret Jones	10/24/14 02:32 PM
Carlos Blanco	Patricia Beetham	Biotechnologist (Permit)	Margaret Jones	10/27/14 08:37 AM
Margaret Jones	Patricia Beetham	ERAD1	Patricia Beetham	10/27/14 09:16 AM
Monica Galli	John Turner	RegOps	Donna Lalli	11/10/14 08:42 AM

Folder

Unlock Application | Tracking Sheet | Messages | Add Note | Workflow Document | Assignment Sheet

Application Summary

Received Date: 10/24/2013
Application Form: BRS Permit
Application Category: New
Application Number: 13-297-102r
Applicant: Shelton, Anthony
Organization: Cornell University/NYSAES
Telephone: 3(b)(6)
Printable Version: Original PDF | Current PDF | Current HTML

Submission Date: 10/24/2013
Submission Level: 2
Prepared By:
Submitted By: Shelton, Anthony

Open Tasks

No Open Tasks

Collaboration Tasks

No Collaboration Tasks

Waiting Responses

No Waiting Response Tasks

Permits/Responses	Issuing Scientist	Exp. Date	Issuance Date
BRS Permit - 13-297-102r	Turner, John	11/10/2017	11/10/2014

Permit Supporting Document(s)

File Description	Submitted By	Include in Final Permit	Include in State Package
BRS Importation permit (Exp.9/2013) (10/18/2013)	Applicant	No	No
Cornell University proposed field release site (10/15/2013)	Applicant	No	No
OX4319L allele persistence report (10/17/2013)	Applicant	No	No
OX4319L chlortetracycline sensitivity report (10/17/2013)	Applicant	No	No
OX4319L construct sequencing report (10/17/2013)	Applicant	No	No
OX4319L molecular characterisation report (10/17/2013)	Applicant	No	No
OX4319L population suppression cages report (10/17/2013)	Applicant	No	No
OX4319L resistance management report (10/17/2013)	Applicant	No	No
Peer-reviewed publication (Jin et al. 2013) describing development/testing of RIDL DBM (09/26/2013)	Applicant	No	No
Table of genetic elements, OX4319 and OX4767 (10/15/2013)	Applicant	No	No
Threatened or endangered species (10/15/2013)	Applicant	No	No
tTAV expression levels report (10/17/2013)	Applicant	No	No
13-297-102_mpcl.docx (05/13/2014)	*BRS*	*No*	*No*
13-297-102r_first_FR notice_APHIS-2014-0056-0001.pdf (11/04/2014)	*BRS*	*No*	*Yes*
13-297-102r_rpcl.doc (05/13/2014)	*BRS*	*No*	*No*
13-297-102r_sl.doc (10/31/2014)	*BRS*	*No*	*Yes*
13_297102r_dea_Part1.pdf (10/30/2014)	*BRS*	*No*	*Yes*

13_297102r_dea_Part2.pdf (10/30/2014)	BRS	No	Yes	
13_297102r_dea_Part3.pdf (10/30/2014)	BRS	No	Yes	

State Review Responses

State Reviewer	Response	Review Log	Date of Action	Conditions?
New York	State has reviewed the APHIS determination and has no comment or additional regulatory requirements.	Jan Morawski (11/05/14)	11/05/2014	No

Application Supporting Document(s)

BRS Importation permit (Exp.9/2013) (10/18/2013)

Cornell University proposed field release site (10/15/2013)

OX4319L allele persistence report (10/17/2013)

OX4319L chlortetracycline sensitivity report (10/17/2013)

OX4319L construct sequencing report (10/17/2013)

OX4319L molecular characterisation report (10/17/2013)

OX4319L population suppression cages report (10/17/2013)

OX4319L resistance management report (10/17/2013)

Peer-reviewed publication (Jin et al. 2013) describing development/testing of RIDL DBM (9/26/2013)

Table of genetic elements, OX4319 and OX4767 (10/15/2013)

Threatened or endangered species (10/15/2013)

tTAV expression levels report (10/17/2013)

Task Documents

No Task Attachments

Submitted Reports

Reports Summary

No Submitted Reports

Submitted Notices

No Submitted Notices

Notice Task Notes

No Notice Task Notes

Task Notes

General Comment - 11/5/2014 @ 7:12 AM
Margaret Jones: Emails 102414 - 102714 were stored in the DocWar due to size limitations.

General Comment - 10/27/2014 @ 9:13 AM
Patricia Beetham: The EA link uploaded is the incorrect draft EA out for public comment. The correct one will be uploaded shortly, along with the FR notice for public comments.

General Comment - 10/21/2014 @ 7:57 AM
Carlos Blanco: The Environmental Assessment document has been changed for a short version that only contains the cover pages, table of contents, figures and tables and a link to download the document from Internet.

Environmental Assessment Documents - 10/20/2014 @ 1:00 PM
Carlos Blanco: The EA document is too long to upload. I temporarily added the application to this step to move forward.

General Comment - 12/2/2013 @ 7:47 AM
Thomas Nesbitt: Permit reassigned to Carlos. Proceed with processing of permit. Once draft permit conditions have been prepared, permit will need discussion with BEAB to determine adequate NEPA analysis (catex vs EA).

General Comment - 11/5/2013 @ 10:31 AM
Patricia Beetham: This permit needs extensive review from PPQ and BRS. At the minimum, an EA needs to be done. As it stands, there is not enough information to make an informed decision on this unconfined field trial proposed by the applicant. The applicant has not been contacted as of 11/06/13, but will be contacted as soon as Colin Stewart of PPQ returns to the office after November 12th.

Task Collaboration Messages and Responses

No Task Collaboration Messages

Deficiency Correspondence

No Messages

Messages

No Messages

Application Lock/Unlock Log

No Application Lock/Unlock Logs

Messages

Folder New Message

Received Messages

View Sent Messages

No Messages

Messages

Folder

Sent Messages

View Received Messages

	▼ Date
Applicant Acceptance of Draft Permit Conditions	Tue 11/04/14 9:44 AM

Message

Folder

Message Detail

Back to Sent Messages

BRS Permit for the Introduction of Genetically Engineered Organisms
Application Number 13-297-102r

Sent: Tue, 11/04/14 9:44 AM
To: Anthony Shelton
Subject: Applicant Acceptance of Draft Permit Conditions

Tracking Sheet

Folder

Application Form: BRS Permit
Application Number: 13-297-102r
Applicant: Shelton, Anthony
Organization: Cornell University/NYSAES

Application Duration: 383 days

Bold text denotes **open** tasks, and *italic* text denotes the closed tasks.

✓ 1.	*Receive and Assign Application*	10/24/13 08:40 AM	10/24/13 10:14 AM	Anita Drummond	
✓ 2.	*Completeness Check*	10/24/13 10:14 AM	10/25/13 01:49 PM	Kimberly Diggs	
3.	Waiting for Applicant Response				
4.	Review Applicant Response				
✓ 5.	*Assign Biotechnologist and Managers*	10/25/13 01:49 PM	10/25/13 01:50 PM	Kimberly Diggs	
✓ 6.	*Review BRS Permit Application*	10/25/13 01:50 PM ↶ 12/02/13 07:44 AM	✗ 12/02/13 07:44 AM 05/06/14 07:12 AM	Thomas Nesbitt Carlos Blanco	
✓ 7.	*Review Article and Donors*	05/06/14 07:12 AM	05/06/14 07:13 AM	Carlos Blanco	
✓ 8.	*Biotechnologist Initial Review*	05/06/14 07:13 AM	05/06/14 07:13 AM	Carlos Blanco	
9.	Generate and Send Denial Letter				
10.	Generate and Send Application Deficiency Letter				
11.	Waiting for Applicant Response				
12.	Review Applicant Response				
✓ 13.	*Complete Biotechnologist Review Forms*	05/06/14 07:13 AM ↶ 10/27/14 09:17 AM	10/20/14 08:55 AM 11/04/14 09:32 AM	Carlos Blanco Patricia Beetham	
✓ 14.	*Determine if an Environmental Assessment is Required*	10/20/14 08:55 AM 11/04/14 09:32 AM	10/20/14 12:43 PM 11/04/14 09:32 AM	Carlos Blanco Patricia Beetham	
✓ 15.	*NEPA Decision Summary and TES Review*	10/20/14 12:43 PM 11/04/14 09:32 AM	10/24/14 02:28 PM 11/04/14 09:33 AM	Margaret Jones Patricia Beetham	
✓ 16.	*Environmental Assessment Documents*	10/20/14 12:43 PM 11/04/14 09:32 AM ↶ 11/04/14 09:43 AM	10/20/14 01:00 PM 11/04/14 09:33 AM 11/04/14 09:43 AM	Carlos Blanco Patricia Beetham Patricia Beetham	
✓ 17.	*Determine Supplemental Conditions for Release*	11/04/14 09:32 AM	11/04/14 09:43 AM	Patricia Beetham	
18.	Determine Supplemental Conditions for Movements				
19.	Determine if a Facility Inspection is Required				
20.	Facility Inspection				
✓ 21.	*Management Review of Draft Conditions*	11/04/14 09:43 AM	11/04/14 09:44 AM	Patricia Beetham	
22.	Modify Draft Conditions prior to applicant review and approval				
23.	Prepare and send Applicant Draft Conditions				
✓ 24.	*Waiting for Applicant's Response to Draft Conditions*	11/04/14 09:44 AM	11/04/14 09:47 AM	Patricia Beetham	
25.	Review Applicant's Response to Draft Conditions				
✓ 26.	*Select Effective and Expiration Dates for Permit*	11/04/14 09:47 AM	11/04/14 09:47 AM	Patricia Beetham	
✓ 27.	*State Review Confirmation*	11/04/14 09:47 AM	11/04/14 09:47 AM	Patricia Beetham	
✓ 28.	*Generate Permit Package for State*	11/04/14 09:47 AM	11/04/14 09:59 AM	Kimberly Diggs	

	Review				
✓ 29.	*Management Review of State Package*	11/04/14 09:59 AM	11/04/14 10:39 AM	Patricia Beetham	
30.	Generate and Send Letter to State Official				
31.	Notify State				
✓ 32.	*State Regulatory Official Review*	11/04/14 10:39 AM	11/05/14 09:50 AM	Jan Morawski	
✓ 33.	*Biotechnologist Final Review*	11/05/14 09:50 AM	11/05/14 10:03 AM	Patricia Beetham	
✓ 34.	*Generate BRS Permit*	11/05/14 10:03 AM	11/05/14 11:04 AM	Kimberly Diggs	
✓ 35.	*Final ERAD BRS Permit Review*	11/05/14 11:04 AM	11/07/14 03:15 PM	John Turner	
✓ 36.	*Final RegOps BRS Permit Review*	11/07/14 03:15 PM	11/10/14 09:07 AM	John Turner	
✓ 37.	*Issue BRS Permit*	11/10/14 09:07 AM	11/10/14 09:39 AM	Kimberly Diggs	
38.	Assign Inspection Station and Determine Labels				
39.	Generate and Print Shipment Labels				
40.	Shipment Label Print Confirmation				
41.	Void Labels				

Received Date: 10/24/2013
Application Form: BRS Permit
Application Category: New
Application Number: 13-297-102r
Applicant: Shelton, Anthony
Organization: Cornell University/NYSAES
Submission Level: 2

Original	Current

Task Open Date	Task Close Date
10/25/2013	10/25/2013

Assign Biotechnologist and Managers

* Indicates a required field

Biotechnologist (Permit):* Beetham, Patricia

ERAD1:* Beetham, Patricia
NEPA Decision Summary, TES, Permit denial review

ERAD2:* Beetham, Patricia
Management Review of State Package

ERAD3:* Turner, John
Final ERAD BRS Permit Review

Summary of total application for each biotechnologist or manager that are pending or has been processed within the last 120 days.

Abel, Sidney	0	0
Beaman, Jeffrey	0	0
Beetham, Patricia	2	109
Bennett, Steven	0	1
Beyene, Nebiyu	0	0
Blanchette, Michael	0	0
Bodnar, Anastasia	0	0
Boulais, Virginia	12	118
Butler, Tracye	0	0
Chestnut, Evan	0	0
De Sa Snow, Patricia	3	5
Diggs, Kimberly	0	40
Divan, Charles	0	0
Doley, William	0	0
Drummond, Anita	2	48
Eck, Cynthia	0	33
Ermalinski, Jeffrey	0	0
Glen, Ian	0	0
Grant, Douglas	0	0
Hegde, Subray	29	5
Jin, Wendy	0	0
Jones, Margaret	0	17
Koehler, Susan	0	2
Lalli, Donna	0	0
Lightle, Helen	4	47
Liu, Zhaowei	0	0

McRae, Denise	0	13
Nesbitt, Thomas	0	0
Pardoe, Linda	0	0
Pearson, Alan	14	126
Rappaport, Kate	10	22
Samboju, Narasimha	2	7
Serrano Miranda, Eddie	0	0
Sethuraman, Karthik	0	0
Sethuraman, Karthik	0	0
Smelley, Anna	2	9
Spaine, Pauline	4	5
Turner, John	0	7
Vieglais, Christina	3	54
Vongpaseuth, Khamkeo	11	9
Wanex, Miranda	0	0
Weinsetel, Natalia	0	0

BRSPMT-0005

Received Date: 10/24/2013
Application Form: BRS Permit
Application Category: New
Application Number: 13-297-102r
Applicant: Shelton, Anthony
Organization: Cornell University/NYSAES
Submission Level: 2

Original	Current

Task Open Date	Task Close Date
11/5/2014	11/5/2014

Biotechnologist Final Review

View Permit Package

New York	State has reviewed the APHIS determination and has no comment or additional regulatory requirements.	Jan Morawski (11/05/14)	11/05/2014	No	

BRS Permit Application Versions

BRS Permit Application Version 1 Nov 4 2014 1039AM.pdf
BRS Permit Application Original Oct 24 2013.pdf

BRSPMT-0035

Received Date:	10/24/2013
Application Form:	BRS Permit
Application Category:	New
Application Number:	13-297-102r
Applicant:	Shelton, Anthony
Organization:	Cornell University/NYSAES
Submission Level:	2

Original	Current

Task Open Date	Task Close Date
11/5/2014	11/5/2014

Biotechnologist Final Review

New York's Response

Date of Action: 11/05/2014

Response: State has reviewed the APHIS determination and has no comment or additional regulatory requirements.

Comments:

Add Condition To add a Condition to the list below select **Add Condition**.

State has not determined any Condition(s)

Update Response **Cancel**

BRSPMT-0035

Received Date: 10/24/2013
Application Form: BRS Permit
Application Category: New
Application Number: 13-297-102r
Applicant: Shelton, Anthony
Organization: Cornell University/NYSAES
Submission Level: 2

Original	Current

Task Open Date	Task Close Date
5/6/2014	5/6/2014

Biotechnologist Initial Review

The application is:

- Incomplete
- Complete and further review is NOT needed (Amendments Only)
- • Complete
- Denied

NOTE: DO NOT INCLUDE CBI INFORMATION IN THESE COMMENTS

Reason for Incomplete: (Enter if Incomplete. NOTE: Text entered here will show up on the Deficiency Letter.)

Reason for Denial: (Enter if Denied. NOTE: Text entered here will show up on the Denial Letter.)

Biotechnologist's Comment: (NOTE: For BRS Internal Use)

The following is a list of review forms you may use in your review process:

- APHIS HR BMP Recommendation for Plant Release Permits (effective 12/11/2014)
- EPA Notification Form for Microorganisms 021014 (effective 02/10/2014)
- Movement Permit Checklist 060412 (effective 06/04/2012)
- NEPA Decision Summary (effective 04/12/2007)
- NEPA ESA Decision Worksheet for Permits 042413 (effective 04/24/2013)
- PMP Equipment cleaning SOP Checklist (effective 11/22/2006)
- PMP Review Training Checklist (effective 11/22/2006)
- Pharma, industrial and phyto checklist (effective 11/22/2006)
- Release Permit Checklist 060412 (effective 06/04/2012)
- State Letter Template (effective 05/07/2010)
- VS Review of Import permits containing animal genes (effective 11/22/2006)

Received Date: 10/24/2013
Application Form: BRS Permit
Application Category: New
Application Number: 13-297-102r
Applicant: Shelton, Anthony
Organization: Cornell University/NYSAES
Submission Level: 2

Original	Current

Task Open Date	Task Close Date
5/6/2014	10/20/2014
10/27/2014	11/4/2014

Complete Biotechnologist Review Forms

The following review forms: • are complete
could not complete, additional information is needed

13-297-102_mpcl.docx	05/13/2014
13-297-102r_rpcl.doc	05/13/2014
13_297102r_dea_Part1.pdf	10/30/2014
13_297102r_dea_Part2.pdf	10/30/2014
13_297102r_dea_Part3.pdf	10/30/2014
13-297-102r_sl.doc	10/31/2014
13-297-102r_first_FR notice_APHIS-2014-0056-0001.pdf	11/04/2014

BRSPMT-0015

Received Date:	10/24/2013
Application Form:	BRS Permit
Application Category:	New
Application Number:	13-297-102r
Applicant:	Shelton, Anthony
Organization:	Cornell University/NYSAES
Submission Level:	2

Original	Current

Task Open Date	Task Close Date
10/24/2013	10/25/2013

Completeness Check

Application is:

- Incomplete
- Complete and ready to issue (Courtesy Permit)
- Complete and further review is NOT needed (Amendments Only)
- • Complete and ready for biotechnologist review

If the application is incomplete, please indicate what area(s) need to be addressed by selecting one or more of the appropriate areas.

Your complete Street Address (P.O. Box is not acceptable)

Your telephone number where you can be reached during business hours

Other: (Please specify below) **NOTE: DO NOT INCLUDE CBI INFORMATION IN THIS COMMENT**

BRSPMT-0002

Received Date:	10/24/2013
Application Form:	BRS Permit
Application Category:	New
Application Number:	13-297-102r
Applicant:	Shelton, Anthony
Organization:	Cornell University/NYSAES
Submission Level:	2

Original	Current

Task Open Date	Task Close Date
10/20/2014	10/20/2014
11/4/2014	11/4/2014

Determine if an Environmental Assessment is Required

An environmental assessment is: NOT Required

• Required

13-297-102_mpcl.docx	05/13/2014
13-297-102r_rpcl.doc	05/13/2014
13_297102r_dea_Part1.pdf	10/30/2014
13_297102r_dea_Part2.pdf	10/30/2014
13_297102r_dea_Part3.pdf	10/30/2014
13-297-102r_sl.doc	10/31/2014
13-297-102r_first_FR notice_APHIS-2014-0056-0001.pdf	11/04/2014

BRSPMT-0017

Received Date:	10/24/2013
Application Form:	BRS Permit
Application Category:	New
Application Number:	13-297-102r
Applicant:	Shelton, Anthony
Organization:	Cornell University/NYSAES
Submission Level:	2

Original	Current

Task Open Date	Task Close Date
11/4/2014	11/4/2014

Determine Supplemental Conditions for Release

Supplemental Conditions for Release

1. All persons working with the genetically-engineered (GE) diamondback moth must be informed of these permit conditions. Anyone working with these insects must agree to and sign/ initial these conditions before beginning work. These signed conditions do not need to be submitted to USDA/ APHIS but must be readily accessible in the event of an inspection and presented upon request.

