Oxitec Experimental Use Permit for OX5034 *Ae. aegypti*

ED_006741_00004899-00001

Summary of Oxitec's EUP proposal

Oxitec Ltd. proposed the following parameters for the EUP to evaluate efficacy of OX5034 mosquitoes to suppress populations of wild *Aedes aegypti* mosquitoes:

- Testing in FL and TX
- 6600 total acres
- Max. rate of 0.000056 g active ingredient (tTAV-OX5034)/acre/week, equivalent to 20,000 male OX5034/acre/wk
- 2 years of testing

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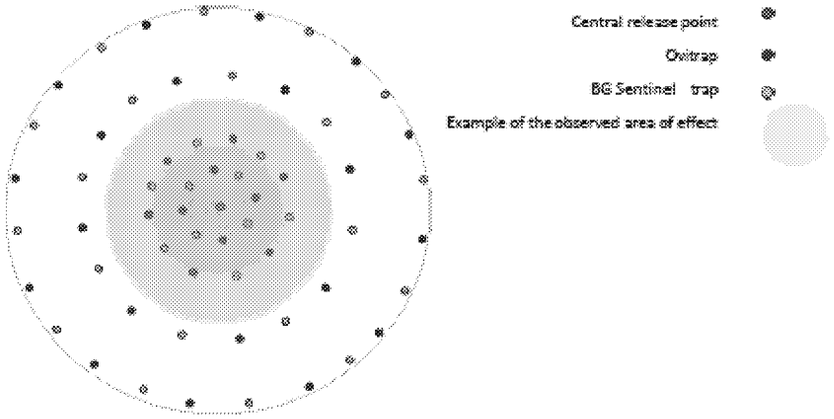
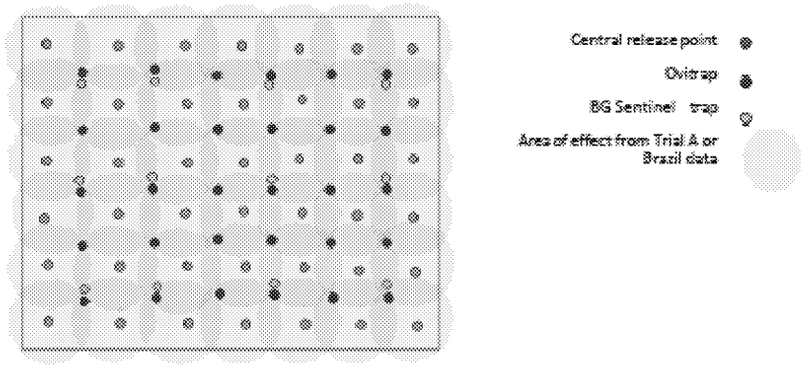
EUP experimental program summary

Two trials (Trial A and Trial B)

