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INTRODUCTION TO OXITEC 2ND GENERATION MOSQUITOES

TECHNOLOGY SUMMARY - ROLL BACK MALARIA VECTOR CONTROL WORKING GROUP - GENEVA– 30th Jan – 1st Feb, 2019

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Oxitec 2nd Generation Friendly™ Mosquito technology adds a variety of superior benefits over other traditional and novel solutions.

- ✓ First-ever solution that combines **superior suppression AND insecticide resistance reversal** in one application.
- ✓ First-ever solution to offer **self-limiting insects with multi-generational suppression**
- ✓ Multiple commercial applications, **enabling flexible solutions for new and existing customers.**
- ✓ Currently being deployed via pilot in Indaiatuba, Brazil with support by local regulators and communities.

BILL & MELINDA
GATES foundation



- Oxitec has entered into an agreement with Bill and Melinda Gates Foundation (BMGF) to transfer the self-limiting technology to two malaria vectors.
- A 2-3 year multi-million dollar project funded by BMGF was launched to develop a next generation, self-limiting *An. albimanus* and *An. stephensi* strains using Oxitec's 2nd Generation technology.



Overview – Oxitec’s New 2nd Generation Friendly™ *Aedes aegypti*

- **Increased performance** as male progeny also mate and pass the self-limiting gene to a proportion of their own offspring.
- **Greater accuracy in sex sorting; reduced costs** as less labor is required.
- **Easier monitoring** as the fluorescent marker is highly visible in larvae AND adults.
- **Prolonged life of insecticides** as insecticide-susceptible genes in Oxitec males dilute resistance genes in the wild.

SELF-LIMITING GENE



- **Inherited traits** and offspring do not survive to adulthood.
- After releases stop, **self-limiting genes do not persist** in the environment for long.

FLUORESCENT MARKER GENE



- **Efficient tracking** with fluorescent protein detected by microscope identifies **Oxitec insects vs. wild.**
- More accurate estimation of pest population sizes and **monitoring of suppression in real time.**
- **Flexible releases** can be adjusted based on tracking data.

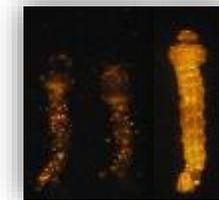
1ST GEN: SELF-LIMITING

Physical sorting of males. Self-limiting gene lethal to both male and female offspring.



2ND GEN: MALE-SELECTING

Genetic sorting of males. Self-limiting gene lethal only in females.