   Note: these conditions may be copied and stored electronically for electronic signature and initialing provided that the permit number, authorized organisms and life stages, release locations, and authorization statement all appear on the document with the permit number. The residency condition does not need to be signed. Signing these conditions only indicates that the person working under this permit has read them; the permit holder is the sole responsible party under this permit.
2. Modifications to the containment of these organisms must be approved prior to making changes by applying for an amendment in ePermits.
3. This permit does not authorize movement or use of plant pathogens listed in the Public Health Security and Bioterrorism Preparedness and Response Act of 2002. If any organism listed as a Select Agent is identified from materials associated with this research, the permit holder is required to notify APHIS, Agricultural Select Agent Program (ASAP) immediately by phone at 301-851-3300, and within seven (7) days submit APHIS/CDC Form 4 (Report of Identification of a Select Agent or Toxin in a Clinical or Diagnostic Laboratory) to APHIS, ASAP; 4700 River Rd, Unit 2, Riverdale, MD 20737 (see instructions at: http://www.aphis.usda.gov/programs/ag_selectagent/index.shtml). Failure to comply with this requirement is a violation of the Agricultural Bioterrorism Protection Act of 2002.
4. If organisms that are not authorized in this permit are received, the permit holder must take all prudent measures to contain the organism(s) and notify the BRS Compliance Staff by contacting a compliance officer immediately (that is, within one business day) by calling 301-851-3935 or by e-mail to BRSCompliance@aphis.usda.gov. The permit holder must immediately notify the permit unit of the destruction of regulated organisms received under this permit, as above. Similarly, the permit holder must immediately notify the permit unit if facilities are destroyed or decommissioned for any reason.
5. The permit holder must maintain an official permanent work assignment at the address identified on this permit. If the permit holder ceases assignment/affiliation at the address identified on this permit, or personnel circumstances change in any way, then a compliance officer must be notified at the BRS Compliance Staff immediately (that is, within one business day) by either (a) email to BRSCompliance@aphis.usda.gov, (b) verbal communication at 301-851-3935, or (c) conventional mail to BRS Compliance Staff, 4700 River Road, Riverdale, Unit 91, MD 20737. The permit holder must destroy all regulated organisms prior to departure unless the permit holder either (a) requests cancellation of this permit and complies with all permit-specific termination conditions, (b) applies for and receives a permit to move the organisms to a new facility, or (c) relinquishes control of the regulated organisms to a qualified individual who obtained a permit for the continued use of these regulated organisms prior to this permit holder's departure.
6. All laboratories and growth chambers where this genetically engineered insect is employed will be locked with limited access by authorized personnel. In each area where a regulated genetically engineered organism is used, at least one sign must be posted stating that a regulated genetically engineered organism is being used.
7. Upon completion of research all engineered insects (except those retained for future research), will be disposed of by freezing at -20 for 24 hours/or autoclaving.
8. There is to be no further distribution of these genetically engineered insects under this permit without prior approval from State (intrastate movement) and Federal regulatory officials.
9. THIS AUTHORIZATION IS VALID FOR THE RELEASE INTO THE ENVIRONMENT OF THIS GENETICALLY ENGINEERED INSECT ONLY IN THE AREAS DESCRIBED IN THE APPLICATION.
10. THIS IS A CROP DESTRUCT TRIAL. Brassicas will be destroyed at the end of the research field trial. No plants of produce shall be used for food or feed. Upon completion of the experiment, the insecticide shall be sprayed on the plants and surrounding area within a 100 m radius of treated fields to kill remaining diamondback moth larvae.
11. Without prior notice and during reasonable hours authorized Plant Protection and Quarantine and State regulatory officials shall be allowed to inspect the conditions under which this genetically engineered insect is being kept.
12. Reporting an Unauthorized or Accidental Release

a. According to the regulation in 7 CFR § 340.4(f)(10)(i), APHIS shall be notified orally immediately upon discovery and notified in writing within 24 hours in the event of any accidental or unauthorized release of the regulated article.

- For immediate verbal notification, contact APHIS BRS Compliance Staff at (301) 851-3935 and ask to speak to a Compliance and Inspection staff member. Leave a verbal report on voicemail if the phone is not answered by a Compliance Officer.
- In addition, in the event of an emergency in which you need to speak immediately to APHIS personnel regarding the situation, you may call:

The APHIS/BRS Regional Biotechnologist assigned in the region where the field test occurs:
For Western Region, contact the Western Region Biotechnologist at (970) 494-7513
or e-mail: BRSWRBT@aphis.usda.gov
For Eastern Region, contact the Eastern Region Biotechnologist at (919) 855-7622 or e-mail: BRSERBT@aphis.usda.gov Or

The APHIS State Plant Health Director for the state where the unauthorized release occurred. The list of APHIS State Plant Health Directors is available at:
http://www.aphis.usda.gov/services/report_pest_disease/report_pest_disease.shtml.
or
http://pest.ceris.purdue.edu/stateselect.html

b. Written notification should be sent by one of the following means:

By e-mail:
BRSCompliance@aphis.usda.gov

By mail:
Biotechnology Regulatory Services (BRS)
Regulatory Operations Program
USDA/APHIS
4700 River Rd. Unit 91
Riverdale, MD 20737

Additional instructions for reporting compliance incidents may be found at
http://www.aphis.usda.gov/biotechnology/compliance_incident.shtml

13. No person shall move a regulated article interstate unless the number of the limited permit appears on the outside of the shipping container.

Standard Conditions

1. The regulated article shall be maintained and disposed of (when necessary) in a manner so as to prevent the dissemination and establishment of plant pests.
2. All packaging material, shipping containers, and any other material accompanying the regulated article shall be treated or disposed of in such a manner as to prevent the dissemination and establishment of plant pests.
3. The regulated article shall be kept separate from other organisms, except as specifically allowed in the permit.
4. The regulated article shall be maintained only in areas and premises specified in the permit.
5. An inspector shall be allowed access, during regular business hours, to the place where the regulated article is located and to any records relating to the introduction of a regulated article.
6. The regulated article shall, when possible, be kept identified with a label showing the name of the regulated article, and the date of importation.
7. The regulated article shall be subject to the application of measures determined by the Administrator to be necessary to prevent the accidental or unauthorized release of the regulated article.
8. The regulated article shall be subject to the application of remedial measures (including disposal) determined by the administrator to be necessary to prevent the spread of plant pests.
9. A person who has been issued a permit shall submit to APHIS a field test report within 6 months after the termination of the field test. A field test report shall include the APHIS reference number, methods of observation, resulting data, and analysis regarding all deleterious effects on plants, nontarget organisms, or the environment.
10. APHIS shall be notified within the time periods and manner specified below, in the event of the following occurrences:

    (i) Orally notified immediately upon discovery and notify in writing within 24 hours in the event of any accidental or unauthorized release of the regulated article;

    (ii) In writing as soon as possible but not later than within 5 working days if the regulated article or associated host organism is found to have characteristics substantially different from those listed in the application for a permit or suffers any unusual occurrence (excessive mortality or morbidity, or unanticipated effect on non-target organisms).
11. A permittee or his/her agent and any person who seeks to import a regulated article into the United States shall:

    (i) Import or offer the regulated article for entry only through any USDA plant inspection station listed in 7 CFR 319.37-14;

    (ii)Notify APHIS promptly upon arrival of any regulated article at a port of entry, of its arrival by such means as a manifest,

customs entry document, commercial invoice, waybill, a broker's document, or a notice form provided for such purpose; and

(iii) Mark and identify the regulated article in accordance with 7 CFR 340.7.

Rev. January 1, 2010

Attachments to include with State Package

13_297102r_dea_Part1.pdf (10/30/2014)
13_297102r_dea_Part2.pdf (10/30/2014)
13_297102r_dea_Part3.pdf (10/30/2014)
13-297-102r_sl.doc (10/31/2014)
13-297-102r_first_FR notice_APHIS-2014-0056-0001.pdf (11/04/2014)

BRSPMT-0021

Received Date:	10/24/2013
Application Form:	BRS Permit
Application Category:	New
Application Number:	13-297-102r
Applicant:	Shelton, Anthony
Organization:	Cornell University/NYSAES
Submission Level:	2

Original	Current

Task Open Date	Task Close Date
10/20/2014	10/20/2014
11/4/2014	11/4/2014
11/4/2014	11/4/2014

Environmental Assessment Documents

13-297-102_mpcl.docx	05/13/2014
13-297-102r_rpcl.doc	05/13/2014
13_297102r_dea_Part1.pdf	10/30/2014
13_297102r_dea_Part2.pdf	10/30/2014
13_297102r_dea_Part3.pdf	10/30/2014
13-297-102r_sl.doc	10/31/2014
13-297-102r_first_FR notice_APHIS-2014-0056-0001.pdf	11/04/2014

Task Notes

10/20/2014 @ 1:00 PM
Carlos Blanco : The EA document is too long to upload. I temporarily added the application to this step to move forward.

BRSPMT-0019

Received Date:	10/24/2013
Application Form:	BRS Permit
Application Category:	New
Application Number:	13-297-102r
Applicant:	Shelton, Anthony
Organization:	Cornell University/NYSAES
Submission Level:	2

Original	Current

Task Open Date	Task Close Date
11/5/2014	11/7/2014

Final ERAD BRS Permit Review

Permit Dates

Effective Date:	11/10/2014
Expiration Date:	11/10/2017

Supplemental Conditions for Release

1. All persons working with the genetically-engineered (GE) diamondback moth must be informed of these permit conditions. Anyone working with these insects must agree to and sign/ initial these conditions before beginning work. These signed conditions do not need to be submitted to USDA/ APHIS but must be readily accessible in the event of an inspection and presented upon request.

   Note: these conditions may be copied and stored electronically for electronic signature and initialing provided that the permit number, authorized organisms and life stages, release locations, and authorization statement all appear on the document with the permit number. The residency condition does not need to be signed. Signing these conditions only indicates that the person working under this permit has read them; the permit holder is the sole responsible party under this permit.
2. Modifications to the containment of these organisms must be approved prior to making changes by applying for an amendment in ePermits.
3. This permit does not authorize movement or use of plant pathogens listed in the Public Health Security and Bioterrorism Preparedness and Response Act of 2002. If any organism listed as a Select Agent is identified from materials associated with this research, the permit holder is required to notify APHIS, Agricultural Select Agent Program (ASAP) immediately by phone at 301-851-3300, and within seven (7) days submit APHIS/CDC Form 4 (Report of Identification of a Select Agent or Toxin in a Clinical or Diagnostic Laboratory) to APHIS, ASAP; 4700 River Rd, Unit 2, Riverdale, MD 20737 (see instructions at: http://www.aphis.usda.gov/programs/ag_selectagent/index.shtml). Failure to comply with this requirement is a violation of the Agricultural Bioterrorism Protection Act of 2002.
4. If organisms that are not authorized in this permit are received, the permit holder must take all prudent measures to contain the organism(s) and notify the BRS Compliance Staff by contacting a compliance officer immediately (that is, within one business day) by calling 301-851-3935 or by e-mail to BRSCompliance@aphis.usda.gov. The permit holder must immediately notify the permit unit of the destruction of regulated organisms received under this permit, as above. Similarly, the permit holder must immediately notify the permit unit if facilities are destroyed or decommissioned for any reason.
5. The permit holder must maintain an official permanent work assignment at the address identified on this permit. If the permit holder ceases assignment/affiliation at the address identified on this permit, or personnel circumstances change in any way, then a compliance officer must be notified at the BRS Compliance Staff immediately (that is, within one business day) by either (a) email to BRSCompliance@aphis.usda.gov, (b) verbal communication at 301-851-3935, or (c) conventional mail to BRS Compliance Staff, 4700 River Road, Riverdale, Unit 91, MD 20737. The permit holder must destroy all regulated organisms prior to departure unless the permit holder either (a) requests cancellation of this permit and complies with all permit-specific termination conditions, (b) applies for and receives a permit to move the organisms to a new facility, or (c) relinquishes control of the regulated organisms to a qualified individual who obtained a permit for the continued use of these regulated organisms prior to this permit holder's departure.
6. All laboratories and growth chambers where this genetically engineered insect is employed will be locked with limited access by authorized personnel. In each area where a regulated genetically engineered organism is used, at least one sign must be posted stating that a regulated genetically engineered organism is being used.
7. Upon completion of research all engineered insects (except those retained for future research), will be disposed of by freezing at -20 for 24 hours/or autoclaving.
8. There is to be no further distribution of these genetically engineered insects under this permit without prior approval from State (intrastate movement) and Federal regulatory officials.
9. THIS AUTHORIZATION IS VALID FOR THE RELEASE INTO THE ENVIRONMENT OF THIS GENETICALLY ENGINEERED INSECT ONLY IN THE AREAS DESCRIBED IN THE APPLICATION.
10. THIS IS A CROP DESTRUCT TRIAL. Brassicas will be destroyed at the end of the research field trial. No plants of produce shall be used for food or feed. Upon completion of the experiment, the insecticide shall be sprayed on the plants and surrounding area within a 100 m radius of treated fields to kill remaining diamondback moth larvae.

11. Without prior notice and during reasonable hours authorized Plant Protection and Quarantine and State regulatory officials shall be allowed to inspect the conditions under which this genetically engineered insect is being kept.
12. Reporting an Unauthorized or Accidental Release

    a. According to the regulation in 7 CFR § 340.4(f)(10)(i), APHIS shall be notified orally immediately upon discovery and notified in writing within 24 hours in the event of any accidental or unauthorized release of the regulated article.

    - For immediate verbal notification, contact APHIS BRS Compliance Staff at (301) 851-3935 and ask to speak to a Compliance and Inspection staff member. Leave a verbal report on voicemail if the phone is not answered by a Compliance Officer.
    - In addition, in the event of an emergency in which you need to speak immediately to APHIS personnel regarding the situation, you may call:

    The APHIS/BRS Regional Biotechnologist assigned in the region where the field test occurs:
    For Western Region, contact the Western Region Biotechnologist at (970) 494-7513
    or e-mail: BRSWRBT@aphis.usda.gov
    For Eastern Region, contact the Eastern Region Biotechnologist at (919) 855-7622 or e-mail: BRSERBT@aphis.usda.gov Or

    The APHIS State Plant Health Director for the state where the unauthorized release occurred. The list of APHIS State Plant Health Directors is available at:
    http://www.aphis.usda.gov/services/report_pest_disease/report_pest_disease.shtml.
    or
    http://pest.ceris.purdue.edu/stateselect.html

    b. Written notification should be sent by one of the following means:

    By e-mail:
    BRSCompliance@aphis.usda.gov

    By mail:
    Biotechnology Regulatory Services (BRS)
    Regulatory Operations Program
    USDA/APHIS
    4700 River Rd. Unit 91
    Riverdale, MD 20737

    Additional instructions for reporting compliance incidents may be found at
    http://www.aphis.usda.gov/biotechnology/compliance_incident.shtml
13. No person shall move a regulated article interstate unless the number of the limited permit appears on the outside of the shipping container.

Standard Conditions

1. The regulated article shall be maintained and disposed of (when necessary) in a manner so as to prevent the dissemination and establishment of plant pests.
2. All packaging material, shipping containers, and any other material accompanying the regulated article shall be treated or disposed of in such a manner as to prevent the dissemination and establishment of plant pests.
3. The regulated article shall be kept separate from other organisms, except as specifically allowed in the permit.
4. The regulated article shall be maintained only in areas and premises specified in the permit.
5. An inspector shall be allowed access, during regular business hours, to the place where the regulated article is located and to any records relating to the introduction of a regulated article.
6. The regulated article shall, when possible, be kept identified with a label showing the name of the regulated article, and the date of importation.
7. The regulated article shall be subject to the application of measures determined by the Administrator to be necessary to prevent the accidental or unauthorized release of the regulated article.
8. The regulated article shall be subject to the application of remedial measures (including disposal) determined by the administrator to be necessary to prevent the spread of plant pests.
9. A person who has been issued a permit shall submit to APHIS a field test report within 6 months after the termination of the field test. A field test report shall include the APHIS reference number, methods of observation, resulting data, and analysis regarding all deleterious effects on plants, nontarget organisms, or the environment.
10. APHIS shall be notified within the time periods and manner specified below, in the event of the following occurrences:

    (i) Orally notified immediately upon discovery and notify in writing within 24 hours in the event of any accidental or unauthorized release of the regulated article;

    (ii) In writing as soon as possible but not later than within 5 working days if the regulated article or associated host organism is found to have characteristics substantially different from those listed in the application for a permit or suffers any unusual occurrence (excessive mortality or morbidity, or unanticipated effect on non-target organisms).
11. A permittee or his/her agent and any person who seeks to import a regulated article into the United States shall:

(i) Import or offer the regulated article for entry only through any USDA plant inspection station listed in 7 CFR 319.37-14;

(ii)Notify APHIS promptly upon arrival of any regulated article at a port of entry, of its arrival by such means as a manifest, customs entry document, commercial invoice, waybill, a broker's document, or a notice form provided for such purpose; and

(iii) Mark and identify the regulated article in accordance with 7 CFR 340.7.

Rev. January 1, 2010

State Conditions

New York

No State Conditions have been added for New York.

Attachments to include with Permit Package

No attachments included.