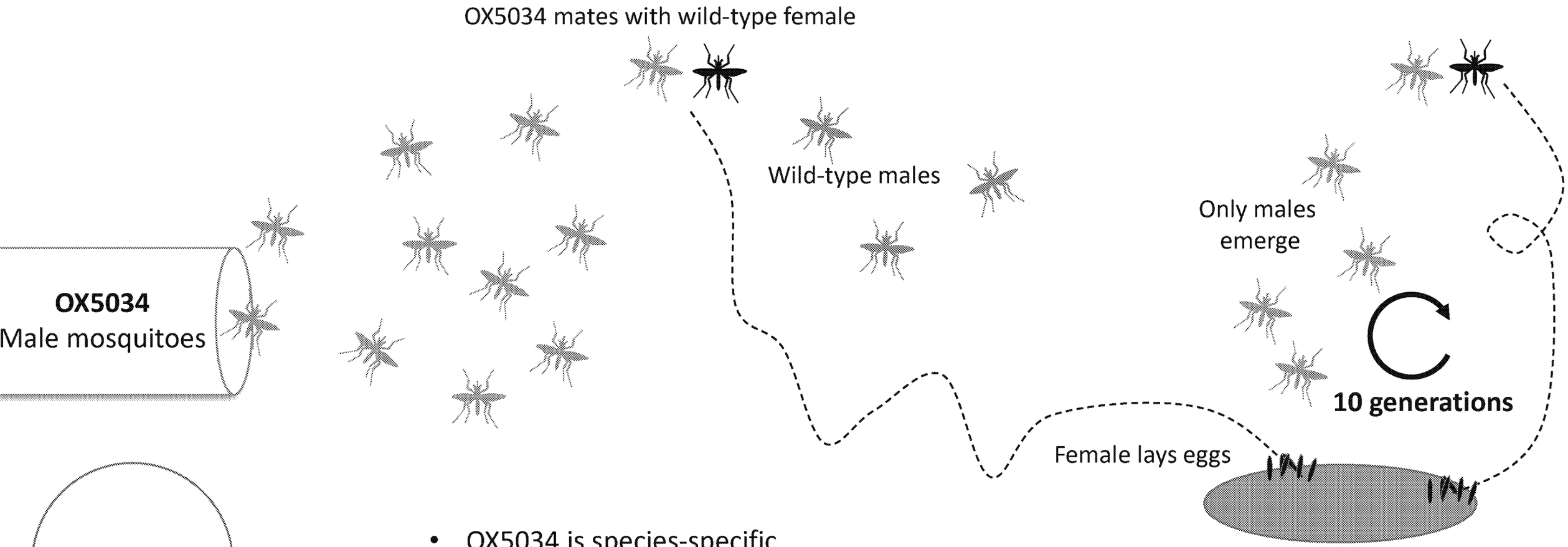
Trial A – single release point intended to measure dispersal to inform Trial B

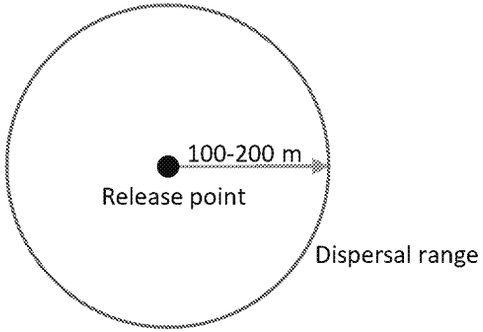
Trial B – multiple release point to evaluate efficacy, area covered by a single release point will be considered the mean/median distance found in trial A