OX513A OX5034



OX5034



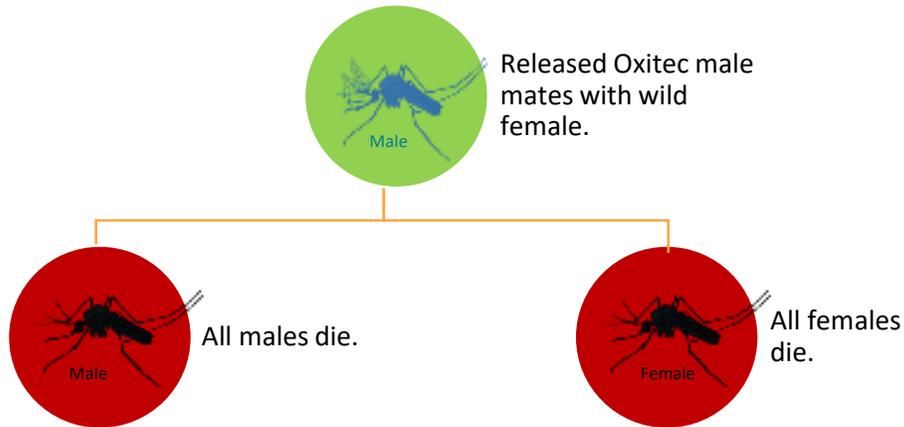
OX5034



Next Generation - Same Mechanism but Acts on Females Only

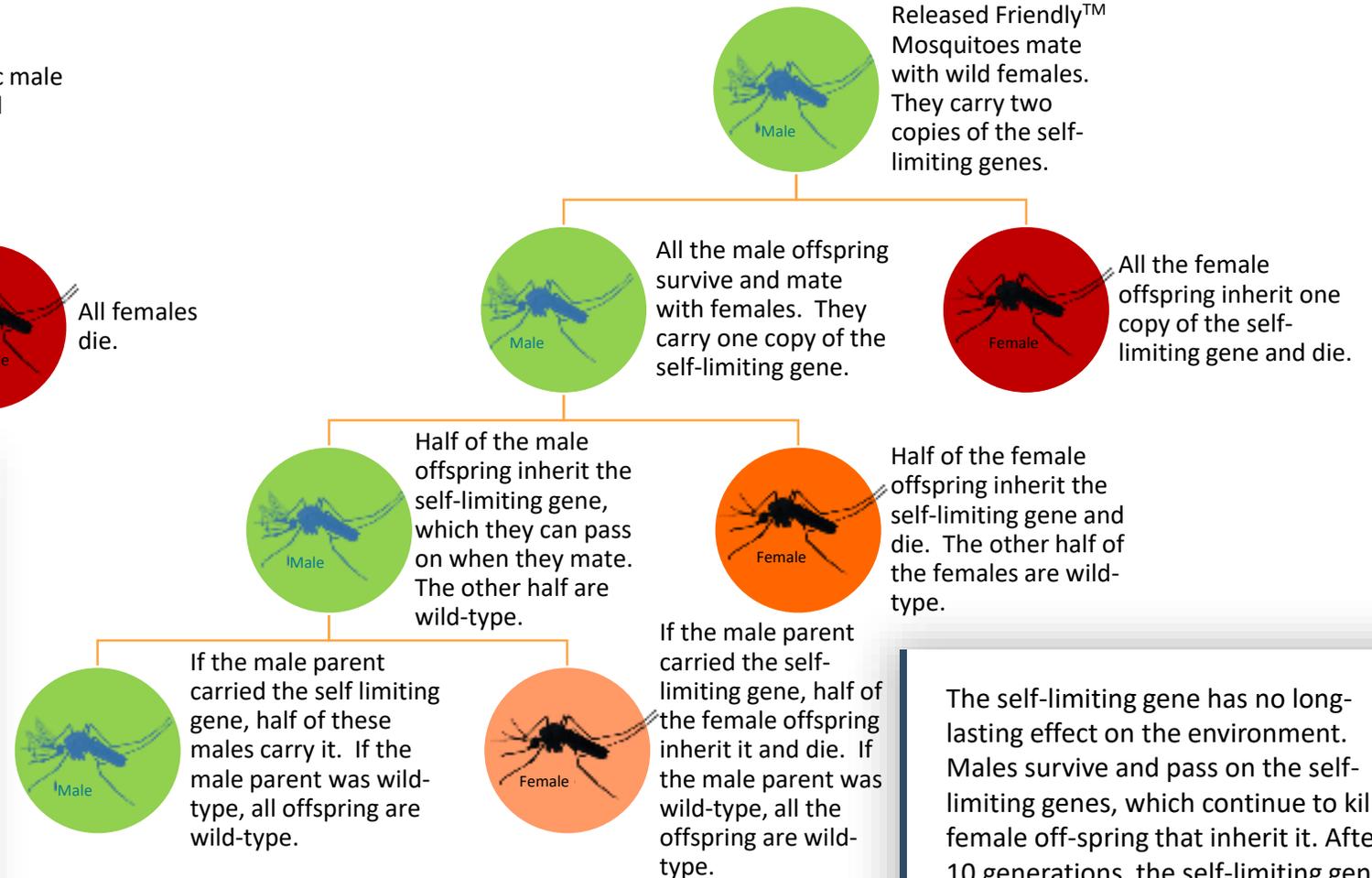
1ST GEN: SELF-LIMITING

Male and female survival on tetracyclines – requires sex sort



2ND GEN: MALE-SELECTING

Male-only survival off tetracyclines – no manual sex sort required



OX5034 achieves **genetic sexing** by expressing tTAV only in females using sex-specific alternative splicing:

