Aedes aegypti control programmes in Brazil

Margareth L. Capurro
mcapurro@icb.usp.br
Figure 1. Annual incidence dengue fever (DF) and dengue hemorrhagic fever (DHF) and the premises index, Singapore, 1966–2005. DHF was made a notifiable disease in 1966, while DF became a notifiable disease in 1977. The annual incidences of DF and DHF reported in this figure were calculated from the number of reported cases each year from 1966 to 2004. The annual premises index is expressed as a percentage of the premises in which Aedes aegypti or A. albopictus larvae were found divided by the number of premises visited by environmental health officers.
Integrate Control for *Aedes aegypti* Population Suppression

- Mechanical Control: Remove breeding sites
  - Ovitraps
  - Adult traps
  - Massive Collection
- Education: Community Engagement and Responsability
- Larvicides
  - Bti
  - Fishes
  - Auto dissemination Pyriproxyfen
- Adulticides
  - Chemicals
- Population Suppression
  - SIT
  - BT/SIT
  - Transgenic
Integrate Control for *Aedes aegypti* Population Supression

- Mechanical Control: Remove breeding sites
  - Ovitraps
  - Adult traps
  - Massive Collection
  - Larvicides
    - Bt
    - Fishes
    - Auto dissemination: Pyriproxyfen

- Adulticides
- Chemicals
- Education: Community Engagement and Responsability
- Population Suppression
  - SIT
  - IIT/SIT
  - Transgenic
Integrate Control for Aedes aegypti Population Suppression

- Ovitraps
- Adult traps
- Larvicides
- Bti
- Fishes
- Auto dissemination Pynproxylfen

Mechanical Control: Remove breeding sites

Education: Community Engagement and Responsibility

Adulticides

Population Suppression:
- Chemicals
- SIT
- IIT/SIT
- Transgenic
Integrate Control for *Aedes aegypti* Population Supression

Mechanical Control
- Remove breeding sites

- Ovitraps
- Adult traps

- Massive Collection

Larvicides
- Bti
- Fishes
- Auto dissemination Pyriproxyfen

Adulticides

Chemicals

Education:
- Community Engagement and Responsibility

Population Suppression
- SIT
- SIT/SIT
- Transgenic

80%
Integrate Control for *Aedes aegypti* Population Suppression

- Ovitraps
- Adult traps
- Massive Collection
- Larvicides: Bti, Fishes, Auto dissemination Pyriproxyfen
- Adulticides
- Mechanical Control: Remove breeding sites
- Chemicals
- Education: Community Engagement and Responsability
- Population Suppression: SIT, BT/SIT, Transgenic

80%
Integrate Control for *Aedes aegypti* Population Suppression

**Mechanical Control:** Remove breeding sites
- Ovitraps
- Adult traps

**Larvicides:**
- Bti
- Fishes
- Auto dissemination Pyriproxyfen

**Adulticides:**
- Chemicals

**Education:** Community Engagement and Responsibility

**Population Suppression:**
- SIT
- IIT/SIT
- Transgenic

80%
Step 1. Suppression

Natural Population

Step 2. Replacement
Open Field Release of **OX513A Aedes aegypti** Transgenic line evaluation
Repressive of Insects carrying a Dominant Lethal gene (RIDL)

Thomas et al. 2000 Science 287: 2474-6
Mass Production of Genetically Modified *Aedes aegypti* for Field Releases in Brazil

Danilo O. Carvalho¹², Derric Nimmo¹, Neil Naish¹, Andrew R. McKemey¹, Pam Gray¹, André B. B. Wilke², Mauro T. Marrelli³, Jair F. Virginio⁴, Luke Alphey¹⁵, Margareth L. Capurro²⁶

COLONY
4 to 6 million eggs/week

Males for releases
1,5 million/week
Suppression of a Field Population of Aedes aegypti in Brazil by Sustained Release of Transgenic Male Mosquitoes

Danilo O. Carvalho\textsuperscript{1,2,6}, Andrew R. McKemey\textsuperscript{1,6,7}, Luiza Garziera\textsuperscript{3}, Renaud Lacroix\textsuperscript{4}, Christl A. Donnelly\textsuperscript{4}, Luke Alphey\textsuperscript{1,5,6}, Aldo Malavasi\textsuperscript{3}, Margareth L. Capurro\textsuperscript{2,7}
Project Phase 2 – Jacobina - Bahia
Pupa Transportation

25000 males
Pupa transportation (LEMI)

- C25
- BOD 16°C ON
- 180,000 per container

Arriving at LEMI
Emergency, Monitoring and Information Lab

Preparation for release
What do we learn?