Principles of Oxitec's OX5034 technology



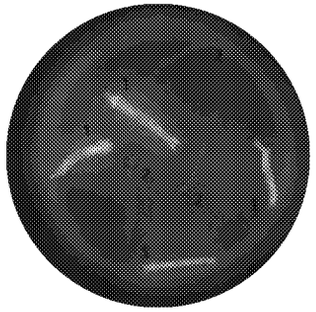


- OX5034 is species-specific
- Results in emergence of all-male offspring

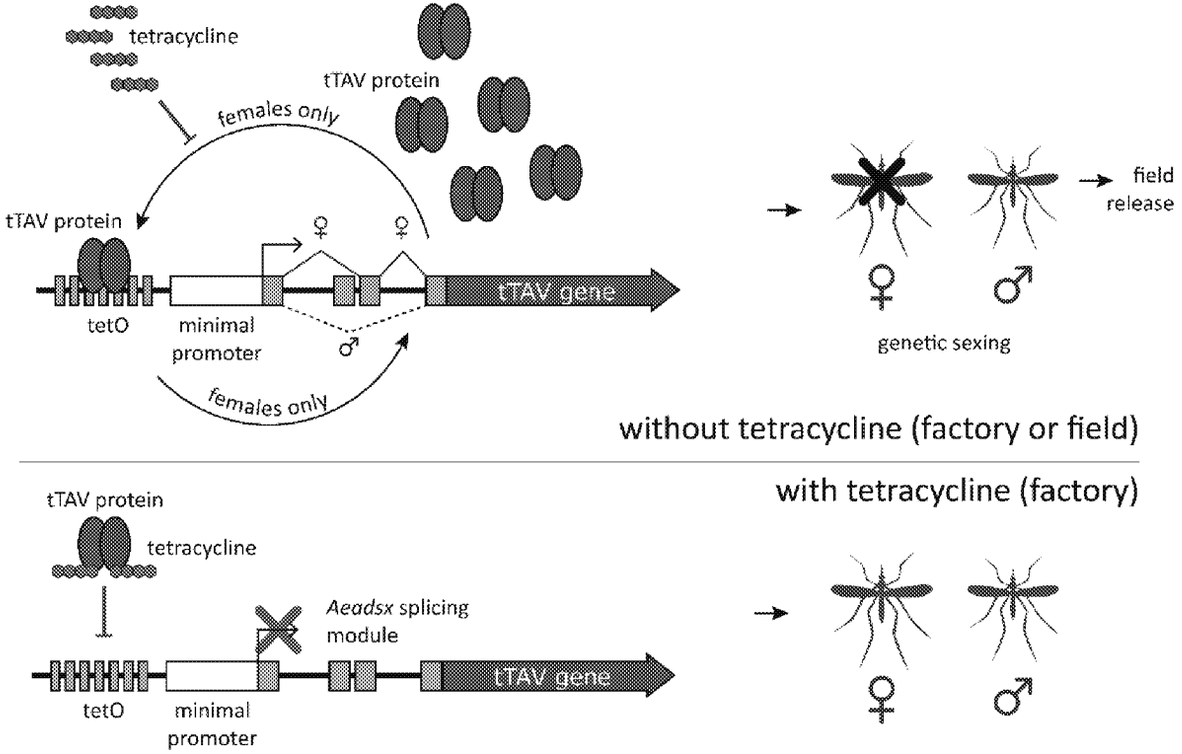
Principles of Oxitec's OX5034 technology

tTAV-OX5034 (active ingredient)
is selectively expressed in female *Ae. aegypti*
in absence of tetracycline

DsRed2-OX5034 (inert ingredient)
is expressed in both males and females



Fluorescence used to identify individuals collected from the test areas with OX5034 genetic construct.



Risk assessment

EPA evaluated likelihood of exposure to female OX5034

Penetrance for the OX5034 mosquitoes refers to proportion of female insects that die before reaching adulthood (i.e., does it consistently work).

Two components of evaluation to assess penetrance of lethal trait:

1. Laboratory and field studies confirming 100% female lethality
   - Reciprocal crosses between OX5034 and WT mosquitoes confirmed 100% female lethality. In these studies OX5034 and the WT strain originate from the same genetic background.
   - 100% female lethality found offspring of OX5034 mosquitoes and WT mosquitoes of a different genetic background.
2. Surveys of possible tetracycline sources in the environment
   - Literature survey indicates low likelihood of tetracycline levels high enough to result in female rescue.
   - 500 m restrictions around distance of release sites to wastewater treatment facilities and citrus groves to further reduce likelihood.

Risk assessment cont.

EPA evaluated the risk of OX5034 mosquitoes to human health

A determination of the toxicity and allergenicity of the two substances in Oxitec's OX5034 mosquitoes that 1) kill female mosquitoes, tTAV-OX5034, and 2) allow trained personnel to identify OX5034 via fluorescence, DsRed2-OX5034, has not been made.

However, because no OX5034 female mosquitoes are being released or are expected to emerge in the environment, exposure is negligible and therefore, so is the potential risk from tTAV-OX5034 and DsRed2-OX5034 (Risk = Exposure x Hazard).

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Risk assessment cont.

Rearing and shipping

OX5034 production (males and females) will take place in the UK. Only eggs shipped to FL and TX.

In US:

1) Packaging into egg release containers
2) Hatching and packaging of males for adult releases on foot and vehicle.