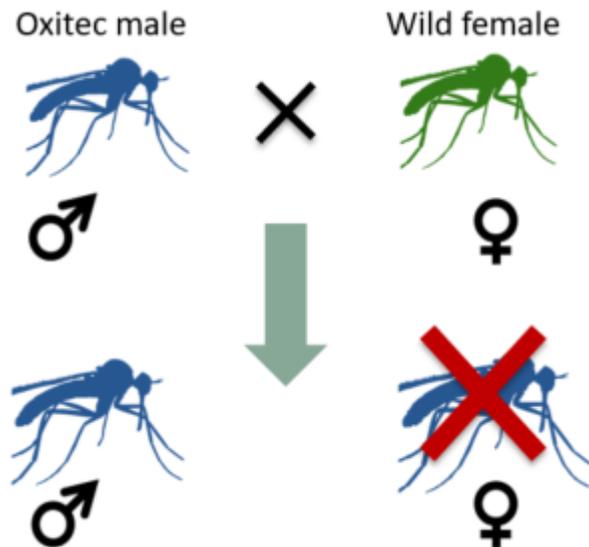
- Female homozygotes die when reared without tetracycline to produce male-only release cohorts.
- Female hemizygotes (offspring of OX5034 males and wild females) die because of lack of tetracycline in the field.
- Alternative splicing module is a natural *Aedes aegypti* sequence coupled to tTAV.

The self-limiting gene has no long-lasting effect on the environment. Males survive and pass on the self-limiting genes, which continue to kill female off-spring that inherit it. After 10 generations, the self-limiting gene is virtually extinguished from the environment.



Self-Limiting, With Diminishing Residual Effect

- In the field, female offspring with 2nd-gen gene do not survive.
- Males do survive, so the self-limiting gene persists for a few generations, offering a potential supplemental vector suppression effect.
- After releases cease, the self-limiting gene declines to extinction, decreasing each generation by half.