• We need Genetic Sexing Strain (GSS)
• Producing Sterile male strain (no Larvae)
• Use of tetracycline only in colonies
Sterility Conditional Construct - SCC

No Antidote

♀
♂

No Viable Eggs

Antidote (Tet +)

♀
♂

Viable Eggs
SCC Transgenes

- Two Effector molecules:
  
  Endonuclease

  ![Diagram of 3XP3, DsRed, ttO, Endonuclease, B2tubulin, tTAV, SV40]

  IAP Antagonist

  ![Diagram of 3XP3, DsRed, ttO, Michelob_X, B2tubulin, tTAV, SV40]
### Transgenic Lines

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<td>AF</td>
<td>L2</td>
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150 pb  350 pb  150 pb
Sterile Conditional Construct (SCC)

60% Homozygous
80% Reduction
Improving transgenic lines
*Aedes aegypti* and *Aedes albopictus*

- Producing Sterile male strain (no Larvae) ✔
- Producing GSS (Genetic Sexing Strain)
Genomic organization and splicing evolution of the doublesex gene, a Drosophila regulator of sexual differentiation, in the dengue and yellow fever mosquito Aedes aegypti

Marco Salverini, Umberto Mauro, Fabrício Lombardo, Andreina Milano, Vincenzo Zazzaro, Bruno Arcà, Lino C. Polito, Giuseppe Saccone

Dsx knockout
Gene Introduction
Virus-regulated mosquito gene
Suicidal Model (Double death model)

Natural Population
Gene Introduction
Virus-regulated mosquito gene
Suicidal Model (Double death model)

Natural Population +
Introduction
Gene Introduction
Virus-regulated mosquito gene
Suicidal Model (Double death model)

Natural Population
+ Introduction

Virus Infection
Gene Introduction
Virus-regulated mosquito gene
Suicidal Model (Double death model)

Virus Infection

Natural Population
+

Introduction
Gene Introduction
Virus-regulated mosquito gene
Suicidal Model (Double death model)

Step 2. Replacement

NS3 Cleavage site

Constitutive Promoter
Lethal protein
ER localization

RRRRSAG
• Double death model - Infection phenotypes in dengue challenged mosquito?
**C6/36 cells - Transfection and D2 challenge:**

- **3 days after DEN2**

  - GFP filter
  - Light
  - Merge
  - ER marker red dye

- **Sec61 – Mx – EGFP**

  - DAPI
  - GFP filter Light
  - Merge

- **PIE – EGFP**

  - GFP filter
  - Light
  - Merge

- **Sec61 – Mx – EGFP**

  - DAPI
  - GFP filter Light
  - Merge
Survival rates of wild type (Higgs-strain) and DUI-Y transgenic line challenged with DENV-2 via intrathoracic injection. Five-to-seven day old females were injected with control supernatant (CS) and DENV-2 infected supernatant (IS) (vial load = 7.0 x 10^6 genomic copies). Females were recovered during 24hs after injection and survived females were maintained on 10% sucrose solution and censored during 14 days after injection (14DPI). Solid lines are showing survival curves of females injected with CS and dotted lines are showing survival curves of females injected with IS. Survival curves were statistically analyzed by Log-rank (Mantel-Cox) test.
Bringing new technology to the field
How the Sterility works?
How the Sterile Mosquito Works?
Why releasing male mosquitoes you kill mosquitoes?
Before releasing mosquitoes

- Site selection
  - Public Engagement:
    - Evolving Public Authorities (Government and agencies);
    - Local ones (community engagement);
    - Local people - explain what we are going to do in that area.
## Community Engagement

<table>
<thead>
<tr>
<th>Action</th>
<th>Period</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Pre-release</td>
</tr>
<tr>
<td>Domiciliary visit</td>
<td>2010*</td>
</tr>
<tr>
<td>Internet</td>
<td>Social Network</td>
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<tr>
<td></td>
<td>Web site</td>
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<tr>
<td>Interviews / appearances</td>
<td>TV</td>
</tr>
<tr>
<td></td>
<td>Radio</td>
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<tr>
<td></td>
<td>Newspaper</td>
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<tr>
<td></td>
<td>Magazines</td>
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<tr>
<td>Jingle broadcast</td>
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<tr>
<td>Leaflets distribution</td>
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<tr>
<td>Meeting local leaders</td>
<td></td>
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<tr>
<td>Questionnaires</td>
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<tr>
<td>School presentations / lectures</td>
<td></td>
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<tr>
<td>Monitoring system</td>
<td></td>
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<tr>
<td>Truck loudspeakers</td>
<td></td>
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* - In both years, the columns are representing the last two semesters and the first two respectively.
Community Engagement

Total people 17,101,269 in Brazil – Based on the Brazilian Institute of Public Opinion and Statistics (IBOPE) data
Talks and Lectures

City Hall Public Hearing
Leaflet distribution

PROJETO AEDES TRANSGÊNICO

1. Os mosquitos transgênicos são produzidos em laboratório.


3. O macho transgênico ao cruzar com a fêmea selvagem, passa o gene mortal e os mosquitos gerados morrem ainda na fase de larva ou pupa.