Insecticide susceptibility

OX5034 was shown to be susceptible to WHO recommended discriminating doses of the larvicide temephos and the three adulticides permethrin, deltamethrin, and malathion.

There is indication for OX5034 resistance to propoxur.

- Will not affect current mosquito control practices as propoxur uses for mosquito control were cancelled by EPA in 1997.

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Risk assessment cont.

Introgression of background genetics into the local mosquito population

EPA considered if releases of OX5034 mosquitoes were likely to increase the ability of wild mosquitoes in the release area to vector/transmit disease, result in larger populations numbers, or result in more robust mosquitoes. EPA found this impact is unlikely.

EPA collaborated with the US Centers for Disease Control and Prevention (CDC) in reviewing laboratory data, a meta-analysis, and rationale submitted by the applicant comparing the vectorial capacity of OX5034 mosquitoes to that of wild mosquitoes.

Persistence of the transgene in the local mosquito population

As hemizygous male offspring survive and go on to reproduce, EPA evaluated how long the transgene is expected to remain in the local mosquito population once releases have ceased.

Experimental caged populations confirmed modeling expectations of trait disappearance within 10 generations.

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Risk assessment cont.

EPA evaluated the risk of OX5034 mosquitoes to non-target organisms (bats, amphibians, etc.).

No direct adverse effects due to consumption of OX5034 males by non-target organisms is expected based on acute oral toxicity studies and bioinformatics analyses.

Ae. aegypti mosquitoes are not a sole or critical food source for non-target organisms, so no indirect adverse effects are expected should there be a decrease in the local mosquito population.

Overall risk assessment conclusion:

EPA determined that there will be no unreasonable adverse effects to humans or the environment as a result of the experimental permit to release Oxitec's OX5034 male mosquitoes.

EUP terms and conditions associated with the RA

1) Potential tetracycline sources: Releases must not occur within 500 meters of sewage treatment facilities and any farm producing citrus crops.

2) Genetic resistance monitoring: From reared field-collected individuals, Oxitec must confirm the absence of the genetic cassette in a minimum of 150 non-fluorescent adult female *Ae. aegypti* once per month.

3) Absence of arboviruses: If evidence is found of invasive *Aedes* spp. or arboviruses principally vectored by *Ae. aegypti* becoming established in the UK, colony related testing will be required.