BRSPMT-0037

Received Date:	10/24/2013
Application Form:	BRS Permit
Application Category:	New
Application Number:	13-297-102r
Applicant:	Shelton, Anthony
Organization:	Cornell University/NYSAES
Submission Level:	2

Original	Current

Task Open Date	Task Close Date
11/7/2014	11/10/2014

Final RegOps BRS Permit Review

Permit Dates

Effective Date:	11/10/2014
Expiration Date:	11/10/2017

Supplemental Conditions for Release

1. All persons working with the genetically-engineered (GE) diamondback moth must be informed of these permit conditions. Anyone working with these insects must agree to and sign/ initial these conditions before beginning work. These signed conditions do not need to be submitted to USDA/ APHIS but must be readily accessible in the event of an inspection and presented upon request.

   Note: these conditions may be copied and stored electronically for electronic signature and initialing provided that the permit number, authorized organisms and life stages, release locations, and authorization statement all appear on the document with the permit number. The residency condition does not need to be signed. Signing these conditions only indicates that the person working under this permit has read them; the permit holder is the sole responsible party under this permit.
2. Modifications to the containment of these organisms must be approved prior to making changes by applying for an amendment in ePermits.
3. This permit does not authorize movement or use of plant pathogens listed in the Public Health Security and Bioterrorism Preparedness and Response Act of 2002. If any organism listed as a Select Agent is identified from materials associated with this research, the permit holder is required to notify APHIS, Agricultural Select Agent Program (ASAP) immediately by phone at 301-851-3300, and within seven (7) days submit APHIS/CDC Form 4 (Report of Identification of a Select Agent or Toxin in a Clinical or Diagnostic Laboratory) to APHIS, ASAP; 4700 River Rd, Unit 2, Riverdale, MD 20737 (see instructions at: http://www.aphis.usda.gov/programs/ag_selectagent/index.shtml). Failure to comply with this requirement is a violation of the Agricultural Bioterrorism Protection Act of 2002.
4. If organisms that are not authorized in this permit are received, the permit holder must take all prudent measures to contain the organism(s) and notify the BRS Compliance Staff by contacting a compliance officer immediately (that is, within one business day) by calling 301-851-3935 or by e-mail to BRSCompliance@aphis.usda.gov. The permit holder must immediately notify the permit unit of the destruction of regulated organisms received under this permit, as above. Similarly, the permit holder must immediately notify the permit unit if facilities are destroyed or decommissioned for any reason.
5. The permit holder must maintain an official permanent work assignment at the address identified on this permit. If the permit holder ceases assignment/affiliation at the address identified on this permit, or personnel circumstances change in any way, then a compliance officer must be notified at the BRS Compliance Staff immediately (that is, within one business day) by either (a) email to BRSCompliance@aphis.usda.gov, (b) verbal communication at 301-851-3935, or (c) conventional mail to BRS Compliance Staff, 4700 River Road, Riverdale, Unit 91, MD 20737. The permit holder must destroy all regulated organisms prior to departure unless the permit holder either (a) requests cancellation of this permit and complies with all permit-specific termination conditions, (b) applies for and receives a permit to move the organisms to a new facility, or (c) relinquishes control of the regulated organisms to a qualified individual who obtained a permit for the continued use of these regulated organisms prior to this permit holder's departure.
6. All laboratories and growth chambers where this genetically engineered insect is employed will be locked with limited access by authorized personnel. In each area where a regulated genetically engineered organism is used, at least one sign must be posted stating that a regulated genetically engineered organism is being used.
7. Upon completion of research all engineered insects (except those retained for future research), will be disposed of by freezing at -20 for 24 hours/or autoclaving.
8. There is to be no further distribution of these genetically engineered insects under this permit without prior approval from State (intrastate movement) and Federal regulatory officials.
9. THIS AUTHORIZATION IS VALID FOR THE RELEASE INTO THE ENVIRONMENT OF THIS GENETICALLY ENGINEERED INSECT ONLY IN THE AREAS DESCRIBED IN THE APPLICATION.
10. THIS IS A CROP DESTRUCT TRIAL. Brassicas will be destroyed at the end of the research field trial. No plants of produce shall be used for food or feed. Upon completion of the experiment, the insecticide shall be sprayed on the plants and surrounding area within a 100 m radius of treated fields to kill remaining diamondback moth larvae.

11. Without prior notice and during reasonable hours authorized Plant Protection and Quarantine and State regulatory officials shall be allowed to inspect the conditions under which this genetically engineered insect is being kept.
12. Reporting an Unauthorized or Accidental Release

    a. According to the regulation in 7 CFR § 340.4(f)(10)(i), APHIS shall be notified orally immediately upon discovery and notified in writing within 24 hours in the event of any accidental or unauthorized release of the regulated article.

    - For immediate verbal notification, contact APHIS BRS Compliance Staff at (301) 851-3935 and ask to speak to a Compliance and Inspection staff member. Leave a verbal report on voicemail if the phone is not answered by a Compliance Officer.
    - In addition, in the event of an emergency in which you need to speak immediately to APHIS personnel regarding the situation, you may call:

    The APHIS/BRS Regional Biotechnologist assigned in the region where the field test occurs:
    For Western Region, contact the Western Region Biotechnologist at (970) 494-7513
    or e-mail: BRSWRBT@aphis.usda.gov
    For Eastern Region, contact the Eastern Region Biotechnologist at (919) 855-7622 or e-mail: BRSERBT@aphis.usda.gov Or

    The APHIS State Plant Health Director for the state where the unauthorized release occurred. The list of APHIS State Plant Health Directors is available at:
    http://www.aphis.usda.gov/services/report_pest_disease/report_pest_disease.shtml.
    or
    http://pest.ceris.purdue.edu/stateselect.html

    b. Written notification should be sent by one of the following means:

    By e-mail:
    BRSCompliance@aphis.usda.gov

    By mail:
    Biotechnology Regulatory Services (BRS)
    Regulatory Operations Program
    USDA/APHIS
    4700 River Rd. Unit 91
    Riverdale, MD 20737

    Additional instructions for reporting compliance incidents may be found at
    http://www.aphis.usda.gov/biotechnology/compliance_incident.shtml
13. No person shall move a regulated article interstate unless the number of the limited permit appears on the outside of the shipping container.

Standard Conditions

1. The regulated article shall be maintained and disposed of (when necessary) in a manner so as to prevent the dissemination and establishment of plant pests.
2. All packaging material, shipping containers, and any other material accompanying the regulated article shall be treated or disposed of in such a manner as to prevent the dissemination and establishment of plant pests.
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5. An inspector shall be allowed access, during regular business hours, to the place where the regulated article is located and to any records relating to the introduction of a regulated article.
6. The regulated article shall, when possible, be kept identified with a label showing the name of the regulated article, and the date of importation.
7. The regulated article shall be subject to the application of measures determined by the Administrator to be necessary to prevent the accidental or unauthorized release of the regulated article.
8. The regulated article shall be subject to the application of remedial measures (including disposal) determined by the administrator to be necessary to prevent the spread of plant pests.
9. A person who has been issued a permit shall submit to APHIS a field test report within 6 months after the termination of the field test. A field test report shall include the APHIS reference number, methods of observation, resulting data, and analysis regarding all deleterious effects on plants, nontarget organisms, or the environment.
10. APHIS shall be notified within the time periods and manner specified below, in the event of the following occurrences:

    (i) Orally notified immediately upon discovery and notify in writing within 24 hours in the event of any accidental or unauthorized release of the regulated article;

    (ii) In writing as soon as possible but not later than within 5 working days if the regulated article or associated host organism is found to have characteristics substantially different from those listed in the application for a permit or suffers any unusual occurrence (excessive mortality or morbidity, or unanticipated effect on non-target organisms).
11. A permittee or his/her agent and any person who seeks to import a regulated article into the United States shall:

(i) Import or offer the regulated article for entry only through any USDA plant inspection station listed in 7 CFR 319.37-14;

(ii)Notify APHIS promptly upon arrival of any regulated article at a port of entry, of its arrival by such means as a manifest, customs entry document, commercial invoice, waybill, a broker's document, or a notice form provided for such purpose; and

(iii) Mark and identify the regulated article in accordance with 7 CFR 340.7.

Rev. January 1, 2010

State Conditions

New York

No State Conditions have been added for New York.

Attachments to include with Permit Package

No attachments included.

BRSPMT-0038

Received Date:	10/24/2013
Application Form:	BRS Permit
Application Category:	New
Application Number:	13-297-102r
Applicant:	Shelton, Anthony
Organization:	Cornell University/NYSAES
Submission Level:	2

Original	Current

Task Open Date	Task Close Date
11/5/2014	11/5/2014

Generate BRS Permit

Permit Dates

Effective Date:	11/10/2014
Expiration Date:	11/10/2017

Supplemental Conditions for Release

1. All persons working with the genetically-engineered (GE) diamondback moth must be informed of these permit conditions. Anyone working with these insects must agree to and sign/ initial these conditions before beginning work. These signed conditions do not need to be submitted to USDA/ APHIS but must be readily accessible in the event of an inspection and presented upon request.

   Note: these conditions may be copied and stored electronically for electronic signature and initialing provided that the permit number, authorized organisms and life stages, release locations, and authorization statement all appear on the document with the permit number. The residency condition does not need to be signed. Signing these conditions only indicates that the person working under this permit has read them; the permit holder is the sole responsible party under this permit.
2. Modifications to the containment of these organisms must be approved prior to making changes by applying for an amendment in ePermits.
3. This permit does not authorize movement or use of plant pathogens listed in the Public Health Security and Bioterrorism Preparedness and Response Act of 2002. If any organism listed as a Select Agent is identified from materials associated with this research, the permit holder is required to notify APHIS, Agricultural Select Agent Program (ASAP) immediately by phone at 301-851-3300, and within seven (7) days submit APHIS/CDC Form 4 (Report of Identification of a Select Agent or Toxin in a Clinical or Diagnostic Laboratory) to APHIS, ASAP; 4700 River Rd, Unit 2, Riverdale, MD 20737 (see instructions at: http://www.aphis.usda.gov/programs/ag_selectagent/index.shtml). Failure to comply with this requirement is a violation of the Agricultural Bioterrorism Protection Act of 2002.
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    or
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    Regulatory Operations Program
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9. A person who has been issued a permit shall submit to APHIS a field test report within 6 months after the termination of the field test. A field test report shall include the APHIS reference number, methods of observation, resulting data, and analysis regarding all deleterious effects on plants, nontarget organisms, or the environment.
10. APHIS shall be notified within the time periods and manner specified below, in the event of the following occurrences:

    (i) Orally notified immediately upon discovery and notify in writing within 24 hours in the event of any accidental or unauthorized release of the regulated article;

    (ii) In writing as soon as possible but not later than within 5 working days if the regulated article or associated host organism is found to have characteristics substantially different from those listed in the application for a permit or suffers any unusual occurrence (excessive mortality or morbidity, or unanticipated effect on non-target organisms).
11. A permittee or his/her agent and any person who seeks to import a regulated article into the United States shall:

(i) Import or offer the regulated article for entry only through any USDA plant inspection station listed in 7 CFR 319.37-14;

(ii)Notify APHIS promptly upon arrival of any regulated article at a port of entry, of its arrival by such means as a manifest, customs entry document, commercial invoice, waybill, a broker's document, or a notice form provided for such purpose; and

(iii) Mark and identify the regulated article in accordance with 7 CFR 340.7.

Rev. January 1, 2010

State Conditions

New York

No State Conditions have been added for New York.

Attachments to include with Permit Package

No attachments included.

BRSPMT-0036

Received Date:	10/24/2013
Application Form:	BRS Permit
Application Category:	New
Application Number:	13-297-102r
Applicant:	Shelton, Anthony
Organization:	Cornell University/NYSAES
Submission Level:	2

Original	Current

Task Open Date	Task Close Date
11/4/2014	11/4/2014

Generate Permit Package for State Review

Permit Dates

Effective Date:	11/10/2014
Expiration Date:	11/10/2017

Supplemental Conditions for Release

1. All persons working with the genetically-engineered (GE) diamondback moth must be informed of these permit conditions. Anyone working with these insects must agree to and sign/ initial these conditions before beginning work. These signed conditions do not need to be submitted to USDA/ APHIS but must be readily accessible in the event of an inspection and presented upon request.

   Note: these conditions may be copied and stored electronically for electronic signature and initialing provided that the permit number, authorized organisms and life stages, release locations, and authorization statement all appear on the document with the permit number. The residency condition does not need to be signed. Signing these conditions only indicates that the person working under this permit has read them; the permit holder is the sole responsible party under this permit.
2. Modifications to the containment of these organisms must be approved prior to making changes by applying for an amendment in ePermits.
3. This permit does not authorize movement or use of plant pathogens listed in the Public Health Security and Bioterrorism Preparedness and Response Act of 2002. If any organism listed as a Select Agent is identified from materials associated with this research, the permit holder is required to notify APHIS, Agricultural Select Agent Program (ASAP) immediately by phone at 301-851-3300, and within seven (7) days submit APHIS/CDC Form 4 (Report of Identification of a Select Agent or Toxin in a Clinical or Diagnostic Laboratory) to APHIS, ASAP; 4700 River Rd, Unit 2, Riverdale, MD 20737 (see instructions at: http://www.aphis.usda.gov/programs/ag_selectagent/index.shtml). Failure to comply with this requirement is a violation of the Agricultural Bioterrorism Protection Act of 2002.
4. If organisms that are not authorized in this permit are received, the permit holder must take all prudent measures to contain the organism(s) and notify the BRS Compliance Staff by contacting a compliance officer immediately (that is, within one business day) by calling 301-851-3935 or by e-mail to BRSCompliance@aphis.usda.gov. The permit holder must immediately notify the permit unit of the destruction of regulated organisms received under this permit, as above. Similarly, the permit holder must immediately notify the permit unit if facilities are destroyed or decommissioned for any reason.
5. The permit holder must maintain an official permanent work assignment at the address identified on this permit. If the permit holder ceases assignment/affiliation at the address identified on this permit, or personnel circumstances change in any way, then a compliance officer must be notified at the BRS Compliance Staff immediately (that is, within one business day) by either (a) email to BRSCompliance@aphis.usda.gov, (b) verbal communication at 301-851-3935, or (c) conventional mail to BRS Compliance Staff, 4700 River Road, Riverdale, Unit 91, MD 20737. The permit holder must destroy all regulated organisms prior to departure unless the permit holder either (a) requests cancellation of this permit and complies with all permit-specific termination conditions, (b) applies for and receives a permit to move the organisms to a new facility, or (c) relinquishes control of the regulated organisms to a qualified individual who obtained a permit for the continued use of these regulated organisms prior to this permit holder's departure.
6. All laboratories and growth chambers where this genetically engineered insect is employed will be locked with limited access by authorized personnel. In each area where a regulated genetically engineered organism is used, at least one sign must be posted stating that a regulated genetically engineered organism is being used.
7. Upon completion of research all engineered insects (except those retained for future research), will be disposed of by freezing at -20 for 24 hours/or autoclaving.
8. There is to be no further distribution of these genetically engineered insects under this permit without prior approval from State (intrastate movement) and Federal regulatory officials.
9. THIS AUTHORIZATION IS VALID FOR THE RELEASE INTO THE ENVIRONMENT OF THIS GENETICALLY ENGINEERED INSECT ONLY IN THE AREAS DESCRIBED IN THE APPLICATION.
10. THIS IS A CROP DESTRUCT TRIAL. Brassicas will be destroyed at the end of the research field trial. No plants of produce shall be used for food or feed. Upon completion of the experiment, the insecticide shall be sprayed on the plants and surrounding area within a 100 m radius of treated fields to kill remaining diamondback moth larvae.

11. Without prior notice and during reasonable hours authorized Plant Protection and Quarantine and State regulatory officials shall be allowed to inspect the conditions under which this genetically engineered insect is being kept.
12. Reporting an Unauthorized or Accidental Release

    a. According to the regulation in 7 CFR § 340.4(f)(10)(i), APHIS shall be notified orally immediately upon discovery and notified in writing within 24 hours in the event of any accidental or unauthorized release of the regulated article.

    - For immediate verbal notification, contact APHIS BRS Compliance Staff at (301) 851-3935 and ask to speak to a Compliance and Inspection staff member. Leave a verbal report on voicemail if the phone is not answered by a Compliance Officer.
    - In addition, in the event of an emergency in which you need to speak immediately to APHIS personnel regarding the situation, you may call:

    The APHIS/BRS Regional Biotechnologist assigned in the region where the field test occurs:
    For Western Region, contact the Western Region Biotechnologist at (970) 494-7513
    or e-mail: BRSWRBT@aphis.usda.gov
    For Eastern Region, contact the Eastern Region Biotechnologist at (919) 855-7622 or e-mail: BRSERBT@aphis.usda.gov Or

    The APHIS State Plant Health Director for the state where the unauthorized release occurred. The list of APHIS State Plant Health Directors is available at:
    http://www.aphis.usda.gov/services/report_pest_disease/report_pest_disease.shtml.
    or
    http://pest.ceris.purdue.edu/stateselect.html

    b. Written notification should be sent by one of the following means:

    By e-mail:
    BRSCompliance@aphis.usda.gov

    By mail:
    Biotechnology Regulatory Services (BRS)
    Regulatory Operations Program
    USDA/APHIS
    4700 River Rd. Unit 91
    Riverdale, MD 20737

    Additional instructions for reporting compliance incidents may be found at
    http://www.aphis.usda.gov/biotechnology/compliance_incident.shtml
13. No person shall move a regulated article interstate unless the number of the limited permit appears on the outside of the shipping container.

Standard Conditions

1. The regulated article shall be maintained and disposed of (when necessary) in a manner so as to prevent the dissemination and establishment of plant pests.
2. All packaging material, shipping containers, and any other material accompanying the regulated article shall be treated or disposed of in such a manner as to prevent the dissemination and establishment of plant pests.
3. The regulated article shall be kept separate from other organisms, except as specifically allowed in the permit.
4. The regulated article shall be maintained only in areas and premises specified in the permit.
5. An inspector shall be allowed access, during regular business hours, to the place where the regulated article is located and to any records relating to the introduction of a regulated article.
6. The regulated article shall, when possible, be kept identified with a label showing the name of the regulated article, and the date of importation.
7. The regulated article shall be subject to the application of measures determined by the Administrator to be necessary to prevent the accidental or unauthorized release of the regulated article.
8. The regulated article shall be subject to the application of remedial measures (including disposal) determined by the administrator to be necessary to prevent the spread of plant pests.
9. A person who has been issued a permit shall submit to APHIS a field test report within 6 months after the termination of the field test. A field test report shall include the APHIS reference number, methods of observation, resulting data, and analysis regarding all deleterious effects on plants, nontarget organisms, or the environment.
10. APHIS shall be notified within the time periods and manner specified below, in the event of the following occurrences:

    (i) Orally notified immediately upon discovery and notify in writing within 24 hours in the event of any accidental or unauthorized release of the regulated article;

    (ii) In writing as soon as possible but not later than within 5 working days if the regulated article or associated host organism is found to have characteristics substantially different from those listed in the application for a permit or suffers any unusual occurrence (excessive mortality or morbidity, or unanticipated effect on non-target organisms).
11. A permittee or his/her agent and any person who seeks to import a regulated article into the United States shall:

(i) Import or offer the regulated article for entry only through any USDA plant inspection station listed in 7 CFR 319.37-14;

(ii)Notify APHIS promptly upon arrival of any regulated article at a port of entry, of its arrival by such means as a manifest, customs entry document, commercial invoice, waybill, a broker's document, or a notice form provided for such purpose; and

(iii) Mark and identify the regulated article in accordance with 7 CFR 340.7.

