Arboviruses: resurgence of a past neglected problem

New anti-mosquito and larvicidal agents in the lab and in the field

Dr. Raquel M. Gleiser

CREAN-IMBIV, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and Universidad Nacional de Córdoba, Argentina

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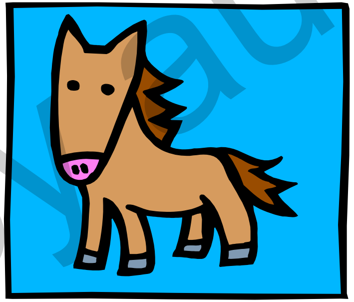
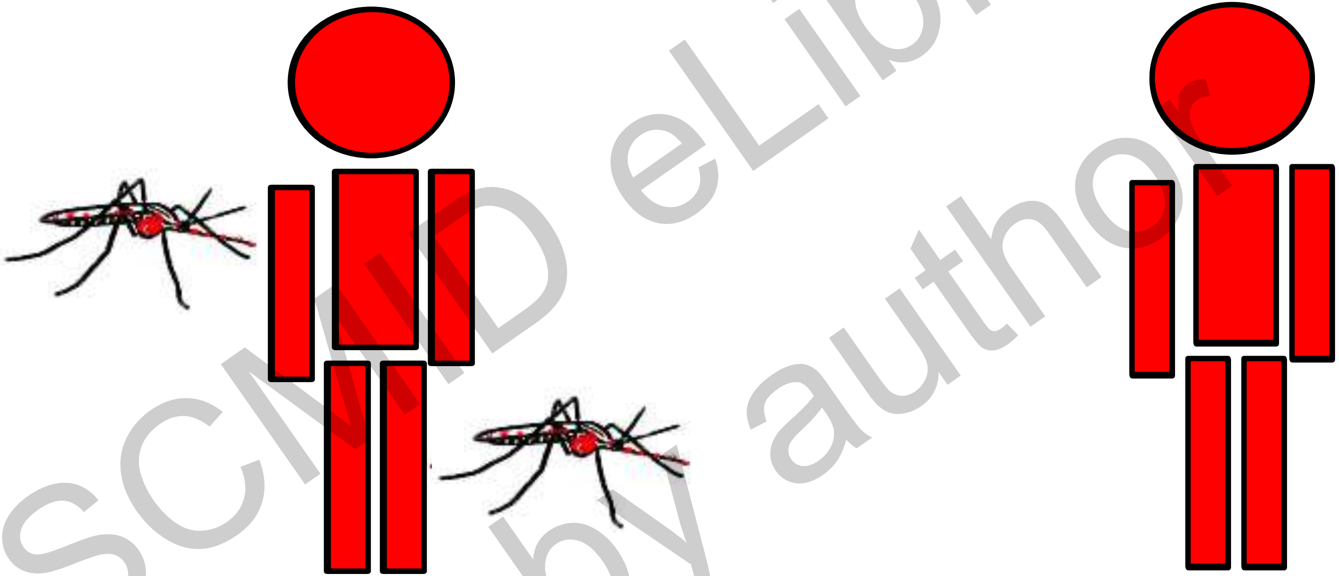
Arbovirus

Arthropod borne virus agents that persist in nature in complex cycles involving birds and mammals



Saint Louis encephalitis
West Nile fever

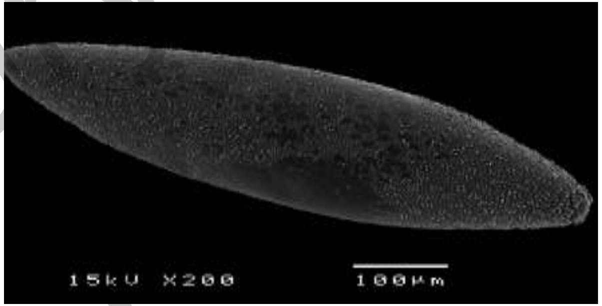
Dengue transmission



Preventing arbovirus transmission

… but still no clear evidence of quantifiable association between vector density and disease transmission

Developmental stages



Egg

Larvae (4 stadium)

Adult

Pupae

Mosquito control

- How depends on/is related with:
  - Why? (prevention? Outbreak interruption?)
  - What species?
  - Where?
  - What stage?