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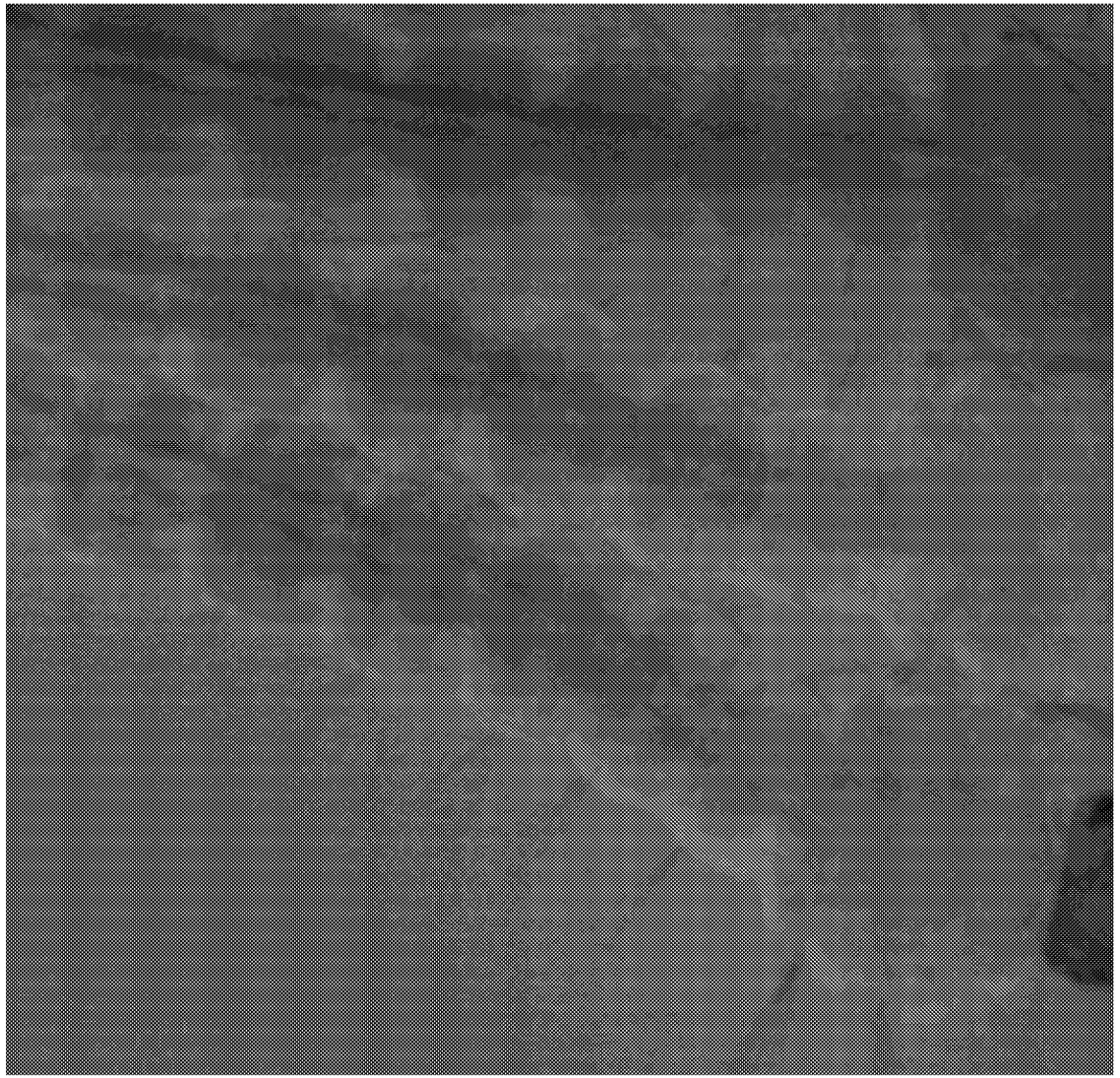
Trial measurements

Ovitrap

- Fluorescent female larval mortality
- The proportion of treated (i.e., fluorescent) individuals trapped (i.e., mating fraction)
- Persistence of the transgene post-release (i.e., distance the transgene disperses, duration of residual activity)

