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Animal and Plant
Health Inspection
Service

Proposal to permit the field release of genetically engineered diamondback moth in New York

Environmental Assessment, May 2014

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACRONYMS AND ABBREVIATIONS

°F	Degrees Fahrenheit
APHIS	Animal and Plant Health Inspection Service
BRS	Biotechnology Regulatory Services
CAA	Clean Air Act
CFR	Code of Federal Regulations
CWA	Clean Water Act
CH_4	Methane
CO_2	Carbon Dioxide
EA	Environmental Assessments
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
FFDCA	Federal Food, Drug, and Cosmetic Act
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FR	Federal Register
FQPA	Food Quality Protection Act
GE	Genetically Engineered
GHG	Greenhouse Gas
N_2O	Nitrous Oxide
NAAQS	National Ambient Air Quality Standards
NEPA	National Environmental Policy Act
NPS	Non-Point Source
NY	New York
NYSAES	New York State Agricultural Experiment Station
OSHA	Occupational Safety and Health Administration
OSTP	Office of Science and Technology Policy
PDP	Pesticide Data Program
PIP	Plant Incorporated Protectant
PM	Particulate Matter
PPA	Plant Protection Act of 2000
SIP	State Implementation Plan

SIT	Sterile Insect Technique
TSCA	Toxic Substances Control Act
US	United States
USC	United States Code
USDA	United States Department of Agriculture
WPA	Worker Protection Standard

1 EXECUTIVE SUMMARY

United States Department of Agriculture (USDA), Animal Plant Health Inspection Service (APHIS) has prepared an environmental assessment (EA) in response to an environmental release permit application (APHIS Number 13-297-102r) received from Dr. Anthony Shelton of Cornell University[1] to allow the field release of genetically engineered (GE) diamondback moth strains OX4319L-Pxy, OX4319N-Pxy, and OX4767A-Pxy on release sites within the grounds of the Cornell University New York State Agricultural Experiment Station (NYSAES). A maximum of six release sites is being requested by the applicant, with total acreage not exceeding 10 acres per site (60 acres in total). GE diamondback moth strains OX4319L-Pxy, OX4319N-Pxy, and OX4767A-Pxy have been genetically engineered with a single construct each to confer red fluorescence and repressible female lethality.

The purpose of the requested field release is for the applicant to assess the efficacy of GE diamondback moth strains OX4319L-Pxy, OX4319N-Pxy, and OX4767A-Pxy in reducing pest populations of non-GE diamondback moths. According to the applicant, these GE diamondback moths may serve as an insecticide-free means of controlling field populations of diamondback moths in a species-specific manner.

APHIS has previously issued the applicant a permit authorizing the importation of GE diamondback moth strains OX4319L-Pxy, OX4319N-Pxy, and OX4767A-Pxy from the United Kingdom to the NYSAES (APHIS Number 12-227-102m). This permit authorizing the importation of GE diamondback moth strains expired on August 14th, 2012.

In summary of this EA, APHIS has concluded that potential impacts of APHIS issuing a permit for the field release of GE diamondback moth strains OX4319L-Pxy, OX4319N-Pxy, and OX4767A-Pxy on the physical environment (e.g., soil resources, water resources, air quality, and climate change); the biological environment (e.g., wildlife, plant communities, and biological diversity); and the human health environment (e.g., farmworker health and health of the general public) are unlikely (Section 5). Additionally, APHIS concluded that cumulative impacts are unlikely (Section 6), and that APHIS' action will have no effect on listed Threatened and Endangered species or species proposed for listing, and will not affect designated habitat or habitat proposed for designation (Section 7).

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[1] referred to as the applicant, hereinafter

2 PURPOSE AND NEED

2.1 Regulatory Authority

"Protecting American agriculture" is the basic charge of the United States Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS). APHIS provides leadership in ensuring the health and care of plants and animals. The agency improves agricultural productivity and competitiveness, and contributes to the national economy and the public health. USDA asserts that all methods of agricultural production (conventional, organic, or the use of genetically engineered varieties) can provide benefits to the environment, consumers, and farm income.

Since 1986, the United States (US) government has regulated genetically engineered (GE) organisms pursuant to a regulatory framework known as the Coordinated Framework for the Regulation of Biotechnology (Coordinated Framework) (51 FR 23302, 57 FR 22984). The Coordinated Framework, published by the Office of Science and Technology Policy, describes the comprehensive federal regulatory policy for ensuring the safety of biotechnology research and products and explains how federal agencies will use existing Federal statutes in a manner to ensure public health and environmental safety while maintaining regulatory flexibility to avoid impeding the growth of the biotechnology industry. The Coordinated Framework is based on several important guiding principles: (1) agencies should define those transgenic organisms subject to review to the extent permitted by their respective statutory authorities; (2) agencies are required to focus on the characteristics and risks of the biotechnology product, not the process by which it is created; (3) agencies are mandated to exercise oversight of GE organisms only when there is evidence of "unreasonable" risk.

The Coordinated Framework explains the regulatory roles and authorities for the three major agencies involved in regulating GE organisms: USDA's Animal and Plant Health Inspection Service (APHIS), the US Food and Drug Administration (FDA), and the US Environmental Protection Agency (EPA).

APHIS is responsible for regulating GE organisms and plants under the plant pest authorities in the Plant Protection Act of 2000, as amended (7 USC § 7701 *et seq*.) to ensure that they do not pose a plant pest risk to the environment.

The regulation of GE organisms by FDA and EPA are further discussed in Section 1.5.

2.2 Regulated Organisms

The APHIS Biotechnology Regulatory Service's (BRS) mission is to protect America's agriculture and environment using a dynamic and science-based regulatory framework that allows for the safe development and use of GE organisms. APHIS regulations at 7 Code of Federal Regulations (CFR) part 340, which were promulgated pursuant to authority granted by the Plant Protection Act (PPA), as amended (7 United States Code (U.S.C.) 7701–7772), regulate the introduction (importation, interstate movement, or release into the environment) of certain GE organisms and products. A GE organism is considered a regulated article if the donor organism, recipient organism, vector, or vector agent used in engineering the organism belongs to one of the taxa listed in the regulation (7 CFR 340.2) and is also considered a plant pest. A GE organism is also regulated under part 340 when APHIS has reason to believe that the GE organism may be a plant pest or APHIS does not have information to determine if the GE organism is unlikely to pose a plant pest risk.

2.3 APHIS Response to a Permit Application for a Field Release

The PPA directs the USDA to facilitate imports and interstate commerce in agricultural products in ways that will reduce, to the extent practicable, the risk of dissemination of plant pests. Under APHIS regulations, the APHIS Administrator has authority to regulate any organism or product altered or produced through genetic engineering that the Administrator determines is a plant pest or has reason to believe is a plant pest. When APHIS receives an application for a permit for environmental release, the application is evaluated to determine whether the environmental release, with appropriate conditions imposed, can be carried out while preventing the dissemination and establishment of plant pests. The receipt of a permit application to introduce a GE organism requires a response from the Administrator:

> *Administrative action on applications*. After receipt and review by APHIS of the application and the data submitted pursuant to paragraph (a) of this section, including any additional information requested by APHIS, a permit shall be granted or denied (7 CFR 340.4(e)).

The applicant has provided the required information associated with this request in the permit application (APHIS Number 13-297-102r). This information is summarized below in Section 2.4 of this Environmental Assessment (EA). Additionally, this information has been reviewed and analyzed in this EA.

2.4 Description and Purpose of the Research

The following information is from the applicant's permit application, 13-297-102r.

The GE diamondback moth strains OX4319L-Pxy, OX4319N-Pxy, and OX4767A-Pxy[2] are genetically engineered to show a phenotype consisting of two introduced traits:

- Red fluorescence; and
- Tetracycline-repressible female lethality.

The red fluorescence trait is conferred by activity of an introduced red fluorescent protein, DsRed2. Activity of DsRed2 in GE diamondback moth is intended to facilitate identification of GE from non-GE diamondback moths during field trials.

Tetracycline-repressible female lethality, also known as female autocide, is conferred by activity of an expressed protein, tTAV. The female autocidal trait permits the selection of male

[2] Collectively referred to as GE diamondback moth, hereinafter

diamondback moths during rearing, as all females incur mortality unless provided in their diet a repressor compound[3]. Additionally, the female autocidal trait is anticipated to decrease the number of diamondback moth offspring following field release through elimination of female moths. Any female progeny produced from GE diamondback moth males and non-GE diamondback moth females is likely to die (Jin *et al.*, 2013).

The purpose of the requested permit is basic research to assess the feasibility and efficacy of this GE diamondback moth in reducing pest populations of non-GE diamondback moths. According to the applicant, these GE diamondback moths may serve as an insecticide-free means of controlling field populations of diamondback moths in a species-specific manner. The release of these GE diamondback moths will allow the applicant to gauge efficacy of this system in reducing pest diamondback moth populations.

This release of GE male-sterile diamondback moths is anticipated to oversaturate breeding populations of non-GE diamondback moths with GE males. Successful mating between GE male diamondback moths and non-GE female diamondback moths will not produce viable female larvae because females would all bear the autocidal trait. Continued presence of either progeny males or introduced GE males with the sterility gene will become a repeated cycle during the growing season of that planted field, and will result in a net reduction of the feral diamondback moth population (Figure 1).

Under the permit application submitted by the applicant, two sites will be planted with a cruciferous crop (e.g., cabbage, broccoli, etc.) and subject to the release of male GE diamondback moths. In one site three plots (not exceeding 10 acres per site) will be used as control sites (i.e., no release of GE diamondback moth will occur). During a permitted field trial, the applicant will release GE male diamondback moths into three plots previously planted with a cruciferous crop (20,000 GE diamondback moths per release per site; up to 100,000 GE diamondback moths per week per site). Some of these released GE diamondback moth males may be marked with Day-Glo fluorescent dusts[4] in order to distinguish released GE diamondback moth males from the male progeny of GE diamondback moth males. Monitoring of diamondback moths in the study sites will be undertaken using sticky traps baited with a synthetic sex pheromone specific for diamondback moth. For each experiment, release and monitoring of GE and non-GE diamondback moths will take place for the duration of the cruciferous crop planting cycle (anticipated to range from 3 to 4 months). At the conclusion of each experiment, the release sites will be devitalized of any remaining diamondback moths through the application of the EPA-registered insecticide, Coragen (chlorantraniliprole). Post-experiment monitoring of diamondback moths with the traps will continue for 2 weeks after the conclusion of each experiment to monitor field longevity of GE diamondback moth. If this permit is issued by APHIS, the permitted field trial may not exceed three years in length.

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[3] i.e., tetracycline

[4] Day-Glo Color Corp., Cleveland, OH. http://www.dayglo.com/ Last accessed April, 2014

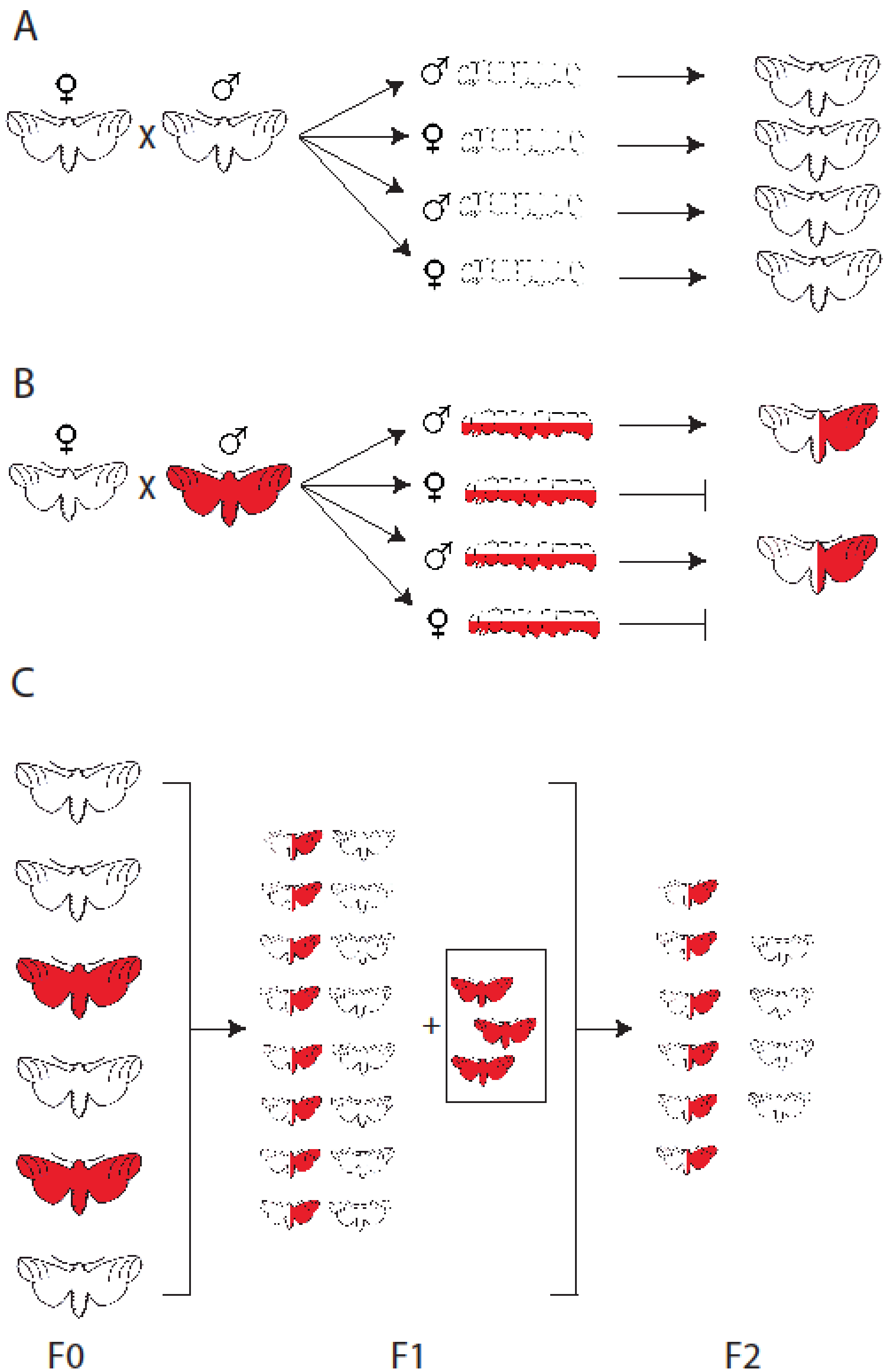

Figure 1. Diamondback moth reproductive cycle in the absence/presence of the female autocidal trait.

Caption for Figure 1 on the previous page. (A) non-GE [white moths] diamondback moth reproductive cycle. (B) non-GE [white moths] and GE [red moths] diamondback moth reproductive cycle. After mating between a GE male and a non-GE female, all progeny larvae carry the female autocidal trait [half white/red larvae]. As a result, all female larvae die and only male larvae mature into adult moths. (C) Simplified model showing the overall reduction in diamondback moth population as a result of GE diamondback moth introduction. At the start of a permitted field trial, there will be a combination of non-GE [white moths] and GE [red moths] diamondback moths following field release. In every successive generation [i.e., F_1, F_2, etc.], adult male moths containing the female autocidal trait is anticipated to be present, either as progeny [half white/red moths] from the successful mating of a non-GE female and GE male or the continuous introduction of GE [boxed red moths] diamondback moths. Mating of either of these males with non-GE females causes the overall diamondback moth population to decrease over time. Furthermore, in every successive generation, male diamondback moths containing the female autocidal trait is anticipated to outnumber non-GE males, due to the weekly introduction of GE diamondback moth males and the male diamondback moth progeny that also carry the female autocidal trait.

2.5 Coordinated Framework Review and Regulatory Review

The US government has regulated GE organisms since 1986 under Federal regulations published in the *Federal Register* (51 FR 23302; 57 FR 22984) entitled "The Coordinated Framework for the Regulation of Biotechnology" (henceforth referred to here as the Coordinated Framework).

The Coordinated Framework, published by the Office of Science and Technology Policy (OSTP), describes the comprehensive Federal regulatory policy for ensuring the safety of biotechnology research and products. It also explains how Federal agencies will use existing Federal statutes to ensure public health and environmental safety while maintaining regulatory flexibility to avoid impeding the growth of the biotechnology industry.

Three central guiding principles form the basis for the Coordinated Framework:

1) Agencies should define those transgenic organisms subject to review to the extent permitted by their respective statutory authorities;
2) Agencies are required to focus on the characteristics and risks of a biotechnology product, not the process by which it was created;
3) Agencies are mandated to exercise oversight of GE organisms only when there is evidence of "unreasonable" risk.

The Coordinated Framework explains the regulatory roles and authorities for the three major agencies involved in regulating GE organisms: USDA-APHIS, the Environmental Protection Agency (EPA), and the Food and Drug Administration (FDA). A summary of each role follows.

2.5.1 USDA-APHIS

As noted in Section 1.2, the PPA authorizes and mandates USDA-APHIS to regulate, manage and control plant pests. This directive includes regulatory authority over the introduction (i.e., importation, interstate movement, or release into the environment) of certain GE organisms and products. A GE organism is considered a regulated article if the donor organism, recipient organism, vector, or vector agent used in engineering the organism belongs to one of the taxa listed in the regulation (7 CFR part 340.2) and is also considered a plant pest. A GE organism is also regulated under 7 CFR part 340, when USDA-APHIS has reason to believe that the GE organism may be a plant pest or USDA-APHIS does not have sufficient information to determine if the GE organism is unlikely to pose a plant pest risk. A GE organism is no longer subject to the plant pest provisions of the PPA or to the regulatory requirements of 7 CFR part 340, when APHIS determines that it is unlikely to pose a plant pest risk.

An individual may petition the Agency for a determination that a particular regulated article is unlikely to pose a plant pest risk, and should not be regulated under the plant pest provisions of the PPA or the regulations at 7 CFR part 340. Under §340.6(c)(4), the petitioner must provide information related to plant pest risk that the Agency can use to determine whether or not a regulated article poses a plant pest risk. A GE organism or other regulated article is subject to the regulatory requirements of 7 CFR part 340 of the PPA until USDA-APHIS determines that it is unlikely to pose a plant pest risk.

2.5.2 FDA

The FDA regulates GE organisms under the authority of the Federal Food, Drug, and Cosmetic Act (FFDCA). The FDA is responsible for ensuring the safety and proper labeling of all plant-derived foods and feeds, including those that are genetically engineered. To help developers of food and feed derived from GE crops comply with their obligations under Federal food safety laws, FDA encourages them to participate in a voluntary consultation process. All food and feed derived from GE crops currently on the market in the US have successfully completed this consultation process. The FDA policy statement concerning regulation of products derived from new plant varieties, including those genetically engineered, was published in the *Federal Register* (FR) on May 29, 1992 (57 FR 22984-23005). Under this policy, FDA uses what is termed a consultation process to ensure that human food and animal feed safety issues or other regulatory issues (e.g., labeling) are resolved prior to commercial distribution of bioengineered food.

The permit applicant did not undergo this voluntary consultation because GE diamondback moth is not anticipated to yield food or feed.

2.5.3 EPA

The EPA regulates plant-incorporated protectants (PIPs) under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and certain biological control organisms under the Toxic Substances Control Act (TSCA).

The EPA is responsible for regulating the sale, distribution and use of pesticides, including pesticides that are produced by an organism through techniques of modern biotechnology. Such pesticides are regulated by EPA as PIPS under FIFRA (7 U.S.C. 136 *et seq.*). EPA also regulates certain biological control organisms under the Toxic Substances Control Act (15 U.S.C. 53 *et seq.*). Before planting a crop containing a PIP, an individual or company must seek an experimental use permit from EPA. Commercial production of crops containing PIPs for purposes of seed increase and sale requires a FIFRA Section 3 registration with EPA.

Any herbicide (or any other pesticide) in the United States must be registered by the EPA prior to any specific use in the United States. EPA regulates pesticide use under authority granted by FIFRA (see 21 U.S.C. § 301 et seq.). EPA defines pesticide registration as:

> … a scientific, legal, and administrative procedure through which EPA examines the ingredients of the pesticide; the particular site or crop on which it is to be used; the amount, frequency, and timing of its use; and store and disposal practices. In evaluating a pesticide registration application, EPA assesses a wide variety of potential human health and environmental effects associated with the use of the product (EPA, 2013c).

EPA requires a variety of pre-defined tests in a pesticide registration package. The potential pesticide registrant must provide this data, according to EPA guidelines (EPA, 2013c). The data resulting from these tests is used by the EPA to produce an ecological risk assessment and human health risk assessment in order to:

> …evaluate whether a pesticide has the potential to cause adverse effects on humans, wildlife, fish, and plants, including endangered species and non-target organisms, as well as possible contamination of surface water or ground water from leaching, runoff, and spray drift. Potential human risks range from short-term toxicity to long-term effects such as cancer and reproductive system disorders (EPA, 2013c).

Following submission of a complete pesticide registration package, EPA may decide to register or not register a pesticide. If EPA decides to register a pesticide, then the pesticide can only be used:

> …legally according to the directions on the labeling accompanying it at the time of sale. Following label instructions carefully and precisely is necessary to ensure safe use (EPA, 2013c).

As a result of this pesticide registration process by EPA, any EPA-registered pesticide used in the United States:

> …if used in accordance with specifications, they will not cause unreasonable harm to the environment (EPA, 2013c).

EPA did not review these GE diamondback moth strains because it neither contains PIPs nor does it require use of any new pesticides that otherwise would not be used on other non-GE diamondback moths.

2.6 Public Involvement

APHIS routinely seeks public comment on EAs prepared in response to permits for field release of a regulated GE organism. APHIS does this through a notice published in the *Federal Register*. This EA is available for public comment for a period of 30-days. Comments received by the end of the 30-day period will be analyzed and used to identify potential substantive issues that APHIS will consider in the evaluation of this permit application and associated NEPA documents.

2.7 Issues Considered

The list of resource areas considered in this EA were developed by APHIS through experience in considering public concerns and issues raised in public comments submitted for other NEPA documents of GE organisms (USDA-APHIS, 2014b), including NEPA documents for the release of GE insects (USDA-APHIS, 2008; 2009; 2011a). The resource areas considered also address concerns raised in previous and unrelated lawsuits, as well as issues that have been raised by various stakeholders in the past. The resource areas considered in this EA are:

Environmental Considerations:

- Soil resources;
- Water resources;
- Air quality;
- Climate change;
- Plant communities;
- Wildlife; and
- Biological diversity.

Human Population Considerations:

- Farmworker health; and
- Health of the general public.

3 AFFECTED ENVIRONMENT

3.1 Introduction

The diamondback moth (*Plutella xylostella*) is an important pest of cruciferous crops[5] throughout New York State and the rest of the world (Talekar and Shelton, 1993; Shelton, 2001a). New York State is ranked as the third largest cabbage and cauliflower producer within the United States (NY Department of Agriculture and Markets, 2014). Though economic impact from the diamondback moth may vary from year to year, a severe outbreak of the pest is estimated to decrease New York crucifer crop values between $10-20 million (Personal Communication, A. Shelton). In the United States, management costs were estimated to be between $1.3 and $2.3 billion in 2012 (Zalucki *et al.*, 2012). However, if the economic impact from residual pest damage is included with management costs, then the economic impact of this pest in 2012 rises increases approximately $4 and $5 billion (Zalucki *et al.*, 2012).

On October 24th, 2013, APHIS received a permit application from an applicant seeking the permitted field release of three GE diamondback moth strains, OX4319L-Pxy, OX4319N-Pxy, and OX4767A-Pxy, at the Cornell University New York State Agricultural Experiment Station[6]. These GE diamondback moth strains are genetically engineered to exhibit red fluorescence and repressible-female lethality (Section 2.4).

The purpose of the field release is basic research to assess the feasibility and efficacy of GE diamondback moth in reducing pest populations of non-GE diamondback moth under field conditions. According to the applicant, these GE diamondback moths may serve as an insecticide-free means of controlling non-GE diamondback moth in a species-specific manner (Section 2.4). The field release of these GE diamondback moths will allow the applicant to gauge efficacy of this system in reducing pest diamondback moth populations.

The following sections describe the action area and aspects of the human environment[7] considered in this EA. Collectively, the action area and considered aspects of the human environment will constitute the Affected Environment of this EA.

3.2 EA Action Area

The primary action area for this EA consists of six potential release sites[8] described within the permit application #13-297-102r (Section 2.4). The action area is contained within the NYSAES

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[5] e.g., including but not limited to cabbage, broccoli, cauliflower, collards, rape, mustard, and Chinese cabbage

[6] Referred to as NYSAES hereinafter

[7] The human environment, as defined by CEQ (40 CFR 1508.14), "shall be interpreted to comprehensively include the natural and physical environments and the relationship of people with that environment." See http://www.gpo.gov/fdsys/pkg/CFR-2012-title40-vol34/pdf/CFR-2012-title40-vol34-sec1508-14.pdf . Last accessed March, 2014

[8] Total acreage for these potential release sites is not to exceed 60 acres

in Geneva, NY. The NYSAES itself consists of 870 total acres and is located on the north-western edge of Geneva, NY, approximately 2 miles from suburban/urban areas (Figure 2).

The potential release sites are generally surrounded by other agricultural fields (Figure 2). The action area, like much of the land managed by the NYSAES, has been subject to constant agricultural activities for much of its 134-year history (NYSAES, 2014). In the present day, over 700 acres of the NYSAES is planted to row/vegetables crops, orchards, and vineyards (NYSAES, 2014), including the proposed field release sites.

Despite reports of diamondback moths moving long distances[9] (Talekar and Shelton, 1993; Hopkinson and Soroka, 2010), this EA will not consider the long-distance dispersal of GE diamondback moth in the description of the relevant resource areas (Sections 3.3, 3.4, 3.5, and 3.6), or the evaluation of Potential Environmental Consequences (Section 5). This exclusion of long-distance dispersal of GE diamondback moth is based on:

- The general characterization of diamondback moth as a weak flyer, a characteristic that strongly limits its ability to disperse long distances (Talekar and Shelton, 1993, Appendix A; Shelton, 2001a);
- Observations from the peer-reviewed literature that long-distance dispersal of diamondback moth, when and where it occurs, is facilitated by strong wind currents across geographic regions (Hopkinson and Soroka, 2010);
- Predominant wind currents across the New England region of the United States, and thereby, across the potential release sites, is primarily west to east (towards a destination which would not change the climatic zone) or south to north (American Meteorological Society, 2012; WeatherSpark, 2014) (which would further assure inability to overwinter) during cruciferous crop planting seasons; and
- The inability of diamondback moth to overwinter at similar latitudes or to the north of the potential release sites (Talekar and Shelton, 1993; Appendix A; Hopkinson and Soroka, 2010).

In summary of the points listed directly above, diamondback moth is generally characterized as a weak flyer incapable of long-distance dispersal. Long-distance dispersal of diamondback moth observed in the peer-reviewed literature is generally regarded as the result of strong wind currents. Predominant wind patterns over New York State when release of GE diamondback moth may occur will generally preclude the movement of any diamondback moth, GE or non-GE, into regions where it may successfully overwinter. As a result of these observations and the ubiquity of non-GE diamondback moth in North America (Andaloro, 1983; Talekar and Shelton, 1993; Shelton, 2001a), the long-distance dispersal of diamondback moth into areas where it may overwinter is not considered likely, and thus, will not be considered in the establishment of the action area (Section 3.2), the description of the relevant resource areas (Sections 3.3, 3.4, 3.5, and 3.6), or the evaluation of Potential Environmental Consequences (Section 5).

[9] Defined as greater than 100 km

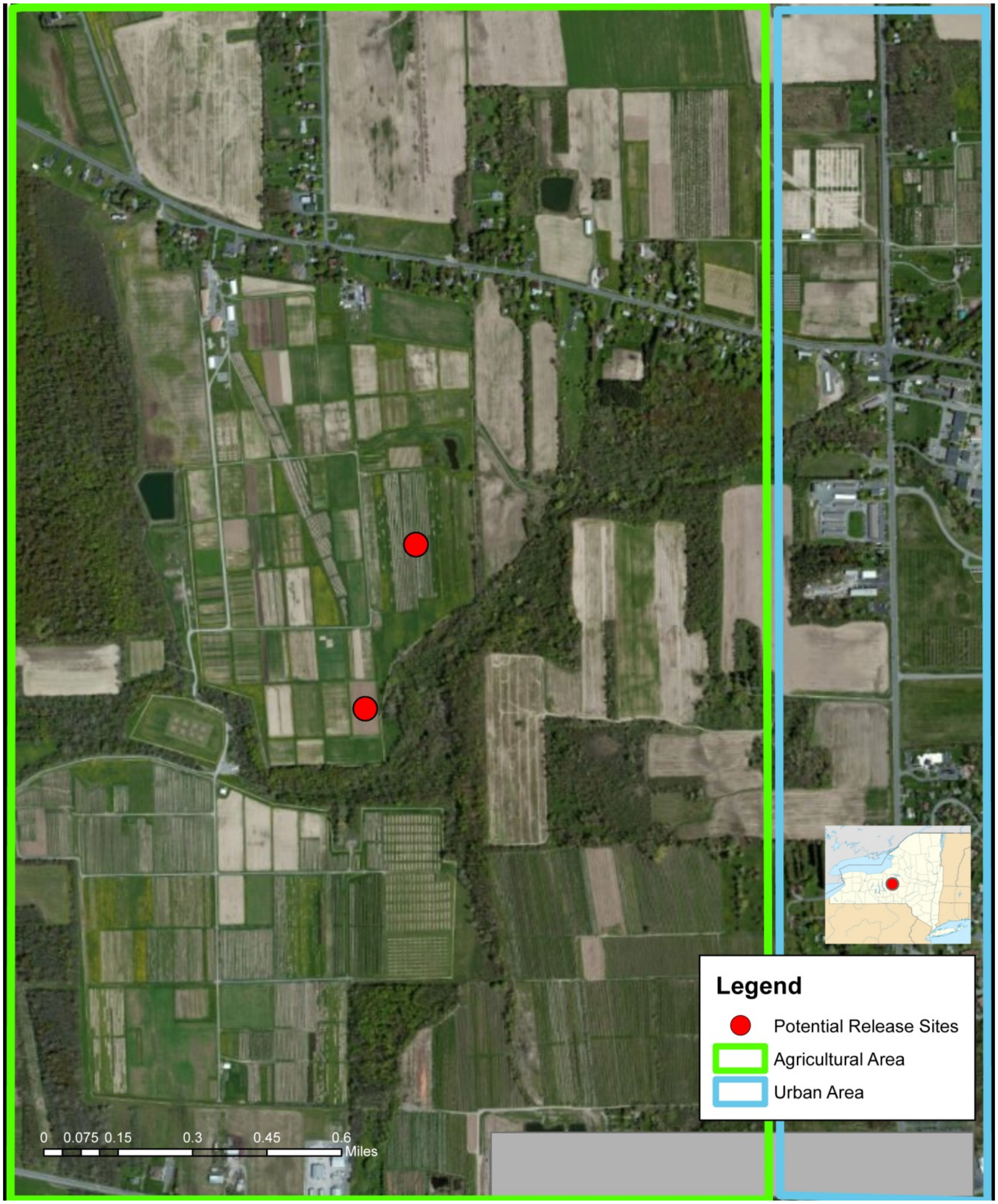

Figure 2. Action area of this Environmental Assessment.
The action area consists of six sites (3 release sites and 3 control sites). The upper red dot represents five release sites that are directly adjacent to one another, while the lower red dot represents a single release site.