Rev. January 1, 2010

Attachments to include with State Package

13_297102r_dea_Part1.pdf (10/30/2014)
13_297102r_dea_Part2.pdf (10/30/2014)
13_297102r_dea_Part3.pdf (10/30/2014)
13-297-102r_sl.doc (10/31/2014)
13-297-102r_first_FR notice_APHIS-2014-0056-0001.pdf (11/04/2014)

BRSPMT-0028

Received Date:	10/24/2013
Application Form:	BRS Permit
Application Category:	New
Application Number:	13-297-102r
Applicant:	Shelton, Anthony
Organization:	Cornell University/NYSAES
Submission Level:	2

Original	Current

Task Open Date	Task Close Date
11/10/2014	11/10/2014

Issue BRS Permit

Permit Document

BRS Permit - 13-297-102r

Attachments Included with Permit Package

No attachments included.

Date Sent:	11/10/2014
Effective Date:	:11/10/2014:
Expiration Date:	11/10/2017

BRSPMT-0055

Received Date:	10/24/2013
Application Form:	BRS Permit
Application Category:	New
Application Number:	13-297-102r
Applicant:	Shelton, Anthony
Organization:	Cornell University/NYSAES
Submission Level:	2

Original	Current
○	○

Task Open Date	Task Close Date
11/4/2014	11/4/2014

Management Review of Draft Conditions

The draft conditions are: • Ready for applicant review and approval

Not ready, require modification prior to applicant review and approval

Instructions/Comments: (Enter information for the Biotechnologist regarding any necessary modifications.)

Supplemental Conditions for Release

1. All persons working with the genetically-engineered (GE) diamondback moth must be informed of these permit conditions. Anyone working with these insects must agree to and sign/ initial these conditions before beginning work. These signed conditions do not need to be submitted to USDA/ APHIS but must be readily accessible in the event of an inspection and presented upon request.

   Note: these conditions may be copied and stored electronically for electronic signature and initialing provided that the permit number, authorized organisms and life stages, release locations, and authorization statement all appear on the document with the permit number. The residency condition does not need to be signed. Signing these conditions only indicates that the person working under this permit has read them; the permit holder is the sole responsible party under this permit.

2. Modifications to the containment of these organisms must be approved prior to making changes by applying for an amendment in ePermits.

3. This permit does not authorize movement or use of plant pathogens listed in the Public Health Security and Bioterrorism Preparedness and Response Act of 2002. If any organism listed as a Select Agent is identified from materials associated with this research, the permit holder is required to notify APHIS, Agricultural Select Agent Program (ASAP) immediately by phone at 301-851-3300, and within seven (7) days submit APHIS/CDC Form 4 (Report of Identification of a Select Agent or Toxin in a Clinical or Diagnostic Laboratory) to APHIS, ASAP; 4700 River Rd, Unit 2, Riverdale, MD 20737 (see instructions at: http://www.aphis.usda.gov/programs/ag_selectagent/index.shtml). Failure to comply with this requirement is a violation of the Agricultural Bioterrorism Protection Act of 2002.

4. If organisms that are not authorized in this permit are received, the permit holder must take all prudent measures to contain the organism(s) and notify the BRS Compliance Staff by contacting a compliance officer immediately (that is, within one business day) by calling 301-851-3935 or by e-mail to BRSCompliance@aphis.usda.gov. The permit holder must immediately notify the permit unit of the destruction of regulated organisms received under this permit, as above. Similarly, the permit holder must immediately notify the permit unit if facilities are destroyed or decommissioned for any reason.

5. The permit holder must maintain an official permanent work assignment at the address identified on this permit. If the permit holder ceases assignment/affiliation at the address identified on this permit, or personnel circumstances change in any way, then a compliance officer must be notified at the BRS Compliance Staff immediately (that is, within one business day) by either (a) email to BRSCompliance@aphis.usda.gov, (b) verbal communication at 301-851-3935, or (c) conventional mail to BRS Compliance Staff, 4700 River Road, Riverdale, Unit 91, MD 20737. The permit holder must destroy all regulated organisms prior to departure unless the permit holder either (a) requests cancellation of this permit and complies with all permit-specific termination conditions, (b) applies for and receives a permit to move the organisms to a new facility, or (c) relinquishes control of the regulated organisms to a qualified individual who obtained a permit for the continued use of these regulated organisms prior to this permit holder's departure.

6. All laboratories and growth chambers where this genetically engineered insect is employed will be locked with limited access by authorized personnel. In each area where a regulated genetically engineered organism is used, at least one sign must be posted stating that a regulated genetically engineered organism is being used.

7. Upon completion of research all engineered insects (except those retained for future research), will be disposed of by freezing at -20 for 24 hours/or autoclaving.

8. There is to be no further distribution of these genetically engineered insects under this permit without prior approval from State (intrastate movement) and Federal regulatory officials.

9. THIS AUTHORIZATION IS VALID FOR THE RELEASE INTO THE ENVIRONMENT OF THIS GENETICALLY ENGINEERED INSECT ONLY IN THE AREAS DESCRIBED IN THE APPLICATION.

10. THIS IS A CROP DESTRUCT TRIAL. Brassicas will be destroyed at the end of the research field trial. No plants of produce

shall be used for food or feed. Upon completion of the experiment, the insecticide shall be sprayed on the plants and surrounding area within a 100 m radius of treated fields to kill remaining diamondback moth larvae.

11. Without prior notice and during reasonable hours authorized Plant Protection and Quarantine and State regulatory officials shall be allowed to inspect the conditions under which this genetically engineered insect is being kept.

12. Reporting an Unauthorized or Accidental Release

a. According to the regulation in 7 CFR § 340.4(f)(10)(i), APHIS shall be notified orally immediately upon discovery and notified in writing within 24 hours in the event of any accidental or unauthorized release of the regulated article.

- For immediate verbal notification, contact APHIS BRS Compliance Staff at (301) 851-3935 and ask to speak to a Compliance and Inspection staff member. Leave a verbal report on voicemail if the phone is not answered by a Compliance Officer.
- In addition, in the event of an emergency in which you need to speak immediately to APHIS personnel regarding the situation, you may call:

The APHIS/BRS Regional Biotechnologist assigned in the region where the field test occurs:
For Western Region, contact the Western Region Biotechnologist at (970) 494-7513
or e-mail: BRSWRBT@aphis.usda.gov
For Eastern Region, contact the Eastern Region Biotechnologist at (919) 855-7622 or e-mail: BRSERBT@aphis.usda.gov Or

The APHIS State Plant Health Director for the state where the unauthorized release occurred. The list of APHIS State Plant Health Directors is available at:
http://www.aphis.usda.gov/services/report_pest_disease/report_pest_disease.shtml.
or
http://pest.ceris.purdue.edu/stateselect.html

b. Written notification should be sent by one of the following means:

By e-mail:
BRSCompliance@aphis.usda.gov

By mail:
Biotechnology Regulatory Services (BRS)
Regulatory Operations Program
USDA/APHIS
4700 River Rd. Unit 91
Riverdale, MD 20737

Additional instructions for reporting compliance incidents may be found at
http://www.aphis.usda.gov/biotechnology/compliance_incident.shtml

13. No person shall move a regulated article interstate unless the number of the limited permit appears on the outside of the shipping container.

Standard Permit Conditions

1. The regulated article shall be maintained and disposed of (when necessary) in a manner so as to prevent the dissemination and establishment of plant pests.

2. All packaging material, shipping containers, and any other material accompanying the regulated article shall be treated or disposed of in such a manner as to prevent the dissemination and establishment of plant pests.

3. The regulated article shall be kept separate from other organisms, except as specifically allowed in the permit.

4. The regulated article shall be maintained only in areas and premises specified in the permit.

5. An inspector shall be allowed access, during regular business hours, to the place where the regulated article is located and to any records relating to the introduction of a regulated article.

6. The regulated article shall, when possible, be kept identified with a label showing the name of the regulated article, and the date of importation.

7. The regulated article shall be subject to the application of measures determined by the Administrator to be necessary to prevent the accidental or unauthorized release of the regulated article.

8. The regulated article shall be subject to the application of remedial measures (including disposal) determined by the administrator to be necessary to prevent the spread of plant pests.

9. A person who has been issued a permit shall submit to APHIS a field test report within 6 months after the termination of the field test. A field test report shall include the APHIS reference number, methods of observation, resulting data, and analysis regarding all deleterious effects on plants, nontarget organisms, or the environment.

10. APHIS shall be notified within the time periods and manner specified below, in the event of the following occurrences:

(i) Orally notified immediately upon discovery and notify in writing within 24 hours in the event of any accidental or unauthorized release of the regulated article;

(ii) In writing as soon as possible but not later than within 5 working days if the regulated article or associated host organism is found to have characteristics substantially different from those listed in the application for a permit or suffers any unusual occurrence (excessive mortality or morbidity, or unanticipated effect on non-target organisms).

11. A permittee or his/her agent and any person who seeks to import a regulated article into the United States shall:

(i) Import or offer the regulated article for entry only through any USDA plant inspection station listed in 7 CFR 319.37-14;

(ii)Notify APHIS promptly upon arrival of any regulated article at a port of entry, of its arrival by such means as a manifest, customs entry document, commercial invoice, waybill, a broker's document, or a notice form provided for such purpose; and

(iii) Mark and identify the regulated article in accordance with 7 CFR 340.7.

Rev. January 1, 2010

BRSPMT-0072

Received Date:	10/24/2013
Application Form:	BRS Permit
Application Category:	New
Application Number:	13-297-102r
Applicant:	Shelton, Anthony
Organization:	Cornell University/NYSAES
Submission Level:	2

Original	Current

Task Open Date	Task Close Date
11/4/2014	11/4/2014

Management Review of State Package

Permit Dates

Effective Date: 11/10/2014

Expiration Date: 11/10/2017

Supplemental Conditions for Release

1. All persons working with the genetically-engineered (GE) diamondback moth must be informed of these permit conditions. Anyone working with these insects must agree to and sign/ initial these conditions before beginning work. These signed conditions do not need to be submitted to USDA/ APHIS but must be readily accessible in the event of an inspection and presented upon request.

   Note: these conditions may be copied and stored electronically for electronic signature and initialing provided that the permit number, authorized organisms and life stages, release locations, and authorization statement all appear on the document with the permit number. The residency condition does not need to be signed. Signing these conditions only indicates that the person working under this permit has read them; the permit holder is the sole responsible party under this permit.
2. Modifications to the containment of these organisms must be approved prior to making changes by applying for an amendment in ePermits.
3. This permit does not authorize movement or use of plant pathogens listed in the Public Health Security and Bioterrorism Preparedness and Response Act of 2002. If any organism listed as a Select Agent is identified from materials associated with this research, the permit holder is required to notify APHIS, Agricultural Select Agent Program (ASAP) immediately by phone at 301-851-3300, and within seven (7) days submit APHIS/CDC Form 4 (Report of Identification of a Select Agent or Toxin in a Clinical or Diagnostic Laboratory) to APHIS, ASAP; 4700 River Rd, Unit 2, Riverdale, MD 20737 (see instructions at: http://www.aphis.usda.gov/programs/ag_selectagent/index.shtml). Failure to comply with this requirement is a violation of the Agricultural Bioterrorism Protection Act of 2002.
4. If organisms that are not authorized in this permit are received, the permit holder must take all prudent measures to contain the organism(s) and notify the BRS Compliance Staff by contacting a compliance officer immediately (that is, within one business day) by calling 301-851-3935 or by e-mail to BRSCompliance@aphis.usda.gov. The permit holder must immediately notify the permit unit of the destruction of regulated organisms received under this permit, as above. Similarly, the permit holder must immediately notify the permit unit if facilities are destroyed or decommissioned for any reason.
5. The permit holder must maintain an official permanent work assignment at the address identified on this permit. If the permit holder ceases assignment/affiliation at the address identified on this permit, or personnel circumstances change in any way, then a compliance officer must be notified at the BRS Compliance Staff immediately (that is, within one business day) by either (a) email to BRSCompliance@aphis.usda.gov, (b) verbal communication at 301-851-3935, or (c) conventional mail to BRS Compliance Staff, 4700 River Road, Riverdale, Unit 91, MD 20737. The permit holder must destroy all regulated organisms prior to departure unless the permit holder either (a) requests cancellation of this permit and complies with all permit-specific termination conditions, (b) applies for and receives a permit to move the organisms to a new facility, or (c) relinquishes control of the regulated organisms to a qualified individual who obtained a permit for the continued use of these regulated organisms prior to this permit holder's departure.
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10. THIS IS A CROP DESTRUCT TRIAL. Brassicas will be destroyed at the end of the research field trial. No plants of produce shall be used for food or feed. Upon completion of the experiment, the insecticide shall be sprayed on the plants and surrounding area within a 100 m radius of treated fields to kill remaining diamondback moth larvae.

11. Without prior notice and during reasonable hours authorized Plant Protection and Quarantine and State regulatory officials shall be allowed to inspect the conditions under which this genetically engineered insect is being kept.
12. Reporting an Unauthorized or Accidental Release

    a. According to the regulation in 7 CFR § 340.4(f)(10)(i), APHIS shall be notified orally immediately upon discovery and notified in writing within 24 hours in the event of any accidental or unauthorized release of the regulated article.

    - For immediate verbal notification, contact APHIS BRS Compliance Staff at (301) 851-3935 and ask to speak to a Compliance and Inspection staff member. Leave a verbal report on voicemail if the phone is not answered by a Compliance Officer.
    - In addition, in the event of an emergency in which you need to speak immediately to APHIS personnel regarding the situation, you may call:

    The APHIS/BRS Regional Biotechnologist assigned in the region where the field test occurs:
    For Western Region, contact the Western Region Biotechnologist at (970) 494-7513
    or e-mail: BRSWRBT@aphis.usda.gov
    For Eastern Region, contact the Eastern Region Biotechnologist at (919) 855-7622 or e-mail: BRSERBT@aphis.usda.gov Or

    The APHIS State Plant Health Director for the state where the unauthorized release occurred. The list of APHIS State Plant Health Directors is available at:
    http://www.aphis.usda.gov/services/report_pest_disease/report_pest_disease.shtml.
    or
    http://pest.ceris.purdue.edu/stateselect.html

    b. Written notification should be sent by one of the following means:

    By e-mail:
    BRSCompliance@aphis.usda.gov

    By mail:
    Biotechnology Regulatory Services (BRS)
    Regulatory Operations Program
    USDA/APHIS
    4700 River Rd. Unit 91
    Riverdale, MD 20737

    Additional instructions for reporting compliance incidents may be found at
    http://www.aphis.usda.gov/biotechnology/compliance_incident.shtml
13. No person shall move a regulated article interstate unless the number of the limited permit appears on the outside of the shipping container.

Standard Conditions

1. The regulated article shall be maintained and disposed of (when necessary) in a manner so as to prevent the dissemination and establishment of plant pests.
2. All packaging material, shipping containers, and any other material accompanying the regulated article shall be treated or disposed of in such a manner as to prevent the dissemination and establishment of plant pests.
3. The regulated article shall be kept separate from other organisms, except as specifically allowed in the permit.
4. The regulated article shall be maintained only in areas and premises specified in the permit.
5. An inspector shall be allowed access, during regular business hours, to the place where the regulated article is located and to any records relating to the introduction of a regulated article.
6. The regulated article shall, when possible, be kept identified with a label showing the name of the regulated article, and the date of importation.
7. The regulated article shall be subject to the application of measures determined by the Administrator to be necessary to prevent the accidental or unauthorized release of the regulated article.
8. The regulated article shall be subject to the application of remedial measures (including disposal) determined by the administrator to be necessary to prevent the spread of plant pests.
9. A person who has been issued a permit shall submit to APHIS a field test report within 6 months after the termination of the field test. A field test report shall include the APHIS reference number, methods of observation, resulting data, and analysis regarding all deleterious effects on plants, nontarget organisms, or the environment.
10. APHIS shall be notified within the time periods and manner specified below, in the event of the following occurrences:

    (i) Orally notified immediately upon discovery and notify in writing within 24 hours in the event of any accidental or unauthorized release of the regulated article;

    (ii) In writing as soon as possible but not later than within 5 working days if the regulated article or associated host organism is found to have characteristics substantially different from those listed in the application for a permit or suffers any unusual occurrence (excessive mortality or morbidity, or unanticipated effect on non-target organisms).
11. A permittee or his/her agent and any person who seeks to import a regulated article into the United States shall:

(i) Import or offer the regulated article for entry only through any USDA plant inspection station listed in 7 CFR 319.37-14;

(ii)Notify APHIS promptly upon arrival of any regulated article at a port of entry, of its arrival by such means as a manifest, customs entry document, commercial invoice, waybill, a broker's document, or a notice form provided for such purpose; and

(iii) Mark and identify the regulated article in accordance with 7 CFR 340.7.