Lab study demonstrating decline of 2nd-Generation gene with each generation

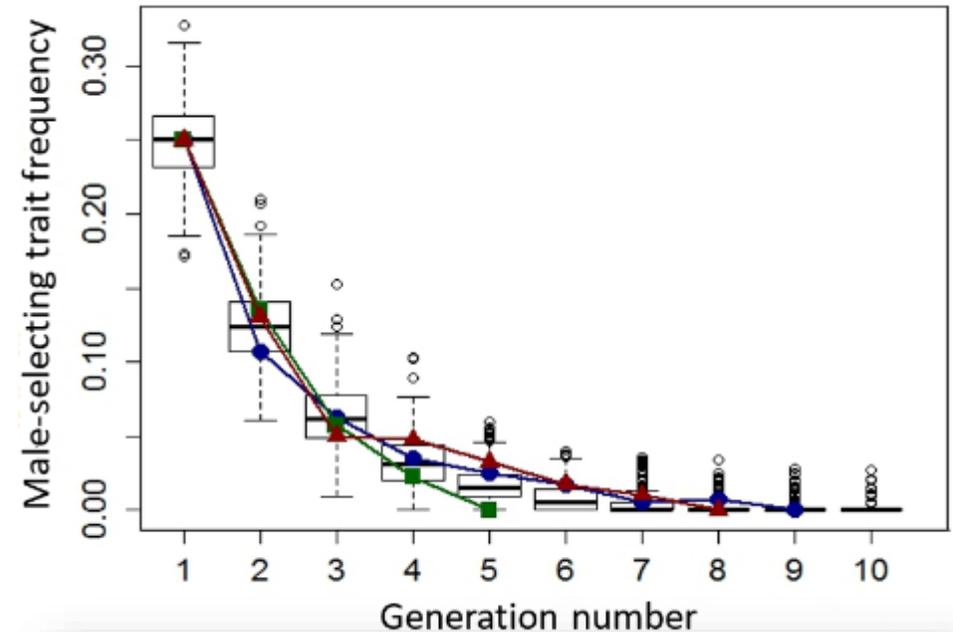
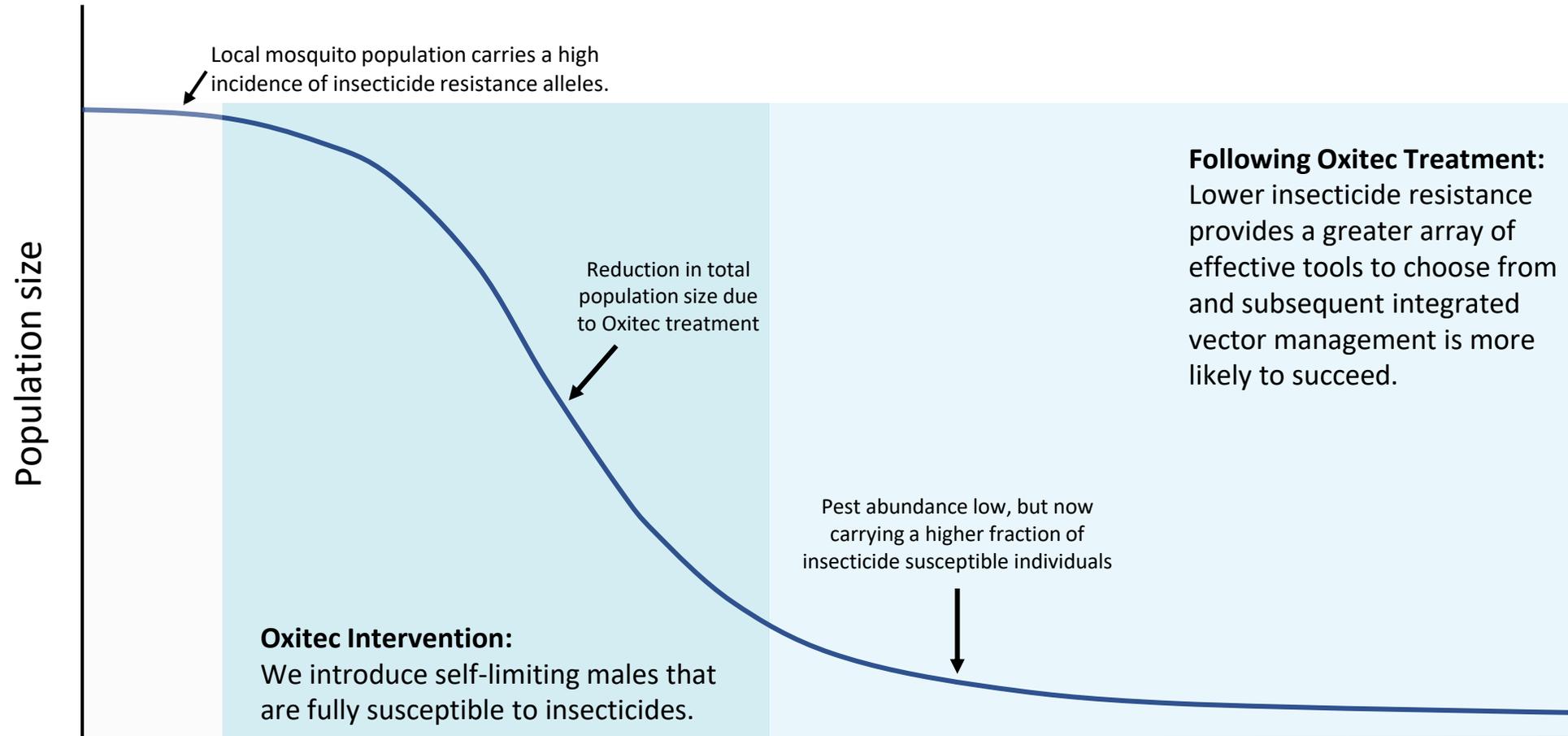


Figure 1. Boxplots showing the results from 500 iterations of a stochastic model simulating the extinction of a male-selecting genetic trait under restrictive conditions. Overlaid onto the box plots are lines (red, blue and green) showing male-selecting trait frequency changes from three replicates of caged experiments. Generation 1 represents a post-field population with a trait frequency of 0.25.

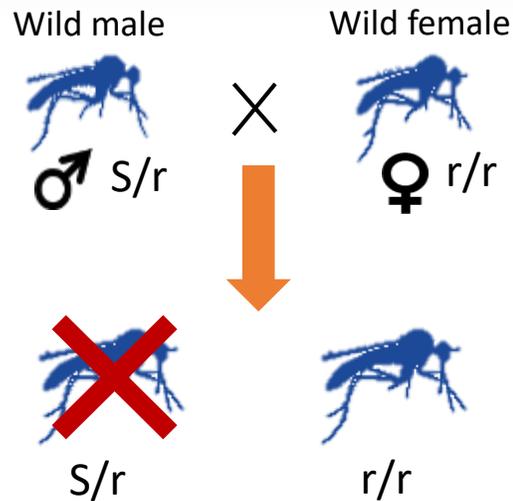
Effect of Oxitec Male Release on Insecticide Susceptibility in the Wild

Oxitec's technology confers an additional benefit of restoring natural insecticide susceptibility, even when releases of OX5034 stop and the self-limiting gene disappears from the environment.