3.3 Resource Areas

A resource area is a relevant component of the human environment. The human environment may include, but not be limited to, aspects of the natural (e.g., soil, water, wildlife, etc.) and human (e.g., economics, social values, etc.) environment. For meaningful environmental analysis of the proposed action, the range of resource areas analyzed in this EA are identified as those areas that have the potential to be impacted by an agency decision.

The list of resource areas considered in this EA were developed by APHIS through experience in considering public concerns and issues raised in public comments submitted for other NEPA documents of GE organisms (USDA-APHIS, 2014b), including NEPA documents for the release of GE insects (USDA-APHIS, 2008; 2009; 2011a). The resource areas considered also address concerns raised in previous and unrelated lawsuits, as well as issues that have been raised by various stakeholders in the past. The resource areas considered in this EA are: Soil resources;

- Water resources;
- Air quality;
- Climate change;
- Plant communities;
- Wildlife;
- Biological Diversity;
- Farm worker health; and
- Health of the general public.

In the following subsections, each specific resource area will be characterized as a component of Physical[10], Biological[11], or Human Health environments[12]. Additionally, brief descriptions will be provided for each specific resource area. Analyses of the potential impact on each specific resource area as a result of an Alternative will be undertaken in Section 4.

3.4 Physical Environment

The physical environment consists of abiotic[13] components within the action area. For the purposes of this EA, components of the physical environment include soil resources, water resources, air quality, and climate change.

3.4.1 Soil Resources

Soil consists of solids (minerals and organic matter), liquids, and gases. This aggregation of inorganic and organic matter is home to a wide variety of fungi, bacteria, and arthropods, as well as the growth medium for terrestrial plant life (USDA-NRCS, 2004). Soil is characterized by its

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[10] i.e., land use and soil resources; water resources; and air quality and climate change

[11] i.e., plant communities; wildlife and insects; and biological diversity

[12] i.e., farm worker health and general population health

[13] i.e., non-living

layers that can be distinguished from the initial parent material due to additions, losses, transfers, and transformations of energy and matter (USDA-NRCS, 1999). It is further distinguished by its ability to support rooted plants in a natural environment. Soil plays a key role in determining the capacity of a site for biomass vigor and plant productivity by physical support, inclusion of air and water, ability to moderate temperature, protect from toxins, and make nutrients available. Soils also determine a site's susceptibility to erosion by wind and water, and a site's flood attenuation capacity.

Furthermore, soil properties change over time; temperature, pH, soluble salts, amount of organic matter, the carbon-nitrogen ratio, numbers of microorganisms and soil fauna all vary seasonally, as well as over extended periods of time (USDA-NRCS, 1999). Soil texture and organic matter levels directly influence its shear strength, nutrient holding capacity, and permeability. Soil taxonomy was established to classify soils according to the relationship between soils and the factors responsible for their character (USDA-NRCS, 1999).

Soils are classified taxonomically into soil orders based on observable properties such as organic matter content and degree of soil profile development (BCAP, 2010). Alfisols and Inceptisols are the primary soil types within the action area (EPA, 2012d). Alfisols result from a variety of weathering processes that leach constituents from the surface layer into the subsoil, while inceptisols are soils of semiarid environments that show a moderate level of soil weathering and development (BCAP, 2010). Both soil types function as good agricultural soils (USDA-NRCS, 2004). Further description of these two soil types may be found in USDA-NRCS (1999).

3.4.2 Water Resources

Water is essential for life and plays a vital role in the proper functioning of the Earth's ecosystems. Water pollution has a substantial impact on all living creatures, and can negatively affect the use of water for drinking, household needs, recreation, fishing, transportation and commerce. Water resources may be considered as either surface or groundwater (USGS, 2013; 2014).

Surface water[14] is water contained within rivers, streams, creeks, lakes, and reservoirs (USGS, 2014). Surface waters support everyday life through the provision of water for drinking and other public uses. Surface water quality is determined by the natural, physical, and chemical properties of the land that surrounds the water body (USGS, 2014). When land use affects one or more of these natural physical characteristics of the land, water quality is almost always impacted to some extent. These impacts may be positive or negative, depending on the type, duration, and extent of land use.

Groundwater is water that flows underground and is stored in natural geologic formations called aquifers (USGS, 2013). In the United States, approximately 47 percent of the population depends on groundwater for its drinking water supply (NGWA, 2010). Groundwater is ecologically important because it sustains ecosystems by releasing a constant supply of water into wetlands and contributes a sizeable amount of flow to permanent streams and rivers (USDA-FSA, 2010). Currently, the largest use of groundwater in the United States is irrigation, representing approximately 67 percent of all the groundwater pumped each day (McCray, 2012).

[14] i.e., freshwater surface water

Agricultural practices have the potential to impact water use through irrigation practices. Additionally, agricultural practices have the potential to substantively impact water quality due to the vast amount of acreage devoted to farming nationwide and the physical and chemical demands that agricultural use imposes on the land. The most common types of agricultural pollutants include excess sediment, fertilizers, animal manure, pesticides and herbicides. Agricultural nonpoint source (NPS) pollution is the leading source of impacts to surveyed rivers and lakes, the third largest source of impairment to estuaries, and a major source of impairment to groundwater and wetlands (USDA-NRCS, 2011). The principal law governing pollution of the nation's water resources is the Federal Water Pollution Control Act of 1972, better known as the Clean Water Act (CWA).

3.4.3 Air Quality

Dry air consists of about 78% nitrogen, 21% oxygen, 0.9% argon and 0.03% carbon dioxide. It also contains small amounts of water vapor and particulate matter (Darley and Middleton, 1966). Air quality is the capability of the atmosphere to sustain life, enabling living organisms to respire, and to buffer life on earth from the extremes of temperature variations (BCAP, 2010).

As defined by the EPA pursuant to the Clean Air Act (CAA) and the National Ambient Air Quality Standards (NAAQS), air quality impairments may represent ozone (O_3); nitrogen dioxide (NO_2); carbon monoxide (CO); sulfur dioxide (SO_2), lead (Pb); or inhalable particulates (coarse particulate matter [PM] greater than 2.5 micrometers and less than 10 micrometers in diameter [PM_{10}] and fine particles less than 2.5 micrometers in diameter [$PM_{2.5}$]) (BCAP, 2010)

3.4.4 Climate Change

The climate of the action area is broadly representative of the larger Northeastern United States and is characterized as humid continental type (NY State Climate Office, n.d.). Approximately 30 – 45 inches of precipitation falls every year, and temperatures range from 16 – 80°F (EPA, 2012d).

Climate and climate change are discreet conditions. Climate may be defined as the average weather, or rigorously, as the statistical description in terms of the mean and variability of relevant measurable units over a period of time in both the short- and long-term scales (EPA, 2013b). On the other hand, climate change represents a stastical change in global climate conditions, including shifts in the frequency of extreme weather (Cook *et al.*, 2008; Karl *et al.*, 2008).

Climate change represents a sustained, statistically significant change in average weather conditions over a broad region. EPA has identified CO_2, methane (CH_4), and nitrous oxide (N_2O) as the most important greenhouse gases (GHG) contributing to climate change. While each of these occurs naturally in the atmosphere, human activity has been a major contributor to the increase of their concentrations since the beginning of the industrial revolution. The level of human-produced gases has been accelerating since the end of World War II, when industrial and consumer consumption expanded greatly. Since the advent of the industrial age, the increase in the concentration of some important GHGs are as follows: CO_2, 36%; CH_4, 148% and N_2O, 18% (EPA, 2011a).

3.5 Biological Environment

3.5.1 Wildlife

The biological environment consists of biotic[15] components within the action area. For the purposes of this EA, components of the biological environment include plant communities, wildlife and insects, and biological diversity.

Wildlife is the totality of all animals in a specific area, including those wildlife species that are native, introduced, desirable, and undesirable (BCAP, 2010). Wildlife species may be generally characterized as mammals, birds, reptiles, amphibians, fish, and molluscs (NatureServe, 2013). Descriptions of each wildlife type may be found in Cambell (1999).

Agricultural fields may be host to a variety of wildlife species for the purposes of habitat or feed. Although agricultural fields are generally considered poor habitat for birds and mammals in comparison with uncultivated land because of continual disturbances associted with typical cultivation activities, the use of these fields by some wildlife is not uncommon (Vercauteren and Hygnostrom, 1993; Patterson and Best, 1996; Palmer *et al.*, 2011) For example, some mammals that utilize cornfields are ground-feeding ominvores that feed on the corn remaining in the field following harvest (Vercauteren and Hygnostrom, 1993; Krapu *et al.*, 2004; Palmer *et al.*, 2011).

Additionally, a number of insects may be found within an agricultural field (NY State IPM Program, 2013). The most relevant of these insects, however, are those insect pests that feed upon the cultivated crop and the insects that prey on these insect pests (Robertson *et al.*, 2012). In particular, a major cruciferous pest within the action area is diamondback moth (Andaloro, 1983; Talekar and Shelton, 1993; Shelton, 2001a) (Figure 3), due to the significant production of cruciferous crops in New York (NY Department of Agriculture and Markets, 2014). Additional information regarding diamondback moth within the action area may be found in Section 2.5.1 and Appendix A.

[15] i.e., living

Figure 3. Diamondback moth adult (A), larvae (B), and damage on a cruciferous crop from diamondback moth larvae.
Individual images derived from Cornell University (n.d.).

3.5.2 Plant Communities

The plant community within an area is the totality of plants in a particular area, including native, introduced, desirable, and undesirable plants (BCAP, 2010). The plant species in the action area may represent a diverse variety of plant species, including forbs, vines, succulents, ferns, grasses, shrubs, and trees (BONAP, 2013). Definitions for these plant types may be found in BONAP (2013). Additionally, for the purposes of this EA, the discussion of plant communities will focus on the Brassicaceae[16], as this is the plant family most likely to be impacted by any decision by USDA-APHIS to deny or issue the applicant's permit application.

The Brassicaceae is a large plant family, containing over 338 genera and 3709 species (Al-Shehbaz, 1984; OECD, 2012). The Brassicaceae constitute some of the world's most

[16] Also known as the Cruciferae

economically important plants, in addition to also containing significant agricultural weeds (OECD, 2012).

Domesticated Brassicaceae include vegetables crops and oilseed crops (OECD, 2012). New York produces many domesticated Brassicaceae (Cornell University Cooperative Extension, 2013). Of the domesticated Brassicaceae, New York is ranked as the third largest cabbage and cauliflower producer within the United States (NY Department of Agriculture and Markets, 2014).

There are numerous weedy Brassicaceae. However, those with the greatest interest to agriculture include *Sinapis arvensis* (wild mustard or charlock), *Raphanus raphanistrum* (wild radish), *Brassica rapus* (wild or bird rape), and *Hirschfeldia incana* (hoary mustard) due to their propensity to cross-pollinate with domesticated *B. napus* (OECD, 2012).

A detailed review of the biology and ecology of both domesticated and non-domesticated Brassicas can be found in OECD (2012).

3.5.3 Biological Diversity

Biological diversity generally refers to the variety and variability of living organisms and the ecosystems where they occur (BCAP, 2010). The degree of biodiversity in an agroecosystem depends on four primary characteristics: (1) diversity of vegetation within and around the agroecosystem; (2) permanence of various crops within the system; (3) intensity of management; and (4) extent of isolation of the agroecosystem from natural vegetation (Altieri, 1999).

The primary function of biological diversity is to contribute to ecosystem services. These ecosystem services may include: pollination, genetic introgression, biological control, nutrient recycling, competition against natural enemies, soil structure, soil and water conservation, disease suppression, control of local microclimate, control of local hydrological processes, and detoxification of noxious chemicals (Altieri, 1999). In general, the loss of biological diversity may result in a need for costly management practices in order to provide these functions (Altieri, 1999).

3.6 Human Health Environment

The human health environment consists primarily of farm worker health and health of the general public. Characterization of human health into these two components is primarily due to the route of exposure to the agricultural activities that are common within the action area and the rest of the NYSAES. Farmworkers are most often directly exposed to agricultural activities. In contrast, the general public is directly exposed to agricultural activities to a much lesser extent, with indirect exposure to the products of those agricultural activities occurring much more frequently.

3.6.1 Farmworker Health

Agriculture is one of the most hazardous industries for US workers. Approximately 3.1 million people in the United States are reported as farm workers, representing approximately 1 percent of the total US population (EPA, 2014a).

Farm workers are exposed to a variety of hazards as a result of common agricultural activities, such as accidents related to production machinery or agricultural inputs. As a result, Congress directed the National Institute of Occupational Safety and Health to develop a program to address high-risk issues related to occupational workers. In consideration of the risk of pesticide exposure to field workers, EPA's Worker Protection Standard (WPS) (40 CFR Part170) was published in 1992 to require actions to reduce the risk of pesticide poisonings and injuries among agricultural workers and pesticide handlers. The WPS offers protections to more than two and a half million agricultural workers who work with pesticides at more than 560,000 workplaces on farms, forests, nurseries, and greenhouses. The WPS contains requirements for pesticide safety training, notification of pesticide applications, use of personal protective equipment, restricted entry intervals following pesticide application, decontamination supplies, and emergency medical assistance; furthermore, the Occupational Safety and Health Administration (OSHA[17]) require all employers to protect their employees from hazards associated with pesticides and herbicides.

Pesticides[18] are used on most agricultural acreage in the United States. Under FIFRA, all pesticides, sold or distributed in the United States must be registered by the EPA (EPA, 2005b). During the registration decision, the EPA must find that a pesticide does not cause unreasonable adverse effects to human health or the environment if used in accordance with the approved label instructions (OSTP, 2001).

EPA labels for pesticides include use restrictions and safety measures to mitigate exposure risks (EPA, 2014c). Growers are required to use registered pesticides consistent with the application instructions provided on the EPA-approved pesticide labels. Worker safety precautions and use restrictions are clearly noted on pesticide registration labels. EPA labels for registered herbicides have been designed to reduce the risks of illness or injury resulting from workers' and handlers' occupational exposures to pesticides used in the production of agricultural plants on farms (EPA, 2014c).

3.6.2 Health of the General Public

Direct exposure of the general population to agricultural activities is limited to personal use of pesticides on personal property or public areas[19]. In scenarios such as this, safe use of pesticides is facilitated much in the same as described directly above for farm workers (Section 2.6.1). The amount of pesticide residues that may remain on agricultural commodities is regulated by EPA and are called pesticide "tolerances" in the United States (EPA, 2014d).

Pursuant to the Federal Food, Drug, and Cosmetic Act (FFDCA), EPA must establish the tolerance value for pesticide residues that can remain on the crop or in foods processed from that crop (EPA, 2010b). In addition, the FDA and the USDA monitor foods for pesticide residues and enforce these tolerances (USDA-AMS, 2010). If pesticide residues are found to exceed the tolerance value, the food is considered adulterated and may be seized. The USDA has implemented the Pesticide

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[17] https://www.osha.gov/ Last accessed March 14, 2014

[18] i.e., herbicides, insecticides, and fungicides

[19] e.g., state forests, county parks, etc.

Data Program (PDP) in order to collect data on pesticides residues on food (USDA-AMS, 2010). The EPA uses PDP data to prepare pesticide dietary exposure assessments pursuant to the 1996 Food Quality Protection Act (FQPA). Pesticide tolerance levels for various pesticides have been established for a wide variety of commodities, including soybean, and are published in the *Federal Register*, CFR, and the *Indexes to Part 180 Tolerance Information for Pesticide Chemicals in Food and Feed Commodities* (EPA, 2011b).

4 ALTERNATIVES

This EA analyzes the potential environmental consequences of APHIS' response to an environmental release (APHIS Number 13-297-102r) received from an applicant to allow the release of GE diamondback moths at the Cornell University New York State Agricultural Research Station. A total of 60 acres (all 6 sites combined) is being requested by the applicant. These GE diamondback moths possess the introduced traits of red fluorescence, repressible-female lethality, and male-sterility. The purpose of the environmental release is for the applicant to assess the efficacy of GE diamondback moths in reducing the population of non-GE diamondback moths in a release site. See the APHIS (2013) permit 13-297-102r and Appendix A for more information about the APHIS permit and GE diamondback moths, respectively.

Under APHIS regulations, the Administrator must either deny or grant permits properly submitted under 7 CFR part 340. Based upon the permit application submitted by the applicant, two alternatives are considered and analyzed in this EA: (1) deny the permit and (2) approve permit application request and issue the APHIS permit.

4.1 No Action Alternative – Deny the Permit

Under the No Action Alternative APHIS would deny the permit application (APHIS Number 13-297-102r) submitted by the applicant. The applicant would not be authorized to release the GE diamondback moth strains OX4319L-Pxy, OX4319N-Pxy and OX4767A-Pxy. APHIS may choose this alternative if there were sufficient evidence to demonstrate that these GE diamondback moth strains would not increase the already existent plant pest risk or allow the establishment and persistence in the environment.

4.2 Preferred Alternative – Issue the APHIS Permit

Under the Preferred Alternative, APHIS would issue an environmental release permit to the applicant in accordance with 7 CFR part 340 to allow the release of GE diamondback strains OX4319L-Pxy, OX4319N-Pxy and OX4767A-Pxy over a maximum field area of 60 acres. APHIS may choose this alternative if there were sufficient evidence to demonstrate that these GE diamondback moth strains would not increase the already existent plant pest risk or allow the establishment and persistence in the environment. If APHIS chooses this alternative, then the permit will be subject to the conditions described in 7 CFR part 340.4[20].

Under the Preferred Alternative, the permit would be valid for a three-year period. The permit will need to be renewed by the applicant and subsequently approved by APHIS to allow any additional release of GE diamondback moths beyond the three-year time period specified in the permit application. Additionally, under the Preferred Alternative, the applicant would be allowed to gather data on performance of GE diamondback moths in reducing populations of non-GE diamondback moths over a multi-year period.

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[20] http://www.gpo.gov/fdsys/granule/CFR-2012-title7-vol5/CFR-2012-title7-vol5-sec340-4/content-detail.html Last accessed May, 2014

4.3 Comparison of Alternatives

Attribute / Measure	Alternative A: No Action Alternative Deny the permit request	Alternative B: Preferred Alternative Grant the permit request
Meets Purpose and Need and Objectives	**No**	**Yes**
Unlikely to pose a plant pest risk	No plant pest risk.	Satisfied through use of regulated field trials, including APHIS imposed permit conditions and monitoring for compliance. Impacts would be similar to the no action alternative.
Physical Environment		
Soil Quality	Common agricultural activities related to field preparation/maintenance that impact soil (e.g., tillage, pesticide application, etc.) will continue under the No Action Alternative.	The permitted field release of GE diamondback moth is not anticipated to change common agricultural activities related to preparing and maintaining an agricultural field that is already occurring under the No Action Alternative. Transfer of non-native DNA from decomposing GE diamondback moth to other soil microflora is not likely under the Preferred Alternative. Thus, impact on soil resources would be similar to the no action alternative.
Water Resources	Agronomic practices that could impact water resources (e.g., irrigation, tillage practices, and the application of agronomic inputs) would be expected to continue under the No Action Alternative. The use of pesticides in accordance with EPA-approved label directions assure no unreasonable risks to water quality from their use	The permitted field release of GE diamondback moth is not anticipated to change common agricultural activities related to preparing and maintaining an agricultural field that is already occurring under the No Action Alternative. Thus, impact on water resources would be similar to the no action alternative.
Air Quality	Common agricultural activities having the potential to impact air quality such as tillage, the application of pesticides and fertilizer, and use of particulate- and pollutant -emitting agricultural equipment would continue under the No Action Alternative. The use of pesticides in accordance with EPA-approved labels minimizes drift and reduces environmental impacts.	The permitted field release of GE diamondback moth is not anticipated to change common agricultural activities related to preparing and maintaining an agricultural field that is already occurring under the No Action Alternative. Thus, impact on air quality would be similar to the no action alternative.

Attribute / Measure	Alternative A: No Action Alternative Deny the permit request	Alternative B: Preferred Alternative Grant the permit request
Climate Change	Common agricultural activities possess the potential to impact climate change, through the release of CO_2 to the atmosphere from tillage; machinery powered by fossil fuel; and NO_2 emissions associated with nitrogen fertilizers use. These activities are already occurring, and are likely to continue occurring, under the No Action Alternative.	The permitted field release of GE diamondback moth is not anticipated to change common agricultural activities related to preparing and maintaining an agricultural field that is already occurring under the No Action Alternative. Thus, the impact on GHG emissions and climate change would be similar to the no action alternative.
Biological Environment		
Wildlife	Common agricultural activities such as such as tillage, cultivation, pesticide and fertilizer applications, and the use of agricultural equipment would continue to impact wildlife communities. The use of EPA-registered pesticides and herbicides in accordance with EPA-approved labels minimize potential impacts to animal communities.	The permitted field release of GE diamondback moth is not anticipated to change common agricultural activities related to preparing and maintaining an agricultural field that is already occurring under the No Action Alternative. The introduced traits in GE diamondback moth do not encode for any known allergen or toxin, and GE diamondback moth is not anticipated to persist within the action area due to its inability to overwinter. Additionally, horizontal gene transfer of DNA from GE diamondback moth to wildlife that may consume it is unlikely. Thus, impact to wildlife would be similar to the no action alternative.

Attribute / Measure	Alternative A: No Action Alternative Deny the permit request	Alternative B: Preferred Alternative Grant the permit request
Plant Communities	Under the No Action Alternative, the plant community within the action area will continue to generally consist of planted crops (cruciferous and non-cruciferous) and weeds of those planted crops. As a result of this simplified agricultural ecosystem, planted crops will continue to be potentially harmed by pests and weeds, and growers will continue to manage the population of pests and weeds.	The permitted field release of GE diamondback moth is not anticipated to change common agricultural activities related to preparing and maintaining an agricultural field that is already occurring under the No Action Alternative. Adult diamondback moths do not damage plant tissues and diamondback moth larvae only feed upon cruciferous plants. Damage from GE diamondback moth larvae on planted cruciferous plants is not anticipated to be substantial, because of the ubiquity of diamondback moth in the action area and its inability to persist within the action area. Damage from GE diamondback moth larvae on cruciferous weeds is also not anticipated to be substantial, because these cruciferous weeds are likely to be managed through cultural or chemical methods; the damage from GE diamondback moth larvae is unlikely to be more than the approaches land managers are likely taking to eradicate these cruciferous weeds from fields within the action area. Thus, impact to plant communities would be similar to the no action alternative.
Biological Diversity	Under the No Action Alternative, biological diversity within the action area is reduced and will continue to be reduced when compared to environments that are less intensively managed.	The permitted field release of GE diamondback moth is not anticipated to change common agricultural activities related to preparing and maintaining an agricultural field that is already occurring under the No Action Alternative. Thus, impacts to biological diversity from common agricultural activities would be similar to the no action alternative. The release of GE diamondback moth is not anticipated to substantially affect biological diversity because non-GE diamondback moth is already targeted for management/control within the action area; and because

Attribute / Measure	Alternative A: No Action Alternative Deny the permit request	Alternative B: Preferred Alternative Grant the permit request
		GE diamondback moth is unlikely to persist within the action area after the end of the calendar year, similar to non-GE diamondback moth.
Human Health Environment		
Human Health	No changes are anticipated to currently-adopted agricultural activities under the No Action Alternative. As a result, human exposure (e.g., farmworkers or the general human population) to risks and hazards as a result of these common agricultural activities are also anticipated to continue occurring under the No Action Alternative. A variety of EPA-approved pesticides would continue to be used for pest management within the action area. Use of registered pesticides in accordance with EPA-approved labels protects human health and worker safety. EPA also establishes tolerances for pesticide residue that give a reasonable certainty of no harm to the general population and any subgroup from the use of pesticides at the approved levels and methods of application.	The permitted field release of GE diamondback moth is not anticipated to change common agricultural activities related to preparing and maintaining an agricultural field that is already occurring under the No Action Alternative. Thus, impacts to human health (e.g., farmworkers and the general human population) from common agricultural activities would be similar to the no action alternative. Previous NEPA documents have analyzed and concluded that there is no unreasonable risk to humans associated with the introduced traits in GE diamondback moth. Thus, GE diamondback moth itself is not anticipated to substantially affect human health under the Preferred Alternative. Additionally, GE diamondback moth is not a member of any lepidopteran family that may generally cause allergic reactions to humans from exposure to scales or hairs.
Compliance with Other Laws		
CWA, CAA, EOs	Fully compliant	Fully compliant

Table 1. Comparison of Alternatives

5 POTENTIAL ENVIRONMENTAL CONSEQUENCES

This analysis of potential environmental consequences addresses the potential impact to the human environment from the alternatives analyzed in this EA, namely taking No Action (i.e., deny the permit) or the Preferred Alternative (i.e., issue the permit). The Alternatives presented in this EA are discussed further in Section 3. Potential environmental impacts within the action area from the No Action Alternative and the Preferred Alternative for GE diamondback moth are described in detail throughout this section.

5.1 Scope of the Analysis

Potential environmental impacts from the No Action Alternative and the Preferred Alternative for GE diamondback moth are described in detail throughout this section. These potential environmental impacts are described within the context of the resource areas described in the Affected Environment (Section 2).

An impact would be any change, positive or negative, from the existing (baseline) conditions of the affected environment. This baseline condition is described in the No Action Alternative analysis for each resource area. Impacts may be categorized as direct, indirect, or cumulative. A direct impact is an effect that results solely from a proposed action without intermediate steps or processes. Examples include soil disturbance, air emissions, and water use. An indirect impact may be an effect that is related to but removed from a proposed action by an intermediate step or process. Examples include surface water quality changes resulting from soil erosion due to increased tillage, and worker safety impacts resulting from an increase in herbicide use.

A cumulative effects analysis is also included for each environmental issue. A cumulative impact may be an effect on the environment which results from the incremental impact of the proposed action when added to other past, present, and reasonably foreseeable future actions. An example includes potential future field releases of GE diamondback moth. If there are no direct or indirect impacts identified for a resource area, then there can be no cumulative impacts. Cumulative impacts are discussed in Section 5.

Where it is not possible to quantify impacts, APHIS provides a qualitative assessment of potential impacts. Certain aspects of this permit may be no different between the alternatives; those are described below.

Because this is an analysis for a permitted field release, APHIS will limit the environmental analysis to those areas where the potential field release would occur. Additionally, APHIS will also consider those areas adjacent to the potential release sites when appropriate. Collectively, the potential release sites and areas adjacent to it are considered the action area. The action area is further described in the Affected Environment (Section 2).

5.2 Physical Environment

5.2.1 No Action Alternative: Soil Resources, Water resources, Air Quality, and Climate Change.

Summary of potential impacts

Under the No Action Alternative, common agricultural activities are currently and will continue to occur within the action area. These common agricultural include activities related to field preparation (e.g., tillage) and field maintenance (e.g., tillage, irrigation, and the application of agricultural inputs). Irresponsible use of these common agricultural activities has the potential to negatively affect soil resources, water resources, air quality, and climate change. For example, irresponsible use of tillage may lead soil erosion, which in turn not only impacts soil quality, but also contributes particles that can impact water (e.g., sedimentation) and air quality (e.g., air-borne dust). Furthermore, the irresponsible use of agricultural inputs can also negatively affect water resources and air quality through the off-site movement of these agricultural inputs.

However, common agricultural practices and regulations also exist to preserve soil resources, water resources, air quality, and the climate. Under the No Action Alternative, these practices and regulations currently and will continue to be in place to mitigate agricultural impacts to the each aspect of the physical environment.

Background

The physical environment consists of soil resources, water resources, air quality, and climate change (Section 2). Each individual aspect of the physical environment may be substantially affected by the anthropogenic activities that occur on it.

As previously discussed in the Affected Environment (Section 2), the action area is located within the NYSAES in Geneva, NY. The action area, similar to rest of the NYSAES-owned land that surrounds it, is land that has been maintained under some form of agricultural management for much of its 134-year history (NYSAES, 2014). Consideration of historical land use patterns and the NYSAES mission[21] strongly suggests that present-day agricultural activities within the action area will continue under the No Action Alternative. Consequently, any current impact on the physical environment as a result of these agricultural activities will also continue under the No Action Alternative.

Common agricultural activities (Delahaut and Newenhouse, 1997; Seaman, 2013) are facilitated by the use of motorized farm equipment[22] and include tillage and the use of agricultural inputs (Personal Communication, A. Shelton). Tillage and the use of agricultural inputs possesses the potential to directly and indirectly affect the physical environment if not properly used (USDA-

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[21] The NYAES was established by the New York State Legislature for "…the purpose of promoting agriculture in its various branches by scientific investigation and experiment." See http://www.nysaes.cornell.edu/cals/nysaes/about /history.cfm. Last accessed March, 2014

[22] e.g., tractors, plows, etc.

NRCS, 2001). For example, tillage and the use of motorized farm equipment may directly or indirectly affect components of the physical environment through the release of soil particles and the emission of various gases (EPA, 2012b). These potential impacts for each component of the physical environment are presented in the following subsections.

Additionally, the use of agricultural inputs may also directly or indirectly affect components of the physical environment (Leistra *et al.*, 2006; Tong, 2009). However, the use of any EPA-registered pesticide within the United States is unlikely to cause adverse effects on the environment if used according to the specifications on the label (See Section 2.5.3 and EPA, 2013c). Therefore, the use of any EPA-registered pesticide is unlikely to have a significant impact on individual components of the physical environment and will not be discussed further.

Soil resources

Modern agricultural activities possess the potential to modify soil quality. While practices such as tillage and the use of agricultural inputs can improve soil health, they can also cause substantial damage if not properly used (USDA-NRCS, 2001). Several concerns relating to common agricultural activities include concerns relating to soil structure[23] and soil composition[24] (USDA-NRCS, 2001).

Soil is generally characterized by the structure and composition of organic/inorganic materials (USDA-NRCS, 1999). Accordingly, any agricultural activity that modifies the structure or composition of soils may affect the quality of the soil (USDA-NRCS, 2001).

Conventional tillage is the intentional disturbance of the soil to achieve a variety of objectives, including weed control, incorporation of agricultural inputs into the soil, and modification of soil aeration/water drainage properties (Hoeft *et al.*, 2000). The intensity of soil disturbance during tillage is a primary factor affecting soil quality (Hoeft *et al.*, 2000; Smith and Conen, 2004), as conventional tillage generally exposes the upper layers of soil to the environment, making it more susceptible to degradation from wind- and water-mediated erosion (NCGA, 2007). Additionally, the use of machinery to till a field may potentially compact the soil (i.e., compaction) (Delahaut and Newenhouse, 1997). Compacted soil possesses a reduced number and size of air spaces in soil, ultimately leading to decreased aeration and water-holding capacity in that soil (USDA-NRCS, 2001). Conservation tillage practices manages the soil erosion and structural concerns of conventional tillage by leaving undisturbed plant residues in the field at the conclusion of the growing season, relying exclusively on herbicide application to control weeds following planting (Markus, 1997; O'Brien, 1998; Hoeft *et al.*, 2000) .