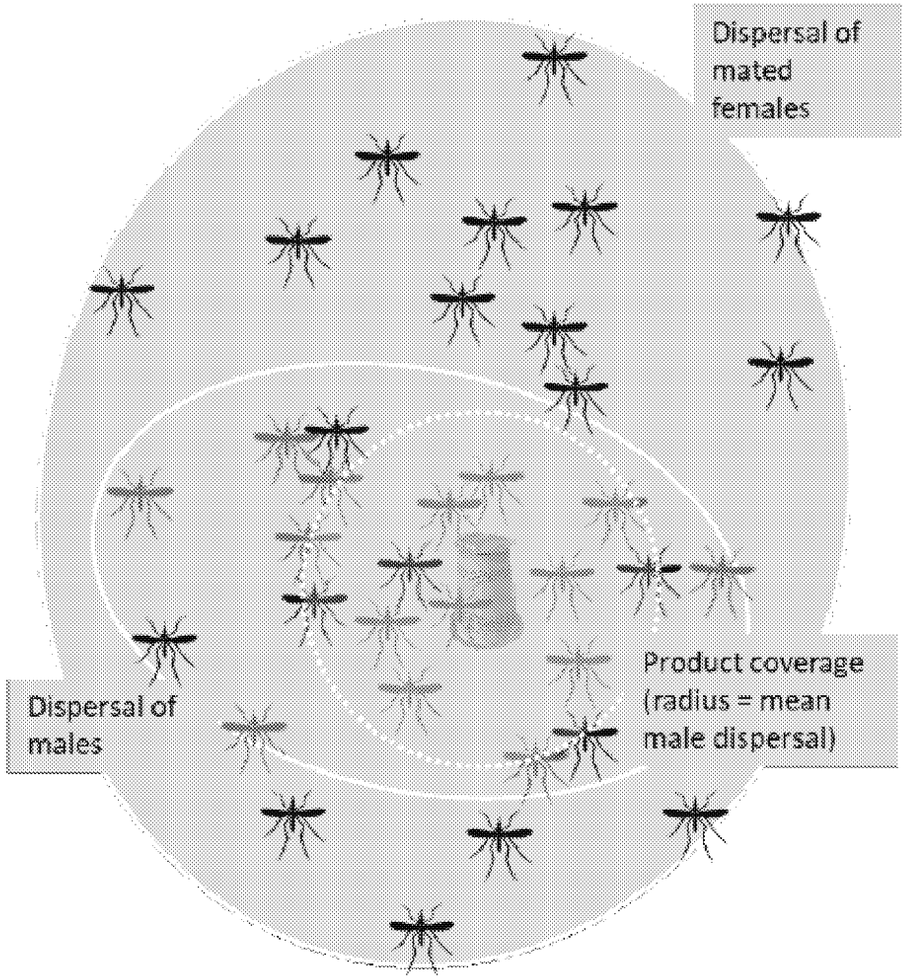
BG Sentinel (adult) traps

- Adult over-flooding ratio achieved (i.e., OX5034 males to wild males)
- *Trial A only:* Dispersal of OX5034 males in the field