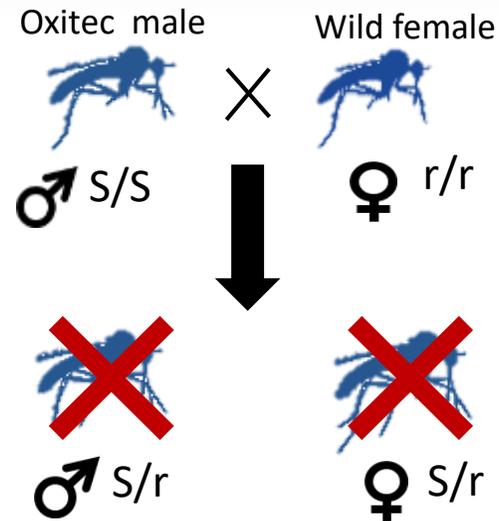
Second Generation insects can potentially protect or restore insecticide efficacy.

No Oxitec males; with insecticide



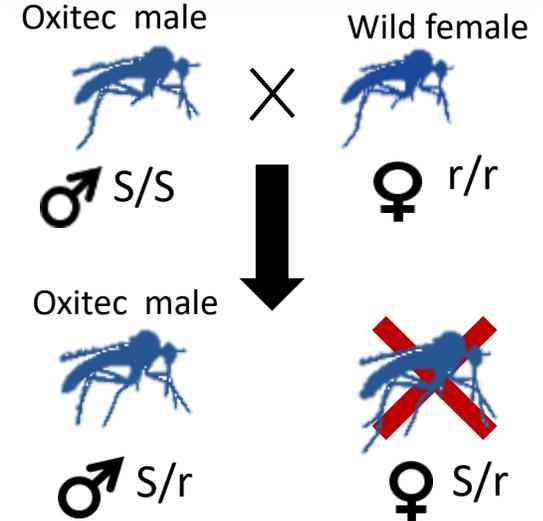
Resistance (r) spreads through the population, leading to reduced efficacy of all insecticide applications.

Oxitec males; with insecticide



Male-selecting trait (blue) combined with insecticide susceptibility (S) offers 'genetic sterility' killing females & slowing the spread of resistance by making male offspring susceptible to insecticides once more.