The use of agricultural inputs is an important aspect of modern agriculture (Heiniger, 2000; Farnham, 2001; University of Arkansas, 2006; USDA-NASS, 2007; NSRL, n.d.). Two primary types of agricultural inputs used in modern agriculture are fertilization and pesticide application. Fertilization is generally used to compensate for deficiencies or imbalances of soil micro/macro nutrients (Delahaut and Newenhouse, 1997; USDA-NASS, 2007; Seaman, 2013; NSRL, n.d.),

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[23] i.e., erosion and compaction

[24] i.e., nutrient imbalance or the presence of synthetic chemicals

while pesticide application is used to manage agricultural pests[25] that decrease crop yields (Talekar and Shelton, 1993; Anonymous, 1999; Hoeft *et al.*, 2000; Farnham, 2001; USDA-ERS, 2005; USDA-NASS, 2007; Boucher, 2012). The use of both types of agricultural inputs may potentially impact soil quality by adding additional components to the soil, thereby potentially altering soil composition.

For example, growers may choose a variety of methods to control pests in an agricultural field, though the specific method will ultimately be dependent on the nature of the pest itself and grower want and need (USDA-ERS, 2005; 2010). For example, growers may choose certain pesticides based on weed, insect and disease pressures, cost of seed and other inputs, technology fees, human safety, potential for crop injury, and ease and flexibility of use (Heiniger, 2000; Farnham, 2001; University of Arkansas, 2006). The environmental risks of pesticide use on soil resources are assessed by EPA in the pesticide registration process and are regularly reevaluated by EPA for each pesticide to maintain its registered status under FIFRA (EPA, 2014c). When used according to label directions, pesticides can be used without posing unreasonable risk to the environment, including soil quality (EPA, 2014c).

Water resources

Water resources generally incudes the amount of water available for use and the quality of water available for use. Common agricultural activities possess the potential to affect water resources, either through direct use for irrigation or indirectly through the contribution of non-point source (NPS) pollutants.

Within the action area, agricultural use of water through irrigation is only used when needed; the source of the irrigation within the NYSAES is an irrigation pond found on the property of the NYSAES (Personal Communication, A. Shelton).

Tillage and the use of motorized farm equipment may result in soil disturbances (USDA-NRCS, 2001). The intensity and frequency of this disturbance is especially relevant for water quality, as any resulting erosion may facilitate the release of sediments in water bodies. At present, sediments represent the primary source of agricultural NPS pollution in the United States (EPA, 2005a; 2012e). Associated with the potential release of sediments into water bodies following the use of tillage or motorized farm equipment, is the release of agricultural inputs that may have adhered with soil particles into these same bodies of water (Whitney, 1997; EPA, 2005a; USDA-NASS, 2007; EPA, 2012e; NSRL, n.d.).

While tillage or the use motorized farm equipment may facilitate the release of sediments or agricultural inputs adhered to sediments into water bodies, agricultural practices that reduce soil disturbances may also reduce the potential impact on water quality (Hoeft *et al.*, 2000; NCGA, 2007).

While sediments represent the most common cause of agricultural water quality impairments, it is not the only source (EPA, 2005a). The off-site movement[26] of agricultural inputs, such as

[25] i.e., weeds, insect pests, or microbial pests

[26] i.e., agricultural runoff

fertilizer or pesticides, also represent common water quality impairments (EPA, 2012e). In the United States, nutrients and pesticides ranked as the 3rd and 16th most important causes of impairments in assessed water bodies, respectively (EPA, 2012e).

Water quality in the United States is overseen by the EPA under authority of the Clean Water Act (CWA). The CWA authorizes the establishment of water quality standards, permit requirements, and monitoring to establish a legal framework to protect and enhance domestic water quality. The EPA sets standards for water pollution abatement for all waters of the U.S. under the authority of this enabling legislation. In most cases, EPA extends to qualifying states the authority to issue and enforce permits. The CWA (33 U.S.C. 1251 et seq.) authorizes regulation of discharges of pollutants into the waters of the U.S. and the establishment of quality standards for surface waters. It is the principal US legislation for safeguarding surface water, but it does not directly address groundwater.

Accordingly, the EPA oversees groundwater and drinking water through the Safe Drinking Water Act (SDWA) of 1974 (Public Law 93-523, 42 U.S.C. 300 *et seq*.) and the Sole Source Aquifer (SSA) designation under the SDWA (US-EPA, 2011). Under the SDWA, the EPA sets national health-based standards for drinking water quality to protect against both naturally-occurring and man-made contaminants that may be found in drinking water.

<u>Air quality</u>

Air quality is the capability of the atmosphere to sustain and buffer life on earth from the extremes of temperature variations (BCAP, 2010). Common agricultural activities may generate each of the criteria pollutants for air quality established under the Clean Air Act (CAA) and the National Ambient Quality Standards (NAAQS) (BCAP, 2010), though in particular, common agricultural activities primarily possess the potential to generate inhalable particulates. Additionally, common agricultural activities may also contribute other air quality impairments, primarily due to the off-site movement/volatization of agricultural inputs.

Dust represents the primary form of particulate matter that may impair air quality in agriculture (EPA, 2013a). Dust, consisting of soil particles suspended in the air, may be generated directly or indirectly following tillage or any other agricultural activity that requires the use of motorized farm equipment (e.g., tractors, plows, etc.) (Fawcett and Towery, 2002). Wind-mediated erosion and the release of particulates into the air following the use of tillage or motorized farm equipment generally represents an indirect cause of air impairment from common agricultural activities (Fawcett and Towery, 2002).

As previously discussed for soil and water resources, the use of conservation tillage possesses the potential to decrease both direct and indirect causes of agricultural-derived dust, due to the lower intensity of intentional and direct soil disturbance (Fawcett and Towery, 2002).

Common agricultural activities, including the use of mechanized equipment and the application of agricultural inputs, may result in agricultural emissions that may consist of gases (e.g., carbon monoxide) or inhalable particulates (e.g., smoke). Agricultural emissions may derive from exhaust from the use of motorized farm equipment or the aerial movement/volatilization of agricultural inputs (Fawcett and Towery, 2002), such as fertilizers and pesticides (FOCUS, 2008; USDA-ARS, 2011).

There are, however, many options to improve air quality within an agricultural setting. These include conservation tillage, residue management, wind breaks, road treatments, burn management, prunings shredding, feed management, manure management, integrated pest management, chemical storage, nutrient management, fertilizer injection, chemigation and fertigation (inclusion in irrigation systems), conservation irrigation, scrubbers, and equipment calibration (USDA-NRCS, 2006).

Air quality within the United States is overseen by the EPA pursuant to the CAA and the NAAQS. Under the CAA, the respective states are required to achieve and maintain the NAAQS and to prepare a State Implementation Plan (SIP) identifying strategies to achieve and maintain the national standard of air quality within the state" (BCAP, 2010).

The environmental risks of pesticide applications are assessed by EPA in the pesticide registration process. Additionally, pesticides are regularly reevaluated by the EPA to maintain its registered status (EPA, 2014c). When used in accordance with registered uses and EPA-approved labels, glyphosate poses minimal risks to air quality (EPA, 2014c). With regard to pesticide movement (i.e., drift), the EPA is currently evaluating new regulations for pesticide drift labeling and the identification of best management practices to control such drift (EPA, 2009).

<u>Climate change</u>

Climate change represents a statistical change in global climate conditions, including shifts in the frequency of extreme weather, that may be measured across time and space (Cook *et al.*, 2008; Karl *et al.*, 2008). Agriculture is recognized as a direct (e.g., exhaust from equipment) and indirect (e.g., agricultural-related soil disturbance) source of GHG emissions (Rosenzweig and Parry, 1994; Dale, 1997; Fargione *et al.*, 2008). GHGs, including CO_2, methane (CH_4), and N_2O, function as retainers of solar radiation (Aneja *et al.*, 2009). The US agricultural sector is second only to energy production as a contributor to GHG emissions (EPA, 2010a).

US agriculture may influence climate change through various facets of the production process and conversion of land to agriculture (Horowitz and Gottlieb, 2010). Additionally, tillage contributes to GHG production because it releases CO_2 sequestered in soil and promotes oxidation of soil organic matter (Baker *et al.*, 2005). CH_4 and N_2O are the primary GHGs emitted by agricultural activities, including emissions from the use of motorized equipment and soil N_2O emissions (Hoeft *et al.*, 2000; Robertson *et al.*, 2000; Del Grosso *et al.*, 2002; West and Marland, 2002; Aneja *et al.*, 2009; EPA, 2011a). The major sources of GHG emissions associated with crop production are soil N_2O emissions, soil CO_2 and CH_4 fluxes, and CO_2 emissions associated with farm equipment operation (Adler *et al.*, 2007).

The contribution of agriculture to climate change largely is dependent on the production practices employed to grow various commodities, the region in which the commodities are grown, and the individual choices made by growers. For example, emissions of nitrous oxide, produced naturally in soils through microbial nitrification and denitrification, can be influenced dramatically by fertilization, introduction of grazing animals, cultivation of nitrogen-fixing crops and forage (e.g., alfalfa), retention of crop residues (i.e., no-till conservation), irrigation, and fallowing of land (EPA, 2012a). These same agricultural practices can influence the decomposition of carbon-containing organic matter sequestered in soil, resulting in conversion to carbon dioxide and subsequent loss to the atmosphere (EPA, 2012a). Conversion of crop land to pasture results in an increase in carbon and nitrogen sequestration in soils (EPA, 2012a).

Additionally, one outcome of the potential effects of agricultural production on climate change is the potential effect of the climate change on agriculture itself. In response to climate change, the current range of weeds and pests of agriculture is expected to increase. Current agricultural practices will need to adapt in response to these changes in the ranges of weeds and pests of agriculture (Field *et al.*, 2007).

5.2.2 Preferred Alternative: Soil Resources, Water Resources, Air Quality, and Climate Change

Under the Preferred Alternative, impacts to the physical environment, including impacts to soil resources, water resources, air quality, and climate change would be similar to the no action alternative. The nature of the activities associated with the Preferred Alternative, the magnitude of these activities, and the size of the potential release fields all represents current agricultural activities that have and will continue within the action area.

Under the Preferred Alternative, six field sites not exceeding 60 total acres will be planted with a cruciferous crop (e.g., broccoli or cabbage) (Section 2.4). The agricultural activities used to plant and maintain these cruciferous crop fields are the same as those agricultural activities (e.g., tillage or pesticide application) that are already occurring and described under the No Action Alternative (Section 4.2.1). Releases of the GE diamondback moth will likely utilize roadways and other access systems already present and utilized within the NYSAES. Consequently, the potential impacts on the physical environment, including soil resources, water resources, air quality, and climate change as a result of these agricultural activities are also the same as those potential impacts described under the No Action Alternative (Section 4.2.1).

The release of GE diamondback moth is not anticipated to substantially affect individual or multiple components of the physical environment, as non-GE diamondback moth is already ubiquitous in the action area (Shelton, 2001b). While the applicant will release GE diamondback moths, these GE diamondback moths are functionally equivalent to non-GE diamondback moth, with the exception of the introduced traits[27] and a slight decrease in lab-observed fitness (Jin *et al.*, 2013). These traits are not anticipated to have an effect on the physical environment, as these traits affect the biology of diamondback moth only (Jin *et al.*, 2013).

Concern has been raised about the potential transfer of DNA, particularly DNA of the introduced traits, from decomposing GE diamondback moth to individual soil microflora. While the transfer of DNA between soil microorganisms is common (Keese, 2008; McDaniel *et al.*, 2010), biodegradation of any organisms after death is likely to result in fragmentation of DNA strands into small pieces (Lerat *et al.*, 2007; Levy-Booth *et al.*, 2008; Hart *et al.*, 2009). The transfer is functioning DNA for these introduced traits from decomposing GE diamondback moth to soil microorganisms is remote and unlikely (Appendix A).

[27] i.e., red fluorescence and repressible-female lethality (i.e., female autocide)

5.3 Biological Environment

5.3.1 No Action Alternative: Wildlife, Plant Communities, and Biological Diversity

Summary of potential impacts

Under the No Action Alternative, common agricultural activities are currently and will continue to occur within the action area. These common agricultural include activities related to field preparation (e.g., tillage) and field maintenance (e.g., tillage, irrigation, and the application of agricultural inputs). As a result of the current and continued practice of these common agricultural activities, individual aspects of the biological environment, including wildlife, plant communities, and biological diversity will continue being impacted under the No Action Alternative.

In general, agricultural environments are not ideal habitats for wildlife and plant communities. As a result, biological diversity is generally lower in these agricultural environments when compared to more natural, less intensively-managed areas. This general impact on wildlife, plant communities, and biological diversity is currently on-going under the No Action Alternative. Additionally, given the likelihood of continued agricultural activities within the action area, it is likely that these general impacts will continue under the No Action Alternative.

Background

The biological environment of the action area consists of wildlife, plant communities in and around the potential release fields, and biological diversity (Section 2).

As previously discussed in the Affected Environment (Section 2), the action area is located within the NYSAES in Geneva, NY. The action area, similar to rest of the NYSAES-owned land that surrounds it, is land that has been maintained under some form of agricultural management for much of its 134-year history (NYSAES, 2014). Consideration of historical land use patterns and the NYSAES mission[28] strongly suggests that present-day agricultural activities within the action area will continue under the No Action Alternative.

Accordingly, the potential impacts to individual components of the biological environment under the No Action Alternative are those potential impacts that may result from the continuation of existing agricultural activities within the action area. A discussion of these potential impacts as a result of the No Action Alternative on individual components of the biological environment is presented below.

Additionally, the use of agricultural inputs may also directly or indirectly affect components of the biological environment (Leistra *et al.*, 2006; Tong, 2009). However, the use of any EPA-registered pesticide within the United States is unlikely to cause adverse effects on the environment if used according to the specifications on the label (See Section 2.5.3 and EPA,

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[28] The NYAES was established by the New York State Legislature for "…the purpose of promoting agriculture in its various branches by scientific investigation and experiment." See http://www.nysaes.cornell.edu/cals/nysaes/about /history.cfm. Last accessed March, 2014.

2013c). Therefore, the use of any EPA-registered pesticide is unlikely to have a significant impact on individual components of the biological environment and will not be discussed further.

Wildlife

In general, land that is under modern agricultural management provides less suitable habitat for wildlife uses than fallow fields or natural areas (Lovett *et al.*, 2003; Landis *et al.*, 2005). As such, the number and types of animal species found in fields under modern agricultural management are less diverse by comparison (Harlan, 1975). Some animals may be associated with cruciferous crop fields, such as marmots. Additionally, deer (Curtis *et al.*, 1994) and red-winged blackbird may also be found in or around a cruciferous crop fields (Bollinger and Caslick, 1985; Curtis *et al.*, 1994)

Invertebrate organisms that feed on cruciferous crops within the action area include beneficial and pest insects. Beneficial insects include pollinators, such as honey bees and bumblebees (OECD, 2012). Other beneficial insects may also include predatory insects that feed on other insects, particularly insect pests, within the agricultural field. These insect predators may include spiders, lady bird beetles, hover flies, and various parasitoid flies (Table 2). Dietary assessments of predator/prey organisms consuming insectivore diets have shown that they are largely generalist organisms and only a small fraction of their diets is a single insect species (Blum *et al.*, 1997). Pest insects include cabbage root maggot (*Delia radicum*); flea beetle (*Phyllotreta striolata* and *P. cruciferae*); diamondback moth (*P. xystella*); imported cabbage worm (*Pieris rapae*); cabbage looper (*Trichoplusia ni*); cabbage and green peach aphids (*Brevicoryne brassicae* and *Myzus persicae*, respectively); onion thrip (*Thips tabaci*); and Swede midge (*Contarinia nasturii*) (NY State IPM Program, 2013). In particular, diamondback moth is a particularly troublesome pest of cruciferous crops, such as cabbage and broccoli (Andaloro, 1983; Talekar and Shelton, 1993), and will be further discussed directly below.

Order Mesostigmata		**Order Diptera**	
Cheiroseius sp.	Ascidae	*Mesograpta marginata*	Syrphidae
Order Araneidae		*Metasyrphus americanus*	Syrphidae
Araniella displicata	Araneidae	*Sphaerophoria cylindrica*	Syrphidae
Chiracanthium inclusum	Clubionidae	*Syrphus rectus*	Syrphidae
Clubiona obsea	Clubionidae	**Order Hemiptera**	
Dictyna hentzi	Dictynidae	*Orius insidiosus*	Anthocoridae
Dictyna volucripes	Dictynidae	*Nabis* spp.	Nabidae
Ceraticelus emertoni	Linyphiidae	**Order Hymenoptera**	
Erigone atra	Linyphiidae	*Vespula* sp.	Vespidae
Hypselistes florens	Linyphiidae	*Apanteles* sp.	Braconidae
Microlinyphia mandibulata	Linyphiidae	*Aspilota* sp.	Braconidae
Metaphidippus protervus	Salticidae	*Dacnusa* sp.	Braconidae
Tetragnatha laboriosa	Tetragnathidae	*Diaeretiella rapae*	Braconidae

Theridion albidum	Theridiidae	*Microctonus vittatae*	Braconidae
Theridion murarium	Theridiidae	*Synaldis* sp.	Braconidae
Tibellus oblongus	Thomisidae	*Ceraphron* sp.	Ceraphronidae
Order Opiliones		*Alloxysta* sp.	Figitidae
Phalangium opilio	Phalangida	*Alloxysta brassicae*	Figitidae
Order Coleoptera		*Hexacola websteri*	Figitidae
Anthicus cervinus	Anthicidae	*Copidosoma truncatellum*	Encyrtidae
Ceratomegilla maculata	Coccinellidae	*Tetrastichus sinope*	Eulophidae
Coccinella novemnotata	Coccinellidae	*Polynema* sp.	Mymaridae
Hippodamia convergens	Coccinellidae	*Leptacis* sp.	Platygastridae
Photinus sp.	Lampyridae	*Asaphes* sp.	Pteromalidae
Collops quadrimaculatus	Melyridae	*Macroglenes penetrans*	Pteromalidae
Orthoperus glaber	Orthoperidae		
Stilbus apicalis	Phalacridae		
Deleaster sp.	Staphylinidae		
Heterothops sp.	Staphylinidae		

Table 2. Beneficial insects found in conjunction with cultivated Brassica species.
Table derived from Root (1973).

Diamondback moth biology and ecology within the action area

The following information about diamondback moth biology and ecology is summarized from Appendix A, unless otherwise stated. Additional and more detailed discussion about diamondback moth within the action area may be found in Appendix A.

Diamondback moth, originally introduced from Europe in 1854, only infests cruciferous plants in its larval form, including commercial cruciferous crops such as canola (*Brassica napus* L.), cabbage (*Brassica oleracea* L.), broccoli (*Brassica oleracea* var. *italica* L.), cauliflower (*Brassica oleracea* var. *botrytis* L.), Chinese cabbage (*Brassica pekinensis* Lour.), and Indian mustard (*Brassica juncea* L.). The current range of diamondback moth in the United States includes all states were cruciferous crops are produced, though damage is most severe in Hawaii and Southern US States where yearly temperatures permit it be present throughout the calendar year. In general, diamondback moths are weak flyers unable to travel long distances by spontaneous flight; however, they are also known to be transported long distances[29] by wind currents.

In general, the lifespan of adult diamondback moths spans between of 12-16 days[30]. Adult diamondback moths do not cause any herbivory damage on cruciferous plants, subsisting on dew

[29] i.e., hundreds of kilometers

[30] Dependent on sex of the diamondback moth

and/or water droplets. It is during this time period that adult diamondback moths mates and reproduces. Within the action area and the United States, diamondback moth is only able to reproduce with other diamondback moths, indicating an absence of sexually-compatible relatives. After mating, female diamondback moths can lay on average 160 eggs over about 10 days on a selected cruciferous plant.

After a pair of diamondback moths mate and reproduce, the eggs are laid individually or in groups of 2-8 on the upper leaf surface and hatch within 4-8 days. After hatching, the diamondback moth larvae go through four instars[31] before pupation. The diamondback moth pupal stage can last between 5-15 days, depending on environmental conditions.

As a result of this lifecycle, multiple generations of diamondback moth can overlap and all four larval stages of diamondback moth can be present in the cruciferous crop field at the same time.

Plant communities

Plants associated with plant agricultural production, particularly those plants associated with cruciferous crop production, include within-field and adjacent-field plant communities. Within-field plant communities generally consist of the planted crop and any weeds associated with the planted crop. Adjacent-field plant communities within the action area are also anticipated to consist of planted crops and any weeds[32] associated with planted crops, due to its use as agriculturally-managed land by the NYSAES (Figure 2).

Due to the location and use of the potential release fields and its adjacent land in this EA, within-field and adjacent-field plant communities are anticipated to be similar within the action area, in that it will be a mixture of cultivated crops and weeds of those cultivated crops[33].

Domesticated crops that may be found within the action area include fruits, field crops, and vegetables (NYSAES, 2014). In particular, a variety of domesticated cruciferous crops may be planted in the action area, such as cabbage or broccoli (Table 3). Surrounding domesticated crops around the potential release fields generally consists of field corn.

Non-domesticated plants within an agricultural setting are generally regarded as potential weeds. There may be numerous non-domesticated plants within the action area; however, the most relevant, given the proposed action in this No Action analysis, are those non-domesticated plants that are also in the Crucifer family. These non-domesticated cruciferous plants span 50 species in 25 genera (Table 4). If present within an agricultural field, it is likely that these 50 species of crucifers would be intentionally managed, like any other weed present in that agricultural field, through the use of common agricultural activities (e.g., herbicide spraying).

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[31] A developmental stage of the diamondback moth represented by larvae of varying sizes/stages in development

[32] Weeds may consist of non-cultivated and non-domesticated field plants and volunteer plants from the previous planting

[33] The potential release fields are adjacent to lands/fields already subjected to agricultural management, because of the location and use of the land by the NYSAES

In general, all individuals within the plant community may be subject to herbivory. In particular, cruciferous plants, whether domesticated or non-domesticated, may be subject to herbivory from diamondback moth larvae (Andaloro, 1983; Talekar and Shelton, 1993; Shelton, 2001a). Within an agricultural setting, damage from diamondback moth larvae is generally not noted if it occurs on non-domesticated cruciferous plants (i.e., weeds that are crucifers). However, damage on cultivated cruciferous plants may be noted by the manager of that field. If certain thresholds[34] are met, the manager of that agricultural field may choose to manage the diamondback moth population causing damage to the cultivated cruciferous plant. In general, if the population of larval diamondback moth exceeds a pre-determined threshold, then insecticide spraying is generally the only viable option (Andaloro, 1983; Talekar and Shelton, 1993).

Plant communities within agroecosystems are generally less diverse than plant communities within other ecosystems. This lack of diversity is attributable to ecological selection that is imposed by crop production practices, such as tillage and herbicide use (Gianessi and Reigner, 2007; Owen, 2008), that aims to maximize crop production (Green and Owen, 2011). Beyond the crop plant that is intentionally planted and cultivated, agricultural practices affect plant communities by exerting selection pressures that influence the type and composition of plants present in a community. For example, natural selection in frequently disturbed environments enables colonization by plants exhibiting early germination and rapid growth from seedling to sexual maturity, and the ability to reproduce sexually and asexually (Baucom and Holt, 2009). These weedy characteristics enable such plants to spread rapidly into areas undesired by humans.

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[34] For example, the Canadian Canola Council website (www.canolacouncil.org/canola-encyclopedia/insects/diamondback-moth/) provides advice on detecting the DBM in the growing season. Farmers are advised to scout their fields early on in the growing season and checking throughout July and August, monitoring crops at least twice a week. Farmers need to take crop samples from a $0.1m^2$ area, beat them onto a clean surface and count the number of larvae dislodged. When 20-30 larvae/$0.1m^2$ are present at the advanced pod stage it is recommended to spray an approved insecticide.

Common domesticated vegetable brassicas	
Armoracia rusticana (horseradish) *Brassica juncea* (brown and oriental mustard) *B. napa* (Rutabaga) *B. nigra* (black mustard) *B. oleracea* (cabbage, broccoli, cauliflower, Brussel sprouts, kohlrabi, collards, and kale) *B. rapa* (Chinese cabbage, bok choy, pai-tsai, mizuna, Chinese mustard, broccoli raab, and turnip)	*Eutrena japonica* (wasabi) *Lepidium sativum* (garden cress) *Nasturtium officinale* (watercress) *Raphanus sativus* (radish) *Sinapis alba* (yellow mustard)
Common domesticated oilseed brassicas	
B. napus (oilseed rape) *B.juncea* (oriental mustard)	*B. rapa* (partially canola oil) *B. carinata* (Ethiopian mustard)

Table 3. Domesticated cruciferous crops.
Table derived from OECD (2012).

Alliaria	***Descurainia***
Alliaria petiolata (Garlic mustard)	*Descurainia pinnata* (Western tansymustard)
Alyssum	***Draba***
Alyssum alyssoides (Pale madwort)	*Draba arabisans* (Rock draba)
Arabidopsis	*Draba verna* (Spring draba)
Arabidopsis lyrata (Lyre-leaved rock-cress)	***Erucastrum***
Arabidopsis thaliana (Thale cress)	*Erucastrum gallicum* (Common dogmustard)
Arabis	***Erysimum***
Arabis pycnocarpa (Creamflower rockcress)	*Erysimum cheiranthoides* (Wormseed wallflower)
Armoracia	***Hesperis***
Armoracia rusticana (Horseradish)	*Hesperis matronalis* (Dames rocket)
Barbarea	***Lepidium***
Barbarea vulgaris (Garden yellowrocket)	*Lepidium campestre* (Field pepperweed)
Berteroa	*Lepidium densiflorum* (Common pepperweed)
Berteroa incana (Hoary alyssum)	*Lepidium draba* (Heart-pod Hoarycress)
Boechera	*Lepidium virginicum* (Virginia pepperweed)
Boechera canadensis (Sicklepod)	***Microthlaspi***
Boechera grahamii (Spreadingpod rock-cress)	*Microthlaspi perfoliatum* (Claspleaf pennycress)
Boechera laevigata (Smooth rockcress)	***Nasturtium***
Boechera stricta (Drummond's rockcress)	*Nasturtium officinale* (Watercress)
Brassica	***Rorippa***
Brassica juncea (Brown mustard)	*Rorippa aquatica* (Lakecress)
Brassica nigra (Black mustard)	*Rorippa palustris* (Bog yellowcress)
Brassica rapa (Field mustard)	*Rorippa sylvestris* (Creeping yellowcress)
Camelina	***Sinapis***
Camelina microcarpa (Littlepod false flax)	*Sinapis alba* (White mustard)
Camelina sativa (Gold-of-pleasure)	*Sinapis arvensis* (Wild mustard)
Capsella	***Sisymbrium***
Capsella bursa-pastoris (Shepherd's purse)	*Sisymbrium altissimum* (Tall tumblemustard)
Cardamine	*Sisymbrium loeselii* (Small tumbleweed mustard)
Cardamine bulbosa (Bulbous bittercress)	*Sisymbrium officinale* (Hedgemustard)
Cardamine concatenata (Cutleaf toothwort)	***Thlaspi***
Cardamine diphylla (Crinkleroot)	*Thlaspi arvense* (Field pennycress)
Cardamine douglassii (Limestone bittercress)	***Turritis***
Cardamine hirsuta (Hairy bittercress)	*Turritis glabra* (Tower mustard)
Cardamine impatiens (Narrowleaf bittercress)	
Cardamine parviflora (Sand bittercress)	

***Cardamine* continued**	
Cardamine pensylvanica (Pennsylvania bittercress) *Cardamine pratensis* (Cuckoo flower) *Cardamine rotundifolia* (American bittercress)	

Table 4. Non-domesticated brassicas in Ontario County, New York.
Table derived from BONAP (2014).

Biological diversity

Biodiversity refers to all plants, animals, and microorganisms interacting in an ecosystem (Wilson, 1988). Biodiversity provides valuable genetic resources for crop improvement (Harlan, 1975) and also provides other functions beyond food, fiber, fuel, and income. These include pollination, genetic introgression, biological control, nutrient recycling, competition against natural enemies, soil structure, soil and water conservation, disease suppression, control of local microclimate, control of local hydrological processes, and detoxification of noxious chemicals (Altieri, 1999). The loss of biodiversity can result in a need for costly management practices in order to provide these functions to the crop (Altieri, 1999).

The degree of biodiversity in an agroecosystem depends on four primary characteristics: 1) diversity of vegetation within and around the agroecosystem; 2) permanence of various crops within the system; 3) intensity of management; and 4) extent of isolation of the agroecosystem from natural vegetation (Harlan, 1975).

The action area, similar to any land subject to common agricultural management practices, generally has low levels of biodiversity compared with natural areas. Modern agriculture generally impacts biodiversity because its establishment represents conversion of natural habitats to monocultures (Ammann, 2005). Common agricultural practices related to field establishment and maintenance of that agricultural field, such as tillage, seed bed preparation, planting of a monoculture crop, pesticide use, fertilizer use, and harvesting all simplify the landscape and limit the diversity of plants and animals (Lovett *et al.*, 2003; Landis *et al.*, 2005).

Biodiversity can be maintained or reintroduced into agro-ecosystems through the targeted management of field edges/land adjacent to the field or the use of contour-strip cropping (Altieri and Letourneau, 1982; Landis *et al.*, 2005; Sharpe, 2010).

For example, field edges are often the least productive areas in a farm field and in some cases and the cost of producing crop areas along field edges exceeds the value of the crop produced (Sharpe, 2010). While allowing these field edges to be colonized by non-domesticated vegetation will contribute to weed seeds in the agricultural field, they may also facilitate its use by birds or beneficial arthropods (Altieri and Letourneau, 1982; Altieri, 1999; Sharpe, 2010). Additionally, the management of land adjacent to the field, such as drainage ditches, hedgerows, riparian areas, or woodlands may provide cover, nesting sites, and forage areas for wildlife populations (Sharpe, 2010; Palmer *et al.*, 2011).

Additionally, contour-strip cropping is another management practice that can be used to promote wildlife habitat. This practice alternates strips of row crops with strips of solid stand crops (i.e., grasses, legumes, or small grains) with the strips following the contour of the land (Sharpe, 2010). The primary purpose of contour-strip cropping is to reduce soil erosion and water runoff, but the solid stand crop also provides nesting and roosting cover for wildlife (Sharpe, 2010).

5.3.2 Preferred Alternative: Wildlife, Plant Communities, and Biological Diversity

The nature of the activities associated with the Preferred Alternative, the magnitude of these activities, and the size of the potential release fields all represents current agricultural activities that have and will continue within the action area. As a result, the only true difference between the Preferred and No Action Alternatives is exposure of GE diamondback moth to other organisms, and any resulting potential impact this exposure may have on the wildlife, plant communities, and biological diversity within the action area.

In the following subsections, potential impacts on each aspect of the biological environment as a result of the Preferred Alternative will be described.

Wildlife

Under the Preferred Alternative, the common agricultural activities used to prepare and maintain the potential release fields are the same common agricultural activities that are already occurring within the action area under the No Action Alternative. Consequently, the potential impact to wildlife, such as mammals, birds, and beneficial insects, from field preparation and management under the Preferred Alternative is the same as the No Action Alternative, as these potential impacts to wildlife are moderated by agricultural activities. Accordingly, the only difference between the Preferred and No Action Alternative, with respect to wildlife, is the potential exposure to GE diamondback moth.

Two types of wildlife are most likely to be exposed to GE diamondback moth: 1) Sexually-compatible insects that may mate with GE diamondback moth; and 2) Vertebrate or invertebrate insectivores that may consume GE diamondback moth. These two distinct types of wildlife will be discussed below, along with any potential impact under the Preferred Alternative.