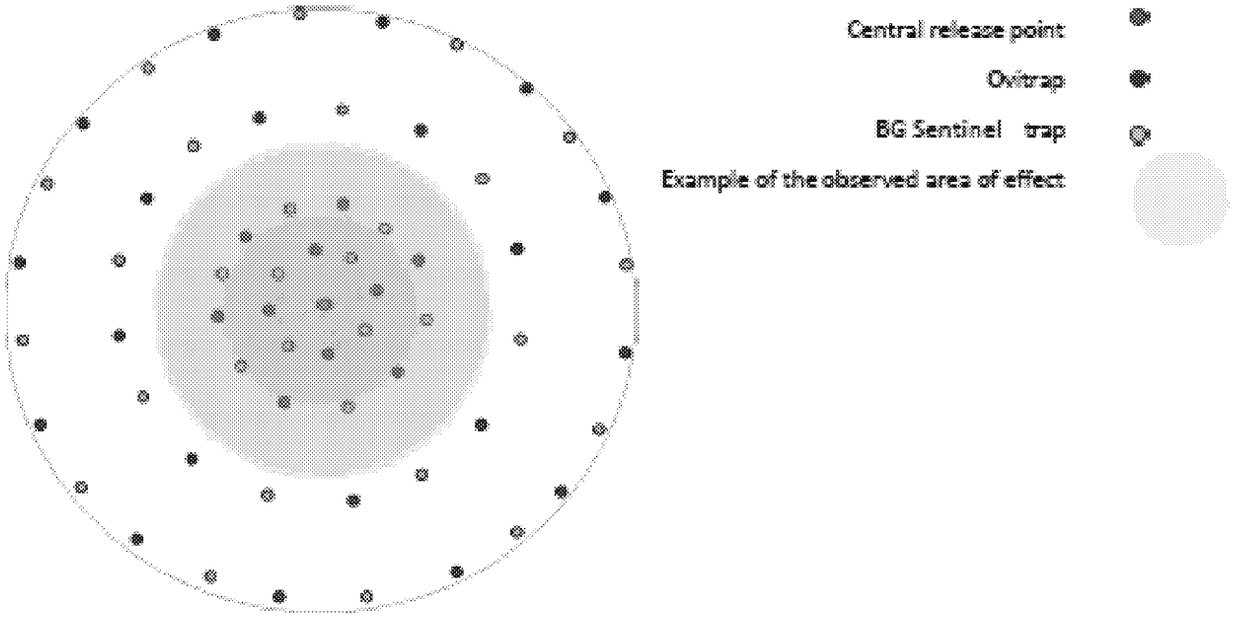
Quality control

- OX5034 mosquitoes and resulting offspring will be identified by fluorescent markers visible in all life stages.
- Eggs from ovitraps will be reared in laboratory settings to adult emergence and fluorescence will be confirmed. Additional PCR analysis will be conducted on a minimum of 40 fluorescent and non-fluorescent individuals to confirm accurate identification.
- Traps are checked every 7-9 days max
- Sites are separated by at least 500 meters



Trial A: single release

- **Objective**: quantify the trial parameters from a single release point
  - Measures efficacy as well as product coverage
- Max 20,000 males released/week. Either adults *OR* eggs
- Product coverage measurements will inform in Trial B



Trial	Control replicates	Low dose replicates	Medium dose replicates	High dose replicates (optional)	Max acreage per trial site	Maximum total acreage
Trial A	3	3	3	3	200	4800

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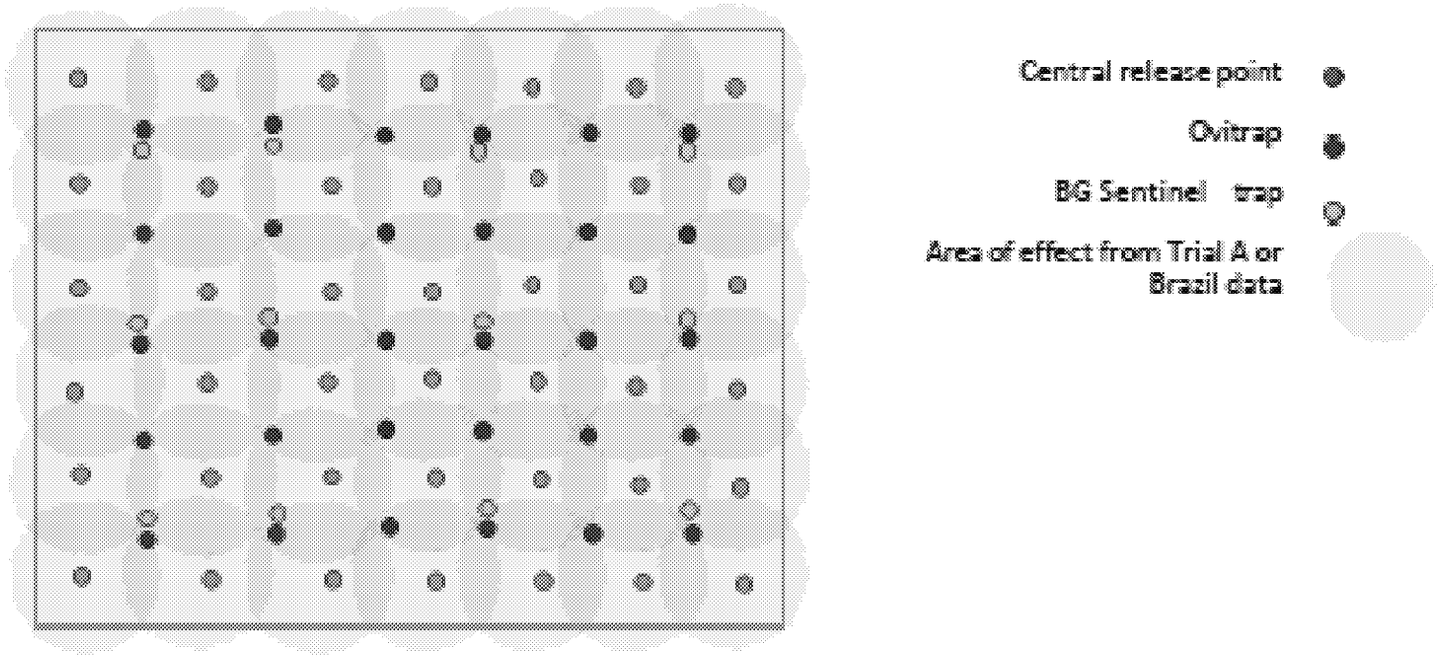
Trial B: multi-point