Oxitec males; no insecticide

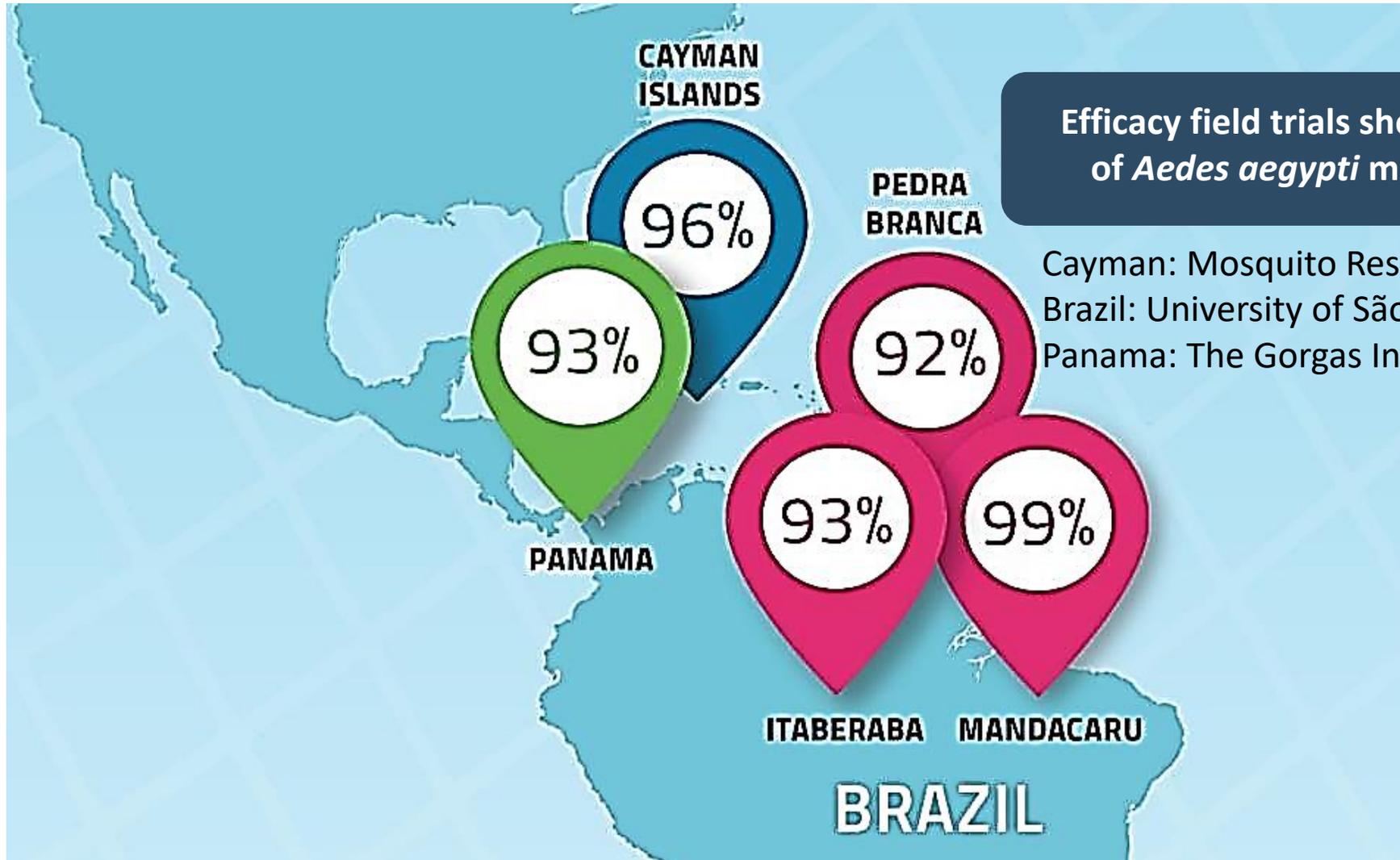


Male-selecting trait (blue) reduces female numbers dramatically and dilutes insecticide resistance genetics, by pushing insecticide susceptibility genes (S) into the population offering resistance reversal.



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Oxitec's 1st Generation Technology Achieved Population Suppression



Efficacy field trials show > 90% suppression of *Aedes aegypti* mosquito populations

Cayman: Mosquito Research and Control Unit
Brazil: University of São Paulo and Moscamed
Panama: The Gorgas Institute



OX5034 Biosafety and Efficacy Testing in Brazil



Indaiatuba, São Paulo, Brazil

- Started in May 2018 (permission for environmental release granted by CTNBio in 2017).
- Completion due in Q2 2019.
- Two application rates + controls (2 replicates of each).
- Targeting biosafety and efficacy.
- Mortality of larvae in the field.
- Dispersal of released homozygote OX5034 males.
- Longevity of released homozygote OX5034 males.
- Mating capacity of OX5034 with local *Aedes aegypti*.
- Dispersal of transgene.
- Numbers of males and females released.



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Oxitec's 2nd Generation Technology for Malaria Vector Control

Anopheles albimanus & Anopheles stephensi



Collaboration with BMGF Launched in July 2018

Project Summary

- Oxitec has entered into an agreement with Bill and Melinda Gates Foundation (BMGF) to develop new tools for malaria vector control.
- Oxitec is developing 2nd Generation self-limiting strains of *Anopheles albimanus* & *An. stephensi* ready for field release. The project is running for 2.7 years.
- *Anopheles albimanus* & *Anopheles stephensi* are important vectors of malaria in meso America and Asia, respectively. *An. stephensi* is also spreading in Africa where it is considered an invasive species.

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Strategy to Transform Anopheline mosquitoes

REARING *An. albimanus* & *An. stephensi*

- ✓ Colonies established at Oxitec

DESIGN OF MOLECULAR TOOLS

✓ Self-limiting strategy

- Conditional female-specific expression of self-limiting tTAV exploiting *An. albimanus* & *An. stephensi* Dsx splicing
- Strategy proven in *Aedes* mosquitoes (Oxitec)

✓ Fluorescent marker-only cassette

- To assess transformation efficiency
- To assess DsRed2 marker expression
- To develop molecular tools (e.g. genotyping assays)

2nd GENERATION CANDIDATE STRAINS

- ✓ To be ready for field release by 2021





Thank you