Within the action area, the only sexually-compatible insects that can mate with the released GE diamondback moth males are non-GE diamondback moth females (Section 2.4). Diamondback moths may only mate with other diamondback moths, thus indicating that vertical gene transfer[35] will occur only within the diamondback moth species.

As a result of the Preferred Alternative, GE diamondback moth males are likely to mate with non-GE diamondback moth females. Assuming stability of the female autocidal trait, the overall diamondback moth population is anticipated to decrease over time due to an anticipated reduction in reproductive potential of the diamondback moth population (Jin *et al.*, 2013 and Section 2.4). However, if functionality of the female autocidal trait were to deteriorate in subsequent diamondback moth generations during the growing season, the overall diamondback moth

[35] i.e., movement of genes through sexual reproduction

population may not experience an overall decrease (Jin *et al.*, 2013). Each scenario is anticipated to have a transient effect on diamondback moth populations within the action area. However, each scenario is not anticipated to have a long-term and significant impact on diamondback moth populations within the action area due to the ubiquitous nature of non-GE diamondback moth in the action area, facilitated by the continual yearly introduction of non-GE diamondback moth into the action area through diamondback moth-infested seedlings (Shelton, 2001b); the inability of diamondback moth to overwinter in the action area (Shelton, 2001b; Nguyen *et al.*, 2014); and the devitalization of all diamondback moths in the potential release fields at the conclusion of each growing season by the applicant (Section 2.4) These three factors strongly suggest that the local diamondback moth population (GE and non-GE) will be significantly reduced at the end of the growing season or calendar year and that a new population of non-GE diamondback moths will be present the following spring before release of GE diamondback moth begins again. This diamondback moth population pattern is already observed in the No Action Alternative.

Additionally, it is important to keep in mind that local populations of diamondback moth in fields adjacent to the potential release fields may potentially experience fluctuations in population size as a result of the released GE diamondback moth[36]. However, this potential impact on overall diamondback moth populations within these adjacent fields is no different from the No Action Alternative, as land managers are likely already using control methods (e.g., insecticide spraying) to manage diamondback moths and other insect pests in adjacent cruciferous crop fields.

Insectivores within the action area may generally include lower-order invertebrates (e.g., spiders, lady bird beetles, hover flies, and various parasitoid flies [Table 1]). Insectivores that consume GE diamondback moth under the Preferred Alternative are not likely to be impacted by the introduced gene or gene products. As previously discussed in the Purpose and Need (Section 1), the GE diamondback moth contains two introduced genes/gene products, DsRed2 and tTAV. Neither gene/gene product is an allergen or toxin that may negatively affect wildlife that consumes GE diamondback moth (Appendix A). Furthermore, both gene/gene products have been previously evaluated by APHIS NEPA documents and found to not pose a significant risk to wildlife (USDA-APHIS, 2005; 2008; 2011b).

Additionally, insectivores may also consume Day-Glo fluorescent dusts as a result of the Preferred Alternative (Section 2.4). Day-Glo fluorescent dusts are the most common commercial dust used to mark insects and has been used in a variety of other insect monitoring studies (Hagler and Jackson, 2001; Reeve and Cronin, 2010). No potential impact to insectivores is anticipated as a result of potentially consuming Day-Glo fluorescent dusts on GE diamondback moths, primarily due to the history and wide-spread use of Day-Glo fluorescent dusts in a variety of insect and wildlife field studies (Burns *et al.*, 1990; Werner and Holsten, 1997; Hagler and Jackson, 2001; Tupper *et al.*, 2009; Reeve and Cronin, 2010; Dickens and Brant, 2014).

Concern has also been noted about the horizontal transfer of introduced genetic elements into other organisms (CFC, 2007). The concern primarily focuses on the genetic elements[37] used to introduce the DsRed2 and tTAV traits into the GE diamondback moth strains. However, as noted

[36] Fluctuations that are dependent on stability or instability of the female autocidal trait.

[37] i.e., transposable elements

by APHIS (2008) in an EIS for GE pink bollworm and GE fruit fly, movement of the *piggyBac*-derived transposable elements used to genetically engineer insects is not likely. This unlikely movement of the *piggyBac*-derived transposable element is caused by the inactivation of the transposase enzymes required for movement; thus, these transposable elements are incapable of moving themselves or any other introduced gene into other organisms (Thibault *et al.*, 1999; Peloquin *et al.*, 2000; Gomulski *et al.*, 2004; USDA-APHIS, 2005).

Assuming stability of the female autocidal trait, there is likely to be a transient increase in the availability of prey items[38] for insectivores upon release of GE diamondback moth (Section 2.4). This transient increase is anticipated to be followed by a reduction of the overall diamondback moth population within the action area as GE diamondback moth males mate with non-GE diamondback moth females (Section 2.4). This transient increase and subsequent decrease in prey availability is not anticipated to substantially affect insectivores, due to the relatively safety of the introduced traits to insectivores (USDA-APHIS, 2005; 2008; 2011b) and non-specialist nature of the insectivores within the action area that may feed upon diamondback moths (Blum *et al.*, 1997; Appendix A; Nagel and Peveling, 2005). Furthermore, because of the inability of diamondback moth to overwinter within the action area; the continual introduction of diamondback moth into the action area each growing season (Appendix A); and the unlikely complete extinction of diamondback moth as a result of GE SIT (Nagel and Peveling, 2005), generalist insectivores are unlikely to be deprived of diamondback moths prey entirely from year to year.

Assuming instability of the female autocidal trait, there is also likely to be a transient increase in the availability of prey items upon release of GE diamondback moth (Section 2.4). However, following the mating of GE diamondback moth males with non-GE diamondback moth females, overall diamondback moth populations may stay the same or increase, dependent on the number of non-GE diamondback moth females already present. This potential transient increase in overall diamondback moth prey availability is not anticipated to substantially affect insectivores due to the relatively safety of the introduced traits to insectivores (USDA-APHIS, 2005; 2008; 2011b) and may provide a transient increase in prey items during the course of the growing season. This transient increase, however, is not anticipated to result in a significant impact in the action area because of field devitalization by the applicant (Section 2.4), and the inability of diamondback moth to overwinter and the continual introduction of diamondback moth into the action area each growing season (Shelton, 2001b; Nguyen *et al.*, 2014, and Appendix A). Thus, generalist insectivores are likely to continually encounter diamondback moth as prey items in a temporal pattern similar to that pattern that is already occurring under the No Action Alternative.

Two additional concerns related to wildlife arise under the Preferred Alternative. These are discussed directly below.

First, concern has been raised regarding the development of resistance to the autocidal trait[39] of GE SIT insects (USDA-APHIS, 2008). This scenario may occur only if non-GE diamondback moth were to develop resistance to the autocidal trait and if this trait were heritable to subsequent

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[38] This transient increase of prey items is due to the release of the GE diamondback moth itself and the observation that adult diamondback moths only possess a lifespan of several days before dying

[39] In this EA, repressible-female lethality and female autocidal traits are used interchangeably

populations of diamondback moth. While a single instance of a female insect pest population developing "evolved sexual isolating barriers" has been noted in the literature (Hibino and Iwahashi, 1991), this is unlikely to occur under the Preferred Alternative. The development of resistance to the autocidal trait is unlikely to occur under the Preferred Alternative because there are two obstacles that substantially interfere with the long-term ability of progeny to inherit any autocidal resistance, including: 1) the devitalization of the field after each growing season (USDA-APHIS, 2014a); and 2) the inability of diamondback moth to overwinter in the action area (Shelton, 2001b; Nguyen *et al.*, 2014, and Appendix A). Both of these two factors, combined with the slight decrease in fitness of the GE diamondback moth strains[40] (Jin *et al.*, 2013), represent significant and redundant obstacles in the continued heritability of any evolved resistance to the autocidal trait, as both factors largely precludes the genetic continuation of diamondback moths at the end of each growing season and calendar year.

Second, the permit calls for field monitoring of GE and non-GE diamondback moth populations using diamondback moth traps baited with a synthetic insect hormone specific for diamondback moth. Deployment of these diamondback moth traps is likely to use already-existing road infrastructure within the NYSAES, meaning that new road construction and subsequent wildlife disturbances will not occur under the Preferred Alternative. The deployment and use of these traps is not anticipated to produce a significant impact that rises above of other more substantial agricultural activities, such as field preparation and harvest using motorized machinery (Nagel and Peveling, 2005). Additionally, because of the specificity of insect traps baited with synthetic insect pheromones, inadvertent capturing of non-target insects is much less likely than with other insect trap types (Nagel and Peveling, 2005).

<u>Plant communities</u>

Under the Preferred Alternative, the common agricultural activities used to prepare and maintain the potential release fields are the same common agricultural activities that are already occurring within the action area under the No Action Alternative. Consequently, the potential impact to plant communities from field preparation and management under the Preferred Alternative is the same as the No Action Alternative, as these potential impacts to plant communities are moderated by agricultural activities. Accordingly, the only difference between the Preferred and No Action Alternative, with respect to plant communities, is the potential exposure to GE diamondback moth.

Adult diamondback moths do not cause herbivory damage on plants (Talekar and Shelton, 1993). Accordingly, adult male offspring resulting from mating between GE diamondback moth males and non-GE diamondback moth females is not anticipated to have any significant effect on any member of the plant community[41], because adult diamondback moths do not feed on plant tissue nor does it function as a significant pollinator of any other plant (Andaloro, 1983; Talekar and Shelton, 1993).

Diamondback moth larvae only feeds upon cruciferous plants, including domesticated and non-domesticated cruciferous plants (Talekar and Shelton, 1993). Consequently, the only members of

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[40] Decreased fitness observed in laboratory conditions, not field conditions

[41] Including cruciferous and non-cruciferous plants

the plant communities that may be potentially impacted by exposure to GE diamondback moth larvae under the Preferred Alternative are domesticated and non-domesticated cruciferous plants.

Domesticated cruciferous crops, such as cabbage or broccoli, will be planted on the potential release fields as a result of the Preferred Alternative (Section 2.4). Any damage to these cruciferous plants from GE diamondback larvae is not anticipated to yield a significant impact because these cruciferous plants will be explicitly planted for the sole purpose of incurring diamondback moth damage and providing a food source for the local population of diamondback moth during the duration of the permitted field study.

Assuming stability of the autocidal trait in the released GE diamondback moths, cruciferous crops planted on adjacent fields may experience some herbivory damage from the larval offspring of a GE diamondback moth male and a non-GE diamondback moth female. This potential impact on planted cruciferous crops in adjacent fields is not likely to be significant due to the anticipated reduction of the local diamondback moth population through a reduction in reproductive capacity (Jin *et al.*, 2013), the ubiquity of diamondback moth within the action area (i.e., those cruciferous plants are likely already incurring diamondback moth herbivory damage) (Andaloro, 1983; Shelton, 2001a; 2001b), nor is there likely to be a future impact because of the inability of that local diamondback moth to overwinter (Talekar and Shelton, 1993; Shelton, 2001b; Nguyen *et al.*, 2014) within the action area.

With regard to planted cruciferous crops on adjacent fields, this potential overall increase in diamondback moth damage is also not anticipated to be significant, due to the likelihood of existing grower management of insect pests in these adjacent fields (Andaloro, 1983; Shelton, 2001a), the ubiquity of diamondback moth in the action area (Andaloro, 1983; Shelton, 2001a; 2001b), and the inability of diamondback moth to overwinter within the action area (Talekar and Shelton, 1993; Shelton, 2001b; Nguyen *et al.*, 2014).

Non-domesticated cruciferous plants can also act as hosts of diamondback moth and may also incur some level of injury from diamondback moth larvae resulting from the mating of GE diamondback moth males and non-GE diamondback moth females. These potential non-domesticated cruciferous plants are listed in Table 4. However, because the potential release fields and adjacent fields all represent agricultural land (NYSAES, 2014 and Figure 2), it is likely that these non-domesticated cruciferous plants would be considered weeds and would likely be targeted for management by the land manager. Any potential damage from diamondback moth larvae resulting from the mating of GE diamondback moth males and non-GE diamondback moth females is not anticipated to be significant when compared to the management activities (e.g., herbicide application) intended to eliminate these weeds that would likely occur under both the Preferred and No Action Alternatives.

<u>Biological diversity</u>

As described in the No Action Alternative analysis on biological diversity (Section 4.3.1), biological diversity within an agroecosystem is lower relative to natural ecosystems, primarily due to simplification of the landscape and the frequent cycles of disturbances associated with common agricultural activities. This continued simplification of the landscape (i.e., preparation of the field to plant crops primarily in monoculture), in conjunction with the continuity of common agricultural activities (e.g., tillage and pesticide use) under the Preferred Alternative, strongly suggests that those activities that already limit biological diversity within the agroecosystem under the No Action Alternative will continue under the Preferred Alternative. No significant impact to biological diversity is anticipated to occur as a result of releasing GE diamondback moth because the introduced traits are not likely allergenic/toxic to insectivores that may consume GE diamondback moth; GE diamondback moth is not an obligate prey of any insectivore (Appendix A); and the inability of GE diamondback moth to overwinter and establish within the action area (Appendix A).

Furthermore, because the Preferred Alternative represents the continuity of common agricultural practices already occurring under the No Action Alternative, practices designed to increase biological diversity within an agroecosystem, such as the directed management of land adjacent to the agricultural field or contour stripping (Altieri and Letourneau, 1982; Altieri, 1999; Landis *et al.*, 2005; Sharpe, 2010; Palmer *et al.*, 2011), may also function to increase biological diversity under the Preferred Alternative.

The release of GE diamondback moth may actually benefit biological diversity, due to the absence of insecticide application during the growing season in potential release fields. In general, the application of broad-spectrum insecticides is more harmful to non-target wildlife than targeted efforts, such as GE SIT (Nagel and Peveling, 2005). With respect to the availability of diamondback moth as prey items for generalist insectivores, it is prudent to recall that control of this pest is likely to occur under both the No Action and Preferred Alternatives, with similar subsequent impacts on diamondback moth populations and its function as prey items for generalist insectivores.

5.4 Human Health Environment

5.4.1 No Action Alternative: Farmworker Health and Health of the General Public

Summary of potential impacts

Under the No Action Alternative, farmworkers are currently and will continue to be exposed to hazards generally associated with farm work, including hazards associated with the use of typical farm equipment/machinery (e.g., physical injury, noise, etc.) and the application of agricultural inputs. Current measures to mitigate exposure to these hazards includes Section 5(a)(1) of the Occupational Safety and Health Act, EPA's pesticide registration process, and EPA's Worker Protection Standards. Under the No Action Alternative, these measures will continue to protect farmworker health.

Additionally, under the No Action Alternative, the general public is currently and will continue to be indirectly exposed to pesticides used in agricultural production. This indirect exposure of pesticides generally occurs in the form of pesticidal residues. EPA regulates the exposure of the general population to these pesticidal residues through the establishment of pesticide tolerances and its pesticide registration process. For the health of the general population, establishment of pesticidal tolerances by the EPA ensures that there is a certainty of no unreasonable harm to the general population from exposure to these pesticidal residues commonly encountered on agricultural commodities.

Background

The human health environment consists of farmworker health and health of the general public (Section 2). Potential agricultural impacts to farmworker health and health of the general public are generally related to route of exposure and magnitude of exposure.

As previously discussed in the Affected Environment (Section 2), the action area is located within the NYSAES in Geneva, NY. The action area, similar to rest of the NYSAES-owned land that surrounds it, is land that has been maintained under some form of agricultural management for much of its 134-year history (NYSAES, 2014). Consideration of historical land use patterns and the NYSAES mission[42] strongly suggests that present-day agricultural activities within the action area will continue under the No Action Alternative.

Accordingly, the agricultural hazards will be different for farmworkers and the general public, because of differences in route and magnitude of exposure. The route and magnitude of exposure for farmworkers and the general public is described directly below, along with an examination of potential impacts under the No Action Alternative.

[42] The NYAES was established by the New York State Legislature for "…the purpose of promoting agriculture in its various branches by scientific investigation and experiment." See http://www.nysaes.cornell.edu/cals/nysaes/about/history.cfm. Last accessed March, 2014.

Farmworker health

Agriculture is one of the most hazardous industries in the Nation (Farmworker Justice, 2014). About 3.1 million people in the United States are reported as farmworkers, while double of that number live in farms in 2014 (EPA, 2014a). Agricultural workers are exposed to a variety of hazards on a farm; in general, these hazards are related to the use of equipment/farm machinery and agricultural inputs (OSHA, 2014b).

Farmworkers use farm-related equipment such as tractors, combines, and sprayers for field cultivation, irrigation, harvest, and pesticide application. Besides the dangers associated with the movement of parts in mechanical equipment and the operation of such devices, farm workers are also exposed to electricity, falls, traffic on highways, livestock handling, toxic gases, slips / trips, pesticides, etc. (OSHA, 2005; Ministry of Labour Canada, 2006). Additionally, the use of agricultural inputs is common practice on many farms. Pesticide use in farms is based on weed, insect and disease pressures, cost of seed and other inputs, technology fees, human safety, potential for crop injury, and ease and flexibility of the production system (Heiniger, 2000; Farnham, 2001; University of Arkansas, 2006). As a result, farmworkers also come into constant and close contact with fertilizers and pesticides during and after application.

There are several ways to mitigate exposure to common agricultural hazards encountered by the typical farmworker.

Section 5(a)(1) of the Occupational Safety and Health Act requires employers to "furnish to each of his employees employment and a place of employment which are free from recognized hazards that are causing or are likely to cause death or serious physical harm to his employees". Particularly for agricultural workers (29 CFR 1928), OSHA provides guidelines to prevent accidents and protect agricultural workers from hazards (OSHA, 2014b). The occupational safety and health standards for agriculture (OSHA, 2014a) provide specific guidelines (e.g., employee operating instruction, safety for agricultural equipment, and general environmental controls) in order to prevent accidents and hazards in farms.

The use of pesticides[43] on a farm is regulated by the EPA under FIFRA as part of the pesticide registration process. As part of the registration process, the EPA considers human health effects from the use of pesticides and must determine that the pesticide will not cause unreasonable adverse effects on human health. If needed, the EPA will establish label restrictions to mitigate or alleviate potential impacts on human health and the environment. Pesticide registration labels provide the guidelines, application restrictions, and precautions necessary to protect human health. These label restrictions carry the weight of law and are enforced by EPA and the states (Federal Insecticide, Fungicide, and Rodenticide Act 7 USC 136j (a)(2)(G) Unlawful Acts).

Additionally, EPA's Worker Protection Standard (WPS) is regulation aimed at reducing the risk of pesticide poisoning and injury among agricultural workers and pesticide handlers. The current WPS offers occupational protections to over 2 million agricultural workers and pesticide handlers, requiring that owners and employers on agricultural establishments provide protections to workers and handlers from potential pesticide exposure, by pesticide safety training, access to information

[43] e.g., herbicides, insecticides, and fungicides

in pesticide labels and other specific material, measures to keep workers from treated areas and information about the restricted-entry interval, provide applicators and handlers with personal protective equipment, decontamination supplies, monitor handlers that handle certain pesticides, and emergency assistance (EPA, 2014b).

Health of the general public

In contrast to farmworkers, the general public is not likely to encounter the same hazards that farmworkers encounter, primarily due to an absence of direct exposure to agricultural equipment/machinery and the application of agricultural inputs. However, due to the common practice of pesticide use in modern agricultural production, pesticidal residues[44] may remain on agricultural commodities. Consequently, the general population may be indirectly exposed to agricultural pesticides through these pesticidal residues on agricultural commodities.

To ensure the safety of the food supply, EPA regulates the amount of each pesticide that may remain in and on foods (EPA, 2012c). Some of the measures that EPA establishes to ensure that pesticides residues are within the acceptable levels include the mandatory pesticide registration; the establishment of tolerances to ensure food safety; and collaboration with other Agencies such as the Food and Drug Administration and the U.S. Department of Agriculture to enforce the pesticide tolerances in food (EPA, 2012c).

Of particular relevance for the general population are pesticide tolerances, as the general population often reflects an endpoint in the production of an agricultural commodity. These pesticide tolerances are also referred to as maximum residue limits (EPA, 2014d). EPA establishes tolerances for each pesticide based on the potential risks to human health posed by that pesticide. The data is established from field trials, food processing and monitoring studies, and surveillance programs. Pesticide tolerances' risk assessments are based on the assumption that residues will always be present in food at the maximum level permitted by the tolerance, or on the actual or anticipated use residue data, to reflect real-world consumer exposure as closely as possible (EPA, 2014d). Establishment of pesticidal tolerances ensures that there is a reasonable certainty of no harm from the pesticide, as obligated under the Federal Food, Drug, and Cosmetics Act (FFDCA), as amended by the Food Quality Protection Act of 1996 (FQPA).

5.4.2 Preferred Alternative: Farmworker Health and Health of the General Public

With respect to the common agricultural activities related to the establishment and cultivation of crops on a managed field, there are no substantial differences between the No Action and Preferred Alternatives. Accordingly, if there are no substantial differences between agricultural activities under the No Action or Preferred Alternative, there can be no substantial differences in potential impacts on farmworker health or health of the general population, as these potential human health impacts are facilitated by agricultural activities within the action area.

With respect to the human health environment, the only true difference between the two Alternatives is potential exposure to GE diamondback moth under the Preferred Alternative.

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[44] Pesticides that may remain on agricultural commodities in small amounts

Accordingly, the release of GE diamondback moth is not anticipated to significantly affect farmworker health or the health of the general population.

Both farmworkers and the general population may be exposed to live GE diamondback moth. While the GE diamondback moth would not be consumed by humans, some allergic responses have been noted in human exposure to moth hairs and scales (Goddard, 1993). However, these allergic responses have been noted primarily noted in moths within the family Notodontidae, Saturniidae, and Lymantriidae (Goddard, 1993), an insect family that diamondback moth is not a member of (UF-IFAS, 2012). Additionally, because the only difference between GE and non-GE diamondback moth is the phenotype associated with the GE traits (Jin *et al.*, 2013), exposure to scales of GE diamondback moth under the Preferred Alternative is not anticipated to yield any different effect than exposure to scales of non-GE diamondback moth under the No Action Alternative.

GE diamondback moth is genetically engineered to display red fluorescent and female autocidal traits (Section 2.4). The GE trait that causes red fluorescence, DsRed2, has previously been examined in a previous APHIS EA and was found to not resemble an allergen or toxin (USDA-APHIS, 2011b). Furthermore, DsRed2 was not found to be any unreasonable risk to human health (USDA-APHIS, 2011b). The GE trait that causes female autocide, tTAV, has also been examined in a previous APHIS EIS and was not found to resemble an allergen, toxin, or pose any unreasonable risk to human health (USDA-APHIS, 2008). In the unlikely event that GE diamondback moth or larvae is inadvertently consumed through the consumption of a cruciferous crop, no adverse impacts are anticipated due to the characteristics of these two introduced genes and production of their respective proteins.

6 CUMULATIVE IMPACTS

Cumulative impacts are defined as those effects that result when added to past, present, and reasonably foreseeable future actions.

The purpose of the research associated with the proposed action is to determine the feasibility/efficacy of sterile insect technique (SIT) within the action area using sterile diamondback moths produced through genetic engineering (Section 2.4). If positive data is produced from this proposed action, it is reasonably foreseeable that the applicant may request an extension of the permit to further study the feasibility/efficacy of GE diamondback moth in a SIT program within the action area (Personal Communication, C.Beech). Upon receipt of a request to extend the permit from the applicant, potential environmental impacts will be assessed in a separate NEPA document. Consequently, no cumulative impacts are anticipated at this time from the proposed action and future requests to extend the permit from the applicant.

As noted in the applicant's permit application, six fields not totaling more than 60 acres will be utilized for three years during this permitted field release of GE diamondback moth (Section 2.4). Based on past and current land use patterns of land managed by the NYSAES, it is reasonably foreseeable that those lands will return back to currently employed agricultural activities after expiration of the permit[45] (NYSAES, 2014). Use of these six fields for this permitted field release is not anticipated to result in any potential impact to any described aspect of the physical[46], biological[47], and human health[48] environments (Section 4) that would preclude return of those fields back to other agricultural activities that are already performed at the NYSAES.

Analysis of cumulative impacts for the release of other GE SIT insects suggests an absence of significant cumulative effects when considering factors such as chemical control, insect resistance, human health, and environmental impacts (USDA-APHIS, 2008; 2009; 2011a). The proposed action may lead to additional management activities that may complement current control measures of diamondback moth. The proposed action may reduce the need for insecticide treatments if diamondback moths are detected in fields of cruciferous crops in the future, based on other APHIS analyses of GE SIT insects (USDA-APHIS, 2008). The release of sterile GE diamondback moth may reduce non-GE diamondback moth populations from increasing to a level that would require insecticide treatment, similar to potential outcomes of other GE SIT insect introductions (Klassen, 2005).

Collectively, the absence of direct and indirect impacts on the physical environment, biological environment, and human health environments from the proposed action (Section 4); the past, current, and reasonably foreseeable use of land managed by the NYSAES (NYSAES, 2014); previous APHIS experience with GE SIT insect introductions (USDA-APHIS, 2008; 2009; 2011a) strongly suggests that no cumulative impacts would occur that reduce the long-term productivity or sustainability of the human environment associated with the action area.

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[45] Assuming a permit extension is not requested by the applicant

[46] i.e., soil resources, water resources, air quality, and climate change

[47] i.e., plant communities, wildlife and insects, and biological diversity

[48] i.e., farmworker health and health of the general population

7 THREATENED AND ENDANGERED SPECIES

The Endangered Species Act (ESA) of 1973, as amended, is one of the most far-reaching wildlife conservation laws ever enacted by any nation. Congress passed the ESA to prevent extinctions facing many species of fish, wildlife and plants. The purpose of the ESA is to conserve endangered and threatened species and the ecosystems on which they depend as key components of America's heritage. To implement the ESA, the U.S. Fish & Wildlife Service (USFWS) works in cooperation with the National Marine Fisheries Service (NMFS), other Federal, State, and local agencies, Tribes, non-governmental organizations, and private citizens. Before a plant or animal species can receive the protection provided by the ESA, it must first be added to the Federal list of threatened and endangered wildlife and plants.

A species is added to the list when it is determined by the USFWS/NMFS to be endangered or threatened because of any of the following factors:

- The present or threatened destruction, modification, or curtailment of its habitat or range;
- Overutilization for commercial, recreational, scientific, or educational purposes;
- Disease or predation;
- The inadequacy of existing regulatory mechanisms; and
- The natural or manmade factors affecting its survival.

Once an animal or plant is added to the list, protective measures apply to the species and its habitat. These measures include protection from adverse effects of federal activities.

Section 7 (a)(2) of the ESA requires that federal agencies, in consultation with USFWS and/or the NMFS, ensure that any action they authorize, fund, or carry out is "not likely to jeopardize the continued existence of a listed species or result in the destruction or adverse modification of designated critical habitat." It is the responsibility of the federal agency taking the action to assess the effects of their action and to consult with the USFWS and NMFS if it is determined that the action "may affect" listed species or designated critical habitat. The request before APHIS is an application for a permit, and the issuance of a permit is considered an agency action whose effects must be assessed.

APHIS met with USFWS officials on June 15, 2011, to discuss whether APHIS has any obligations under the ESA regarding analyzing the effects of pesticide use associated with field trials and production of GE organisms. As a result of these joint discussions, USFWS and APHIS have agreed that it is not necessary for APHIS to perform an ESA effects analysis on pesticide use because EPA has both regulatory authority over the labeling of pesticides and the necessary technical expertise to assess pesticide effects on the environment under FIFRA. APHIS has no statutory authority to authorize or regulate the use of pesticides by any party, including applications under permitted field trials. Under APHIS' current Part 340 regulations, APHIS only has the authority to regulate GE organisms as long as APHIS believes they may pose a plant pest risk (7 CFR § 340.1). APHIS has no regulatory jurisdiction over any other risks associated with

GE organisms including risks resulting from the use of pesticides on those organisms, or used for other purposes.

As discussed elsewhere in this EA, the use of sterile insect technology in the GE diamondback moth strains, OX4319L-Pxy, OX4319N-Pxy, and OX4767A-Pxy, mitigates many of the possible theoretical hazards and risks associated with insect genetic engineering. However, APHIS considered the following potential threats in its effects analysis:

- the transfer of transgenes to other insects, especially listed insects;
- the effect on availability of food to insectivores;
- the potential for toxicity and allergenicity of genetically engineered diamondback moth strains, OX4319L-Pxy, OX4319N-Pxy, and OX4767A-Pxy as a result of the transformation; and
- the potential for the genetically engineered insects to attack/feed on listed plants.

APHIS considered the potential for the movement of the transgenes to other insects, especially listed insects. As discussed in section 4.3 Biological Environment, and the applicants Environmental Risk Assessment, transfer of genes from the GE moths to other species of moths, including listed species, is not possible. This is because reproduction of diamondback moth is specific to diamondback moth. There are no related species which are sexually compatible.

Based upon the scope of the EA and release area identified in the Affected Environment section of the EA, APHIS reviewed the USFWS list of TES species (listed and proposed). The search found that there are no listed species in Ontario County, NY but there is one proposed species. The northern long-eared bat (*Myotis septentrionalis*) is proposed as endangered without critical habitat (USFWS, 2013). During summer, northern long-eared bats roost singly or in colonies underneath bark, in cavities, or in crevices of both live and dead trees. It is opportunistic in selecting roosts, using tree species based on suitability to retain bark or provide cavities or crevices. It may also roost in cooler places, like caves and mines, and rarely, in structures like barns and sheds (USFWS, 2014b). Northern long-eared bats emerge at dusk to fly through the understory of forested hillsides and ridges feeding on moths, flies, leafhoppers, caddisflies, and beetles, which they catch while in flight using echolocation. It also feeds by gleaning motionless insects from vegetation and water surfaces (USFWS, 2014b). Although the release site is not primary habitat for the bat, it is possible that the GE diamondback moths could enter wooded areas in the vicinity and be consumed by a northern long eared bat.

APHIS considered the possibility that the inserted genetic material could adversely affect insectivores, like the northern long-eared bat, that may feed on the moths. As previously discussed in the Purpose and Need (Section 1), the GE diamondback moth contains two introduced genes/gene products, DsRed2 resulting in display to red fluorescence, and tTAV resulting in female autocidal traits in the absence of tetracycline. The GE trait that causes red fluorescence, DsRed2, has been examined in a previous APHIS EA and was found to not resemble an allergen or toxin (USDA-APHIS, 2011b). Furthermore, DsRed2 was not found to pose any unreasonable risk to human health (Richards *et al.*, 2003; USDA-APHIS, 2011b). The GE trait that causes female autocide, tTAV, has also been examined in a previous APHIS EIS and was not found to resemble an allergen, toxin, or pose any unreasonable risk to human health (USDA-APHIS, 2008). In the event that GE diamondback moth or larvae are consumed purposefully by insectivores, like the northern big-eared bat, or incidentally through the consumption of a cruciferous crop, no adverse impacts are anticipated as a result of the two introduced genes and production of their respective proteins. The applicant's Environmental Risk Assessment reached these same conclusions (Appendix A).