4. No laboratório os machos são mantidos para liberação e as fêmeas eliminadas.

5. Na comunidade: • Colocadas as ovitrampas (armadilhas). • é feita a identificação dos mosquitos capturados. • a equipe faz a liberação dos mosquitos transgênicos.

6. Os agentes do PAT realizam o monitoramento para avaliação e análise da redução populacional dos insetos capturados.

Os machos transgênicos não picam. São mosquitos parceiros, que te protegem da dengue.
Mosquito Aedes \ Dengue

Pica durante o dia (bite during the day)

Muriçoca (Culex)

Pica durante a noite (bite during the night)
Bar – Blood for Sale!

Only females (girls) bite

Nectar

Fonte: Google
<table>
<thead>
<tr>
<th>ACTION</th>
<th>TARGET POPULATION LEVEL</th>
<th># EVENTS</th>
<th># PEOPLE</th>
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</thead>
<tbody>
<tr>
<td>Presentations/Lectures</td>
<td>Local/Regional</td>
<td>10</td>
<td>962</td>
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<tr>
<td>Leaflets (^{(1)})</td>
<td>Local</td>
<td>-</td>
<td>10,000</td>
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<tr>
<td>Jingle (^{(1)})</td>
<td>Local</td>
<td>-</td>
<td>-</td>
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<td>Meetings</td>
<td>National/International</td>
<td>39</td>
<td>6,020</td>
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<tr>
<td>Interviews (radio)</td>
<td>Regional</td>
<td>15</td>
<td>1,500</td>
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<td>Interviews (TV)</td>
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<td>09</td>
<td>17,094,000 (^{(2)})</td>
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<td>Interviews (newspaper/magazine)</td>
<td>Local/Regional/National</td>
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<td>Internet (website / social network)</td>
<td>Regional/National</td>
<td>24</td>
<td>_-(^{(3)})</td>
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<td>Houses visited/interviewed with residents</td>
<td>Local</td>
<td>581</td>
<td>2,341</td>
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<tr>
<td>Meetings with local leaders, health agents</td>
<td>Local</td>
<td>16</td>
<td>820</td>
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<tr>
<td>Presentations at elementary and middle school</td>
<td>Local</td>
<td>08</td>
<td>452</td>
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<tr>
<td>Presentation at community center/city hall/others</td>
<td>Local</td>
<td>06</td>
<td>456</td>
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<tr>
<td>Driving truck with loudspeakers in the releasing area</td>
<td>Local</td>
<td>-</td>
<td>500</td>
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<tr>
<td>Spots, jingles and short messages broadcasted in local radio station</td>
<td>Local</td>
<td>52</td>
<td>1,200 (^{(4)})</td>
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<tr>
<td>TOTAL</td>
<td></td>
<td>17</td>
<td>17,101,269</td>
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<tr>
<td>STRATEGIES</td>
<td>Mandatory</td>
<td>Recommended</td>
<td>Suggested</td>
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<tr>
<td>- Visit/interview sample/every house in the target area</td>
<td>- Lectures at community centers/churches – targeting adults</td>
<td>- Action within a local event (parade, carnival, street fairs)</td>
<td></td>
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<tr>
<td>- Meetings with local leaders, school principals, district managers</td>
<td>- Radio spots, jingles and messages broadcasted</td>
<td>- Driving truck with loudspeakers in the targeting area – jingle and messages</td>
<td></td>
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<tr>
<td>- Lectures at schools – targeting kids/teens</td>
<td>- Press releases by Moscamed journalists</td>
<td>- Use of social media: Facebook and twitter</td>
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<tr>
<td>- Press coverage at local/regional level of PAT activities: production, releases</td>
<td>- PAT technical personnel interviewed by local/regional/(inter)national radio stations</td>
<td>- Press coverage at international level of PAT activities: releases</td>
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<tr>
<td></td>
<td>- Press coverage at national level of PAT activities: production, releases</td>
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Spot and Jingle
To control dengue Moscamed is releasing in this community
A large amount of TRANSGENIC MOSQUITOES.
We would like to recall that this mosquitoes are not the well known CULEX
They are transgenic MALES and they DON’T BITE.
They are good fellows that will give you protection against dengue.
For more information call a health agent or get in touch with
MOSCAMED
By the phone
(74) 3612-5399
PAT –AEDES TRANSGENIC PROJECT
This one makes the difference.
Jingle Transgenic *Aedes*