Rev. January 1, 2010

Attachments to include with State Package

13_297102r_dea_Part1.pdf (10/30/2014)
13_297102r_dea_Part2.pdf (10/30/2014)
13_297102r_dea_Part3.pdf (10/30/2014)
13-297-102r_sl.doc (10/31/2014)
13-297-102r_first_FR notice_APHIS-2014-0056-0001.pdf (11/04/2014)

BRSPMT-0033

Received Date: 10/24/2013
Application Form: BRS Permit
Application Category: New
Application Number: 13-297-102r
Applicant: Shelton, Anthony
Organization: Cornell University/NYSAES
Submission Level: 2

Original	Current

Task Open Date	Task Close Date
10/20/2014	10/24/2014
11/4/2014	11/4/2014

NEPA Decision Summary and TES Review

13-297-102_mpcl.docx	05/13/2014
13-297-102r_rpcl.doc	05/13/2014
13_297102r_dea_Part1.pdf	10/30/2014
13_297102r_dea_Part2.pdf	10/30/2014
13_297102r_dea_Part3.pdf	10/30/2014
13-297-102r_sl.doc	10/31/2014
13-297-102r_first_FR notice_APHIS-2014-0056-0001.pdf	11/04/2014

BRSPMT-0018

Received Date:	10/24/2013
Application Form:	BRS Permit
Application Category:	New
Application Number:	13-297-102r
Applicant:	Shelton, Anthony
Organization:	Cornell University/NYSAES
Submission Level:	2

Original	Current

Task Open Date	Task Close Date
10/24/2013	10/24/2013

Receive and Assign Application

Program Specialist: Diggs, Kimberly ▼

BRSPMT-0001

Received Date: 10/24/2013
Application Form: BRS Permit
Application Category: New
Application Number: 13-297-102r
Applicant: Shelton, Anthony
Organization: Cornell University/NYSAES
Submission Level: 2

Original	Current

Task Open Date	Task Close Date
5/6/2014	5/6/2014

Review Article and Donors

Article Name: Plutella xylostella (UNLISTED ARTICLE NAME)

Donors

Repressible lethality	
Escherichia coli (bacterium) and Herpes simplex (virus)	Unlisted Donor
Pink bollworm, Pectinophora gossypiella	Unlisted Donor
Screenable Marker	
Discosoma sp.	Listed Donor
piggyBac from Trichoplusia ni (moth)	Unlisted Donor

BRSPMT-0007

Received Date:	10/24/2013
Application Form:	BRS Permit
Application Category:	New
Application Number:	13-297-102r
Applicant:	Shelton, Anthony
Organization:	Cornell University/NYSAES
Submission Level:	2

Original	Current

Task Open Date	Task Close Date
11/4/2014	11/4/2014

Select Effective and Expiration Dates for Permit

Effective Date:: *	11/10/2014:	Earliest "Proposed Start Date" in the application - 04/01/2014
Expiration Date:: *	11/10/2017:	Latest "Proposed End Date" in the application - 03/31/2017

BRSPMT-0047

Received Date: 10/24/2013
Application Form: BRS Permit
Application Category: New
Application Number: 13-297-102r
Applicant: Shelton, Anthony
Organization: Cornell University/NYSAES
Submission Level: 2

Original	Current

Task Open Date	Task Close Date
11/4/2014	11/5/2014

State Regulatory Official Review

View Permit Package

New York	State has reviewed the APHIS determination and has no comment or additional regulatory requirements.	Jan Morawski (11/05/14)	11/05/2014	No	

BRS Permit Application Versions

BRS Permit Application Version 1 Nov 4 2014 1039AM.pdf
BRS Permit Application Original Oct 24 2013.pdf

BRSPMT-0034

Received Date: 10/24/2013
Application Form: BRS Permit
Application Category: New
Application Number: 13-297-102r
Applicant: Shelton, Anthony
Organization: Cornell University/NYSAES
Submission Level: 2

Original	Current

Task Open Date	Task Close Date
11/4/2014	11/5/2014

State Regulatory Official Review

New York's Response

Date of Action: 11/05/2014

Response: State has reviewed the APHIS determination and has no comment or additional regulatory requirements.

Comments:

Add Condition To add a Condition to the list below select **Add Condition**.

State has not determined any Condition(s)

Update Response **Cancel**

BRSPMT-0034

Received Date:	10/24/2013
Application Form:	BRS Permit
Application Category:	New
Application Number:	13-297-102r
Applicant:	Shelton, Anthony
Organization:	Cornell University/NYSAES
Submission Level:	2

Original	Current

Task Open Date	Task Close Date
11/4/2014	11/4/2014

State Review Confirmation

A state regulatory official response is: NOT Required

• Required

If a State does not need to review this application, please enter a brief justification in the provided area below. (Max. 2000 characters)

BRSPMT-0067

Received Date:	10/24/2013
Application Form:	BRS Permit
Application Category:	New
Application Number:	13-297-102r
Applicant:	Shelton, Anthony
Organization:	Cornell University/NYSAES
Submission Level:	2

Original	Current
○	○

Task Open Date	Task Close Date
11/4/2014	11/4/2014

Waiting for Applicant's Response to Draft Conditions

Applicant Response Received Due Date:* 11/04/2014

Supplemental Conditions for Release

1.	All persons working with the genetically-engineered (GE) diamondback moth must be informed of these permit conditions. Anyone working with these insects must agree to and sign/ initial these conditions before beginning work. These signed conditions do not need to be submitted to USDA/ APHIS but must be readily accessible in the event of an inspection and presented upon request. Note: these conditions may be copied and stored electronically for electronic signature and initialing provided that the permit number, authorized organisms and life stages, release locations, and authorization statement all appear on the document with the permit number. The residency condition does not need to be signed. Signing these conditions only indicates that the person working under this permit has read them; the permit holder is the sole responsible party under this permit.	Agree •	Disagree
2.	Modifications to the containment of these organisms must be approved prior to making changes by applying for an amendment in ePermits.	Agree •	Disagree
3.	This permit does not authorize movement or use of plant pathogens listed in the Public Health Security and Bioterrorism Preparedness and Response Act of 2002. If any organism listed as a Select Agent is identified from materials associated with this research, the permit holder is required to notify APHIS, Agricultural Select Agent Program (ASAP) immediately by phone at 301-851-3300, and within seven (7) days submit APHIS/CDC Form 4 (Report of Identification of a Select Agent or Toxin in a Clinical or Diagnostic Laboratory) to APHIS, ASAP; 4700 River Rd, Unit 2, Riverdale, MD 20737 (see instructions at: http://www.aphis.usda.gov/programs/ag_selectagent/index.shtml). Failure to comply with this requirement is a violation of the Agricultural Bioterrorism Protection Act of 2002.	Agree •	Disagree
4.	If organisms that are not authorized in this permit are received, the permit holder must take all prudent measures to contain the organism(s) and notify the BRS Compliance Staff by contacting a compliance officer immediately (that is, within one business day) by calling 301-851-3935 or by e-mail to BRSCompliance@aphis.usda.gov. The permit holder must immediately notify the permit unit of the destruction of regulated organisms received under this permit, as above. Similarly, the permit holder must immediately notify the permit unit if facilities are destroyed or decommissioned for any reason.	Agree •	Disagree
5.	The permit holder must maintain an official permanent work assignment at the address identified on this permit. If the permit holder ceases assignment/affiliation at the address identified on this permit, or personnel circumstances change in any way, then a compliance officer must be notified at the BRS Compliance Staff immediately (that is, within one business day) by either (a) email to BRSCompliance@aphis.usda.gov, (b) verbal communication at 301-851-3935, or (c) conventional mail to BRS Compliance Staff, 4700 River Road, Riverdale, Unit 91, MD 20737. The permit holder must destroy all regulated organisms prior to departure unless the permit holder either (a) requests cancellation of this permit and complies with all permit-specific termination conditions, (b) applies for and receives a permit to move the organisms to a new facility, or (c) relinquishes control of the regulated organisms to a qualified individual who obtained a permit for the continued use of these regulated organisms prior to this permit holder's departure.	Agree •	Disagree
6.	All laboratories and growth chambers where this genetically engineered insect is employed will be locked with limited access by authorized personnel. In each area where a regulated genetically engineered organism is used, at least one sign must be posted stating that a regulated genetically engineered organism is being used.	Agree •	Disagree
7.	Upon completion of research all engineered insects (except those retained for future research), will be disposed of by freezing at -20 for 24 hours/or autoclaving.	Agree •	Disagree
8.	There is to be no further distribution of these genetically engineered insects under this permit without prior approval from State (intrastate movement) and Federal regulatory officials.	Agree •	Disagree
9.	THIS AUTHORIZATION IS VALID FOR THE RELEASE INTO THE ENVIRONMENT OF THIS	Agree	Disagree

	GENETICALLY ENGINEERED INSECT ONLY IN THE AREAS DESCRIBED IN THE APPLICATION.	•	
10.	THIS IS A CROP DESTRUCT TRIAL. Brassicas will be destroyed at the end of the research field trial. No plants of produce shall be used for food or feed. Upon completion of the experiment, the insecticide shall be sprayed on the plants and surrounding area within a 100 m radius of treated fields to kill remaining diamondback moth larvae.	Agree •	Disagree
11.	Without prior notice and during reasonable hours authorized Plant Protection and Quarantine and State regulatory officials shall be allowed to inspect the conditions under which this genetically engineered insect is being kept.	Agree •	Disagree
12.	Reporting an Unauthorized or Accidental Release a. According to the regulation in 7 CFR § 340.4(f)(10)(i), APHIS shall be notified orally immediately upon discovery and notified in writing within 24 hours in the event of any accidental or unauthorized release of the regulated article. - For immediate verbal notification, contact APHIS BRS Compliance Staff at (301) 851-3935 and ask to speak to a Compliance and Inspection staff member. Leave a verbal report on voicemail if the phone is not answered by a Compliance Officer. - In addition, in the event of an emergency in which you need to speak immediately to APHIS personnel regarding the situation, you may call: The APHIS/BRS Regional Biotechnologist assigned in the region where the field test occurs: For Western Region, contact the Western Region Biotechnologist at (970) 494-7513 or e-mail: BRSWRBT@aphis.usda.gov For Eastern Region, contact the Eastern Region Biotechnologist at (919) 855-7622 or e-mail: BRSERBT@aphis.usda.gov Or The APHIS State Plant Health Director for the state where the unauthorized release occurred. The list of APHIS State Plant Health Directors is available at: http://www.aphis.usda.gov/services/report_pest_disease/report_pest_disease.shtml. or http://pest.ceris.purdue.edu/stateselect.html b. Written notification should be sent by one of the following means: By e-mail: BRSCompliance@aphis.usda.gov By mail: Biotechnology Regulatory Services (BRS) Regulatory Operations Program USDA/APHIS 4700 River Rd. Unit 91 Riverdale, MD 20737 Additional instructions for reporting compliance incidents may be found at http://www.aphis.usda.gov/biotechnology/compliance_incident.shtml	Agree •	Disagree
13.	No person shall move a regulated article interstate unless the number of the limited permit appears on the outside of the shipping container.	Agree •	Disagree

Standard Permit Conditions

	I, Anthony Shelton, accept the Standard Permit Conditions listed below. Standard Permit Conditions are set by the regulations and you must accept them before you will be issued a permit.	Agree •	Disagree
1.	The regulated article shall be maintained and disposed of (when necessary) in a manner so as to prevent the dissemination and establishment of plant pests.		
2.	All packaging material, shipping containers, and any other material accompanying the regulated article shall be treated or disposed of in such a manner as to prevent the dissemination and establishment of plant pests.		
3.	The regulated article shall be kept separate from other organisms, except as specifically allowed in the permit.		
4.	The regulated article shall be maintained only in areas and premises specified in the permit.		
5.	An inspector shall be allowed access, during regular business hours, to the place where the regulated article is located and to any records relating to the introduction of a regulated article.		
6.	The regulated article shall, when possible, be kept identified with a label showing the name of the regulated article, and the date of importation.		
7.	The regulated article shall be subject to the application of measures determined by the Administrator to be necessary to prevent the accidental or unauthorized release of the regulated article.		
8.	The regulated article shall be subject to the application of remedial measures (including disposal) determined by the administrator to be necessary to prevent the spread of plant		

pests.

9. A person who has been issued a permit shall submit to APHIS a field test report within 6 months after the termination of the field test. A field test report shall include the APHIS reference number, methods of observation, resulting data, and analysis regarding all deleterious effects on plants, nontarget organisms, or the environment.

10. APHIS shall be notified within the time periods and manner specified below, in the event of the following occurrences:

    (i) Orally notified immediately upon discovery and notify in writing within 24 hours in the event of any accidental or unauthorized release of the regulated article;

    (ii) In writing as soon as possible but not later than within 5 working days if the regulated article or associated host organism is found to have characteristics substantially different from those listed in the application for a permit or suffers any unusual occurrence (excessive mortality or morbidity, or unanticipated effect on non-target organisms).

11. A permittee or his/her agent and any person who seeks to import a regulated article into the United States shall:

    (i) Import or offer the regulated article for entry only through any USDA plant inspection station listed in 7 CFR 319.37-14;

    (ii)Notify APHIS promptly upon arrival of any regulated article at a port of entry, of its arrival by such means as a manifest, customs entry document, commercial invoice, waybill, a broker's document, or a notice form provided for such purpose; and

    (iii) Mark and identify the regulated article in accordance with 7 CFR 340.7.

    Rev. January 1, 2010

Comments: (Required if the Response is "Disagree" for one or more conditions)

BRSPMT-0076

EPermits Supporting docs

USDA United States Department of Agriculture
APHIS Animal and Plant Health Inspection Service
Biotechnology Regulatory Services
4700 River Road, Unit 147
Riverdale, Maryland
20737-1236

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
BIOTECHNOLOGY REGULATORY SERVICE

PERMIT UNDER 7 CFR 340

(Genetically Engineered Organisms or Products)

This permit was generated electronically via the ePermits system

Enclosed is the BRS Permit Application

PERMITTEE NAME:	Prof. Anthony Shelton
ORGANIZATION:	Cornell/NYSAES
ADDRESS:	614 W. North St. Geneva, NY 14456
PHONE:	(b)(6)
FAX:	
DESTINATION:	NY

PERMIT NUMBER	12-227-102m
DATE ISSUED:	September 12, 2012
EFFECTIVE:	September 15, 2012
EXPIRES:	**September 15, 2013**
INTRODUCTION TYPE	Importation
PERMIT TYPE	Standard
PURPOSE OF PERMIT	Traditional

Under the conditions specified, this permit authorizes the following:

Regulated Article: Plutella xylostella

Permit Number: 12-227-102m

THIS PERMIT HAS BEEN APPROVED ELECTRONICALLY BY THE FOLLOWING BRS OFFICIAL VIA EPERMITS

SIGNATURE OF BRS OFFICIAL	DATE
Steven M. Bennett	September 12, 2012

The collection of this information is authorized by the Plant Protection Act of 2000. The information will be used to determine eligibility to receive all types of permits. No permit will be issued until this application has been approved.

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
BIOTECHNOLOGY REGULATORY SERVICE

APPLICATIONS FOR PERMIT OR COURTESY PERMIT UNDER 7 CFR 340

(Genetically Engineered Organisms or Products)

1. NAME, ADDRESS, TELEPHONE, AND EMAIL OF APPLICANT

Name: Prof. Anthony Shelton
Position: Professor of Entomology
Organization: Cornell/NYSAES
Organization Unique ID:
Address: 614 W. North St.
Geneva, NY 14456
County/Province:
Township/Island:
Day Telephone: (b)(6)
FAX:
Alternate:
Email 1:
Email 2:

2. INTRODUCTION TYPE

- [x] Importation
- [ ] Interstate Movement
- [ ] Interstate Movement and Release
- [ ] Release

3. PERMIT TYPE

- [x] Standard Permit
- [ ] Courtesy Permit

4. PURPOSE OF PERMIT

- [ ] Industrial Product
- [ ] Pharmaceutical Product
- [ ] Phytoremediation
- [x] Traditional

5. CONFIDENTIAL BUSINESS INFORMATION VERIFICATION (CBI)

Does this application contain CBI? [ ] Yes [x] No
CBI Justification:
N/A

6. REQUEST TYPE

[x] **New** [ ] **Amendment** [ ] **Renewal** [ ] **Variance** [ ] **Amendment, Renewal and/or Variance**

Amendment/Renewal Description:
Previous Permit Number(s):

7. MEANS OF MOVEMENT

Express carrier or in baggage or carry-on luggage

8. VARIANCE VERIFICATION

Have you previously applied for variance(s) that you wish to apply to this permit? [ ] Yes [x] No
Variance Number(s):

If so, describe in a brief summary how the variance will be applied:
N/A

9. REGULATED ARTICLE

Scientific Name: Plutella xylostella

Common Name: Diamondback moth

Cultivar and/or Breeding Line:
Phenotypic designation name: fsRIDL

Any biological material (e.g., culture medium, or host material) accompanying the regulated Article during movement:
Artificial insect diet

Country and locality where the donor organism, recipient organism, and vector or vector agent were collected, developed, and produced:
All final engineering of the transforming constructs was performed at Oxitec Ltd, in the United Kingdom.
The genes used form the donor organisms and the piggyBac-derived portions of the vectors used to build the transforming construct were cloned off-site. The recipient organism is the moth, Plutella xylostella, which is endemic in temperate regions around the world, including the USA.
The recipient Plutella xylostella strain for the transformation was a wild-type strain obtained from Syngenta plc, UK, which has been reared in Oxitec insectaries since 2008.

Processes, Procedures, and Safeguards Description:

10. ARTICLE SUPPLIER AND/OR DEVELOPER

Name	Location	Contact Information
(b) (6)	Oxitec Ltd 71 Milton Park Oxford OX14 4RX United Kingdom **County:** Oxfordshire	**Day Telephone:** (b) (6) **Email:** (b) (6)

11. PHENOTYPES/GENOTYPE

1) Phenotypic Designation Name: fsRIDL

Identifying Line(s):

Construct(s): fsRIDL

Mode of Transformation: Direct injection

Phenotype Description:
A description of the anticipated or actual expression of the altered genetic material in the regulated article and how that expression differs from the expression in the non-modified parental organism.

Genetically modified Plutella xylostella were produced using the transposable element piggyBac, isolated from the cabbage looper moth, Trichiplusia ni. These transposons insert in single or multiple copies in the moth genome. Ie1/Hr5 specific promoters derived from Autographa californica nuclear polyhedrosis virus (AcMNPV) are used to drive the expression of the fluorescent proteins DsRed2, which enables easy visualization of the transgenes.

Phenotype(s)

MG - Pigment composition altered

OC - Sterile

Genotype(s)

Sterile
Gene: Screenable marker Gene **from** Discosoma spp - Allows the expression of a fluorescent protein from Discosoma spp. this allows the expression of a fluorescent protein of the GFP superfamily (DsRed2) under the control of a hr5ie1 promoter/enhancer sequence, which is from Autographa californica nuclear polyhedrosis virus (AcMNPV). A transgenic diamondback moth with the marker gene will fluoresce when excited by illumination of the appropriate wavelength. These fluorescent proteins, which have been used as markers in a wide range of vertebrate and invertebrate species, confer no known competitive advantage or disadvantage to the recipient, and no adverse ecological effect or other consequences resulting from incorporation of these markers into the transgenic diamondback moth are envisioned. The unmodified moths are not strongly fluorescent. Expression of a fluorescent protein will therefore permit all other modified moths to be distinguished from unmodified.