As discussed in Section 4.3.2 Preferred Alternative: Wildlife, Plant Communities, and Biological Diversity, the numbers of diamondback moth adults available to insectivores will fluctuate as a result of the release. Initially there will be more available as the sterile male moths are released (Section 2.4). Later, there will be a reduction in the overall diamondback moth population within the action area as GE diamondback moth males mate with non-GE diamondback moth females (Section 2.4). This transient increase and subsequent decrease in prey availability is not anticipated to significantly affect generalist insectivores like the northern long-eared bat (USDA-APHIS, 2005; 2008; 2009). It is important to realize that consumption of diamondback moth by the northern long eared bat is likely uncommon, given the preferred habitat of the bat, and that control of this pest is likely to occur under both the No Action and Preferred Alternatives, with similar reduction in diamondback moth populations. In addition, the application of broad-spectrum insecticides is more harmful to non-target wildlife, especially insects, than targeted efforts such as GE SIT (Nagel and Peveling, 2005). Therefore, it is reasonable to assume that the fluctuation in diamondback moth prey resulting from release of the transgenic moths would have no effect on the northern long-eared bat. Further, because of the lack of any effects expected from consumption, there are no effects anticipated by the proposed action on the northern big-eared bat.

APHIS considered the possibility that the transgenic insects could be attracted to, and feed on, listed plants. The diamondback moth may feed on many species from the family Brassicaceae (Dosdall *et al.*, 2011). A search of the USFWS database of listed plant species indicates that there are 25 listed species and 5 species proposed for listing that are in the family Brassicaceae (USFWS, 2014a; 2014c). Table 5 listed these species along with the states where they are found:

Common Name	Scientific Name	Where Found	Listing Status
`anaunau	*Lepidium arbuscula*	HI	Endangered
Small-Anthered bittercress	*Cardamine micranthera*	NC, VA	Endangered
Missouri bladderpod	*Physaria filiformis*	AR, MO	Threatened

Common Name	Scientific Name	Where Found	Listing Status
White Bluffs bladderpod	*Physaria douglasii ssp. tuplashensis*	WA	Threatened
Santa Cruz Island fringepod	*Thysanocarpus conchuliferus*	CA	Endangered
Texas Golden Gladecress	*Leavenworthia texana*	TX	Endangered
California jewelflower	*Caulanthus californicus*	CA	Endangered
Metcalf Canyon jewelflower	*Streptanthus albidus ssp. albidus*	CA	Endangered
Tiburon jewelflower	*Streptanthus niger*	CA	Endangered
Carter's mustard	*Warea carteri*	FL	Endangered
Penland alpine fen mustard	*Eutrema penlandii*	CO	Threatened
Slender-Petaled mustard	*Thelypodium stenopetalum*	CA	Endangered
Barneby reed-mustard	*Schoenocrambe barnebyi*	UT	Endangered
Clay reed-mustard	*Schoenocrambe argillacea*	UT	Threatened
Shrubby reed-mustard	*Schoenocrambe suffrutescens*	UT	Endangered
Barneby ridge-cress	*Lepidium barnebyanum*	UT	Endangered
Braun's rock-cress	*Arabis perstellata*	KY, TN	Endangered
Hoffmann's rock-cress	*Arabis hoffmannii*	CA	Endangered
McDonald's rock-	*Arabis*	CA	Endangered

Common Name	Scientific Name	Where Found	Listing Status
cress	*macdonaldiana*		
Santa Cruz Island rockcress	*Sibara filifolia*	CA	Endangered
Shale barren rock cress	*Arabis serotina*	VA, WV	Endangered
Howell's spectacular thelypody	*Thelypodium howellii spectabilis*	OR	Threatened
Dudley Bluffs twinpod	*Physaria obcordata*	CO	Threatened
Wide-Leaf warea	*Warea amplexifolia*	FL	Endangered
Gambel's watercress	*Rorippa gambellii*	FL	Endangered
Short's bladderpod	*Physaria globosa*	IN, KY, TN	Proposed Endangered
Kentucky glade cress	*Leavenworthia exigua laciniata*	KY	Proposed Threatened
[Unnamed] gladecress	*Leavenworthia crassa*	AL	Proposed Endangered
Slickspot peppergrass	*Lepidium papilliferum*	ID	Proposed Endangered
Georgia rockcress	*Arabis georgiana*	AL, GA	Proposed Threatened

Table 5. Listed and species proposed for listing in the Brassicaceae family.
Table derived from USFWS (2014a; 2014c).

As can be seen from the table, none of the Brassicaceae species, either listed or proposed for listing, are found in the northeast region of the United States. All are hundreds of miles away, and are upwind from the prevailing west to east weather pattern of the region (American Meteorological Society, 2012; WeatherSpark, 2014). It is unlikely that release of the GE diamondback moths will result in any exposure to a Brassicaceae species that is listed or proposed for listing. Even if such exposure were to occur, the effects of feeding on the plant would not be expected to be any different than from non-transgenic diamondback moths that are already widespread throughout most of the United States.

Conclusion

After reviewing the possible effects of allowing the environmental release of the three GE diamondback moth strains, OX4319L-Pxy, OX4319N-Pxy, and OX4767A-Pxy, APHIS has not identified any stressor that could affect the reproduction, numbers, or distribution of a listed TES or species proposed for listing. APHIS also considered the potential effect on designated critical habitat or habitat proposed for designation, and could identify no scenario where release of these insects would affect habitat in any way. The diamondback moth adults and larvae are safe for consumption by wildlife, and the female autocidal trait will prevent the inserted genetic material from passing on further than one generation. Based on these factors, APHIS has concluded that the experimental field release of diamondback moth strains, OX4319L-Pxy, OX4319N-Pxy, and OX4767A-Pxy will have no effect on listed species or species proposed for listing, and will not affect designated habitat or habitat proposed for designation. Because of this no-effect determination, consultation under Section 7(a)(2) of the Act or the concurrences of the USFWS or NMFS is not required.

8 CONSIDERATION OF EXECUTIVE ORDERS, STANDARDS, AND TREATIES RELATING TO ENVIRONMENTAL IMPACTS

8.1 Executive Orders with Domestic Implications

The following EOs require consideration of the potential impacts of the Federal action to various segments of the population.

- **EO 12898 (US-NARA, 2010), "Federal Actions to Address Environmental Justice in Minority Populations and Low-Income Populations,"** requires Federal agencies to conduct their programs, policies, and activities that substantially affect human health or the environment in a manner so as not to exclude persons and populations from participation in or benefiting from such programs. It also enforces existing statutes to prevent minority and low-income communities from being subjected to disproportionately high and adverse human health or environmental effects.

- **EO 13045, "Protection of Children from Environmental Health Risks and Safety Risks,"** acknowledges that children may suffer disproportionately from environmental health and safety risks because of their developmental stage, greater metabolic activity levels, and behavior patterns, as compared to adults. The EO (to the extent permitted by law and consistent with the agency's mission) requires each Federal agency to identify, assess, and address environmental health risks and safety risks that may disproportionately affect children.

The No Action and Preferred Alternatives were analyzed with respect to EO 12898 and EO 13045. The environmental and human health impacts are presented in Section 4 of this EA. Neither alternative is expected to have a disproportionate adverse effect on minorities, low-income populations, or children.

The following EO addresses Federal responsibilities regarding the introduction and effects of invasive species:

- **EO 1311 (US-NARA, 2010), "Invasive Species,"** states that Federal agencies take action to prevent the introduction of invasive species, to provide for their control, and to minimize the economic, ecological, and human health impacts that invasive species cause.

Diamondback moth is not listed in the United States as an invasive species by the Federal government but it is listed as an invasive species that is currently present in all U.S. States (CABI, 2014). While diamondback moth is a ubiquitous pest of cruciferous plants, domesticated and non-domesticated, within the action area, it does not persist from year to year (Appendix A). Rather, diamondback moth populations within the action area are primarily the result of repetitive introductions from year to year (Appendix A). The two GE traits engineered in the GE diamondback are not expected to contribute to increased fitness. Other GE insects possessing traits similar to this GE diamondback moth have been genetically engineered and released within the United States (USDA-APHIS, 2008; 2009; 2011a). Based on historical experience with GE insects possessing similar traits (USDA-APHIS, 2008; 2009; 2011a) and the data submitted by the applicant, this particular GE diamondback moth is not anticipated to possess increased fitness characteristics compared to non-GE diamondback moth.

The following executive order requires the protection of migratory bird populations:

- **EO 13186 (US-NARA, 2010), "Responsibilities of Federal Agencies to Protect Migratory Birds,"** states that federal agencies taking actions that have, or are likely to have, a measurable negative effect on migratory bird populations are directed to develop and implement, within two years, a Memorandum of Understanding with the Fish and Wildlife Service that shall promote the conservation of migratory bird populations.

Migratory birds may be found in fields containing cruciferous crops, where they may forage for insects and weed seeds found in and adjacent to the field. As discussed in the Preferred Alternative analysis of Wildlife communities (Section 4.3.2), the introduced proteins in GE diamondback moth are similar to other proteins assessed in APHIS NEPA documents and is not expected to be allergenic, toxic, or pathogenic in animals (USDA-APHIS, 2008).

8.2 Consultation and Coordination with Indian Tribal Governments

- **EO 13175 (US-NARA, 2010), "Consultation and Coordination with Indian Tribal Governments"** was issued to ensure that there would be "meaningful consultation and collaboration with tribal officials in the development of Federal policies that have tribal implications…"

APHIS has given this EO careful consideration and does not expect a significant environmental impact on tribal lands because the action area is not on any land maintained by an Indian Tribal Government.

8.3 Impacts on Unique Characteristics of Geographic Areas

Issuing the permit for GE diamondback moth is not expected to impact unique characteristics of geographic areas such as park lands, prime farmlands, wetlands, wild and scenic areas, or ecologically critical areas.

As discussed in the Environmental Consequences (Section 4), no different agronomic activities within the action area are anticipated as a result of the Preferred Alternative. If the permit is issued, the field release will occur on land already under agricultural management, and is not expected to alter land use patterns within the action area.

There are no proposed major ground disturbances; no new physical destruction or damage to property; no alterations of property, wildlife habitat, or landscapes; and no prescribed sale, lease, or transfer of ownership of any property. This action is limited to issuing a three-year permit for GE diamondback moth release. This action would not convert land use to non-agricultural use and, therefore, would have no adverse impact on prime farmland. Standard agricultural practices for land preparation, planting, irrigation, and harvesting of plants would be used on agricultural lands planted under the Preferred Alternative, including the use of EPA-registered pesticides. The inability of diamondback moth to overwinter in the action area suggests that any remaining GE diamondback moth remaining at the conclusion of the calendar year will not persist into the following calendar year (Section 3.2).

Based on these findings, including the assumption that label use restrictions are in place to protect unique geographic areas and that those label use restrictions are adhered to, issuing a permit for the field release of GE diamondback moth is not expected to impact unique characteristics of geographic areas such as park lands, prime farm lands, wetlands, wild and scenic areas, or ecologically critical areas.

8.4 National Historic Preservation Act (NHPA) of 1966 as Amended

The NHPA of 1966 and its implementing regulations (36 CFR 800) require Federal agencies to: (1) determine whether activities they propose constitute "undertakings" that have the potential to cause effects on historic properties, and (2) if so, to evaluate the effects of such undertakings on such historic resources and consult with the Advisory Council on Historic Preservation (i.e., State Historic Preservation Office, Tribal Historic Preservation Officers), as appropriate.

APHIS' proposed action, issuing a permit for the three-year field release of GE diamondback moth, is not expected to adversely impact cultural resources on tribal properties. Any farming activity that may be taken by farmers on tribal lands would only be conducted at the tribe's request; thus, the tribes would have control over any potential conflict with cultural resources on tribal properties.

APHIS' Preferred Alternative would have no impact on districts, sites, highways, structures, or objects listed in or eligible for listing in the National Register of Historic Places, nor would it likely cause any loss or destruction of significant scientific, cultural, or historical resources. This action is limited to issuing a three-year permit for the field release of GE diamondback moth.

APHIS' proposed action is not an undertaking that may directly or indirectly cause alteration in the character or use of historic properties protected under the NHPA. In general, common agricultural activities conducted under this action do not have the potential to introduce visual, atmospheric, or noise elements to areas in which they are used that could result in effects on the character or use of historic properties. For example, there is potential for increased noise on the use and enjoyment of a historic property during the operation of tractors and other mechanical equipment close to such sites. A built-in mitigating factor for this issue is that virtually all of the methods involved would only have temporary effects on the audible nature of a site and can be ended at any time to restore the audible qualities of such sites to their original condition, with no further adverse effects. Additionally, these cultivation practices are already being conducted throughout the production area. The three-year field release of GE diamondback moth is not expected to change any of these agronomic practices that would result in an adverse impact under the NHPA.

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10 REFERENCES

Adler, PR; Del Grosso, SJ; and Parton, WJ (2007) "Life-cycle assessment of net greenhouse-gas flux for bioenergy cropping systems." *Ecological Applications.* 17 (3): p 675-91.

Al-Shehbaz, L (1984) "The Tribes of the Cruciferae (Brassicaceae) in the Southeastern United States." *Journal of the Arnold Arboretum.* p 343-73. Last Accessed: March, 2014 https://archive.org/details/cbarchive_39786_thetribesofcruciferaebrassicac1972.

Altieri, M (1999) "The ecological role of biodiversity in agroecosystems." *Agriculture, Ecosystems and Environment.* 74 (1-3): p 19-31. http://www.sciencedirect.com/science/article/pii/S0167880999000286.

Altieri, MA and Letourneau, DK (1982) "Vegetation management and biological control in agroecosystems." *Crop Protection.* 1 (4): p 405-30.

American Meteorological Society (2012) "Westerlies - Glossary of Meteorology." American Meteorlogical Society. Last Accessed: May, 2014 http://glossary.ametsoc.org/wiki/Westerlies.

Ammann, K (2005) "Effects of biotechnology on biodiversity: herbicide-tolerant and insect-resistant GM crops." *TRENDS in Biotechnology.* 23 (8): p 388-94.

Andaloro, J (1983) "Insects of Crucifers: Diamondback Moth." New York State Agricultural Experiment Station. Last Accessed: March, 2014 http://nysipm.cornell.edu/factsheets/vegetables/cruc/dm.pdf.

Aneja, VP; Schlesinger, WH; and Erisman, JW (2009) "Effects of agriculture upon the air quality and climate: Research, policy, and regulations." *Environmental Science & Technology.* 43 (12): p 4234–40.

Anonymous (1999) "Crop profile for cabbage in New York." Last Accessed: 09Apr14 http://www.ipmcenters.org/cropprofiles/docs/nycabbage.pdf.

Baker, J; Southard, R; and Mitchell, J (2005) "Agricultural dust production in standard and conservation tillage systems in the San Joaquin Valley." *Journal of Environmental Quality.* 34 p 1260-69. http://www.ncbi.nlm.nih.gov/pubmed/15998847.

Baucom, R and Holt, J (2009) "Weeds of agricultural importance: Bridging the gap between evolutionary ecology and crop and weed science." *New Phytologist.* (184): p 741-43.

BCAP (2010) "Programmatic Environmental Impact Statement on the Biomass Crop Assistance Program." Washington DC. United States Department of Agriculture, Farm Service Agency, Biomass Crop Assistance Program.

Beech, C to: Blanco, C. (2014). 13-297-102e/GE DBM.

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 (1): p 23-9.

Bollinger, EK and Caslick, JW (1985) "Red-winged blackbird predation on northern corn rootworm beetles in field corn." *Journal of Applied Ecology.* 22 p 39-48.

BONAP (2013) "Habit." Biota of North America Program. Last Accessed: August, 2013 http://bonap.org/Help/Habit.htm.

BONAP "BONAP's Taxonomic Data Center (TDC) - Brassicaceae in Ontario County, NY." Biota of North America Project. Last Accessed: April, 2014 http://bonap.net/tdc#.

Boucher, J (2012) "Cole crop "worms" (caterpillar pests)." Last Accessed: 09Apr14 http://ipm.uconn.edu/documents/raw2/html/108.php?display=print.

Burns, R; Connolly, G; and Savarie, P (1990) "Day-Glo Fluorescent Particles as a a Marker for Use in M-44 Cyanide Capsules." *Fourteenth Vertebrate Pest Conference*.

CABI (2014) "*Plutella xylostella.*" CABI.org, Invasive Species Compendium. Last Accessed: 09Apr14 http://www.cabi.org/isc/datasheet/42318.

Cambell, N; Reece, J; and Mitchell, L (1999) *Biology*. Benjamin/Cummins.

CFC. "Re: Public Comment on APHIS Notice of Intent to Prepare an Environmental Impact Statement and Proposed Scope Study Regarding Proposed use of Genetically Engineered Fruit Fly and Pink Bollworm." 2007.

Cook, E; Bartlein, P; Diffenbaugh, N; Seager, R; Shuman, B; Webb, R; Williams, J; and Woodhouse, C. "Hydrological Variability and Change." The U.S. Climate Change Science Program, 2008.

Cornell University (n.d.) Cornell University College of Agriculture and Life Sciences

New York State Agricultural Experiment Station. Last Accessed: March, 2014 http://web.entomology.cornell.edu/shelton/diamondback-moth/.

Cornell University Cooperative Extension (2013) "Production Guide for Organic Cole Crops." Cornell University Cooperative Extension

New York State Department of Agriculture and Markets. Last Accessed: March, 2014 http://nysipm.cornell.edu/organic_guide/cole_crops.pdf.

Curtis, PD; Fargione, MJ; and Richmond, ME (1994) "Preventing deer damage with barrier, electrical, and behavioral fencing systems." *Proceedings of the Sixteenth vertebrate pest conference.:* p 223-27. DigitalCommons@University of Nebraska - Lincoln. Last Accessed: 14Apr14 http://digitalcommons.unl.edu/vpc16/15/.

Dale, VH (1997) "The Relationship Between Land-Use Change and Climate Change." *Ecological applications.* 7 (3): p 753-69. http://www.esajournals.org/doi/abs/10.1890/1051-0761%281997%29007%5B0753%3ATRBLUC%5D2.0.CO%3B2.

Darley, EF and Middleton, JT (1966) "Problems of Air Pollution in Plant Pathology." *Annual Review of Phytopathology.* 4 (1): p 103-18. http://www.annualreviews.org/doi/abs/10.1146/annurev.py.04.090166.000535.

Del Grosso, S; Ojima, D; Parton, W; Mosier, A; Peterson, G; and Schimel, D (2002) "Simulated effects of dryland cropping intensification on soil organic matter and greenhouse gas exchanges using the DAYCENT ecosystem model." *Environmental Pollution.* 116 p S75-S83.

Delahaut, KA and Newenhouse, AC (1997) "Growing broccoli, cauliflower, cabbage, and other cole crops in Wisconsin: a guide for fresh-market growers." http://learningstore.uwex.edu/assets/pdfs/A3684.PDF.

Dickens, B and Brant, H (2014) "Effects of marking methods and fluorescent dusts on Aedes aegypti survival." *Parasites & Vectors.* 7 (1): p 1-9. http://dx.doi.org/10.1186/1756-3305-7-65.

Dosdall; Julie J. Soroka; and Olfert, O (2011) "The Diamondback Moth in Canola and Mustard: Current Pest Status and Future Prospects

" *Prairie Soils & Crops Journal.* 4 p 66-76. Last Accessed: April 21, 2014 http://www.prairiesoilsandcrops.ca/articles/volume-4-8-screen.pdf.

EPA. "Protecting Water Quality from Agricultural Runoff." Washington, DC: Nonpoint Source Control Branch (4503T), EPA 841-F-05-001, 2005a.
EPA (2005b) "Reregistration Eligibility Decision for 2,4-D." U.S. Environmental Protection Agency. http://www.epa.gov/oppsrrd1/REDs/24d_red.pdf.
EPA (2009) "Pesticide Spray and Dust Drift. ." U.S. Environmental Protection Agency. http://www.epa.gov/opp00001/factsheets/spraydrift.htm.
EPA (2010a) "Climate change indicators in the United States." http://www.epa.gov/climatechange/indicators/pdfs/ClimateIndicators_full.pdf.
EPA (2010b) "Pesticide Registration Manual: Chapter 11 - Tolerance Petitions." U.S. Environmental Protection Agency. http://www.epa.gov/pesticides/bluebook/chapter11.html.
EPA (2011a) "Inventory of U.S. Greenhouse Gas Emissions and Sinks: 1990 – 2009." Environmental Protection Agency. Last Accessed: March, 2014 http://www.epa.gov/climatechange/Downloads/ghgemissions/US-GHG-Inventory-2011-Complete_Report.pdf.
EPA (2011b) "Pesticide Tolerance." U.S. Environmental Protection Agency. http://www.epa.gov/pesticides/regulating/tolerances.htm.
EPA (2012a) "Inventory of U.S. Greenhouse Gas Emissions and Sinks: 1990-2010." US-EPA.
EPA (2012b) "Rural roads." Last Accessed: 08Apr14 http://www.epa.gov/agriculture/trur.html.
EPA (2012c) "Setting tolerances for pesticide residues in food." Last Accessed: 25Mar14 http://www.epa.gov/pesticides/factsheets/stprf.htm#other.
EPA (2012d) "Summary Table: Characteristics of the Ecoregions of New York." Environmental Protection Agency. Last Accessed: March, 2014 http://www.epa.gov/wed/pages/ecoregions/ny_eco.htm#Please note:.
EPA (2012e) "Watershed Assessment, Tracking & Environmental Report. National Summary. Causes of Impairment in Assessed Rivers and Streams." http://iaspub.epa.gov/waters10/attains_index.control.
EPA (2013a) "Air Quality." Environmental Protection Agency. Last Accessed: August, 2013 http://www.epa.gov/airquality/cleanair.html.
EPA (2013b) "Glossary of Climate Change Terms." Environmental Protection Agency. Last Accessed: August, 2013 http://www.epa.gov/climatechange/glossary.html#C.
EPA (2013c) "Pesticide Registration." Environmental Protection Agency. Last Accessed: January, 2013 http://www.epa.gov/pesticides/factsheets/registration.htm.
EPA (2014a) "Ag 101: demographics." Last Accessed: 13Mar14 http://www.epa.gov/oecaagct/ag101/demographics.html.
EPA (2014b) "Current agricultural worker protection standard (WPS)." Last Accessed: 20Mar14 http://www.epa.gov/pesticides/health/worker.htm.
EPA (2014c) "Pesticide Product Labels." Environmental Protection Agency. Last Accessed: March, 2014 http://www.epa.gov/pesticides/regulating/labels/product-labels.htm.
EPA (2014d) "Pesticide Tolerances." Environmental Protection Agency. Last Accessed: March, 2014 http://www.epa.gov/pesticides/regulating/tolerances.htm.
Fargione, J; Hill, J; Tilman, D; Polasky, S; and Hawthorne, P (2008) "Land Clearing and the Biofuel Carbon Debt." *Science.* 319 (5867): p 1235-38. http://www.sciencemag.org/content/319/5867/1235.abstract.

Farmworker Justice (2014) "Workplace hazards." Last Accessed: 26Mar14 http://www.farmworkerjustice.org/content/workplace-hazards.
Farnham, D (2001) "Corn Planting Guide." Iowa State University University Extention. http://www.extension.iastate.edu/publications/pm1885.pdf.
Fawcett, R and Towery, D (2002) "Conservation Tillage and Plant Biotechnology: How New Technologies Can Improve the Environment By Reducing the Need to Plow." Conservation Technology Information Center. http://www.whybiotech.com/resources/tps/ConservationTillageandPlantBiotechnology.pdf.
Field, CB; Mortsch, LD; Brklacich, M; Forbes, DL; Kovacs, P; Patz, JA; Running, SW; and Scott, MJ (2007) "North America." *Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge, UK: Cambridge University Press. p 617-52. Last Accessed: December 3, 2010 http://www.ipcc.ch/publications_and_data/ar4/wg2/en/ch14s14-4-4.html.
FOCUS (2008) "Pesticides in Air: Considerations for Exposure Assessment." European Commission Forum for Coordination of Pesticide Fate Models and Their Use Working Group on Pesticides in

Air. http://focus.jrc.ec.europa.eu/ai/docs/FOCUS_AIR_GROUP_REPORT-FINAL.pdf
Gianessi, LP and Reigner, NP (2007) "The value of herbicides in U.S. crop production." *Weed Technology*. (21): p 559-66.
Goddard, J (1993) *Physician's guide to arthropods of medical importance: fifth edition*. CRC Press.
Gomulski, LM; Torti, C; Murelli, V; Bonizzoni, M; Gasperi, G; and Malacrida, AR (2004) "Medfly transposable elements: diversity, evolution, genomic impact and possible applications." *Insect Biochem Mol Biol.* 34 (2): p 139-48.
Green, JM and Owen, MDK (2011) "Herbicide-resistant crops: Utilities and limitations for herbicide-resistant weed management." *Journal of Agricultural and Food Chemistry* 59 p 5819-29.
Hagler, JR and Jackson, CG (2001) "METHODS FOR MARKING INSECTS: Current Techniques and Future Prospects." *Annual Review of Entomology*. 46 (1): p 511-43. http://www.annualreviews.org/doi/abs/10.1146/annurev.ento.46.1.511.
Harlan, JR (1975) "Our vanishing genetic resources." *Science.* 188 (4188): p 618-21.
Hart, M; Powell, J; Gulden, R; Levy-Boot, D; Dunfield, K; Peter Pauls, K; Swanton, C; Klironomos, L; and Trevors, J (2009) "Detection of transgenic cp4 epsps genese in the soil food web." *Agronomy for Sustainable Development.* 29 (4). http://www.agronomy-journal.org/index.php?option=com_article&access=doi&doi=10.1051/agro/2009020&Itemid=129.
Heiniger, R (2000) "NC Corn Production Guide - Chapter 4 - Irrigation and Drought Management " http://www.ces.ncsu.edu/plymouth/cropsci/cornguide/Chapter4.html.
Hibino, Y and Iwahashi, O (1991) "Appearance of wild females unreceptive to sterilized males on Okinawa Island [Japan] in the eradication program of the melon fly, Dacus cucurbitae Coquillett (Diptera: Tephritidae)." *Applied Entomology and Zoology.* 26 p 265-70.
Hoeft, RG; Nafziger, ED; Johnson, RR; and Aldrich, SR (2000) *Modern Corn and Soybean Production (1st Ed)*. Champaign: MCSP Publications.

Hopkinson, RF and Soroka, JJ (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): p 1-11. http://www.sciencedirect.com/science/article/pii/S0168192309001932.

Horowitz, J and Gottlieb, J (2010) "The Role of Agriculture in Reducing Greenhouse Gas Emissions." U.S. Department of Agriculture–Economic Research Service. http://www.ers.usda.gov/Publications/eb15/.

Jin, L; Walker, AS; Fu, G; Harvey-Samuel, T; Dafa'alla, T; Miles, A; Marubbi, T; Granville, D; Humphrey-Jones, N; O'Connell, S; Morrison, NI; and Alphey, L (2013) "Engineered Female-Specific Lethality for Control of Pest Lepidoptera." *ACS Synthetic Biology*. 2 (3): p 160-66. Last Accessed: 2014/03/05 http://dx.doi.org/10.1021/sb300123m.

Karl, T; Meehl, G; Miller, C; Hassol, S; Waple, A; and Murray, W (2008) "Weather and Climate Extremes in a Changing Climate - Regions of Focus: North America, Hawaii, Caribbean, and U.S. Pacific Islands." The U.S. Climate Change Science Program. http://www.climatescience.gov/Library/sap/sap3-3/final-report/.

Keese, P (2008) "Risks from GMOs due to horizontal gene transfer." *Environmental Biosafety Research*. 7 p 123-49.

Klassen, W (2005) "Area-Wide Integrated Pest Management and the Sterile Insect Technique." *Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management*. Dordrecht, Netherlands: Springer. p 39-68.

Krapu, GL; Brandt, DA; and Cox Jr., RR (2004) "Less Waste corn, More Land in Soybeans, and the Switch to Genetically Modified Crops: Trends with Important Implications for Wildlife Management." http://digitalcommons.unl.edu/usgsnpwrc/65.

Landis, DA; Menalled, FD; Costamagna, AC; and Wilkinson, TK (2005) "Manipulating plant resources to enhance beneficial arthropods in agricultural landscapes." *Weed Science*. 53 p 902-08.

Leistra, M; Smelt, JH; Westgate, JH; Berg, Fvd; and Aalderink, R (2006) "Volatilization of the pesticides chlorpyrifos and fenpropimorph from a potato crop." *Environmental Science & Technology*. 40 p 96-102.

Lerat, S; Gulden, R; Hart, M; Powell, J; England, L; Peter Pauls, K; Swanton, C; Klironomis, J; and Trevors, J (2007) "Quantification and persistence of recombinant DNA of Roundup Ready corn and soybean in rotation." *Agricultural and Food Chemistry*. 55 p 10226-91. http://www.planta.cn/forum/files_planta/quantification_and_persistence_of_recombinant_dna_of_roundup_ready_corn_and_soybean_in_rotation_171.pdf.

Levy-Booth, D; Gulden, RH; Campbell, RG; Powell, JR; Klironomos, J; Peter Pauls, K; Swanton, C; Trevors, J; and Dunfield, K (2008) "Roundup Ready soybean gene concentrations in field soil aggregate size classes." *Federation of European Microbiological Societies*. Microbiol Lett 291 (2009) (2): p 175-79. http://onlinelibrary.wiley.com/doi/10.1111/j.1574-6968.2008.01449.x/pdf. .

Lovett, S; Price, P; and Lovett, J (2003) "Managing Riparian Lands in the Cotton Industry." Cotton Research and Development Corporation. http://www.crdc.com.au/uploaded/File/E-Library/E-ENVIRO/RiparianCottonGuide.pdf.

Markus, DJ (1997) "Winter broccoli performance and nutrien quality when grown by conservation tillage." *Subtropical Plant Science*. 49 p 16-21.

McCray, K (2012) "Ground Water: Out of Sight, But Not Out of Mind." http://www.ngwa.org/Events-Education/awareness/Pages/Editorial.aspx.

McDaniel, LD; Young, E; Delaney, J; Ruhnau, F; Ritchie, KB; and Paul, JH (2010) "High frequency of horizontal gene transfer in the oceans." *Science.* 330 (6000): p 50. http://www.sciencemag.org/content/330/6000/50.abstract.

Ministry of Labour Canada (2006) "Occupational Health and Safety Guidelines for Farming Operations in Ontario." Last Accessed: 17Mar14 http://www.labour.gov.on.ca/english/hs/pdf/farming_ohsag.pdf.

Nagel, P and Peveling, R (2005) "Environment and the Sterile Insect Technique." *Sterile Insect Technique: Principles and Practices in Area-Wide Integrated Pest Management*. Dordrecht, Netherlands: Springer. p 499-524.

NatureServe "NatureServe Explorer." Last Accessed: August, 2013 http://www.natureserve.org/explorer/servlet/NatureServe?menuselect=none&nameSpec=&whichTypes=all&nameType=either&noPunct=CHECKED&findTerm=&allSelected=on&taxonomyCommand=&taxonomyLevel=All+Ecological+Units&post_processes=SetTaxon&isSpecies=true&c_wetland_opt=ALL&s_wetland_opt=ALL&s_lf_opt=ALL&sourceTemplate=nameSearchEcol.wmt&refineTarget=nameSearchEcol.wmt&referringPage=nameSearchEcol.wmt&loadTemplate=nameSearchSpecies.wmt&prevTarget=nameSearchEcol.wmt&isEcol=true&post_processes=PostName&searchType=Ecol&jump_to=.

NCGA (2007) "Sustainability - Conserving and Preserving: Soil Management and Tillage." http://www.ncga.com/uploads/useruploads/conservingpreservingsoilmanagement.pdf.