- **Objective**: quantify trial parameters from multiple release points
  - Test for efficacy within cryptic breeding sites
- Max 20,000 mosquitoes/point/week
- Eggs only; tests use of mosquito rearing boxes
- Low and medium dose trials



Trial B	Control replicates	Low dose replicates	Medium dose replicates	Max acreage per trial site	Maximum total acreage
	3	3	3	80	1440

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Risk management decision to approve EUP

Based on the experimental program submitted, this product may be shipped for use under this EUP to the states of Florida and Texas for the next experimental period, effective immediately until 04/30/2022. Post-release activities (e.g., data collection and post-release monitoring) may continue afterwards as necessary in accordance with the experimental program and terms and conditions below.

The acres and amounts permitted per state are as follows:

STATE	ACRES	NUMBER OF MOSQUITOES	POUNDS OF ACTIVE INGREDIENT (mg)
		YEAR April 30, 2020 – April 29, 2021	
Florida	3120	508,560,000	0.0046 (2,085.10 mg)
Texas	0	0	0
TOTAL (YEAR 2020-2021)	3120	508,560,000	0.0046 (2085.10 mg)
		YEAR April 30, 2021 - April 30, 2022	
Florida	3120	508,560,000	0.0046 (2,085.10 mg)
Texas	360	249, 600,000	0.0023 (1,023.36 mg)
TOTAL (YEAR 2021-2022)	3480	758,160,000	0.0069 (3,108.46 mg)
OVERALL PERMIT TOTAL	6,600	1,266,720,000	0.0115 (5193.56 mg)

Label

Directions for 2 methods of release

- Egg-rearing boxes – 20,000 eggs are placed in boxes and allowed to develop and males pupate and emerge as adults and disperse
- Adult release – adult male OX5034 mosquitoes are released

Max. release rate is 20,000 adult male mosquitoes/acre/week, minimum rate is 500 adult male mosquitoes/acre/wk

Restriction: “Do not release within 500 m of commercial citrus growing areas or sewage treatment plants.”

Terms and conditions for the EUP

1) Releases must not occur within 500 meters of sewage treatment facilities and any farm producing citrus crops.

2) Oxitec must conduct continuous weekly monitoring for fluorescent larvae at release sites as indicated in the section G experimental program (sections 5.2.6.1 and 5.9.4.1). From the reared field-collected individuals, Oxitec must determine the presence of the genetic cassette (vector pOX5034) in a minimum of 150 non-fluorescent adult female *Ae. aegypti* following the standard operating procedures QD-R-00109 or QD-R-00108 once per month. If at any time during the course of the EUP Oxitec finds female individuals containing the OX5034 genetic construct surviving to adulthood Oxitec must take the following remediation actions: immediately cease releases of all OX5034 mosquitoes, as soon as practicable apply adulticide and larvicide pesticides to the treated area where the surviving females were detected and continue to monitor for the presence of the OX5034 genetic construct in female *Ae. aegypti* until OX5034 mosquitoes are no longer found for at least two successive mosquito generations, a minimum of 10 weeks. EPA may require additional applications of adulticides and larvicides if fluorescent mosquitoes continue to be found in the treated area after the initial detection.

3) If evidence is found of invasive *Aedes* spp. or arboviruses principally vectored by *Ae. aegypti* becoming established in the UK, colony related testing will be required.

4) As indicated in the section G experimental program (sections 5.3.1 and 5.10), Oxitec must conduct post-release monitoring until no fluorescent OX5034 mosquitoes have been found for at least two successive generations, a minimum of 10 consecutive weeks.