Enhancer: Enhancer **from** Escherichia coli - Tetracycline-repressible transcriptional activator from tTAV is a synthetic fusion of the tetR protein from Escherichia coli with VP16 from a type 1 herpes simplex virus the tTA protein binds to and activates expression from the tetracycline response element (tRE), which includes multiple copies of the specific DNA sequence to which tTA binds (tetO) (Gossen et al., 1994; Gossen & Bujard, 1992). tTAV also binds tetracyclines with high affinity; the tetracycline-bound form of tTAV does not bind DNA. tTAV therefore acts as a tetracycline-regulated switch. In the absence of tetracycline it will induce expression from tRE, whereas in the presence of tetracycline in will not. High-level expression of tTAV is thought to be deleterious to cells as it can repress their normal transcription; low-level expression has no known effect other than activation of tRE (Berger, et al., 1990; Damke et al., 1995; Gillespie et al., 1997; Gong et al., 2005; Gossen and Bujard, 1992; Salghetti et al., 2001). tTAV has been used in fungi, plants, mice and D. melanogaster with no known adverse effects. Unmodified Plutella xylostella do not have a tTAV activity.

Gene: Female-specificity **from** Escherichia coli - tTAV is inserted into genomic regions containing specific sequences enabling the alternative splicing allowing for the expression of tTAV in a sex-specific manner, resulting in a female-lethal system.

12. INTRODUCTION

Point of Origin

Location Name & Description	Location Address	Contact(s)
1) Oxitec Ltd	United Kingdom	

Destination

Location Name & Description	Location Address	Contact(s)
1) Cornell University/NYSAHS - Barton Lab	Room 421 630 W. North St. Geneva, NY 14456 **County:** Ontario **Proposed Start Date:** 9/14/2012 **Proposed End Date:** 9/13/2013 **Quantity:** 5000 Individual Adult, eggs, larvae or pupae **Inspected by BRS or PPQ?** Yes **Previous Permit No.:** 63937	

13. DESIGN PROTOCOLS

Production Design

A detailed description of the purpose for the introduction of the regulated article including detailed description of the proposed experimental and/or production design:

N/A

Destination or Release Description

A detailed description of the intended destination (including final and all intermediate destinations), uses, and/or distribution of the regulated article (e.g., greenhouses, laboratory, or growth chamber location; field trial location, pilot project location; production, propagation, and manufacture location; proposed sale and distribution location):

Shipment of no more than 5,000 Plutella xylostella eggs/larvae/pupae from the United Kingdom to Cornell University will involve packaging the moths into shatter-resistant plastic containers with a further two layers of containment, which will be closed and sealed with tape. The box will be labelled as to its contents, origin, destination and contact telephone numbers.
Upon arrival at the insectaries at Cornell University the shipment of moths (b)(4)

ld

e received insects will be used to populate a laboratory colony that will be used for research purposes within the laboratory and trials in fully contained glasshouses with restricted access.

Confinement Protocols

A detailed description of the proposed procedures, processes, and safeguards which will be used to prevent escape and dissemination of the regulated article at each of the intended destinations:

Transgenic diamondback moths at Cornell University will be housed within Barton lab in room
(b) (4)

(b) (4)

× 1.7 m high) constructed of nylon netting are hung and insects and plants are placed into cages. The mesh of the cages does not permit adults or larvae to move through them. Access into the cages is through sleeves sew into the cages.
Even if there are escapes from the cages and through the greenhouse, it has been well documented that diamondback moth is incapable of surviving the winter in our area. Diamondback moth colonizes cabbage in western New York from the shipment of infested transplants from southern areas or greenhouses, or migrates here on weather patterns.
Diamondback moth in the rearing room and glasshouses:
Diamondback moths will be reared according to our established protocols (http://web.entomology.cornell.edu/shelton/diamondback-moth/)
In the laboratory, moths will be reared at 25oC with a natural photoperiod. Eggs are laid on aluminium foil that has been dipped into cabbage juice and larvae and pupae are kept in plastic rearing containers. Pupae are transferred to cages for subsequent egg collection. In caged glasshouse experiments, diamondback moth with be reared on cabbage or broccoli throughout their life-cycle, with some samples moved to the rearing room to be reared as described above. All

adults are kept in sturdy screened, tightly closed cages within the laboratory or glasshouse. All surplus eggs, larvae, pupae or adults, and associated food material, are killed by freezing at -20oC for at least 12 hours.
Shipment of diamondback moth from the United Kingdom to Cornell University will involve placing the moths into shatter-resistant plastic containers with a further two layers of containment, which will be closed and sealed with tape. The box will be labelled as to its contents, origin, destination and contact telephone numbers. That sealed container will be shipped via TNT or another designated carrier or occasionally carried by a traveller.
Final disposition description:
Transgenic diamondback moths will be reared until such time as experiments pertaining to the specific transgenes under investigation are finished. At such time all remaining eggs, larvae, pupae and adults will be frozen at -20oC for 12 hours.

Final Disposition Method: ☒ Destruction/Devitalization ☐ Other ☐ Storage in Contained Facility

Final Disposition Description: Transgenic diamondback moths will be reared until such time as experiments pertaining to the specific transgenes under investigation are finished. At such time all remaining eggs, larvae, pupae and adults will be frozen at -20oC for 12 hours.

14. ATTACHMENTS

15. ADDITIONAL INFORMATION

Insects will be shipped in a shatter-resistant plastic containers with a further two layers of containment, which will be closed and sealed with tape. The box will be labelled as to its contents, origin, destination and contact telephone numbers.

16. COURTESY JUSTIFICATION

I, *Anthony Shelton*, hereby certify that the information in this application and all attachments is complete and accurate to the best of my knowledge and belief.

I acknowledge this is not an application to move or import select agents, the genes expressing select agents, or the toxins made by the select agents, as described in 9 CFR 121.

I will not introduce the regulated articles described in this application until APHIS has deemed the application complete and has granted the permit. By signing this permit, I agree to comply with any and all state, local, and tribal laws and regulations that may apply to the introduction of the articles described in this applications.

If there are any changes to the information disclosed in this application, I will contact APHIS.

17. SIGNATURE OF RESPONSIBLE PERSON	**18. DATE**
Anthony Shelton	August 14, 2012

SUPPLEMENTAL PERMIT CONDITIONS

For Movement of Plutella xylostella

(1) The Plutella xylostella eggs, larvae, or pupae are to be shipped in containers as specified in 7 CFR Part 340.8(4), for insects, mites, and related organisms or as stated in the permit application.

(2) This authorization is strictly for rearing and research in a controlled laboratory environment.

(3) All laboratories and growth chambers where this genetically engineered insect is employed will be locked with limited access by authorized personnel. In each area where a regulated genetically engineered organism is used, at least one sign must be posted on the door or wall stating that a regulated genetically engineered organism is being used.

(4) Upon completion of research all engineered insects (except those retained for future research), will be disposed of by freezing at -20 for 24 hours/or autoclaving for a minimum of 20 minutes.

(5) This authorization for movement under permit, is valid for execution, for a period of 1 year.

(6) There is to be no further distribution of these genetically engineered insects under this permit without prior approval from State (intrastate movement) and Federal regulatory officials (interstate movement).

(7) THIS AUTHORIZATION IS NOT VALID FOR THE RELEASE INTO THE ENVIRONMENT OF THIS GENETICALLY ENGINEERED INSECT.

All necessary precautions must be taken to prevent escape of these genetically engineered insects.

(8) Without prior notice and during reasonable hours authorized Plant Protection and Quarantine and State regulatory officials shall be allowed to inspect the conditions under which this genetically engineered insect is being kept.

(9) Reporting an Unauthorized or Accidental Release

1. According to the regulation in 7 CFR § 340.4(f)(10)(i), APHIS shall be notified orally immediately upon discovery and notified in writing within 24 hours in the event of any accidental or unauthorized release of the regulated article.

- For immediate verbal notification, contact APHIS BRS Compliance Staff at (301) 851-3935 and ask to speak to a Compliance and Inspection staff member. Leave a verbal report on voicemail if the phone is not answered by a Compliance Officer.
- In addition, in the event of an emergency in which you need to speak immediately to APHIS personnel regarding the situation, you may call:

The APHIS/BRS Regional Biotechnologist assigned in the region where the field test occurs:
For Western Region, contact the Western Region Biotechnologist at (970) 494-7513
or e-mail: BRSWRBT@aphis.usda.gov
For Eastern Region, contact the Eastern Region Biotechnologist at (919) 855-7622 or e-mail: BRSERBT@aphis.usda.gov

Or

The APHIS State Plant Health Director for the state where the unauthorized release occurred. The list of APHIS State Plant Health Directors is available at:
http://www.aphis.usda.gov/services/report_pest_disease/report_pest_disease.shtml.
or
http://pest.ceris.purdue.edu/stateselect.html

2. Written notification should be sent by one of the following means:

By e-mail:
BRSCompliance@aphis.usda.gov

By mail:
Biotechnology Regulatory Services (BRS)
Regulatory Operations Program
USDA/APHIS
4700 River Rd. Unit 91
Riverdale, MD 20737

3. Additional instructions for reporting compliance incidents may be found at http://www.aphis.usda.gov/biotechnology/compliance_incident.shtml

(10) No person shall move a regulated article interstate unless the number of the limited permit appears on the outside of the shipping container.

Permit Number: 12-227-102m

THIS PERMIT HAS BEEN APPROVED ELECTRONICALLY BY THE FOLLOWING BRS OFFICIAL VIA EPERMITS	
SIGNATURE OF BRS OFFICIAL	**DATE**
Steven M. Bennett	September 12, 2012

United States Department of Agriculture
Animal and Plant Health Inspection Service
Biotechnology Regulatory Services
4700 River Road, Unit 147
Riverdale, Maryland
20737-1236

Standard Permit Conditions for the Introduction of a Regulated Article

(7 CFR 340.4 (f))

Permit Conditions: A person who is issued a permit and his/her employees or agents shall comply with the following conditions , and any supplemental conditions which shall be listed on the permit, as deemed by the Deputy Administrator to be necessary to prevent the dissemination and establishment of plant pests:

(1) The regulated article shall be maintained and disposed of (when necessary) in a manner so as to prevent the dissemination and establishment of plant pests.

(2) All packaging material, shipping containers, and any other material accompanying the regulated article shall be treated or disposed of in such a manner as to prevent the dissemination and establishment of plant pests.

(3) The regulated article shall be kept separate from other organisms, except as specifically allowed in the permit.

(4) The regulated article shall be maintained only in areas and premises specified in the permit.

(5) An inspector shall be allowed access, during regular business hours, to the place where the regulated article is located and to any records relating to the introduction of a regulated article.

(6) The regulated article shall, when possible, be kept identified with a label showing the name of the regulated article, and the date of importation.

(7) The regulated article shall be subject to the application of measures determined by the Administrator to be necessary to prevent the accidental or unauthorized release of the regulated article.

(8) The regulated article shall be subject to the application of remedial measures (including disposal) determined by the administrator to be necessary to prevent the spread of plant pests.

(9) A person who has been issued a permit shall submit to APHIS a field test report within 6 months after the termination of the field test. A field test report shall include the APHIS reference number, methods of observation, resulting data, and analysis regarding all deleterious effects on plants, nontarget organisms, or the environment.

(10) APHIS shall be notified within the time periods and manner specified below, in the event of the following occurrences:

(i) Orally notified immediately upon discovery and notify in writing within 24 hours in the event of any accidental or unauthorized release of the regulated article;

(ii) In writing as soon as possible but not later than within 5 working days if the regulated article or associated host organism is found to have characteristics substantially different from those listed in the application for a permit or suffers any unusual occurrence (excessive mortality or morbidity, or unanticipated effect on non-target organisms).

(11) A permittee or his/her agent and any person who seeks to import a regulated article into the United States shall:

(i) Import or offer the regulated article for entry only through any USDA plant inspection station listed in 7 CFR 319.37-14;

(ii)Notify APHIS promptly upon arrival of any regulated article at a port of entry, of its arrival by such means as a manifest, customs entry document, commercial invoice, waybill, a broker's document, or a notice form provided for such purpose; and

(iii) Mark and identify the regulated article in accordance with 7 CFR 340.7.

Rev. January 1, 2010

Permit Number: 12-227-102m

THIS PERMIT HAS BEEN APPROVED ELECTRONICALLY BY THE FOLLOWING BRS OFFICIAL VIA EPERMITS	
SIGNATURE OF BRS OFFICIAL	**DATE**
Steven M. Bennett	September 12, 2012

Any regulated article introduced not in compliance with the requirements of 7 CFR 340 or any standard or supplemental permit conditions, shall be subject to the immediate application of such remedial measures or safeguards as an inspector determines necessary, to prevent the introduction of such plant pests. Any regulated article introduced not in compliance with the requirements of 7 Code of Federal Regulation Part 340 or any standard or supplemental permit conditions, shall be subject to the immediate application of such remedial measures or safeguards as an inspector determines necessary, to prevent the introduction of such plant pests. The responsible party may be subject to fines or penalties as authorized by the Plant Protection Act (7 U.S.C. 7701-7772).

Permit Number: 12-227-102m

THIS PERMIT HAS BEEN APPROVED ELECTRONICALLY BY THE FOLLOWING BRS OFFICIAL VIA EPERMITS	
SIGNATURE OF BRS OFFICIAL	**DATE**
Steven M. Bennett	September 12, 2012

Location of experimental site, boundaries marked as red

(b)(4)

(b)(4)

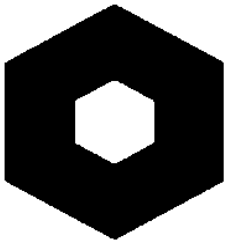
(b)(4)

Longitude/latitude of marked boundary points

(b)(4)

Redact Coordinates from photo above also

Redact Coordinates from photo above also



OXITEC

INTERNAL RESEARCH REPORT

1. **Title**: Investigating the persistence of a tetracycline-repressible, female-specific lethal trait in a captive mixed fsRIDL and wild-type population of diamondback moth (*Plutella xylostella*) reared off tetracycline.

2. **Statement Of Data Confidentiality Claims:**

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4. **Authors:**

Study Coordinator (Signature):	**Study Supervisor (Signature):**
(b6	(b6
Study Coordinator (Name And Position): (b6 Research Scientist	**Study Supervisor (Name And Position):** (b)(6) PhD (b6
Date Signed: 17th September 2013	**Date Signed:** 17th September 2013

Other P

5. **Associated Personnel:**

Name	Tasks
(b6	Study coordination, experimental design, report writing
	Experimental design, data collection and analysis, modelling, report writing
	Modelling
	Experimental design, approval
	Study Sponsor

6. **Test Facility:**

This research was performed at Oxitec's research facilities located at:

71 Milton Park,
Abingdon, Oxfordshire,
OX14 4RX
United Kingdom

7. **Objectives:**

The objective of this study was to determine the rate at which the OX4319L transgene insertion (or allele) disappears after introduction into a stable, caged wild-type population of diamondback moth.

8. **Summary:**

This experiment was designed to test whether the OX4319 transgene will fully select itself out of a wild post release population at a rate predicted by simulation models of natural selection against the transgene. Larvae in this experiment were reared off-tetracycline (restrictive conditions) in order to simulate the anticipated field environment, where insects are not expected to have access to a tetracycline source. A mixed population of OX4319L wild-type diamondback moths (derived from the same progenitor strain as OX4319L strain and therefore sharing non-transgene genetic background) was established by crossing males heterozygous for the OX4319L transgene insertion with wild-type females. The colony was maintained over a number of generations, with larvae reared on non-tetracycline diet and

200 randomly selected pupae used to establish each generation's cage. The number of transgenic pupae - identified by the fluorescent protein marker - in these selected pupal batches was recorded at each generation. Transgene frequency decreased in a manner consistent with model predictions leading to extinction of the transgene from each experimental population. These data are consistent with the hypothesis that the OX4319 transgene will rapidly reduce in frequency and disappear from a wild-type population unless maintained by periodic release of additional transgene-bearing individuals.

9. Introduction:

The diamondback moth strain, OX4319L, is engineered to express two phenotypes: tetracycline-repressible, female-specific lethality; and red fluorescence from expressed DsRed2 protein (Jin et al., 2013). Female lethality permits automated production of male-only cohorts of moths, by withholding tetracycline (or suitable analogues, such as chlortetracycline) from the larval feed. For the insect control strategy, fsRIDL, male-only release is expected to improve efficiency of target pest control, which is induced by fsRIDL males mating with wild counterparts and all female progeny dying in the absence of tetracycline in the host plant. With sustained releases of fsRIDL males, in sufficient numbers, the number of females in the wild drops, and consequently the reproductive potential of the wild population of the target pest crashes.

The RIDL strain of diamondback moth, OX4319L, has undergone a series of laboratory experiments to study the engineered female-lethal phenotype with a view to understanding how the trait will act in the field.

Here we investigated the persistence of the OX4319L allele in a laboratory-reared, mixed population of OX4319L and wild-type moths, reared in the absence of tetracycline. In parallel, we modelled this experiment and compared these results to those from the laboratory experiment.

10. Methods

Strains

OX4319L (female-specific lethal RIDL strain): In the absence of tetracycline, the females of this strain express high levels of the tTAV protein during immature stages through a genetic positive feedback loop, resulting in high mortality. However, when reared in the presence of tetracycline (or suitable analogues), expression of tTAV is repressed, allowing survival (Jin et al., 2013).