Nguyen, C; Bahar, MH; Baker, G; and Andrew, NR (2014) "Thermal Tolerance Limits of Diamondback Moth in Ramping and Plunging Assays." *PLoS ONE.* 9 (1): p e87535. http://dx.doi.org/10.1371%2Fjournal.pone.0087535.

NGWA (2010) "Groundwater Facts." Natinal Groundwater Association. Last Accessed: March, 2014 http://www.ngwa.org/Fundamentals/use/Pages/Groundwater-facts.aspx.

NSRL (n.d.) "Soybean Production Basics." National Soybean Research Laboratory. http://www.nsrl.illinois.edu/general/soyprod.html.

NY Department of Agriculture and Markets (2014) "Ag Facts." New York State Department of Agriculture and Markets. Last Accessed: March, 2014 http://www.agriculture.ny.gov/agfacts.html.

NY State Climate Office (n.d.) "The Climate of New York." New York State Climate Office, Department of Earth and Atmospheric Sciences, Cornell University. Last Accessed: March, 2014 http://nysc.eas.cornell.edu/climate_of_ny.html.

NY State IPM Program (2013) "Integrated Crop and Pest Management Guidelines for Commercial Vegetable Production: Cabbage, Broccoli, Cauliflower, and Brussel Sprouts." Corner Cooperative Extension. Last Accessed: March, 2014 http://veg-guidelines.cce.cornell.edu/15frameset.html.

NYSAES (2014) "New York State Agricultural Experiment Station website." Cornell University, College of Agriculture and Life Sciences

New York State Agricultural Experiment Station. Last Accessed: March, 2014 http://www.nysaes.cornell.edu/.

O'Brien, TJ (1998) "Integration of conservation tilage and cover crops in broccoli production systems of the Pacific Northwest." Oregon State University. *Dissertations and Theses*. https://ir.library.oregonstate.edu/xmlui/handle/1957/25646.
OECD (2012) "Consensus Document on the Biology of the Brassica crops (Brassica spp.)." Organization for Economic Cooperation and Development.
OSHA, OSHA (2005) "Farm safety fact sheet." Last Accessed: 20Mar14 https://www.osha.gov/OshDoc/data_General_Facts/FarmFactS2.pdf.
OSHA, OSHA (2014a) "Occupational safety and health standards for agriculture." Last Accessed: 20Mar14 https://www.osha.gov/pls/oshaweb/owastand.display_standard_group?p_toc_level=1&p_part_number=1928.
OSHA, OSHA (2014b) "Safety and Health Topics. agricultural operations." Last Accessed: 19Mar14 https://www.osha.gov/dsg/topics/agriculturaloperations/standards.html.
OSTP (2001) "Case Studies of Environmental Regulation for Biotechnology: Herbicide-Tolerant Soybean." The White House Office of Science and Technology Policy. http://www.whitehouse.gov/files/documents/ostp/Issues/ceq_ostp_study4.pdf.
Owen, MDK (2008) "Weed species shifts in glyphosate-resistant crops." *Pest Management Science.* 64 p 377-87.
Palmer, WE; Bromley, PT; and Anderson, JR (2011) "Wildlife and Pesticides - Corn." North Carolina Cooperative Extension Service AG-463-2. http://ipm.ncsu.edu/wildlife/corn_wildlife.html.
Patterson, MP and Best, LB (1996) "Bird abundance and nesting success in Iowa CRP fields: The importance of vegetation structure and composition." *American Midland Naturalist.* 135 (1): p 153-67. Last Accessed: May 18, 2011 http://www.jstor.org/stable/2426881 .
Peloquin, JJ; Thibault, ST; Staten, R; and Miller, TA (2000) "Germ-line transformation of pink bollworm (Lepidoptera: gelechiidae) mediated by the piggyBac transposable element." *Insect Mol Biol.* 9 (3): p 323-33.
Reeve, JD and Cronin, JT (2010) "Edge behaviour in a minute parasitic wasp." *Journal of Animal Ecology.* 79 (2): p 483-90. http://dx.doi.org/10.1111/j.1365-2656.2009.01640.x.
Richards, HA; Han, CT; Hopkins, RG; Failla, ML; Ward, WW; and Stewart, CN, Jr. (2003) "Safety assessment of recombinant green fluorescent protein orally administered to weaned rats." *J Nutr.* 133 (6): p 1909-12.
Robertson, BA; Porter, C; Landis, DA; and Schemske, DW (2012) "Agroenergy Crops Influence the Diversity, Biomass, and Guild Structure of Terrestrial Arthropod Communities." *Bioenerg. Res.* 5 p 179-88.
Robertson, GP; Paul, EA; and Harwood, RR (2000) "Greenhouse gases in intensive agriculture: Contributions of individual gases to the radiative forcing of the atmosphere." *Science.* 289 p 1922-25.
Root, R (1973) "Organization of a Plant-Arthropod Association in Simple and Diverse Habitats: The Fauna of Collards (Brassica Oleraceae)." *Ecological Monographs.* 43 (1): p 95-124.
Rosenzweig, C and Parry, ML (1994) "Potential impact of climate change on world food supply." *Nature.* 367 (6459): p 133-38. Last Accessed: March, 2014 http://www.nature.com/nature/journal/v367/n6459/pdf/367133a0.pdf.
Seaman, A (2013) "2013 Production guide for organic cole crops." http://nysipm.cornell.edu/organic_guide/cole_crops.pdf.

Sharpe, T (2010) "Cropland Management (*Chapter 4*)." *Tarheel Wildlife: A Guide for Managing Wildlife on Private Lands in North Carolina*. Raleigh: North Carolina Wildlife Resources Commission. p 26-29. Last Accessed: November 9, 2010 http://www.ncwildlife.org/tarheelwildlife/documents/Tarheel_Wildlife_ch_4.pdf.

Shelton, A (2001a) "Management of the diamondback moth: deja vu all over again?" *4th International Workshop on the Management of Diamondback Moth and Other Crucifer Pests*. Last Accessed: March, 2014 http://www.regional.org.au/au/esa/2001/02/0201shelton.htm?print=1.

Shelton, A (2001b) "Regional outbreaks of diamondback moth due to movement of contaminated plants and favourable climatic conditions." Melbourne, Australia

Shelton, A to: Blanco, C. (2014). Re: Additional information needed.

Smith, KA and Conen, F (2004) "Impacts of land management on fluxes of trace greenhouse gases." *Soil Use and Management.* 20 (2): p 255-63. Last Accessed: March, 2014 http://dx.doi.org/10.1111/j.1475-2743.2004.tb00366.x.

Talekar, NS and Shelton, AM (1993) "Biology, Ecology, and Management of the Diamondback Moth." *Annual Review of Entomology.* 38 (1): p 275-301. http://www.annualreviews.org/doi/abs/10.1146/annurev.en.38.010193.001423.

Thibault, ST; Luu, HT; Vann, N; and Miller, TA (1999) "Precise excision and transposition of piggyBac in pink bollworm embryos." *Insect Mol Biol.* 8 (1): p 119-23.

Tong, C (2009) "Growing cole crops in Minnesota home gardens." Last Accessed: 09Apr14 http://www.extension.umn.edu/garden/yard-garden/vegetables/growing-cole-crops-in-minnesota-home-gardens/.

Tupper, S; Cummings, J; and Engerman, M (2009) "Longevity of DayGlo fluorescent particle marker used to mark birds in flight pen and field." *Wildlife Research.* 36 p 319-23.

UF-IFAS (2012) "Featured Creatures: Diamondback moth." University of Florida - Institute of Food and Agricultural Safety. Last Accessed: March, 2013 http://entnemdept.ifas.ufl.edu/creatures/veg/leaf/diamondback_moth.htm.

University of Arkansas (2006) "Soil and Water Management, Soybeans – Crop Irrigation." http://www.aragriculture.org/soil_water/irrigation/crop/soybeans.htm.

US-EPA (2011) "Sole Source Aquifer Protection Program." U.S. Environmental Protection Agency. http://water.epa.gov/infrastructure/drinkingwater/sourcewater/protection/solesourceaquifer.cfm.

USDA-AMS (2010) "PDP Program Overview." U.S. Department of Agriculture–Agricultural Marketing Service. http://www.ams.usda.gov/AMSv1.0/ams.fetchTemplateData.do?more=G.OptionalText2&template=TemplateG&navID=PDPDownloadNav2Link2&rightNav1=PDPDownloadNav2Link2&topNav=&leftNav=ScienceandLaboratories&page=PDPProgramOverview&resultType=&acct=pestcddataprg.

USDA-APHIS (2005) "Field study of genetically modified pink bollworm, Pectinophora gossypiela (Lepidoptera: Gelechiidae): Environmental Assessment." Riverdale, MD. Service, United States Department of Agricultural - Animal and Plant Health Inspection. Last Accessed: March, 2014 http://www.aphis.usda.gov/brs/biotech_ea_permits.html.

USDA-APHIS (2008) "Use of Genetically Engineered Fruit Fly and Pink Bollworm in APHIS Plant Pest Control Programs: Final Environmental Impact Statement." Riverdale, MD.

USDA-APHIS (2009) "Light Brown Apple Moth Sterile Insect Field Evaluation Projects in Sanoma and Napa, California: Environmental Assessment." Riverdale, MD.
USDA-APHIS (2011a) "Light Brown Apple Moth Sterile Insect Field Evaluation Projects in Long Beach and San Diego, California: Environmental Assessment." Riverdale, MD.
USDA-APHIS (2011b) "Pioneer Hi-Bred International, Inc. Seed Production Technology (SPT) Process DP-32138-1 Corn: Final Environmental Assessment." Service, United States Department of Agriculture - Animal and Plant Health Inspection. Last Accessed: March, 2014 http://www.aphis.usda.gov/biotechnology/petitions_table_pending.shtml.
USDA-APHIS (2014a) "Biotechnology Enviromental Documents for Permits and Petitions." United States Department of Agriculture - Animal and Plant Health Inspection Service. Last Accessed: March, 2014 http://www.aphis.usda.gov/biotechnology/biotech/brs_environmental_assessments.shtml.
USDA-APHIS (2014b) "Petitions for Determination of Nonregulated Status." United States Department of Agriculture - Animal and Plant Health Inspection Service. Last Accessed: March, 2014 http://www.aphis.usda.gov/biotechnology/petitions_table_pending.shtml.
USDA-ARS (2011) "At ARS, the Atmosphere is Right for Air Emission Studies." U.S. Department of Agriculture–Agricultural Research Service. http://www.ars.usda.gov/is/AR/archive/jul11/emissions0711.htm?pf=1.
USDA-ERS (2005) "Agricultural Chemicals and Production Technology: Sustainability and Production Systems." Economic Research Service. http://www.ers.usda.gov/Briefing/AgChemicals/sustainability.htm.
USDA-ERS (2010) "Agricultural Chemicals and Production Technology: Soil Management." http://www.ers.usda.gov/briefing/agchemicals/soilmangement.htm.
USDA-FSA (2010) "Conservation Reserve Program: Supplemental Environmental Impact Statement." Agency, United States Department of Agriculture - Farm Service. Last Accessed: March, 2014 http://www.fsa.usda.gov/Internet/FSA_File/crpfinalseismaster61010.pdf.
USDA-NASS (2007) "Agricultural Chemical Usage 2006 Field Crops Summary." U.S. Department of Agriculture–National Agricultural Statistics Service. http://usda.mannlib.cornell.edu/usda/nass/AgriChemUsFC/2000s/2007/AgriChemUsFC-05-16-2007_revision.pdf.
USDA-NRCS (1999) "Soil Taxonomy - A Basic System of Soil Classification for Making and Interpreting Soil Surveys." United States Department of Agriculture - National Resource Conservation Service. Last Accessed: May, 2013 ftp://ftp-fc.sc.egov.usda.gov/NSSC/Soil_Taxonomy/tax.pdf.
USDA-NRCS (2001) "Soil Quality - Introduction." U.S. Department of Agriculture–Natural Resources Conservation Service. http://soils.usda.gov/sqi/publications/publications.html#agy.
USDA-NRCS (2004) "Soil Biology and Land Management." U.S. Department of Agriculture–Natural Resources Conservation Service. http://soils.usda.gov/sqi/publications/publications.html#atn.
USDA-NRCS (2006) "Conservation Resource Brief: Soil Erosion, Number 0602." National Resources Conservation Service. http://www.nrcs.usda.gov/Internet/FSE_DOCUMENTS/nrcs143_023234.pdf.

USDA-NRCS (2011) "Managing Nonpoint Source Pollution from Agriculture." U.S. Environmental Protection Agency. http://water.epa.gov/polwaste/nps/outreach/point6.cfm.
USFWS (2013) "Endangered and Threatened Wildlife and Plants; Listing the Northern Long-Eared Bat as an Endangered Species." Last Accessed: April 17, 2014 http://www.gpo.gov/fdsys/pkg/FR-2013-12-02/pdf/2013-28705.pdf.
USFWS "Listed Flowering Plant Species." USFWS. Last Accessed: April 21, 2014 http://ecos.fws.gov/tess_public/pub/SpeciesReport.do?groups=Q&listingType=L&mapstatus=1.
USFWS (2014b) "Species Profile: northern long-eared Bat (Myotis septentrionalis)." USFWS. Last Accessed: April 17, 2014 http://ecos.fws.gov/speciesProfile/profile/speciesProfile.action?spcode=A0JE.
USFWS "Species Proposed forListing." USFWS. Last Accessed: April 21, 2014 http://ecos.fws.gov/tess_public/pub/SpeciesReport.do?listingType=P.
USGS (2013) "Groundwater and aquifer FAQs." United States Geological Service. Last Accessed: October, 2013 http://www.usgs.gov/faq/?q=categories/9812/2773.
USGS (2014) "Surface Water Information." United States Geological Survey. Last Accessed: March, 2014 http://water.usgs.gov/edu/mearthsw.html.
Vercauteren, KC and Hygnostrom, SE (1993) "White-tailed deer home range characteristics and impacts relative to field corn damage." p 2. Lincoln, NE. http://digitalcommons.unl.edu/gpwdcwp/354.
WeatherSpark (2014) "Average weather for Ithaca, New York." Last Accessed: May, 2014 https://weatherspark.com/averages/30616/Ithaca-New-York-Unite.
Werner, R and Holsten, E (1997) "Dispersal of the Spruce Beetle, Dendroctonus rufipennis, and the Engraver Beetle, Ips perturbatus, in Alaska." United States Department of Agriculture - Forest Service. Last Accessed: April, 2014 http://www.fs.fed.us/pnw/pubs/pnw_rp501.pdf.
West, TO and Marland, G (2002) "A synthesis of carbon sequestration, carbon emissions, and net carbon flux in agriculture: comparing tillage practices in the United States." *Agriculture, Ecosystems and Environment.* 91 p 217–32.
Whitney, D (1997) "Fertilization-Soybean Production Handbook." Kansas State University. http://www.ksre.ksu.edu/library/crpsl2/c449.pdf.
Wilson, EO (1988) *Biodiversity*. Washington: National Academy Press.
Zalucki, MP; Shabbir, A; Silva, R; Adamson, D; Shu-Sheng, L; and Furlong, MJ (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 (4): p 1115-29. Last Accessed: 2014/03/13 http://dx.doi.org/10.1603/EC12107.

11 APPENDIX A – APPLICANT'S TECHNICAL REPORT

OXITEC

Environmental Risk Assessment

For the Open Field Release of Genetically Engineered Diamondback Moths in the United States

Contents

Field study of Genetically Engineered Diamondback Moth, *Plutella xylostella* (Lepidoptera; Yponomeutidae)

1 PURPOSE AND NEED

1.1 Background

Professor Anthony Shelton, Cornell University (NY) has submitted an application to the U.S. Department of Agriculture, Animal and Plant Health Inspection Service/Biotechnology Regulatory Service (application number 13-297-102r) for open field release of transgenic strain of *Plutella xylostella*, diamondback moth (DBM), the major pest of agricultural brassica crops.

The transgenic strain of DBM OX4319L-Pxy has previously been imported into the US for glass house trials (**Appendix I**) under the permit number 12-227-102m (**Appendix II**).

1.2 Introduction and spread in the USA

DBM is a widespread pest within the US following its introduction from Europe to Illinois in 1854 (Knodel and Ganehiarachchi, 2008). Restricted in its range by adverse overwintering temperatures, DBM is known to seasonally reinfest crops in North American states and Canada from Mexico and southern states such as Georgia and Maryland. Spread of the DBM can be through transplanting of brassica seedlings (Shelton, 2001) but DBM is also known to spread using wind (Hopkinson and Soroka, 2010).

DBM has a short generation time in tropical countries where it can have up to 20 generations per year. This rapid turnover in generations can lead to insecticide-resistant strains developing when control is reliant on insecticides (Talekar and Shelton, 1993). DBM was the first insect to develop resistance to DDT (Sarfraz et al., 2006) and is the only species to develop resistance to *Bacillus thuringiensis* (*Bt*)-based insecticidal sprays (Sarfraz et al., 2006).

Development of insecticide-resistant strains is not expected to occur in New York State. Conversely, insects in warm countries that have a continual cycling population are likely to develop insecticide resistance; these strains are then likely to migrate to new areas. Persistence of insecticide-resistant strains throughout New York State will not occur as the cold winter temperatures prevent any DBM from overwintering (Shelton, 2001).

1.3 Economic importance

DBM is a major crop pest, able to infest all brassica species including commercial crops such as canola, *Brassica napus* L., cabbage, *Brassica oleracea* L., broccoli, *Brassica oleracea* var. *italica* L., cauliflower, *Brassica oleracea* var. *botrytis* L., Chinese cabbage, *Brassica pekinensis* (Lour.), and Indian mustard, *Brassica juncea* (L.) Czern, amongst others. It can infest crops each season, with larvae damaging leaf material in large quantities, leaving the crop unsaleable.

DBM occurs in all states where crucifers are produced and causes severe damage especially in southern states and Hawaii where temperatures allow it to be present year-round.
DBM is estimated to have an economic burden of US$1 billion; however this value was calculated in 1993 (Talekar and Shelton, 1993) and recent revisions suggest that a conservative revised estimate would place this value much higher at US$4-5 billion (Zalucki et al., 2012).

1.4 Current control of DBM

There are no currently viable management options such as crop rotation or specific sanitation methods advised within Integrated Crop and Pest Management Guidelines[1]. However use of trap crops (plants grown around the field to prevent pests moving in) can help in some small areas. Approved insecticides include varieties of *Bacillius thuringiensis (Bt)*, beta-cyfluthrin, chlorantraniliprole, emamectin benzoate, indoxacarb, lambda-cyhalothrin, methomyl, permethrin, spinetoram, spinosad, zeta-cypermethrin[2].

The Canadian Canola Council website (www.canolacouncil.org/canola-encyclopedia/insects/diamondback-moth/) provides advice on detecting the DBM in the growing season. Farmers are advised to scout their fields early on in the growing season and checking throughout July and August, monitoring crops at least twice a week. Farmers need to take crop samples from a $0.1m^2$ area, beat them onto a clean surface and count the number of larvae dislodged. When 20-30 larvae/$0.1m^2$ are present at the advanced pod stage it is recommended to spray an approved insecticide.

DBM will also persist on brassicaceous weeds and a list of host weeds is provided in Talekar and Shelton (1993) in times when their preferred host crop has not yet emerged. Therefore maintaining fields free of volunteer weeds can help reduce the pest population in the area of the field.[3] It has also been suggested that inter-planting crops with plant species that DBM will preferentially lay eggs on could reduce commercial crop damage (Charleston and Kfir, 2000).

At present the global DBM population is known to be resistant to a wide range of insecticides, including carbamates (group 1A), organophosphates (group 1B), phenylpyrazoles (group 2B), Pyrethroids (group 3A), Neonicotinoids (group 4A), Spinosyns (group 5), Avermectins (group 6), Pyrrols (group 13). Benzoylureas (group 15), diacylhydrazines (group 18), Tolfenpyrad (group 21A), Indoxacarb (group 22A), Metaflumizone (group 22B), Diamides (group 28).[4]

In addition to the use of chemical control of the DBM, biological control strategies have been trialed, as outlined below, however; the efficacy of the control is below the economic threshold limits to make this commercially viable.

[1] http://veg-guidelines.cce.cornell.edu/ (Accessed 02/04/2014)

[2] http://veg-guidelines.cce.cornell.edu/15frameset.html (Accessed 02/04/2014)

[3] http://www.canolacouncil.org/canola-encyclopedia/insects/diamondback-moth/ (Accessed 26/03/2014)

[4] http://www.irac-online.org/pests/plutella-xylostella/ (Accessed 28/03/2014)

The parasitoid *Cotesia plutella* was imported and released in Florida in 1990 and since then it has been released into cabbage fields in the state sporadically for control of DBM. Despite these releases the parasitoid did not become established. Another disadvantage of this parasitoid species is that they do no kill their hosts until they are almost fully grown and have already done a lot of feeding (Elwakil and Mossler, 2013).

Experiments with sprays of the fungus, *Beauveria bassiana*, demonstrated a reduction in the 3rd and 4th instars of DBM of 70% in trials, while insecticide application reduced the population by 95% however, the rate of fungal spore release (14×10^{13}/hectare) may be cost prohibitive under field conditions (Vandenberg et al., 1998).

Two components of the sex pheromone of the female DBM have been sprayed to disrupt mating and reduce populations in cabbage fields. Experiments in Florida have shown that the mating disruption sprays in combination with three insecticide sprays resulted in cabbages planted in the interior of the plots reaching US No 1 quality grade. Cabbage at the extreme perimeter of the pheromone-treated area was heavily damaged by DBM larvae (McLaughlin et al., 1994). However, other studies have shown that mating disruption does not work (Schroeder et al., 2000).

Whilst trap crops such as yellow rocket proved to be a good candidate for DBM because it is highly attractive for egg laying but larvae will not persist or feed on this crop. It was however unsuccessful as a control strategy as other imported pests, such as cabbageworm, are able to develop on this plant species (Gianessi, 2009).

The use of entomopathogenic nematodes for controlling the diamondback moths has been assessed. In field trials, nematodes provided 41% control however it was also viewed that repeated applications of nematodes will probably be ineffective in attaining control. Nematodes also require high humidity, moderate temperatures, and protection from ultraviolet light to be most effective which could limit their commercial viability (Baur et al., 1998).

Whilst these biological control strategies have been reviewed with the potential for their implementation in the field, as yet no commercially viable solution for DBM control has been detected other that the use of chemical pesticides, therefore a new solution is required.

1.5 Purpose of Product

The transgenic strain of DBM (*Plutella xylostella*) OX4319L-Pxy has been developed by Oxitec Ltd (UK) in order to develop a female-specific genetically sterile diamondback moth strain for the control of wild populations of DBM, using a method analogous to the Sterile Insect Technique (Alphey, 2002; Alphey et al., 2008; Jin et al., 2013).

The Sterile Insect Technique (SIT) was first pioneered in the 1950s with a programme against the New World Screwworm by Dr R.C. Bushland and Dr E.F. Knipling, who jointly received the 1992 World Food Prize for this work (Dyck, 2005; Vreysen, 2005).

Traditional SIT relies on the release of large numbers of male insects sterilized by irradiation. Mating of released sterile males with native females leads to a decrease in the females' reproductive potential and ultimately, if males are released in sufficient numbers over a sufficient period of time, to the local elimination or suppression of the pest population.

Highly successful, area-wide SIT programmes have eliminated the screwworm fly *Cochliomyia hominivorax* from the USA, Mexico, Central America, and Libya. Other targets of area-wide SIT programs include the Mediterranean fruit fly (Medfly) *Ceratitis capitata* and other tephritid fruit flies in the USA, Central and South America, South Africa, Europe and Asia, the pink bollworm *Pectinophora gossypiella* in the USA, and the codling moth in Canada.

A genetically sterile insect control method has been developed in Medfly (Fu et al., 2007; Gong et al., 2005), Pink Bollworm (Simmons et al., 2011; Walters et al., 2012) and mosquitoes (Phuc et al., 2007). This method of control relies on the inheritance of an engineered synthetic lethal gene to replace the radiation–induced lethal mutations. These strains work through the use of the "tet-off" gene expression system. The tTA transactivator of the tet-off system can be used as a lethal effector; high levels of tTAV expression can be obtained through a positive feedback circuit that is lethal to insects. The system is repressed in the presence of tetracycline, or suitable analogues, which are readily supplied in larval diet. Use of sex-alternative splicing of the sex determination gene *transformer* (*tra*) enables the production of a female-lethal strain with regulated tetracycline-repressible expression (Jin et al., 2013). Following mating between transgenic male insects with the female-specific lethality trait and wild females, only male progeny are produced as daughters die in the absence of tetracycline.

Repeated inundative releases of the transgenic males over time would reduce the number of DBM females in the population, and consequently, the reproductive potential of the wild pest population. Combining this genetic trait with a fluorescent protein marker (Lukyanov et al., 2000; Matz et al., 1999; Shagin et al., 2004) enables genetic sexing, autocidal population control and field monitoring in one insect.

The second gene to be introduced into the insect is a fluorescent marker, DsRed2. This enables successful and accurate environmental and laboratory monitoring of the transgenic insect.

1.6 Biology of the Diamondback moth

Eggs are laid individually or in groups of 2-8 on the upper leaf surface and hatch within 4-8 days. Eggs are small and yellowish-white in color.

First instar larvae bore into leaves where they feed on the leaf mesophyll. Subsequent instars are surface feeders eating leaves, buds, flowers or pods. The different larval instars have an average duration of 4 days (1st instar), 3.6 days (2nd instar), 3.4 days (3nd instar) and 4.2 days (4th instar). Development in DBM is temperature-dependent with low temperatures of 10°C leading to a far longer development time (egg-pupae average duration in days of 81.26 ± 1.17) than in warmer temperatures of 30°C (egg- pupae average duration in days of 11.43 ± 0.10) (Golizadeh et al., 2007).

Larvae pupate in loose mesh cocoons on the leaves and stems or seedpods of plants, as they develop they change from light green to brown as the adult becomes visible. The pupal stage can last between 5-15 days depending on environmental conditions.

Adult moths are small brown moths with a wingspan of 15 mm and a body length of 6 mm. Forewings are brownish-gray and lighter along the anterior margin, with fine dark speckles. A creamy colored stripe with a wavy edge on the posterior margin this can sometimes be constricted to form one or more light-colored diamonds on the back of the moth. Males live for about 12 days whilst females survive for about 16 days. Females lay on average 160 eggs over about 10 days, with more eggs at the start of their egg laying period compared to later.[5]

Insects exchange gametes internally and have specific structures and mating behaviors which prevent the mating with insects other than DBM. Mating in the DBM is also reliant to a large degree to sex pheromones specific to the DBM species (Lee et al., 2005). There is also a degree of photoperiodism in DBM mating as mating is initiated at the onset of dark and lasts for round three hours (Lee et al., 1995).

Multiple generations of DBM can overlap and all four larval stages of DBM can be present in the field at the same time.

DBM are weak fliers not travelling long distances by spontaneous flight; however they are known to be transported by wind currents and can be swept long distances (Hopkinson and Soroka, 2010).

1.7 Coordinated Framework Review and Regulatory Review

1.7.1 USDA-APHIS

Agency completion

1.7.2 EPA

Agency completion

1.7.3 FDA

Agency completion

1.8 Purpose and Need

1.8.1 Proposed action

The proposed action is for APHIS/ Biotechnology Regulatory Services (BRS), to issue a permit for field-testing of DBM genetically modified to express a female-specific lethality trait as well as a genetic marker a red fluorescent protein (DsRed2).

1.8.2 Purpose and need for this EA

The need for this EA is to assess any potential adverse environmental effects of a field research study using transgenic DBM in Geneva, New York State. A permit for importation of the strains was issued on September 12th 2012 (Permit number 12-227-102m) in order to carry out initial caged testing of the DBM strain. The application for an open release permit was received by APHIS/ BRS (Application number 13-297-102r).

[5] http://entnemdept.ifas.ufl.edu/creatures/veg/leaf/diamondback_moth.htm (Accessed 26/03/2014)

1.8.3 Similar EAs

The open field release of transgenic Pink bollworm has previously been evaluated by the USDA- APHIS/BRS in September 2005, culminating in a Finding of No Significant Impact (FONSI) statement. Since 2005, APHIS has conducted an Endangered Species Act section 7 consultation with Fish and Wildlife Services for the National Pink Bollworm Eradication Program in Arizona, New Mexico, and Texas. The use of SIT has been included in this consultation (http://www.aphis.usda.gov/plant_health/ea/downloads/SWPnkBwrm.pdf, page 18).

Furthermore a detailed Environmental Impact Statement on the Use of Genetically Engineered Fruit Fly and Pink Bollworm in APHIS Plant Pest Control Programs has been completed by the USDA-APHIS in October 2008, and a Record of Decision issued in May 2009 (http://www.gpo.gov/fdsys/pkg/FR-2009-05-07/pdf/E9-10633.pdf). In their record of Decision, USDA-APHIS concluded that "the environmentally preferable alternative for the use of sterile insect technique in plant pest control programs is the alternative that minimizes potential impacts to human health, non-target species, and environmental quality. Among the alternatives considered in this EIS, the preferred alternative, which involves integration of genetically engineered insects into programs, is also the environmentally preferable alternative. This alternative is environmentally preferable because the potential environmental impacts of this alternative are minimized by program use of genetically engineered strains of sterile and marker-gene insects maintained in biologically secure containment facilities, by the reduced use of irradiation with its associated hazards, by the reduced need for large numbers of insects due to the release of males that are more competitive in mating, and by the reduced need to apply pesticides from a more effective genetic sterile insect technique and improved monitoring of pest populations through the use of genetic markers."

1.8.4 Need for this action

Under APHIS regulation, the receipt of a permit application to introduce a genetically engineered organism requires a response from the Administrator:

> Administrative action on applications. After receipt and review by APHIS of the application and the data submitted pursuant to paragraph (a) of this section, including any additional information requires by APHIS, a permit shall be granted or denied. 7 CFR 340.5(e)

1.9 Public Involvement

Agency to Complete

1.10 Alternatives

1.10.1 No action

Under APHIS/BRS regulations, the Administrator must either grant or deny permits properly submitted under 7 CFR 340. For the purposes of this Environmental Assessment, the No Action alternative would be the denial of the permit application 13-297-102r.

1.10.2 Issue a permit

Issuing this permit would allow the following research to proceed at the field research site in Geneva, New York State. **Appendix III** contains details of the research plan from the permit application together with supplementary trial design information.

1.10.2.1 Purpose of the research

The proposed release application is for a permit which will enable up to 3 years of seasonal releases (April – October) to be carried out. The 3-year window has been requested due to the seasonal nature of the pest and the variable natural infestation rate. These releases of no more than 100,000 male moths per week will enable the dispersal and persistence of the transgenic strain to be measured, with a view to assessing the value of the strains for wild pest population control.

The transgenic DBM will be reared at the insectaries at Cornell University, Geneva NY and the releases will be carried out in brassica fields in Cornell University field stations.

1.10.2.2 Description of the research

The release of the transgenic DBM strains will be carried out in phases; firstly a mark-release-recapture (MRR) using traps or trap plants to monitor the DBM population. This MRR will allow the transgenic DBM strain to be compared to the unmodified strain in terms of dispersal and longevity throughout a brassica field. Field cage trials will also be used to evaluate the mating competitiveness of the transgenic DBM in comparison to wild DBM in natural settings. Future field investigations will build on existing data and the proposed trials carried out in the first year but will be limited to 100,000 males released per week within the proposed field station.

Further details on the production and release design as outlined in the release permit are outlined in **Appendix III** together with supplementary trial design information.

2 ISSUES CONSIDERED

The issues considered in this Environmental Assessment relate to the issues which have been evaluated in previous EAs assessing the impact of the release of transgenic insects

The test site in Ontario (NY) is within the geographic area that is frequently seasonally infested by DBM. No overwintering of the DBM is known to occur and all DBM, whether transgenic or wild, are expected to die once the temperature drops in the winter months. Whilst slow acclimatization of the DBM can enable it to persist in the environment at extreme low and high temperatures, these are exceptional circumstances. DBM typically do not survive in temperatures below 7°C (Nguyen et al., 2014).

All brassica crop plants and brassica weeds will be cleared within an area of 10 m from the test field site of 10 acres. Traps will be placed up to 1000 m surrounding the release site and will be monitored at least weekly. The surrounding farmland is primarily field corn which is not a host plant of the DBM.

On completion of the experiment, the approved insecticide for use on brassicas, DuPont™ Coragen® Insect Control, with the active ingredient RynaXypyr® (active ingredient, chlorantraniliprole) will be sprayed on the plants and surrounding area (within 100 m radius of treated fields) to kill remaining DBM larvae.

Post-experiment trapping will continue until no transgenic DBM are detected for 2 consecutive weeks.

2.1 Environmental consequences as a result of the proposed release

The environmental consequences are summarized in **Table 1**. and are further detailed in subsequent **Section 2.4**

Table 1. Summary of consequences

Issues	No Action	Issue permit
Possibility of the conditional-lethality failing, resulting in risk to the environment	No effect	No effect
Risk of DsRed2 to the environment	No effect	No effect
Persistence or increased invasiveness of the transgenic DBM compared to the wild DBM	No effect	No effect
Gene transfer to offspring of related species	No effect	Intention of the trait, F_1 female sterility
Horizontal gene transfer to other organisms, such as predators, saprophytes, or parasites	No effect	No effect
Interaction of the transgenic DBM with target organisms	No effect	Intention of the trait, F_1 female sterility
Interaction of the transgenic DBM with non-target organisms	No effect	No effect

Effect of the transgenic DBM on human and animal health	No effect	Worker precautions in place
Impacts on the specific cultivation, management and harvesting techniques	No effect	No effect
Effects on chemical (Pesticide, herbicide, fungicide) load on the environment	No effect	Potential benefit

2.2 Deny the permit application

To deny the permit application would have no expected potential adverse environmental impacts and would prevent this confined field research from proceeding and prevent any benefits derived from it being realized in the future.

2.3 Issuance of the permit

Based on information and data presented in this Environmental Assessment, the proposed action is not expected to have any adverse environmental impacts for the following biological and physical reasons:

- No adverse consequences to non-target organisms or environmental quality are expected to arise from the use of the transgenic DBM in a field setting.
- The OX4319L-Pxy DBM strain has no increased persistence or invasiveness compared to the wild DBM.
- The expression products of the inserted transgenes have been evaluated in a bioinformatics study and indicate no similarity to any allergenic or toxic protein.
- The inserted genes are stable and have not moved throughout the genome over the course of the time in the laboratory. In the field there is unlikely to be exposure to an exogenous source of transposase which could enable the gene to mobilize. No antibiotic marker is present in the DBM.

2.4 Analysis of the issues, consequences and theoretical risks of field research on the female-specific condition lethal DBM strain.

2.4.1 Possibility of the conditional-lethality trait failing, resulting in potential risk to the environment

Risk to the environment in the proposed trial will be reviewed as an increased persistence of the DBM or damage to surrounding crop plants caused by the transgenic DBM in comparison to the unmodified DBM.

The brassica plants used in the proposed experiments will be planted specifically for the project, therefore crop damage to the brassica used in the trial is not considered as an adverse environmental impact.

The DBM is a weak flier and in the presence of the host plant at the release site is not anticipated to spread into surrounding fields. Surrounding the proposed release site are fields planted with corn although there is the possibility that brassica weed plants could be present at sites surrounding the release site. In the event that the transgenic male DBM move into non-target brassica species surrounding the release site they could mate with wild female DBM. The majority of the female offspring (>99%) of any of these crosses would not be anticipated to survive therefore there is the possibility of a population reduction of wild DBM populations in non-target brassica species. As DBM are frequently controlled with insecticides, are seasonally introduced into New York State from southern states and are a serious economic pest species, a negative impact on DBM populations would not pose an environmental risk.

In the event that the female-specific lethality trait fails in the F_1 offspring, no adverse impact is anticipated. The transgenic OX4319L-Pxy DBM has a lower fitness than the unmodified DBM strain in laboratory conditions and the transgenic insects will be selected against in environmental settings (Harvey-Samuel et al., in press). Survival of the OX4319L-Pxy transgene is not expected to persist beyond a few generations in the wild. In laboratory studies persistence of the strain averaged 6 generations with a maximum persistence of 7 generations in the absence of the dietary supplement tetracycline (Harvey-Samuel et al., in press).

Conclusion

The likelihood of harm arising as a result of the lethality trait failing is "highly unlikely". No increase in the DBM population is anticipated even if the specific lethality trait fails; therefore the consequence of harm to the environment arising in the event that conditional lethality trait fails is "marginal". Therefore, there is no reason to suggest that the transgenic DBM would have an adverse impact or longer persistence than the unmodified wild DBM strains in the environment and the risk that the conditional lethality trait fails would be "negligible".

2.4.2 Potential Risk of DsRed2 to the environment

DsRed2 is a marker protein which is expressed constitutively in the larvae pupae and adults of the transgenic DBM strains. DsRed is a naturally occurring fluorescent protein which was originally found in various *Discosoma spp.* DsRed2 was artificially developed from DsRed through a combination of random and site-directed mutagenesis in order to enhance the fluorescence and improve the solubility, which in turn increases the sensitivity of detection (Bevis and Glick, 2002; Ip and Wan, 2004; Lukyanov et al., 2000; Matz et al., 1999; Shagin et al., 2004). The improvement to the solubility of DsRed2 has been achieved through reducing its tendency to form aggregates; these mutations also reduce the time from transfection to detection to only 24 hours. DsRed2 has the benefit of high signal to noise ratio and due to the distinct spectrum in which it fluoresces it can for use in multicolor labelling and can be used in combination with Enhanced-GFP (CLONTECHniques, 2001). The amino acid sequence of the DsRed2 protein is provided in (**Figure 1**).

MASSENVITE FMRFKVRMEG TVNGHEFEIE GEGEGRPYEG HNTVKLKVTK
GGPLPFAWDI LSPQFQYGSK VYVKHPADIP DYKKLSFPEG FKWERVMNFE
DGGVATVTQD SSLQDGCFIY KVKFIGVNFP SDGPVMQKKT MGWEASTERL
YPRDGVLKGE THKALKLKDG GHYLVEFKSI YMAKKPVQLP GYYYVDAKLD
ITSHNEDYTI VEQYERTEGR HHLFL

Figure 1. **Amino acid sequence of DsRed2**

DsRed2 Potential Toxicity and Allergenicity Assessment:

The DsRed2 marker protein has been evaluated in an Early Food Safety of Protein Evaluation by the FDA-CFSAN for human food and animal feed safety (Pavely and Fedorova, 2006). In their response letter, FDA "had no questions" regarding Pioneer Hi-Bred International's conclusion that "that DsRed2 protein would not raise food safety concerns when it is in a new food plant variety that is present at low levels in the food supply" (FDA CFSAN NPC 00004, 29 January, 2010). Pioneer's evaluation involved an assessment of the amino acid sequence for homology to known allergens 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. Additional information on the lack of toxicity of DsRed2 is given in Section 15 of this document, including oral studies in rats (Richards et al., 2003). DsRed2 has been further evaluated in an Environmental Assessment (EA) by the United States Dept of Agriculture[6] which concluded that the corn transformation event that contained the DsRed2 gene was unlikely to become a plant pest risk. Additional EA's on GE pink bollworm expressing fluorescent genes similar to DsRed2 have also been conducted (USDA, 2001)[7] and 2005 and concluded that it was unlikely to present any hazard to the environment:

No adverse consequences to non-target organisms or environmental quality are expected from incorporation of this marker into the pink bollworm.

- Green fluorescent protein is a naturally occurring protein, not known to cause adverse effects (Appendix V). The normal digestive process of predators would preclude transfer of functional genetic material to the predator so such transfer is not expected with eating this insect. In addition, there have been no adverse effects to birds that are naturally exposed to the GFP through feeding directly on jellyfish.
- Released pink bollworms will be sterile adults that are not expected to produce fertile offspring. No adverse consequences are expected to beneficial insects (e.g. natural enemies, decomposers, pollinators) from release of transgenic PBW adults expressing GFP.

[6] http://www.aphis.usda.gov/brs/aphisdocs/08_33801p_dpra.pdf (Accessed 19 March 2013)

[7] http://www.gpo.gov/fdsys/pkg/FR-2006-04-19/html/E6-5878.htm (Accessed 14th March 2013)

Conclusion

The likelihood of harm arising as a result of the expression of the marker protein DsRed2 is "highly unlikely" as the DsRed2 protein sequence has no known homology to any known allergen or toxin. The impact of expression of the DsRed2 to the environment is considered to be "marginal" as the protein is not considered to be toxic or allergenic. Therefore, there is no reason to suppose that the transgenic DBM strain OX4319L-Pxy would have an adverse environmental impact as a result of the expression of the DsRed2 protein when compared to the unmodified wild DBM strains in the environment and the risk is "negligible".

2.4.3 Persistence or increased invasiveness of the transgenic DBM compared to the wild DBM

The DBM moth strain OX4319L-Pxy is not anticipated to have any increased persistence or increased invasiveness in the environment compared to the wild DBM, rather the expression of the lethality trait which is expressed in all females provides a strong negative selection pressure preventing persistence and limiting invasiveness.

Laboratory evaluation of the OX4319L-Pxy strain has established that the inserted genes do not increase persistence in comparison to the unmodified background DBM. (Harvey-Samuel, et. al, accepted). This has been evaluated in both permissive (in the presence of sufficient tetracycline to repress the female-lethality trait) and in restrictive conditions (absence of tetracycline) and is summarized below:

In a caged population in permissive conditions the transgenic allele frequency declined by 63.3% over the course of the experiment (10 generations), which was significantly different to a non-neutral drift distribution of the transgene. In restrictive conditions where no tetracycline was included in the diet, the transgene was lost from the population within a mean of 6 generations with the maximum number of generations until allele extinction of 7 generations for the DBM strain OX4319L-Pxy. Within the field conditions at the release site it is extremely unlikely there will be sufficient environmental levels of tetracycline to enable the DBM strain to persist. A dose response for the amount of chlortetracycline (CTC) in the larval diet required to repress the lethality trait has been assessed. Levels of CTC at 0.1 μg/ml enable survival of small numbers of the heterozygous DBM females to adulthood, but at 0.01 μg/ml no OX4319-L DBM females survived to adulthood. This level of tetracycline far exceeds the level of tetracycline which the DBM might encounter at the field site. A review of the literature indicates that cabbage fertilized with tetracycline-contaminated manure accumulated tetracycline concentrations of <0.004 μg/ml CTC in foliage (Kumar et al., 2005), which is insufficient to repress the lethality trait in the DBM strain OX4319L-Pxy. A review of tetracycline in the environment has been provided in **Appendix IV**.

Conclusion

In the event of any unexpected persistence or invasiveness of the DBM an approved insecticide will be sprayed at weekly intervals over a one month period to control surviving DBM. Unexpected persistence of the DBM will be assessed by the project director but could involve the transgenic DBM surviving beyond the scope of the experiment (surviving into November). Increased invasiveness will be determined as detection of fluorescent DBM in traps set up at 1000m beyond the perimeter of the release site on more than two occasions. Traps which have been set up at the release site and at intervals surrounding the periphery of the release site will be checked at least once per week.

The background strain into which the transgenes were inserted has been laboratory-reared for over 15 years in the absence of pesticides, therefore levels of pesticide resistance common in wild DBM populations is not expected in the transgenic DBM. In *Bt* toxicity assays, the strain has been shown to be highly susceptible **Appendix V**.

In addition to the proposed control methods to devitalize the transgenic DBM it is important to note that there is no overwintering of DBM populations at the release site. DBM only survives in New York State in summer months and reinvades from southern States annually.

The likelihood of any persistence of the strain OX4319L-Pxy is "highly unlikely" and the consequence of any persistence would be "marginal" as the strain is not known to be any more invasive or persistent that its wild type counterpart. The overall risk of the transgenic DBM persisting at the release site is "negligible".

2.4.4 Gene transfer to offspring of relative species

Insects exchange gametes internally and therefore it is the intended effect of the modification that DBM males mate with females of its own species. There are no related lepidopteran species in northern United States with which the DBM can mate.
Vertical transfer of the genetic trait will be analyzed through monitoring of traps or trap plants. As the conditional lethal trait and the fluorescent marker are linked, the expression of the fluorescent marker, DsRed2 will indicate successful vertical transfer of the gene, by visual analysis. Any ambiguous visual results can be sent to Oxitec Ltd (UK) for further molecular analysis.

There is no known close relative of DBM within North America and there is no potential for the DBM to mate with other closely related species. Insects exchange gametes internally and have specific structures and mating behaviors which prevent the mating with insects other than DBM. Mating in the DBM is also reliant to a large degree to sex pheromones specific to the DBM species (Lee et al., 2005). There is also a degree of photoperiodism in DBM mating, as mating is initiated at the onset of dark and lasts for around 3 hours (Lee et al., 1995).

Conclusion

There is not anticipated to be any vertical transfer of the transgene from the released DBM into any species other than wild DBM at the field site. It is the specific and intended affect that crosses with wild female DBM will pass on the transgene to their offspring; however the presence of the female-lethal trait will limit the persistence of the transgene at the release site through strong negative selection against persistence of the trait.

Therefore whilst the likelihood of gene transfer from the transgenic DBM to the wild DBM at the release site is "highly likely" this is not an appropriate measure of harm in this instance as this is the intention of the release. The likelihood that there will be no vertical transfer of the genetic trait between the wild DBM and the released DBM is a more appropriate assessor of risk, the likelihood of no interaction between the released DBM and the wild DBM is "unlikely". The consequence of the mating is intended to result in the majority of female offspring not surviving to adults therefore the consequence will have a "marginal" impact on the environment. Overall considering that the likelihood whilst "unlikely" is a positive attribute of the transgenic DBM and the consequence is "marginal" there is not anticipated to be an adverse impact to the environment.

2.4.5 Horizontal gene transfer to other organisms, such as predators, saprophytes, or parasites

In this area of the assessment the main concern is the potential transfer of recombinant DNA from transgenic DBM to other organisms at the release site. Consequences and likelihood of the movement of the inserted genes into other organisms have also been evaluated.
The horizontal gene transfer (HGT) is defined in the guidance document on the Environmental Risk Assessment of GM animals (EFSA, 2012) as "any process in which an organism incorporates genetic material from another organism into its genome without being the offspring of that organism".

The scope of this environmental assessment covers a time-limited environmental release in a restricted field site of 10 acres, however a scenario in which wider environmental exposure to transgenic DBM through accidental release of viable insects, during transport and handling has been considered. Although the uptake of insect genes by micro-organisms is an extremely rare event it could be hypothetically possible. The hypothesis that HGT between the transgenic DBM and other organisms in the environment will not have an adverse impact on human or animal health or have an adverse environmental effect has been assessed. Further review of the potential for movement of the vector transposon used for the transformation has also been carried out in this section of the assessment where the hypothesis that movement of the transposon would not result in harm to human or animal health or the environment was carried out.

The transgenic DBM strains have been transformed using the non-autonomous transposon, *piggyBac* which enabled stable integration of the inserted genes into the DBM genome. Non-autonomous transposons required the use of an exogenous transposase source for excision and integration into a genome, during the transformation process. This enzyme was supplied on a separate plasmid during the transformation and was not integrated into the DBM genome alongside with the required genetic elements. The phenotype has remained stable during rearing of the insects for over 3 years (approximately 80 generations).

Molecular characterisation of the transgene insertion in the OX4319L-Pxy strain of DBM

In order to determine the genomic DNA sequence flanking the transgene insertion in the OX4319L-Pxy strain and to quantify the number of transgene insertions within this DBM strain the flanking regions of the insertion were sequenced and a Southern blot was performed.

Insertion of a *piggyBac* transposable element such as is found in the OX4313 construct 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. Furthermore, given the very large number of potential insertion sites it is highly unlikely that two independent insertions would insert at the same site. The genomic insertion site of a given transgenic strain therefore provides an event-specific identifier, particularly when combined with knowledge of the sequence of the inserted DNA. For instance, transgene insertion-homozygous colonies are typically generated using PCRs specific for the adjacent sequence (usually termed "flanking sequence") – a PCR reaction using suitable primers binding genomic DNA either side of the insertion will amplify a characteristic band from a wild type (non-insertion) allele at the insertion site but not from homozygotes, for which both alleles have a large insertion between the two primers. These PCRs are also a key quality control activity for each Oxitec product strain, verifying integrity of the homozygous colony. Verifying the number of transgene insertions in a colony is also important, to ensure that all inserted novel sequences are identified and characterized. Southern blots are a well-known means of assessing the number of transgene insertions.

The genomic insertion site of the OX4319L-Pxy insertion was sequenced (**Appendix VI**). Nucleotide and translated nucleotide BLAST analysis detected no similarity to known sequences, indicating that presence of the OX4319L-Pxy insertion is not disrupting gene coding sequence. Primers specific for the 5′ and 3′ flanking sequences were designed and used to PCR-amplify the wild-type locus of the insertion site, confirming that these sequences flank the same OX4319 transgene insertion. The single transgene copy was also indicated by progeny phenotype of test crosses with OX4319L-Pxy-heterozygous male moths and wild-type female moths. When reared on chlortetracycline (which represses engineered female-specific lethality), the ratio of transgenic and wild-type progeny was approximately 1:1, indicating a single transgene locus by Mendelian inheritance.

Furthermore a Southern blot analysis of OX4319L-Pxy gDNA indicated the presence of only a single transgene insertion in this line. (**Appendix VI**)

The OX4319L-Pxy insertion site has been sequenced, providing a means of identifying the strain by PCR and distinguishing it from other transgenic strains and wild-type strains of diamondback moth: a vital quality control tool. Southern blot analysis showed single bands for each lane which, taken together with Mendelian inheritance of the transgenic phenotype, indicates that the strain carries a single transgene insertion.

The inserted traits confer no selective advantage to the insects rather they confer a strong negative selection pressure on the insects, without positive selection any persistence of the genetic traits is not expected.

This assessment will evaluate the impact of the HGT in the environment in two distinct ways and will then evaluate the ability of the transposon to remobilize.

- The transfer of the inserted genes into other insects at the release site

- The transfer of the inserted genes into other bacterial species at the release site

a) HGT between insects.

Transfer of the inserted traits from the transgenic DBM strains to wild DBM present at the release site is the intention of the modification and is a result of vertical gene transfer. HGT of the inserted traits from the transgenic DBM is highly unlikely to occur. Whilst direct mechanisms which enable gene transfer between bacterial species exist, such as homologous recombination and bacterial competence to take up prokaryotic DNA these are not present in eukaryotic organisms. Homologous recombination requires the presence of identical stretches of DNA sequence between the recombination points; therefore the lack of DNA sequence similarity represents a major barrier to inter-domain transfer between bacteria and insects. Therefore the assessment has considered the following:

- Origin and donor organism of the newly inserted genes
- Presence of any antibiotic resistance genes
- Sequence similarities of the newly inserted genes with other genetic components
- Whether expression of the newly inserted genes in transgenic DBM could lead to harm if transferred to micro-organisms
- Whether the expression of the newly inserted genes in transgenic DBM could lead to harm to human, animal health or the environment

Insects exchange gametes internally and have complex mating behaviours and structures to prevent or limit interspecies gene transfer. Exchange of genetic material through any other mechanism by insects at the release site is biologically improbable. In the highly unlikely event that a transfer of the inserted genetic traits, tTAV and DsRed2 to any other insects did occur at the release site the genes would not confer any benefit or selective advantage. In order for HGT to have an impact in a population it would require integration into the germ line of the insect and would need to confer a benefit to the organism in order for it to be selected for. DsRed2 is a neutral fluorescent marker that has no known benefit to any insect and the tTAV expresses a trait that is lethal (i.e. a strong selective disadvantage) in the absence of tetracycline.

Transposable elements (transposons or TEs) are capable of transferring segments of DNA from one site to another and are found in a wide range of eukaryotic genomes, this is likely the result of long evolutionary processes. There are several classes of TEs which differ in their ability to move throughout the genome. The transposon used in the transformation of the insects is a non-autonomous transposon which does not encode the enzyme (transposase) necessary for their own transposition therefore in order for the transposon to move in the genome of the released transgenic DBM it would require exposure to an external source of transposase.

In the unlikely event that the transposon moved from the released insect into another species at the release site detection of the event would be virtually impossible as should the transposon disrupt gene function during insertion the insect would not develop and if the transposon successfully integrated into a non-coding region the impact would be neutral or detrimental to the insect and as such would not be anticipated to persist. It is unlikely that the transposon would move and should the transposon move it is unlikely to persist in the environment or result in harm to human health, animal health or the environment.

In comparison to the wild type DBM there is not predicted to be a negative impact on human or animal health or the environment. The inserted genes present in the transgenic DBM strains will not confer a selective advantage rather they have a strong selective disadvantage and as such are not expected to persist in the environment in the unlikely event that they are transferred to any other organism.

b) HGT to microorganisms.

HGT is known to occur between different bacteria and there is the potential that bacteria at the release site could be exposed to the introduced genetic traits, tTAV and DsRed2. The released DBM will be males which could shed bacteria in faeces and could pass bacteria onto females during mating or other interactions. It is highly unlikely that the inserted genes would have moved via HGT into the gut bacteria of the released DBM. There are many bacterial species which could come into contact with the released males throughout the duration of the trial. This risk assessment has explored three exposure scenarios where bacteria could be exposed to the released DBM at the release site.

Exposure Scenario 1: to the gut micro-flora of predators of the Diamondback moth

At the release site there is the potential for the DBM to interact with non-target organisms such as invertebrates or small mammals. There are a number of insect species which are associated with the DBM and it is likely that some of the released DBM will be eaten by predators. The gut flora of the predators could then be exposed to low levels of fragmented products of the ingested DNA, including recombinant genes. Genomic DNA is a component of the diet of all organisms and becomes substantially degraded during digestion in the gastrointestinal tract and no full-length genes have been detected in the large intestine or in faeces (EFSA, 2009). Dietary assessments of predator/prey organisms consuming insectivore diets have shown that they are largely generalist organisms and only a small fraction of their diets is a single insect species (Blum et al., 1997). In the unlikely event that the gut bacteria take up any of the novel DNA, in order for the genes to persist in the environment they will require positive selection. The inserted genetic traits confer a strong negative selection therefore it is unlikely that they would be capable of persisting in the gut flora of any predator or in the environment.

Exposure Scenario 2: to micro-organisms associated with parasitoids at the release site

There are a number of parasitic wasps such as *Cotesia plutella* which are known to infect DBM as they develop. Any bacteria transferred from the parasitic wasp during ovipositioning into the larvae could come into contact with DNA from the transgenic DBM. As the insertion has been stable for over 80 generations it is unlikely that the stable inserts would move from the DBM into any bacteria at an increased rate than genomic DNA, as the inserted genes are incapable of excision without the presence of the transposase.

Exposure Scenario 3: Soil bacteria could be exposed as the Diamondback moths decompose at the release site.

As the transgenic DBM males decompose/decay at the release site there is the likelihood that soil bacteria could be exposed to the inserted genes. However it is very unlikely that intact DNA will be available for uptake by soil bacteria following this decomposition. Studies that looked at potential soil transfer from GM crop plants (Heuer and Smalla, 2007) indicated that this could happen but likely to be exceedingly rare event in the environment. These routes of exposure would represent very low levels of the genetic material which would be limited temporally and spatially. As the genetic inserts confer a strong negative selection pressure it is unlikely that any bacteria which take up the genetic traits would have a selective advantage or improved survival.

Given the competence of most bacteria to take up foreign DNA, the major barrier to such inter-domain transfer is the lack of sufficient DNA sequence similarities for homologous recombination to occur in bacteria. Non-homologous recombination events are theoretically possible but are thought to only occur at exceptionally low levels. The genetic traits inserted into the DBM are stable in the genome of the transgenic DBM and are not expected to move or have an increased mobility in comparison to the unmodified moth.

Conclusion

The background information collected on horizontal gene transfer, the molecular characterisation data gathered on the transgenic DBM strains and the results of the comparative safety assessment between the transgenic DBM and the wild type DBM have been considered in this section of the assessment. The conclusion is that the inserted genes, tTAV and DsRed2 in the DBM strains are unlikely to be transferred to micro-organisms in the short time period of the trial and even if they were this would not lead to human, animal or environmental harm. Thus the likelihood of horizontal gene transfer resulting in harm to humans, animals or the environment is "highly unlikely" and the consequences considered "marginal". Therefore the overall risk is considered "negligible".

2.4.6 Interaction of the transgenic DBM with target organisms

In this area of the assessment the interaction between the GM insects and the target organisms is considered according to the risk areas specified by the guidance document on conducting an ERA on the GM animals (EFSA, 2012).

The interaction of the transgenic DBM with a target organism (TO) is the intention of the release. In the proposed release the transgenic DBM will be assessed for their ability to mate with the wild DBM at the release site in the first year. Assessments at Oxitec have shown that the transgenic OX4319L-Pxy DBM is capable of competing against wild type males for female moths (Jin et al., 2013); however assessing field mating levels will inform future risk assessments and will provide data for second and third year experiments.

Persistence of the transgenic DBM

The duration of the releases will be for 3 years throughout the breeding season of the DBM (April-October) and the numbers of insects used in the proposed release will be relatively low (between 20,000 and 100,000 males/week). Whilst it is potentially conceivable that some resistance to the lethality trait might develop over a long time period, the releases have a short duration and require low numbers of insects to assess mating compatibility. Furthermore experience with large scale releases of irradiation sterilized insects have only resulted in isolated cases of "behavioral resistance" developing where females are able to identify and avoid mating with released sterile males (Dyck, 2005).

Fitness advantages which could enable establishment in the environment

In the unlikely event that resistance to the lethality trait develops, female DBM expressing the marker protein DsRed2 can still be detected. There is likely to be an adaptation of the colony to laboratory rearing which could confer a fitness penalty when in the harsher conditions of the environment. Bionomic studies have evaluated the OX4319L-Pxy strain in comparison to the wild type DBM laboratory strain. The wild type laboratory strain had a slightly higher longevity (31.7 days ± 1.43 standard error), although this was not significantly different to that of OX4319L-Pxy (25.9 days ± 1.73 standard error). A comparison of the male mating competitiveness of the OX4319L-Pxy was assessed in lab cages, in competition with the wild-type males, the OX4319L-Pxy achieved a relative sterility index (RSI) of 0.41 (McInnis et al., 1996) (an RSI of 0.5 indicated equal mating competitiveness). Mated DBM females do not become fully refractory to re-mating, therefore subsequent tests sought to include factors such as sperm competition and mating competitiveness over time in the assessment of male mating performance to indicate the long term mating competitiveness of the strain. OX4319L-Pxy males and WT-males were kept with the WT females for the duration of the female's reproductive lives. Of the resultant offspring 37% were transgenic. This value is similar to the proportion of observed matings in 1- to 3- hour trials which indicates that the OX4319L-Pxy males perform well in other aspects of their reproductive biology such as re-mating over time and post-copulatory effects (Jin et al., 2013).

Conclusion

The transgenic DBM expresses a female-specific conditional lethality trait which in the absence of tetracycline will prevent >99% of females from progressing to the late larval stages however male DBM survive to pass on the lethality trait. The potential for increased persistence of the DBM and the potential that the released DBM will confer a fitness advantage to the wild population of DBM have been evaluated in this assessment together with an evaluation of the impact of mating competitiveness of the DBM. The interaction between the transgenic DBM and the target organism, wild DBM, is essential in order for the transgenic DBM to be used as a form of pest control therefore whilst there is a "highly likely" likelihood that the target organism with interact with the transgenic DBM this is the specific and intended effect of the genetic engineering and therefore not an appropriate factor for generating the risk estimate. The likelihood that there will be no interaction between the target organism and the released DBM is a more appropriate assessor of risk, the likelihood of no interaction between the released DBM and the wild DBM is "unlikely" and the consequences of such an action is "marginal" therefore the risk that harm to biodiversity could result from the proposed trial is considered "negligible".

2.4.7 *Interaction of the transgenic DBM with non-target organisms*

In this area of the assessment the main environmental concern according to the EFSA 2012 guidance on the ERA for the release of GM animals is that the population level changes in competitors, prey, hosts, symbionts, predators, parasites, and pathogens which interact with the released DBM could have a negative impact on the environment via an adverse impact on ecosystem services.

The transgenic DBM strain differs from the wild type only in regards to the inserted genes; the conditional lethality and the fluorescent marker. There is a slight fitness penalty associated with the inserted genes however studies of the life history characteristics and the mating competitiveness of the organism have indicated that the OX4319L-Pxy DBM is comparative to the non-transgenic DBM. There is also likely to be an adaptation of the transgenic colony to laboratory rearing which could confer a fitness penalty when in the harsher conditions of the environment. The purpose of the release is to determine the mating competitiveness of the transgenic DBM with the wild DBM in a natural environment as well as the longevity of the insect and its persistence and dispersal in the environment.

Mating competitiveness

One of the objectives of the proposed trial is to assess the mating competitiveness of the transgenic DBM with the wild DBM in a natural environment. Previous cage and semi-field trials have indicated that the mating competitiveness of the DBM strain OX4319L-Pxy is sufficient to result in population suppression; further details on the mating competitiveness of the DBM are described by Jin et al. (2013).

Penetrance of the lethality trait

Recent laboratory studies and cage trials carried out at Oxitec have shown that the lethality trait has a high penetrance (>99% female-specific, in >800 individuals tested) females which inherit the trait will not survive beyond the late larval stages in the absence of dietary tetracycline (Oxitec Ltd, unpublished data). Irradiation SIT programmes rarely achieve complete sterility in their insects as the high irradiation levels required for 100% sterility renders the insects unable to compete (Bakri et al., 2005). Despite the lack of complete sterility the insects are still able to result in population suppression at the release site. In the result of complete failure of the lethality trait, the inserted genetic elements are associated with a fitness cost in the insect and will not be selected for, in order for a novel genetic trait to introgress into a population it will require positive selection pressure.

Non-target organisms at the release site

The proposed release site is a Cornell University field station in Ontario (NY). The proposed release site will be planted with brassica species (e.g. cabbage or broccoli) prior to commencing releases in the spring.

It is likely in a managed agricultural environment such as this that other arthropod species such as spiders, millipedes, centipedes and a variety of insect species will be present at the site. Small mammals and a number of bird species could also come into contact with the released transgenic DBM.