Experimental procedures

OX4319L males and females were crossed in single sex cohorts to Wt members of the opposite sex. The resulting heterozygous eggs were reared on-tet and male and female pupae separated by hand. Once eclosed, 100 male heterozygotes were crossed to 100 Wt females in a 30cmx30cmx30cm cage (Bugdorm). Thus, the initial transgene allele frequency in the cage was 0.25, with all transgenics as heterozygous males, representing a post OX4319L release field population. Insects were provided with a non-tet sugar water source and a parafilm artificial leaf on which to oviposit. Both sugar water and parafilm were

replaced 2 days after setting up the experiment, and removed 2 days later. The two egg collections were reared on non-tet diet in plastic deli pots with the first being taken for the next generation and the second acting as a backup. Once all individuals had pupated, 200 pupae were randomly selected from the pot, scored for fluorescence and for sex. As the RIDL phenotype in this line expresses itself at an early larval stage ($1^{st}/2^{nd}$ instar), selecting a constant number of individuals at the pupal stage allowed us to proportionally represent the genotype frequencies of each population in each new generation, whilst keeping the numerical size of each experimental population manageable. Each combination of sex and genotype was kept separately in paper cups with access to non-tet sugar water. Once all individuals had eclosed (in order to prevent possible mating biases based on eclosion time, for example towards those genotypes which eclosed first in each generation) they were combined in the same cage arrangement as their parents, with males being introduced first and females c.2 hours later. This process was repeated for multiple generations, until the transgene was selected out of the population, and for one generation after. Three replicates were conducted.

Once the transgene had gone extinct in all three replicates, the results were compared to deterministic and stochastic models of transgene persistence built in Excel and R, respectively. The deterministic model was based on a constant 50% reduction in transgene allele frequency each generation. The stochastic model used Monte Carlo simulations to generate 101 independent populations, in each of which mating pairs and offspring were randomly selected from the population of the previous generation. Both models assumed fully penetrant lethality in transgenic females but no fitness penalty in transgenic males relative to wild-type males; this corresponds to an overall 50% fitness penalty for the transgene. Therefore, in the deterministic model, the transgene frequency is predicted to halve in each generation. As all larvae were reared off-tet and the penetrance of this construct is almost 100%, it was assumed that all transgenic individuals observed from Generation 2 onwards were heterozygous for OX4319L.

Statistical Analyses

Data were analysed using R (Version 2.15.0) and Excel.

11. RESULTS

The experimental results were entirely consistent with the predictions of the deterministic model. In figure 1, the contribution of both sexes to transgene extinction is explored. It can be seen in panel A) that the transgene initially (generation 2) appears to be being selected out of the population at a rate slower than predicted by the deterministic model. However, when only the male cohort was examined (panel B) it was seen the trend in transgene extinction was extremely similar in both the experimental and modelled data (a slightly higher rate in experimental data was most likely the result of non sex-specific costs of transgenesis not built into the model). A return to the raw data revealed a small number of female transgenics surviving in this second generation. As the deterministic model assumes 100% lethality to female transgenics this explained the departure from modelled data observed. In turn, this may have caused an underestimate of the transgene allele frequency.

However, the very low number of females that were transgenics, and the low allele frequency in these populations as a whole, means that the probability of two transgenic heterozygotes mating was very slim. In addition, the costs of this construct insertion are known to be exacerbated in homozygotes compared to heterozygotes, so the overall fitness and mating competitiveness of any females which did manage to survive to adulthood may be lower; this effect would likely be more severe in the field than in the benign environment of the laboratory. The presence of female transgenic pupae surviving is in keeping with what we might expect from this line, as female survival to pupation is around 10% (all die before adulthood) and very large numbers of individuals were being handled in each generation. The fact that there seems to be no inheritance of this 'lack of penetrance' in females between the generations and that the transgene continues to be selected out at a highly predictable rate, even in the face of small female survival, are both highly positive outcomes.

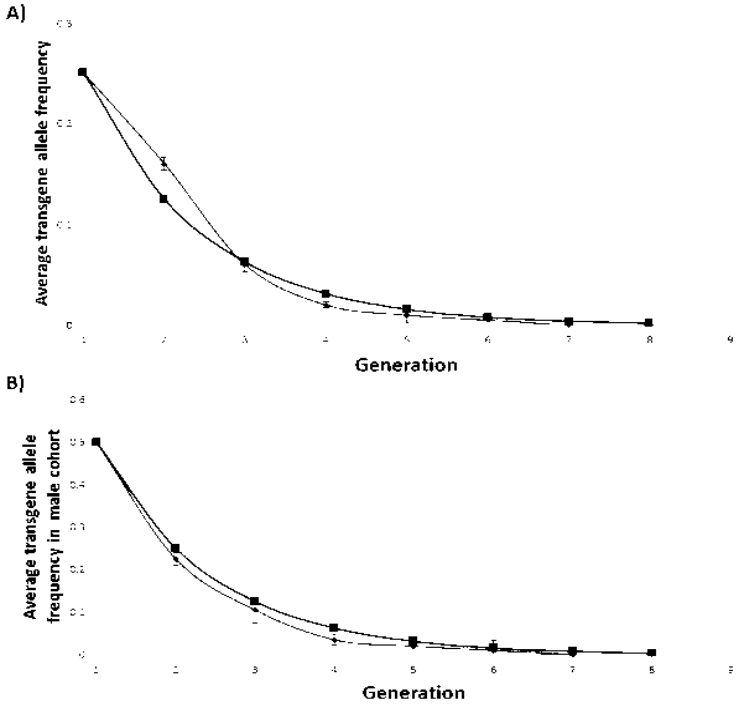

Figure 1: Graphs showing the average of transgene allele extinction in three experimental cages (blue line) and the expected rate of decline based on a deterministic model under restrictive conditions (red line), over multiple generations. Graph A) shows data summed over sexes in each experimental cage, B) shows a subset of the data in which only the male cohort in each cage was included. Generation 1 represents the initial adults used.

In the light of the lack of biological realism inherent in the deterministic model, a second model was built in which stochasticity was built into the rate at which the transgene was selected out of the population (Figure 2). This stochasticity simulates the known non-100% penetrance of the construct, whether due to biotic or abiotic factors. The predictions of the stochastic model closely mirror that of the deterministic model, with the obvious addition of predicted variation between experimental populations, and also closely match the experimental data. The data from experimental cages never exceeded 1.5 x the interquartile range (roughly two standard deviations) of the modelled data and was generally within the

interquartile range, indicating a good fit between the model and the experimental data. In experimental data, the transgene survived in one cage until generation 7 (blue line). By comparison, in generation 8 there were 22 simulated populations in which the transgene persisted (albeit at a very low frequency). A stochastic model is a useful tool for exploring the frequency distribution of outcomes: we modelled 101 cage populations, which would not have been practical experimentally. For comparison, based on the model prediction of 0.22 probability of a cage population having a non-zero transgene frequency in generation 8, the chances of 3 independent populations each having a transgene frequency of zero by this point is $(1-0.22)^3 = 0.47$, in other words this experimental observation is not surprising based on the model predictions. Overall, this comparison shows that the experimentally observed rate of loss of the OX4319L transgene is consistent with a simple model capturing the key known features of the OX4319L phenotype, which can therefore be considered adequately to explain transgene frequency evolution in these experiments. The combined results of these comparisons imply that not only will the OX4319L transgene be fully selected out of a DBM population, but that this selection follows a logical trend predicted by our knowledge of the sex specific fitness costs of fsRIDL.

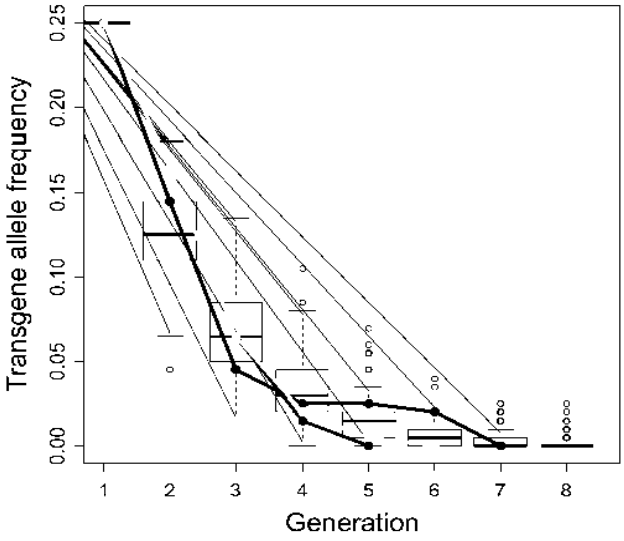

Figure 2: Graph showing the results of a stochastic model simulating the extinction of an fsRIDL transgene (box and whisker plots) under restrictive conditions and the OX4319L transgene allele frequencies in three experimental cages, over multiple generations (red, green and blue lines). Generation 1 represents the initial adults used.

12. **Discussion and Conclusions:**

The laboratory experiments indicate that the engineered female-specific trait in OX4319L confers a highly significant fitness cost that rapidly disappears from a mixed population also comprising non-transgenic moths. These findings agreed closely with those of a simple stochastic model based on the female-specific lethal phenotype of the transgene. Moreover, in field conditions which would typically be much more challenging for the insects, we might

anticipate that any fitness cost seen in the laboratory will be exacerbated and that the OX4319L allele will disappear more rapidly than these experiments indicate.

13. **Literature:**

Jin, L., Walker, A.S., Fu, G., Harvey-Samuel, T., Dafa'alla, T.H., Miles, A., Marubbi, T., Granville, D., Humphrey-Jones, N., O'Connell, S., *et al.* (2013). Engineered female-specific lethality for control of pest lepidoptera. ACS Synthetic Biology *2*, 160-166.

OXITEC

INTERNAL RESEARCH REPORT

1. **Title**: Investigating the expression of the tetracycline-repressible, female-specific lethal trait in the fsRIDL strain, OX4319L-Pxy, in response to different concentrations of chlortetracycline in larval feed.

2. **Statement Of Data Confidentiality Claims:**

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3. **Statement Concerning Good Laboratory Practices:**
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4. **Authors:**

Study Coordinator (Signature): (b6	**Study Supervisor (Signature):** (b6
Study Coordinator (Name And Position): (b6 Research Scientist	**Study Supervisor (Name And Position):** (b)(6) PhD (b6
Date Signed: 17th September 2013	**Date Signed:** 17th September 2013

5. **Associated Personnel:**

	Tasks
(b6	Study coordination, experimental design, report writing, data collection
	Experimental design, approval
	Study Sponsor

6. **Test Facility:**

This research was performed at Oxitec Ltd facilities at:

71 Milton Park,
Abingdon,
Oxfordshire,
OX14 4RX,
UK

7. **Objectives:**

Investigate the effect of dietary chlortetracycline on the penetrance of the female-lethal trait in OX4319L-Pxy-heterozygotes.

8. **Summary:**

This experiment was designed to investigate at which concentration chlortetracycline is able to repress engineered female- specific lethality in OX4319L-Pxy females. There is a possibility of tetracycline availability in the wild, for instance through manure from antibiotic-treated farm animals used to fertilise crops, but this is uncommon and - when detected - concentrations found in crops are low (Hu et al., 2010; Migliore et al., 2010; Seo et al., 2010). We tested survival of male and female larvae, heterozygous for the OX4319L-Pxy transgene insertion, when reared on different chlortetracycline concentrations in artificial diet (Jin et al., 2013).

9. **Introduction:**

The tetracycline-repressible, female-specific mortality in fsRIDL insect strains provides a means of producing large male-only cohorts of insects and a population suppression effect in the target population: reduction of females reduces a population's reproductive potential. Efficacy relies upon high penetrance of the female-lethal trait in the field. In laboratory test crosses, in the absence of dietary tetracycline, OX4319L-Pxy heterozygotes have shown 0% survival of females (i.e. 100% penetrance of the female-lethal phenotype), and high survival of males. A theoretical concern is that environmental tetracyclines could repress the female lethal trait and allow the transgene to persist for longer in the environment. We therefore set out to establish sensitivity concentration-response relationship between dietary chlortetracycline and the penetrance of the female-lethal phenotype in OX4319L-Pxy heterozygotes. We tested OX4319L-Pxy heterozygotes, rather than homozygotes, as we anticipated that one copy of the transgene per cell is likely more susceptible to repression than is two copies, and the larvae present in the field are expected to be heterozygous for the OX4319L-Pxy insertion (the progeny of released homozygous males and wild-type females).

10. Methods

Eggs heterozygous for the OX4319L-Pxy transgene insertion were generation by establishing 10 replicated crosses with OX4319L-Pxy-homozygous males with wild-type females. Artificial diet (Bioserv beet armyworm diet, cat # F9221B) was prepared with six different concentrations of chlortetracycline: 0 µg/ml, 0.01 µg/ml, 0.1 µg/ml, 1.0 µg/ml, 10 µg/ml and 100 µg/ml. Egg collections from each cross were divided between these diets, and the hatched larvae reared to pupation. These pupae were separated by sex then incubated in Petri dishes; survival to adulthood was recorded.

11. RESULTS

As in previous test crosses, OX4319L-Pxy-heterozygous male survival is approximately equivalent to, but slightly lower than, that of wild-type, irrespective of chlortetracycline concentration (Figures 1 & 2). Mortality was well-repressed in females reared on 10 µg/ml and 100 µg/ml. As the chlortetracycline concentrations reduced to 1.0 µg/ml and 0.1 µg/ml, female survival rates dropped sharply, and below this (0.01 µg/ml and 0.0 µg/ml) only very small numbers of females survived to pupation (<2%) and none survived to adulthood.

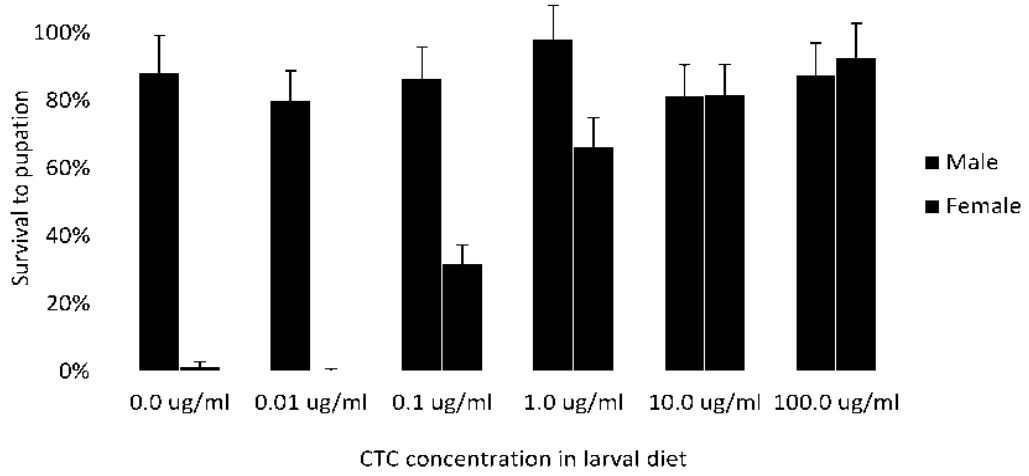

Figure 1: Survival-to-pupation of OX4319L-Pxy-heterozygous diamondback moth reared on different concentrations of chlortetracycline (CTC) in larval diet. Survival is expressed relative to that of wild-type counterparts. Error bars indicate 95% confidence intervals.

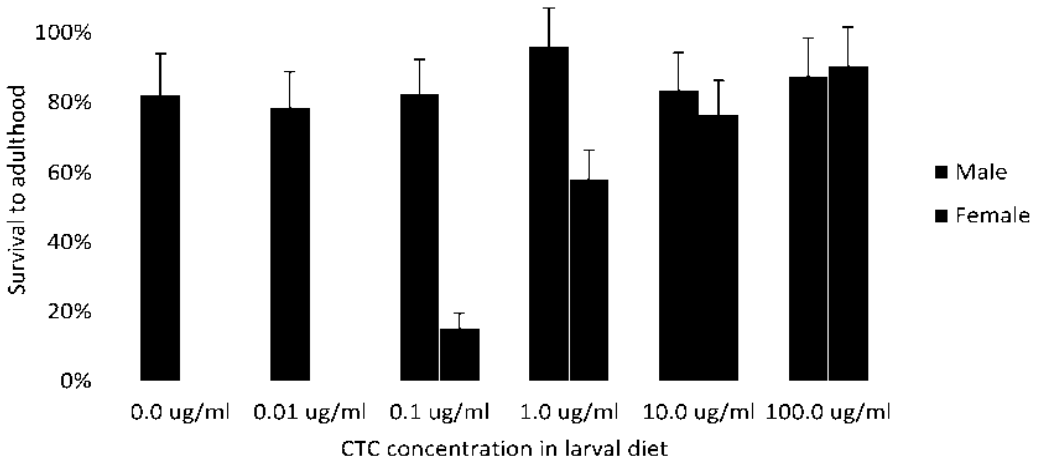

Figure 2: Survival-to-adulthood of OX4319L-Pxy-heterozygous diamondback moth reared on different concentrations of chlortetracycline (CTC) in larval diet. Survival is expressed relative to that of wild-type counterparts. Error bars indicate 95% confidence intervals.

12. Discussion and Conclusions:

No OX4319-Pxy-heterozygous females survived to adulthood on 0.01 µg/ml CTC, while at or above 10 µg/ml CTC OX4319L-Pxy-heterozygous female survival to adulthood, relative to wild-type, was similar to that of males. The level of CTC needed for survival far exceeds that which diamondback moth might be expected to encounter in the wild. For comparison, laboratory experiments growing cabbage on soil artificially contaminated with manure from CTC-fed pigs, and spiked with CTC solution, found <0.004 µg/ml CTC in foliage (Kumar et al., 2005). These results provide evidence that fsRIDL trait-repressing concentrations of tetracycline are highly unlikely to be encountered on host plants in the environment.

13. Literature:

Hu, X., Zhou, Q., and Luo, Y. (2010). Occurrence and source analysis of typical veterinary antibiotics in manure, soil, vegetables and groundwater from organic vegetable bases, northern China. Environmental Pollution *158*, 2992-2998.

Jin, L., Walker, A.S., Fu, G., Harvey-Samuel, T., Dafa'alla, T.H., Miles, A., Marubbi, T., Granville, D., Humphrey-Jones, N., O'Connell, S., *et al.* (2013). Engineered female-specific lethality for control of pest Lepidoptera. ACS Synthetic Biology *2*, 160-166.

Kumar, K., Gupta, S.C., Baidoo, S.K., Chander, Y., and Rosen, C.J. (2005). Antibiotic uptake by plants from soil fertilized with animal manure. Journal of environmental quality *34*, 2082-2085.

Migliore, L., Godeas, F., De Filippis, S., Mantovi, P., Barchi, D., Testa, C., Rubattu, N., and Brambilla, G. (2010). Hormetic effect(s) of tetracyclines as environmental contaminant on *Zea mays*. Environmental Pollution *158*, 129-134.