Comparing the impact of the small-scale time limited release of transgenic DBM to the conventional control system at the release site, namely the application of chemical insecticides such as *Bacillus thuringiensis Bt* or e.g. Coragen® containing RynaXypyr®, there is not anticipated to be an adverse impact. DBM have highly specific mating behaviors therefore the lethality trait will only be expressed in the DBM whereas the use of a non-specific insecticide could result in non-target organisms being adversely affected. Due to a greater number of DBM at the release site throughout the trial, DBM could form a greater proportion of the diet of predator species than in equivalent untreated fields. However as the inserted sequences are not known to express any toxic or allergenic proteins there is not anticipated to be an adverse impact on any organisms consuming transgenic DBM at the release site. Studies with predatory arthropods consuming other insects expressing the same genetic components found no adverse effects (Oreenaiza et al., 2013).

Population dynamics of decomposers at the release site could be affected by a greater number of dead male adult DBM; however decomposer organisms are often opportunistic, feeding on detritus when it is found. Biodiversity in soil ecosystems is generally high with a range of organisms assisting in the breakdown of organic matter. Complex interactions involving many species exist above and below ground, many of these species are microscopic and would be extremely difficult to monitor effectively. A number of decomposers could be involved in the breakdown of the DBM, including but not limited to organisms from classes of, Oligochaeta, Diplopoda, Isopoda, Nematodes, Collembola, Acari and Earthworms as well as species of Protozoa, Fungi and Bacteria. The release site that has been proposed will be a research facility where the crops are regularly treated with insecticides (e.g. Coragen® containing RynaXypyr®) which are more likely to result in damage to the soil invertebrate ecosystem, which could have a subsequent impact on the decomposer population.

Possibility for adverse effects on charismatic or protected species habitat's surrounding the release site that could be affected as a result of accidental release during transport, handling or storage.

The transgenic DBM will be mass reared at the facilities at Oxitec Ltd (UK) and shipped to the insectaries at Cornell University. The proposed release site will be managed agricultural land sown with brassica crops belonging to Cornell University however; usual movement through the agricultural land will be necessary for crop cultivation and release activities. Accidental release of the DBM during transport, handling or storage could occur however the small numbers of DBM sent in each shipment, the use of appropriate containment and labelling as required under CFR 340.7, together with the expression of the conditional lethal trait are expected to limit any adverse impact and prevent establishment of the moth in the environment. The inserted genetic traits encode a strong negative selection pressure which is not expected to enable persistence of the DBM in the environment. All activities are proposed on land owned by Cornell University. A review of the threatened and protected species which could interact with the DBM at the release site in Ontario and at the rearing facility in New York State has been conducted (See **Appendix VII**) and it is highly unlikely that any protected or charismatic species will be affected by the accidental or proposed release of the transgenic DBM. There is no species habitat overlap of the protected or charismatic species in the proposed trial site. In the event that an animal does consume the transgenic DBM, the novel genes have no homology to known toxins or allergens (Goodman, 2013).

In comparison to the current control systems to suppress the wild DBM population at the release site (broad-spectrum insecticides), the interactions between the transgenic DBM and predators or parasitoids are not likely to result in harm to biodiversity or charismatic or key species.

Role of the DBM in ecosystem services

DBM are consumers, larvae feed on the leaves of brassica plants and adults feed on water drops or dew. DBM are not significant decomposers in a habitat and at the proposed release site in New York State, they seasonally invade from southern US states such as Georgia and Maryland. Within the US the DBM are an historically invasive pest species arriving from Europe to Illinois in 1854 (Knodel and Ganehiarachchi, 2008).

Conclusion

The mating competitiveness of the transgenic DBM and the penetrance of the lethality trait are anticipated to enable a species-specific population control with limited non-target impact. The likelihood of the released transgenic DBM having an adverse impact on non-target organisms is considered to be "highly unlikely" whilst the consequence of any interaction is considered "marginal". In conclusion, the likely risk of harm to populations of charismatic, protected species or key species and biodiversity at the release site is considered to be "negligible".

2.4.8 Effect of the transgenic DBM on human and animal health

This assessment has been used to establish whether there are any unintended changes that have occurred in the modified DBM as a result of the genetic engineering which could increase the DBM's potential to harm human or animal health.

DBM adults produce scales on their wings which can lead to respiratory problems. Insect scales are known allergen within the laboratory and current operating procedures in the insectaries at Oxitec Ltd (UK) require all staff to wear masks (half-mask respirator of approved European Standard EN140:1998) whilst working with the adult moths. The adult moths are kept separate from the non-allergenic juvenile life stages to limit worker exposure to the moths. The increased rearing which will be required at Cornell University to provide sufficient DBM for the studies will be reviewed in the insectaries at Cornell however good lab practises and use of personal protective equipment are typically sufficient to prevent any damage or harm to worker health.

Evaluation of transgenic DBM with its non-transgenic comparator have identified no biologically relevant differences except the expression of the inserted transgenes, and a small overall fitness penalty resulting from the intended trait expression and its adaptation to laboratory rearing conditions.

Consequently the risk assessment has focused on the potential toxic or allergenic effects of the inserted proteins in comparison to the use of the commonly used insecticide DuPont™ Coragen® Insect Control with the active ingredient RynaXypyr®. As invertebrate species are also in the Animalia Kingdom, consideration has been given to the impact of the release on other invertebrate species. However this has been comprehensively assessed in the non-target organism (NTO) section and therefore will not be addressed again here. The conclusion from the NTO section of this risk assessment was that potential harms were negligible given the temporal and spatial limitations of the trial.

The scope of this application covers a time-limited environmental release at a University field station however a scenario where wider environmental exposure to transgenic DBM through accidental release in addition to the proposed release has been considered. The hypothesis that the DBM at the release site will result in no harm to human or animal health has been reviewed.

Toxicity and allergenicity of the inserted genetic traits

Neither the transgenic DBM nor any parts of the inserted trait are anticipated to have any toxic or allergenic impact in isolation. The conditional lethality trait works via a tTAV system which elicits cell death is a result of a build-up of proteins within the cells of larvae, this is known as transcriptional squelching (Lin et al., 2007). A study commissioned by Oxitec Ltd used bioinformatics to study the homology between the tTAV and known allergens and known toxins. Using internationally recognised techniques (Codex Alimentarius assessment criteria for allergenic potential), no homology between the tTAV to any allergen or toxin was found (Goodman, 2013). tTA and its variants have been widely used in a large number of mammalian and other animal systems, with over 10,000 papers published on its use[8] without adverse effects.

As stated previously in section 2.4.2., the expressed DsRed2 marker protein has been has been evaluated for human food and animal feed safety in an Early Food Safety Evaluation by FDA- CFSAN, and the weight of evidence developed showed that the protein was unlikely to be allergenic using bioinformatics comparisons of the amino acid sequence of the DsRed2 protein with known or putative protein allergen sequences; evaluation of the stability of the DsRed2 protein using an in vitro gastric digestion model; and assessment of the DsRed2 gene source and history of use and exposure. Further evaluations in the assessment for food safety used bioinformatics to show that the marker showed no homology to any known toxin

[8] http://www.tetsystems.com/science-technology/highlighted-publications/ (Accessed 12 December 2012)

protein, additional information supporting the lack of toxicity to the DsRed2 protein is implied by is homology to the Green Fluorescence Protein (GFP) and the GFP's safety assessment which concluded that when ingested by rats it was non-toxic (Goodman, 2013; Pavely and Fedorova, 2006).

Due to cloning mechanisms in the strain development, there has been the unintended addition of 5 amino acids (3 on one side of the DsRed2 insertion and 2 on the other side, between the protein and the nuclear localisation site). The function of the DsRed2 protein has not been impaired by these additional amino acids and there is no reason to suppose that any adverse unintended effects might arise from the presence of these additional amino acids.

Toxicity of the comparator: Chemical control product Coragen®

DuPont™ Coragen® Insect Control with the active ingredient RynaXypyr® contains the active ingredient chlorantraniliprole. Chlorantraniliprole is a compound in a new class of chemistry, the anthranilic diamides, IRAC Group 28, which controls the economically important pest class Lepidoptera. Coragen® is approved for use on brassicas, curcurbits, fruiting and leafy vegetables along with potatoes. Coragen® can translocate into the leaf following surface application which offers long-lasting crop protection.

RynaXypyr® (*aka* chlorantraniliprole) is the technical active ingredient in Coragen®. RynaXypyr® functions via activation of insect ryanodine receptors (RyRs) where is disrupts calcium channels and prevents muscle movements.

Coragen® works against Lepidoptera species, but can also be used against Coleoptera, Hemiptera and Isoptera. This insect control product has been demonstrated to have a low to no impact on honeybees and bumblebees when exposed to spray applications although it is typically advises that the pesticide is sprayed to avoid direct application whilst bees are foraging.[9]

tTAV toxicity and potential allergenicity assessment: the potential toxicity of the tTAV protein was assessed using a bioinformatics study with the amino acid sequence and publically available protein sequences. The study report is appended (**Appendix VIII**). Potential toxicity was evaluated by comparison of the amino acid sequences of the TetR N-terminal (208 amino acids) and the VP16 C Terminal 129 amino acids against the NCBI database using BLAST and keyword search query limits ("toxic" or "toxin") on 10 August 2013. Sequence matches were identified on the N-terminal side that matched TetR sequences of endogenous genes of bacteria or synthetic genes of recombinant constructs. The most significant match was to the TetR protein of *Escherichia fergusoni,* which is a true homologue of Tn10. The sequence is tagged as a toxin because it is from a toxic organism, however no data could be identified to demonstrate that the protein itself is toxic.

The same study (**Appendix VIII**) evaluated the allergenic potential of tTAV protein. There is no *a priori* reason to believe that tTAV is an inherently allergenic protein, and no evidence of

[9] http://www2.dupont.com/Production_Agriculture/en_US/assets/downloads/pdfs/K-14833.pdf (Accessed 02/04/2014)

amino acid sequence homology has been identified between tTAV and allergenic proteins from a literature search in PubMed.

Against this background there are two questions that emerge:

1. *Does the tTAV protein have a degree of homology with proteins that are known to be allergenic*
2. *If tTAV were found to have allergenic potential, would exposure to the OX4319L-Pxy moth represent a greater risk to human health exposure to an existing moth?*

A systematic bioinformatic analysis conducted by Dr Rick Goodman, a leading expert on allergenicity of genetically modified products based at the University of Nebraska, USA, using an internationally recognised approach (Codex Alimentarius) found no evidence of a level of homology between tTAV and any known protein allergens that would signal a potential hazard. His analyses included:

- an overall BLAST search against NCBI public sequence database using BLASTP algorithm and no query limit to identify any similar sequences known to be associated with allergenic organisms or obviously associated with allergens. All alignments identified with tetracycline controlled regulatory elements or their components.
- a BLAST search against the NCBI public sequence database using the keyword limit of " allerg" and looking for statistically significant overlaps with identified protein allergens or possible allergens. All alignments either identified with tetracycline controlled regulatory elements or was linked to author laboratory affiliation rather than identification of allergenic sequences.
- Consideration of homology with the allergenic salivary protein from *Aedes albopictus* mentioned above.
- Potential for IgE cross-reactivity with similar proteins; The current internationally accepted paradigm is that the threshold for a level of homology that might be relevant for cross-reactivity is 35% amino acid identity over any stretch of an 80 amino acid sequence (Codex, 2003). This is a very conservative guideline, but will probably identify nearly every protein that is sufficiently similar. The complete sequence of the tTAV protein was used to search the allergenic sequences of Version 11 of the Food Allergy Research and Resource Program (FARRP) Allergenonline.org[10] database, the only public, peer reviewed allergen database available for safety evaluation, containing over 1491 protein sequences. In the report cited above and appended **(Appendix VIII)**
- two matches were identified using the FASTA routine;
- Tropomyosin from *Neptunea polycostata (a gastropod)* with an identity score of 22% in an 181 amino acid overlap

[10] http://www.allergenonline.org (accessed 22 Jan 2013)

- Salivary protein of mosquito *Aedes albopictus* with an identity score of 27.5% in a 102-amino acid overlap.

Neither match suggests a risk of cross-reactivity. A second test used the conservative criteria of >35% identity over any 80 amino acid section. No matches were identified demonstrating lack of probable cross-reactivity to any known allergens. A further analysis was conducted using the precautionary search for any match of any eight (8) amino acid segment to any known allergen in the Allergenonline database, which was also negative.

The amino acid sequence of tTAV is given in **Figure 2** below:

```
>tTAV
MGSRLDKSKVINSALELLNEVGIEGLTTRKLAQKLGVEQPTLYWHVKNKRALLDALAIEM
LDRHHTHFCPLEGESWQDFLRNNAKSFRCALLSHRDGAKVHLGTRPTEKQYETLENQLAF
LCQQGFSLENALYALSAVGHFTLGCVLEDQEHQVAKEERETPTTDSMPPLLRQAIELFDH
QGAEPAFLFGLELIICGLEKQLKCESGSGPAYSRARTKNNYGSTIEGLLDLPDDDAPEEA
GLAAPRLSFLPAGHTRRLSTAPPTDVSLGDELHLDGEDVAMAHADALDDFDLDMLGDGDS
PGPGFTPHDSAPYGALDMADFEFEQMFTDALGIDEYGG
```

Figure 2. Amino Acid Sequence of the tTAV protein.

These results together indicated that additional testing was not required to evidence possible cross-reactivity as no hazard was identified.

DsRed2 is a marker protein which is expressed constitutively in all life stages of the OX4319L-Pxy strain. DsRed is a naturally occurring fluorescent protein which was originally found in various *Discosoma spp.* DsRed2 was artificially developed from DsRed through a combination of random and site directed mutagenesis in order to enhance the fluorescence and improve the solubility, which in turn increases the sensitivity of detection (Bevis and Glick, 2002; Ip and Wan, 2004; Lukyanov et al., 2000; Matz et al., 1999; Shagin et al., 2004). The improvement to the solubility of DsRed2 has been achieved through reducing its tendency to form aggregates; these mutations also reduce the time from transfection to detection to only 24 hours. DsRed2 has the benefit of high signal to noise ratio and due to the distinct spectrum in which it fluoresces it can for use in multicolor labelling and can be used in combination with Enhanced-GFP (CLONTECHniques, 2001). The DsRed2 protein is 97% identical to the original DsRed protein isolated from *Discsoma* species, with 6 amino acid changes (**Figure 3**).

MASSENVITE FMRFKVRMEG TVNGHEFEIE GEGEGRPYEG HNTVKLKVTK
GGPLPFAWDI LSPQFQYGSK VYVKHPADIP DYKKLSFPEG FKWERVMNFE
DGGVATVTQD SSLQDGCFIY KVKFIGVNFP SDGPVMQKKT MGWEASTERL
YPRDGVLKGE THKALKLKDG GHYLVEFKSI YMAKKPVQLP GYYYVDAKLD
ITSHNEDYTI VEQYERTEGR HHLFL

Figure 3. DsRed2 amino acid sequence

DsRed2 Potential Toxicity and Allergenicity Assessment:

As stated previously in Sections 2.4.2. and 2.4.8, of this document the DsRed2 marker protein has been evaluated in a New Protein Evaluation by FDA-CFSAN for human food and animal feed safety. CFSAN raised no objections and had "no additional questions" to the conclusion reached by the submitted, Pioneer Hi-Bred International to their conclusion of its safety when used as a selectable marker in food plant. Additional information on the lack of toxicity of DsRed2 is given in Section 15 of this document, including oral studies in rats (Richards et al., 2003). Additionally, as presented in Section 2.4.2 of this document, DsRed2 has been further evaluated in an Environmental Assessment (EA) by the United States Dept of Agriculture[11] which concluded that the corn transformation event that contained the DsRed2 gene was unlikely to become a plant pest risk. Additional EA's on GE pink bollworm expressing fluorescent genes similar to DsRed2 have also been conducted (USDA, 2001)[12] and concluded that it was unlikely to present any hazard to the environment.

Conclusion

The bioinformatics testing of both the tTAV and the DsRed2 protein provide evidence that the lethality of the proteins produced is not as a result of any toxic activity of the protein. Consequently the likelihood of adverse direct or indirect effects from the release of OX4319L-Pxy on other species is considered to be "highly unlikely".

A weight of evidence argument has been presented that indicates the proteins expressed by the inserted rDNA construct in OX4319L-Pxy strain are not intrinsically toxic and are non-toxic to other organisms, therefore the consequence of environmental exposure to the transgenic DBM is considered to be "marginal". However it is the specific and intended effect of the insertion of the rDNA construct for release OX4319L-Pxy and female progeny of matings with male OX4319L-Pxy will die due to over-expression of the tTAV protein and the disruption of the cellular transcriptional activity, in the absence of suitable concentrations of tetracycline or its analogues. Overall the risk associated with the release of the transgenic DBM on human and animal health is considered to be "negligible".

2.4.9 Impacts on the specific cultivation, management and harvesting techniques

The proposed release site is a field research site belonging to Cornell University. The specific management techniques on the field used for evaluation of the transgenic strain would not differ from the practices usually carried out when growing brassica. The field would initially be prepared by spraying of an herbicide, the crop plant would then be planted, traditional hoeing would be implemented if necessary to reduce disturbance to the release site. Insecticides would only be used in the event that the population of DBM increased substantially or the transgenic DBM persisted beyond the length of the trial or spread beyond the range of the field site. The proposed insect control product Coragen® containing RynaXypyr® is currently approved for field use in New York State and will be used at the end of each experiment to devitalize any persisting transgenic DBM. Overall fewer insecticides are expected to be used at the release site than is currently advised for the growing of brassica.

[11] http://www.aphis.usda.gov/brs/aphisdocs/08_33801p_dpra.pdf (Accessed 19 March 2013)

[12] http://www.gpo.gov/fdsys/pkg/FR-2006-04-19/html/E6-5878.htm (Accessed 14th March 2013)

Conclusion

There is not expected to be any negative impact on the cultivation, management and harvesting techniques at the proposed field station therefore the consequence of the release of the transgenic DBM is considered "marginal". There is also the likelihood of a positive outcome as a result of the field trial it is likely that fewer insecticides could be used on the field. Overall the likelihood of an adverse impact occurring as a result of the trial is "highly unlikely" and the risk estimate for adverse impacts on the specific cultivation, management and harvesting at the release site is considered to be "negligible".

2.4.10 Effects on chemical (Pesticide, herbicide, fungicide) load on the environment

Overall the use of insecticides in the environment is likely to be at a lower level throughout the course of these trials than insecticide levels which are currently permitted for the growing of brassica crops in New York State. Coragen® containing RynaXypyr® is a permitted insecticide that maybe used against lepidopteron pests, we propose to only apply this insect control treatment twice at the end of any open release (end of the MRR (year 1) at the end of the field cage experiments and any subsequent open releases such as a range finder).

Insecticides will also be used in the event of inadvertent/accidental release of the transgenic DBM either at the rearing facility or in transit to the field site. In the unlikely event that the transgenic DBM spreads beyond the trial site this is expected to be detected by the sex-pheromone baited sticky traps set up at 1000 m boundaries about the field site. Reapplication of Coragen® containing RynaXypyr® across the field site extending to the 1000 m boundary will be carried out; no overwintering of any DBM is expected in New York State.

Conclusion

Overall the environmental exposure to chemical insecticides at the field site is expected to be lower than is typical for a brassica field site in New York State therefore likelihood of the chemical load increasing is "highly unlikely". The consequence of the use of chemical insecticides at the release site on the environment is likely to be "marginal" when this use is compared to the control of the wild type DBM, no increase in use compared to conventional control of the DBM is expected. There is therefore no increased environment risk from the use of insecticide throughout the proposed experiments and the overall risk estimate is considered "negligible".

3 OVERALL CONCLUSION

An application for field evaluation of the transgenic DBM strain OX4319L-Pxy which has been developed at Oxitec Ltd., for use as a pest control tool has been requested. This strain has been characterized under laboratory conditions at Oxitec and further tested in greenhouse trials both at Rothamsted research facilities in the UK and Cornell University in the United States.

This application has outlined a program of study lasting for three years which includes three specific field seasons for the strain evaluation from April – October, over brassica crops at a field site at Cornell University.

This environmental risk assessment has evaluated the potential for adverse effects to arise in the environment at or surrounding the release site as a result of the release of up to 100,000 transgenic DBM males per week at the release site.

Key issues in this environmental assessment have been considered and the risks attributed to these scenarios evaluated, summaries of the relevant conclusions for risk have been outlined.

The DBM are not expected to have an adverse impact or longer persistence than the unmodified DBM at the release site. The likelihood of increased crop damage is considered to be negligible.

The background strain into which the transgenes were inserted has been lab reared for over 15 years in the absence of pesticides, therefore levels of pesticide resistance which is common in wild DBM populations is not likely in the transgenic DBM. The likelihood of increased pesticide resistance or introgression of resistance traits in the wild population is low to negligible, and release of the lab strain with the low levels of insecticide resistance might introgress susceptible alleles into the population instead, potentially conferring a benefit (Alphey et al., 2007).

The mating competitiveness of the transgenic DBM and the penetrance of the lethality trait are anticipated to enable a species-specific population control with limited non-target impact. We have determined it is likely that the risk of harm to populations of charismatic, protected species or key species and biodiversity at the proposed release site is negligible. The expressed novel genes, DsRed2 and tTAV are not known to be toxic or allergenic bioinformatics testing of both the tTAV and the DsRed2 protein provide evidence that the lethality of the proteins produced is not as a result of any toxic activity of the protein. Further evidence is obtained from the use of these traits in different insect species in predator arthropod feeding studies at 100% of diet with no negative consequences observed. Consequently no direct or indirect effects from the release of OX4319L-Pxy are anticipated on other species and there is not anticipated to be an increased likelihood of harm arising from consumption of a transgenic DBM in comparison to a wild DBM.

There is not expected to be any impact on the cultivation, management and harvesting techniques at the proposed field station. The release site is a field station which is frequently used for research purposes therefore the impact of a release of wild DBM or transgenic DBM is likely to have a low to negligible impact. Overall the environmental exposure to chemical insecticides at the field site is expected to be lower than is typical for a brassica field site in New York State. There is therefore no increased environment risk from the use of insecticide treatment programs throughout the proposed experiments.

In addition to the proposed control methods to devitalize the transgenic DBM it is important to note that there is no overwintering of DBM populations at the release site (Average low temperatures in Ithaca, NY, are at or below freezing from November – March on an annual basis) http://www.weather.com/weather/wxclimatology/monthly/USNY0717. Therefore, DBM only survives in New York State in summer months and reinvades from southern states annually.

4 REFERENCES

(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.

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.

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.

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).

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.

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*.

Harris, A.F., McKemey, A.R., Nimmo, D., Curtis, Z., Black, I., Morgan, S.A., Oviedo, M.N., Lacroix, R., Naish, N., Morrison, N.I., *et al.* (2012). Successful suppression of a field mosquito population by sustained release of engineered male mosquitoes. Nat Biotechnol *30*, 828-830.

Harris, A.F., Nimmo, D., McKemey, A.R., Kelly, N., Scaife, S., Donnelly, C.A., Beech, C., Petrie, W.D., and Alphey, L. (2011). Field performance of engineered male mosquitoes. Nat Biotechnol *29*, 1034-1037.

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

Koukidou, M., Klinakis, A., Reboulakis, C., Zagoraiou, L., Tavernarakis, N., Livadaras, I., Economopoulos, A., and Savakis, C. (2006). Germ line transformation of the olive fly Bactrocera oleae using a versatile transgenesis marker. Insect Mol Biol *15*, 95-103.

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.

Labbé, G.M.C., Nimmo, D.D., and Alphey, L. (2010). *piggyBac*- and PhiC31-Mediated Genetic Transformation of the Asian Tiger Mosquito, *Aedes albopictus* (Skuse). PLoS Negl Trop Dis *4*, e788.

Lacroix, R., McKemey, A.R., Norzahira, R., Lim, K.W., Wong, H.M., Teoh, G.N., Siti Rahidah, A.A., Sawaluddin, S., Selvi, S., Oreenaiza, N., *et al.* (2012). Open field release of genetically engineered sterile male *Aedes aegypti* in Malaysia. PLoS ONE *7*, e42771.

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.

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.

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 (Pioneer: DuPont).

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.

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.

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.

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.

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.

APPENDIX I

Please see OX4319L-Pxy population suppression cages 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

APPENDIX III

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

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

Tetracycline in the environment

April 2014

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 Ganehiarachchi, 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 genes introduced into genetically engineered mosquitos (*Aedes aegypti*), strain OX513A for production of sterile males to reduce vector transmission of important human diseases

Authors

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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 OX513A

5.0 Conclusions

No convincing evidence was found to suggest that the DsRed2 protein or the tTAV protein expressed in the OX513A mosquitos represent risks of allergy or toxicity to humans (or other mammals). Based on the guidelines of the Codex Alimentarius Commission (2003 and 2009), and on common practices for evaluation of potential risks of allergy or toxicity from GMO (plants, animals or microbes), there is no reason to perform additional tests to evaluate potential risks of allergy or toxicity for these proteins. Although the guidelines are intended primarily evaluating potential food safety concerns regarding potential risks from genetically engineered organisms, the same safety evaluation process is scientifically sound as an approach for evaluating other potential routes of exposure, namely via airway (inhalation of insect body parts) or through insect bites (e.g. mosquito saliva). There is no evidence that these proteins pose any risk of eliciting allergic or toxic reactions.

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 Tet repressor alters the 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 neurotoxin A engineered for 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 genetically modified OX513A 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 in human glioma cells 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 and functional genomics. *Plant Physiol* 150(3):1111-1121.

Codex Alimentarius Commission. (2003). Alinorm 03/34: Joint FAO/WHO Food Standard Programme, Codex Alimentarius Commission, Twenty-Fifth Session, Rome, Italy 30 June-5 July, 2003. Appendix III, Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants and Appendix IV, Annex on the assessment of possible allergenicity, pp. 47-60.

Debarry J, Hanuszkiewicz A, Stein K, Holst O, Heine H. (2010). The allergy –protective properties of *Acinetobacter Iwoffii* F78 are 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 higher plants. *Biotechniques* 32(2):286-293.

Dutkiewicz J, Skorska C, Krysinska-Traczyk E, Cholewa G, Sitkowska J, Mianowski J, Gora A. (2002). Precipitin responses 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, Marinotti O, Jasinskiene N, James AA, Alphey L. (2010). Female-specific flightless phenotype for mosquito control. *Proc Natl Acad Sci* USA 107(10):4550-4554.

Gong P, Epton MJ, Fu G, Scaife S, Hiscox A, Condon KC, Condon GC, Morrison NI, Kelly DW, Dafa'alla T, Coleman PG, Alphey L. (2005). A dominant lethal genetic system for autocidal control of the Mediterranean fruitfly. *Nat Biotechnol* 23(4):453-456.

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

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

Goodman RE, Vieths S, Sampson HA, Hill D, Ebisawa M, Taylor SL, van Ree R. (2008). Allergenicity assessment of genetically 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 promoters. *Proc Natl Acad Sci USA* 89:5547-5551.

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

Jadhav S, Sahasrabudhe T, Kalley V, Gandham N. (2013). The microbial colonization profile of respiratory devices and the 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 trafficking using a histone fluorescent protein 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 cdc42 video imaged by fluorescent resonance energy transfer-based single-molecule probes in the membrane of living cells. Mol Cell Biol 22(18):6582-6591.

Lacroix R, McKemey AR, Raduan N, Kwee Wee L, Hong Ming W, Guat Ney T, Rahidah AAS, Salman S, Subramaniam S, Nordin O, Hanum ATN, Angamuthu C, Marlina Mansor S, Lees RS, Naish N, Scaife S, Gray P, Labbe G, Beech C, Nimmo D, Alphey L, Vasan SS, Lim LH, Wasi AN, Murad S. (2012). Open field release of genetically engineered sterile male *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 with CFP or GFP for quantitatively imaging induced activation of Rac, Cdc42 in living cells. *Mol Imaging Biol* 13(3):424-431.

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

Long JZ, Lackan CS, Hadjantojnakis AK. (2005). Genetic and spectrally distinct in vivo imaging: embryonic stem cells and mice with widespread expression of a monomeric red fluorescent protein. BMC Biotechnol 5:20 doi:10.1186/1472-6750-5-20.

Matz MV, Fradkov AF, Labas YA, Savitsky AP, Zaraisky AG, Markelov ML, Lukyanov SA. (1999). Fluorescent proteins 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 DsRed-E5 as a 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, Mori K, Yamaguchi M. (2011). Compensation of depleted neuronal subsets by new neurons in a local area of the adult olfactory bulb. J Neurosci. 31(29):10540-10557.

Nishihara K, Kanemori M, Ktagawa M, Yanagi H, Yura T. (1998). Chaperone coexpression plasmids: differential and synergistic roles of DnaK-DnaJ-GrpE and GroEL-GroES in assisting folding of an allergen of Japanese cedar pollen, Cryj2 in *Escherichia coli*. App. Environ Microbiol 64(5):1694-1699.

Nordin O, Donald W, Ming WH, Ney TG, Mohamed KA, Halim NA, Winskill P, Hadi AA, Muhammad ZS, Lacroix R, Scaif S, McKemey AR, Beech C, Shahnaz M, Alphey L, Nimmo DD, Nazni WA, Lee HL. (2013). Oral ingestion of transgenic RIDL *Ae. aegypti* larvae has no negative effect on two predator Toxorhynchites species. PLOS One 8(3):e58805, doi 10.1371/journal pone.0058805.

Phuc HK, Andreasen MH, Burton RS, Vass C, Epton MJ, Pape G, Fu G, Condon KC, Scaife S, Donnelly CA, Coleman PG, White-Cooper H, Alphey L. (2007). Late-acting dominant lethal genetic systems and mosquito control. *BMC Biol* 20:5-11.

Qiu H, Kuolee R, Harris G, Zhou H, Miller H, Patel GB, Chen W. (2011). *Acinetobacter baumannii* infection inhibits airway eosinophilia and lung patrhology in a mouse model of allergic asthma. PLOS One 6(7):e22004. Doi 10.1371/journal.pone.oo22004.

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

Ryu JY, Siswanto A, Harimoto K, Tagawa YI. (2013). Chimeric analysis of EGFP and DsRed2 transgenic mice demonstrates 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, Iwaki M. (2012). *Corynebacterium ulcerans* 0102 carries the gene encoding diphtheria toxin on a prophage different from the *C. diptheriae* 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. (1997). Protein interactions in the *Herpes simplex* virus type 1 VP16-induced complex: VP16 peptide inhibition and mutational analysis of host cell factor requirements. J Virol 71(5):3886-3894.

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

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

Tawfik VL, Regan MR, Haenggeli C, LaCroix-Fralish ML, Nutile-McMenemy N, Perez N, Rothstein JD, DeLeo JA. (2008). Propentofylline-induced astrocyte modulation leads to alternations in glial glutamate promoter activation following spinal nerve transection. *Neuroscience* 152(4):1086-1092.

Teterina NL, Levenson EA, Ehrenfeld E. (2010). Viable polioviruses that encode 2A proteins with fluorescent protein tags. *J Virol* 84(3):1477-1488.

Thompson SA, Maani EV, Lindell AH, King CJ, McArthur V. (2007). Novel tetracycline resistance determinant isolated from 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 ribosomal DNA in house dust mite cultures. J Allergy Clin Immunol 116(6):1296-1300.

Wenck A, Pugieux C, Turner M, Dunn M, Stacy C, Tiozzo A, Dunder E, van Grinsven E, Khan R, Sigareva M, Wang WC, Reed J, Drayton P, Oliver D, Trafford H, Legris G, Rushton H, Tayab S, Launis K, Chang Y-F, Chen D-F, Melchers L. (2003). Reef-coral proteins as visual, non-destructive reporters for plant transformation. *Plant Cell Reports* 22:244-251.

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

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