Seo, Y., Cho, B., Kang, A., Jeong, B., and Jung, Y.-S. (2010). Antibiotic uptake by plants from soil applied with antibiotic-treated animal manure. Korean J Soil Sci Fert *43*, 466-470.

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INTERNAL RESEARCH REPORT

1. **Title**: (b) (4)
   OX4319L-Pxy.

2. **Statement Of Data Confidentiality Claims:**

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4. **Authors:**

Study Coordinator (Signature):	Study Supervisor (Signature):
(b6	
Study Coordinator (Name And Position): (b6	**Study Supervisor (Name And Position):** (b6
Date Signed: 17th September 2013	**Date Signed:** 17th September 2013

5. Associated Personnel:

Name	Tasks
(b6	Study coordination
	Experimental design, report writing, data collection and analysis
	Data collection and analysis
	Experimental design, approval
	Study Sponsor

6. Test Facility:

This research was performed in Oxitec's laboratories at:

Oxitec Ltd,
71 Milton Park,
Abingdon,
Oxon OX14 4RX,
UK

7. Objectives:

To determine the(b) (4)

8. Summary:

(b) (4)

9. Introduction:

(b) (4)

(b6

10. Methods and Results

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

Raw data:

All the experimental results are included in Oxitec laboratory book (b) (4)

11. (b) (4)

OXITEC

INTERNAL RESEARCH REPORT

1. **Title**: Characterisation of the (b) (4) ,
   OX4319L-Pxy.

2. **Statement Of Data Confidentiality Claims:**

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4. **Authors:**

(b) (6)

Study Supervisor (Signature):

5. Associated Personnel:

(b) (6)

Name	Tasks
	Study coordination, report writing
	Data collection
	Data collection
	Data collection
	Experimental design, approval
	Study Sponsor

6. Test Facility:

This research was performed at Oxitec Ltd facilities at:

71 Milton Park,
Abingdon,
Oxfordshire,
OX14 4RX,
UK

7. Objectives:

To determine the (b)(4)

8. Summary:

The OX4319L-Pxy carries a DsRed2 fluorescent protein marker, which enables workers to track the presence of the transgene for research and quality control purposes. The marker will also be used to monitor the strain in the field. (b)(4)

9. Introduction:

Insertion of a piggyBac transposable element is specific to the sequence TTAA, but there are many such sequences in an arthropod genome, therefore the transgene inserts at any of a large number of potential sites, giving pseudo-random insertion location. (b)(4)

10. Methods

(b)(4)

(b)(4)

(b)(4)

(b)(4)

11. RESULTS

(b)(4)

(b)(4)

(b)(4)

12. Discussion and Conclusions:

(b)(4)

13. Literature:

Sambrook, J., and Russell, D. (2001). Molecular Cloning: A Laboratory Manual; third edition (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press).

OXITEC

INTERNAL RESEARCH REPORT

1. **Reference Number**:

2. **Issuing Date**:

3. **Title**: Investigating the effect on caged populations of diamondback moth (*Plutella xylostella* L.) of sustained release of fsRIDL males.

4. **Statement Of Data Confidentiality Claims:**

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6. **Authors:**

Study Coordinator (Signature):	Study Supervisor (Signature):

(b) (6)

7. Associated Personnel:

(b) (6)

Name	Tasks
	Study coordination, experimental design, report writing, data collection
	Experimental design, data collection and analysis, modelling, report writing
	Data collection
	Data collection
	Data collection
	Experimental design, approval
	Study Sponsor

8. Test Facility:

This research was performed at (b)(4) at:

(b)(4)

The facility comprised a fully air-conditioned, four-compartment glasshouse wing with alarmed/recorded +-2°C temperature control (day/night).

9. Objectives:

(b)(4)

10. Summary:

This experiment was to test the hypothesis that sustained releases of male moths the female-specific RIDL strain of diamondback moth, OX4319L-Pxy, will lead to population suppression. This effect has been previously demonstrated with similar strains of the olive fly, *Bactrocera oleae* (Ant et al., 2012), and the Mediterranean fruit fly, *Ceratitis capitata* (manuscript in preparation). (b)(4)

(b)(4)

11. Introduction:

The tetracycline-repressible, female-specific mortality in fsRIDL insect strains provides a means of producing large male-only cohorts of insects and, *via* release of such males into a target population, a population suppression effect in the target population: reduction of females reduces a population's reproductive potential.

(b)(4)

12. Methods

(b)(4)

(b)(4)

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(adapted fromWise de Valdez et al., 2011).

(b)(4)

(b)(4)

(b)(4)

(b)(4)

(b)(4)

(b)(4)

14. Discussion and Conclusions:

(b)(4)

15. Literature:

Ant, T., Koukidou, M., Rempoulakis, P., Gong, H.-F., Economopoulos, A., Vontas, J., and Alphey, L. (2012). Control of the olive fruit fly using genetics-enhanced sterile insect technique. BMC Biology *10*, 51.

Wise de Valdez, M.R., Nimmo, D., Betz, J., Gong, H.F., James, A.A., Alphey, L., and Black, W.C.t. (2011). Genetic elimination of dengue vector mosquitoes. Proc Natl Acad Sci U S A *108*, 4772-4775.

INTERNAL RESEARCH REPORT

1. **Title**: Investigating the predicted synergistic resistance management benefit of combined use of fsRIDL diamondback moth and transgenic *Bt* broccoli.

2. **Statement Of Data Confidentiality Claims:**

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Date Signed: 17th September 2013	**Date Signed:** 17th September 2013

5. Associated Personnel:

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Tasks
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Experimental design, data collection and analysis, modelling, report writing
Experimental design, approval
Study Sponsor

6. Test Facility:

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9. Introduction:

The RIDL strain of moth, OX4319L-Pxy, shows engineered tetracycline-repressible, female-specific lethality (fsRIDL). fsRIDL males mating with wild females will result in introgression of genetic background alleles (non-transgene alleles, especially alleles not linked to the transgene) from the fsRIDL colony into the target field population (see schematic below). (b)(4) odelling studies indicate that releases of fsRIDL males will thereby provide a potent pesticide resistance management benefit (Alphey et al., 2009; Alphey et al., 2007), in addition to population control through death of female progeny.

Diamondback moth is notorious for its capacity to rapidly develop resistance to pesticides. It was the first agricultural pest to exhibit field-evolved resistance to DDT (Ankersmit, 1953; Johnson, 1953) and to the biopesticide *Bacillus thuringiensis* (*Bt*) (Tabashnik et al., 1990). A major contributing factor to this capability is diamondback moth's short generation time - as low as 12 days in warm climates - permitting rapid selection. In parts of diamondback moth's range, selection for resistance may be accelerated by indiscriminate and unregulated pesticide application (Georghiou and Langunes-Tejeda, 1991; Mota-Sanchez et al., 2002).
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10. Methods

Strains

OX4319L-Pxy (female-specific lethal RIDL strain): In the absence of tetracycline, the females of this strain express high levels of the tTAV protein during immature stages through a genetic positive feedback loop, resulting in high mortality. However, when reared in the presence of tetracycline (or suitable analogues), expression of tTAV is repressed, allowing survival (Jin et al., 2013).

Regulatory

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13. Literature:

Alphey, N. (2009). Modelling optimal strategies for novel genetics-based pest management. In Department of Zoology (Oxford, UK: University of Oxford), pp. 253.
Alphey, N., Bonsall, M., and Alphey, L. (2009). Combining pest control and resistance management: synergy of engineered insects with *Bt* crops. J Econ Entomol *102*, 717-732.
Alphey, N., Coleman, P.G., Donnelly, C.A., and Alphey, L. (2007). Managing insecticide resistance by mass release of engineered insects. J Econ Entomol *100*, 1642-1649.
Ankersmit, G.W. (1953). DDT resistance in *Plutella maculipennis* (Curt.) (Lepidoptera) in Java. Bull Entomol Res *44*, 421-425.
Georghiou, G., and Langunes-Tejeda, A. (1991). The occurrence of resistance to pesticides in arthropods (Rome: Food and Agriculture Organisation of the United Nations).

Jin, L., Walker, A.S., Fu, G., Harvey-Samuel, T., Dafa'alla, T.H., Miles, A., Marubbi, T., Granville, D., Humphrey-Jones, N., O'Connell, S., *et al.* (2013). Engineered female-specific lethality for control of pest Lepidoptera. ACS Synthetic Biology *2*, 160-166.
Johnson, D.R. (1953). *Plutella maculipennis* resistance to DDT in Java. J Econ Entomol *46*, 176.
Mota-Sanchez, D., Bills, P.S., and Whalon, M.E. (2002). Arthropod resistance to pesticides: status and overview. In Pesticides in Agriculture and the Environment, W.B. Wheeler, ed. (New York: Marcel Dekker Inc.), pp. 241-272.
Tabashnik, B., Cushing, N., Finson, N., and Johnson, M. (1990). Field development of resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera: Plutellidae). J Econ Entomol *83*, 1671-1676.

SyntheticBiology

Research Article

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Engineered Female-Specific Lethality for Control of Pest Lepidoptera

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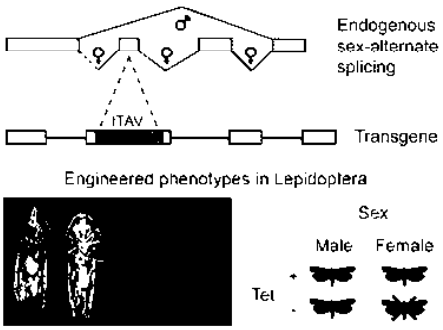
Supporting Information

ABSTRACT: The sterile insect technique (SIT) is a pest control strategy involving the mass release of radiation-sterilized insects, which reduce the target population through nonviable matings. In Lepidoptera, SIT could be more broadly applicable if the deleterious effects of sterilization by irradiation could be avoided. Moreover, male-only release can improve the efficacy of SIT. Adequate methods of male-only production in Lepidoptera are currently lacking, in contrast to some Diptera. We describe a synthetic genetic system that allows male-only moth production for SIT and also replaces radiation sterilization with inherited female-specific lethality. We sequenced and characterized the *doublesex* (*dsx*) gene from the pink bollworm (*Pectinophora gossypiella*). Sex-alternate splicing from *dsx* was used to develop a conditional lethal genetic sexing system in two pest moths: the diamondback moth (*Plutella xylostella*) and pink bollworm. This system shows promise for enhancing existing pink bollworm SIT, as well as broadening SIT-type control to diamondback moth and other Lepidoptera.



KEYWORDS: *sterile insect technique, SIT, RIDL, transgenic, insect, doublesex, moth*

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Description of genetic elements in *Plutella xylostella* strains OX4319L-Pxy, OX4319N-Pxy and OX4767A-Pxy

Components listed as in plasmid maps

Construct component name	Construct component type	Component function	Donor	Detailed description
piggyBac 5'	Vector sequence	Germline transformation	*piggyBac* from *Trichoplusia ni* (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 melanogaster* 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 *T. ni* (moth)	As above (Handler & Beeman, 2003)
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. These fluorescent proteins, which have been used as markers in a wide range of vertebrate and invertebrate species, confer no known competitive advantage or disadvantage to the recipient, and no ecological or other consequences resulting from incorporation of these markers into the transgenicDBM can be envisioned.
Target site A	Synthetic sequence	Target site	synthetic	Target recognition site in construct for integrase or recombinase, which is not used in this particular strain and project.
Intron	Regulatory sequence for 5'UTR	Requirement for translation	*Drosophila melanogaster*	Stabilizes mRNA and required for translation of mRNA
***ie1*/Hr5**	Enhancer /Promoter	Control expression of	*Autographa californica*	Promoter from *immediate-early-1* gene and *hr5* enhancer region

	sequences	DsRed2	nuclear polyhedrosis virus (AcMNPV)	
***tetO*×7**	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*	Contains an alternative splicing intron allowing sex-specific expression of sequence inserted into the female-specific exon (in this case tTAV).
hsp70	promoter sequence	Minimal promoter	*Drosophila melanogaster*	Minimal promoter to enable transcription of gene
PBW *dsx* genomic region	splicing	Gives female specificity	*Pectinophora gossypiella*	Contains an alternative splicing intron allowing sex-specific expression of sequence inserted into the female-specific exon (in this case tTAV).
tetR	Protein coding region (Gene)	Component of tTAV protein	Tn10-specified tetracycline-resistance operon of *E. coli* (Gossen & Bujard, 1992)	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 (Gossen & Bujard, 1992)
tTAV (comprising of tet R and VP16 domains)	Protein coding region (Gene)	Expression tTAV protein	*Escherichia coli* (bacterium) and Herpes simplex (virus)	tTAV is a tet-responsive transcriptional factor. It is a fusion of the 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	*Drosophila melanogaster*	Regulatory sequence that helps stabilize mRNA
VP16	Protein coding region	Component of tTAV protein	Herpes simplex (virus)	Component of synthetic transcription factor tTA

References

ALFRED M HANDLER AND RICHARD W BEEMAN (2003) United States Department of Agriculture—Agricultural Research Service: advances in the molecular genetic analysis of insects and their application to pest management. Pest Manag Sci **59**:728–735

GUOLIANG FU, KIRSTY C CONDON1, MATTHEW J EPTON, PENG GONG, LI JIN, GEORGE C CONDON,

NEIL I MORRISON, TARIG H DAFA'ALLA & LUKE ALPHEY (2007) Female-specific insect lethality engineered using alternative splicing. Nature Biotechnology letters **25**, 3: 353-357

MANFRED GOSSEN AND HERMANN BUJARD (1992) Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. Proc. Nat. Acad. Sci. USA. **89**: 5547-5551

Threatened or endangered species present at the release site

A search was carried out on the IUCN red list of threatened species (http://www.iucnredlist.org/search; accessed 12th August 2013) according to the following search criteria:

Show taxa:
    Species
Search by taxonomy:
    ANIMALIA
Search by location:
    New York
    (Native)
Search by systems:
    Terrestrial
Match any habitat:
    1. Forest
    2. Savanna
    3. Shrubland
    4. Grassland
    5. Wetlands (inland)
    6. Rocky areas (eg. inland cliffs, mountain peaks)
    7. Caves and Subterranean Habitats (non-aquatic)
    8. Desert
    14. Artificial/Terrestrial
    16. Introduced vegetation
    17. Other
    18. Unknown
Match any threat:
    1. Residential & commercial development
    2. Agriculture & aquaculture
    3. Energy production & mining
    4. Transportation & service corridors
    5. Biological resource use
    6. Human intrusions & disturbance
    7. Natural system modifications
    8. Invasive & other problematic species & genes
    9. Pollution
    10. Geological events
    11. Climate change & severe weather
    12. Other options
Search by assessment:
    Categories: CR, EN, VU, DD

This search found only seven species from which only one species, the New Cottontail Rabbit (*Sylvilagus transitionalis*), whilst this is not an aquatic species and has the potential for habitat overlap with the diamondback moth this species this species is a herbivore which is unlikely to directly interact with the released moth.

Further searches on the New York States Department for Environment (http://www.dec.ny.gov/animals/7494.html; accessed 12th August 2013) indicate that there are a number of endangered and threatened animals in the state which are not listed on the IUCN red list. Evaluation of these species for animals which might have a habitat which overlaps with the agricultural pest, diamondback moth, has been carried out and is presented in Table 1.

Overall there are a number of birds which could be present around abandoned agricultural land or nearby open grasslands however occurrence of any special concern bird species in a large highly managed farmland is unlikely. Insects form the diet of many small mammals, reptiles and birds however there is no one species which is reliant on the diamond back moth as a diet source. None of the species listed on the IUCN red list and New York States Department of Environment were reliant on any one species as a food source therefore the impact that this release of diamondback moths would have on the endangered, threatened or special concern animal populations is negligible.

Table 1. Species which could interact with the released Diamondback moths and are present in New York State and are Endangered, threatened or are of special concern.

Common name (*latin name*)	**Distribution**	**Threat**	**New York Status**	**Habitat overlap with diamondback moth**
Loggerhead Shrike (*Lanius ludovicianus*)	Most of Northern America from South Canada to South Mexico.	Threats to this species are unclear however it has been suggested that abandonment of farms and orchards have removed breeding sites. Roadkills and pesticide contamination could also be factors.	Endangered	Feed on beetles, grasshopper sand small rodents therefore it is unlikely that this species will have a direct interaction with the diamondback moth however this species is found in agricultural land.
Vesper Sparrow (*Pooecetes gramineus*)	Open grassy areas in North America	This species requires bare ground as breeding territory, abandonment of farms and regrown of forest areas threaten this species.	Special concern	This species has a diet consisting of insects and seeds. In New York this species is commonly found in the Erie-Ontario Plain and the central Appalachians and is not anticipated to be present in currently managed farmland.
Grasshopper Sparrow (*Ammodramus savannarum*)	Common throughout much of the United States and Southern Canada.	Threats include mowing of grasslands, use of pesticides and loss of grassland by plant succession.	Special concern	This species breeding in meadows, pastures, hayfields and croplands. There could be a habitat overlap between the diamondback moths and this species however interactions are likely to be limited as this is a widespread species and the proposed trial is small.
Golden-Winged Warbler (*Vermivora chrysoptera*)	Breeds throughout north central and north-eastern United States	Maintenance of early successional fields is required to preserve this species.	Special concern	This species breeds in early successional habitats therefore it could be present on any abandoned farmlands near to the release site. This is limited potential for habitat overlap and interaction with this species.

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INTERNAL RESEARCH REPORT

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Date Signed: 17th September 2013	**Date Signed:** 17th September 2013

5. **Associated Personnel:**

(b) (6)

Tasks
Study coordination, experimental design, report writing
Experimental design
Experimental design, data collection, data analysis
Data collection
Experimental design, approval
Study Sponsor

6. **Test Facility:**

This research was performed at Oxitec laboratories at:
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UK

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9. Introduction:

The tetracycline-repressible, female-specific mortality in fsRIDL insect strains provides a means of producing large male-only cohorts of insects and a population suppression effect following release into a target population: pre-reproduction mortality of females reduces a population's reproductive potential.

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10. Methods

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11. RESULTS

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12. Discussion and Conclusions:

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13. Literature:

Jin, L., Walker, A.S., Fu, G., Harvey-Samuel, T., Dafa'alla, T.H., Miles, A., Marubbi, T., Granville, D., Humphrey-Jones, N., O'Connell, S., *et al.* (2013). Engineered female-specific lethality for control of pest Lepidoptera. ACS Synthetic Biology *2*, 160-166.