Aedes aegypti

- Dengue, zika, chikungunya, urban yellow fever
- Urban
- Larval habitat: containers
- Diurnal activity
- Low dispersion

Culex quinquefasciatus

- WNV, arboviral encephalitis
- Urban, rural
- Larval habitat: ground level
- Crepuscular-nocturnal
- Moderate dispersion

Mosquito control

- How depends on/is related with:
  - Why? (prevention? Outbreak interruption?)
  - What species?
  - Where?
  - What stage?
  - Legal requirements, among other

Integrated mosquito control strategies

- Mosquito surveillance
- Public education & Community outreach
- Physical Control (Source Reduction)
- Biological Control
- Chemical /microbiological Control (Larvicides & Adulticides)
- Disease surveillance

Anti-mosquito agents

- **Biorational/biopesticides:** affect limited taxa and/or stages (eg. Bacterial toxins, insects growth regulators)
- **Mid spectrum larvicides:** oils, films, surfactants that cause drowning in air breathing aquatic insects
- **Broad spectrum insecticides:** dosage-dependent toxic effects on most organisms (pyrethroids, organo-phosphates)

Global Trends in the Use of Insecticides to Control Vector-Borne Diseases

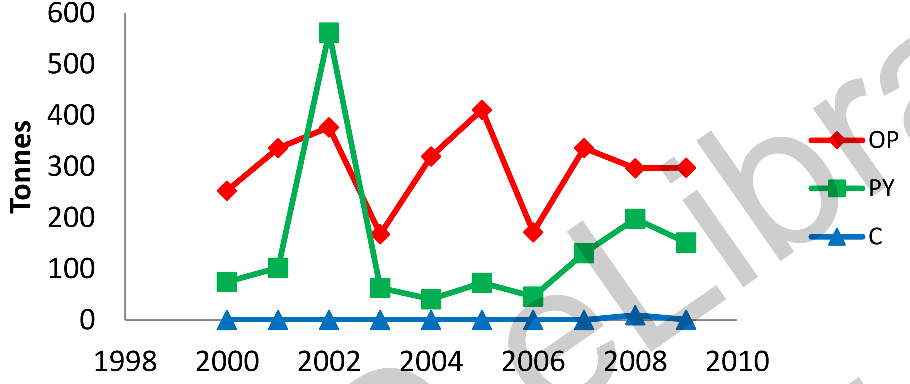
H van den Berg, M Zaim, RS Yadav, A Soares, B Ameneshewa, A Mnzava, J Hii, A Prasad Dash, M Ejov. **Environ Health Perspectives 120**

- 125 countries
- Insecticide application methods: residual spraying, space spraying, treatment of nets*, and larviciding
- Disease targets: Malaria, **dengue**, leishmaniasis, Chagas disease

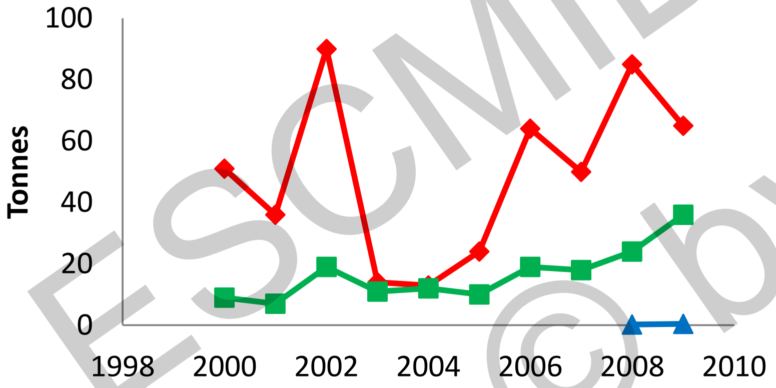
*long lasting nets were excluded

Broad spectrum insecticides for dengue control*

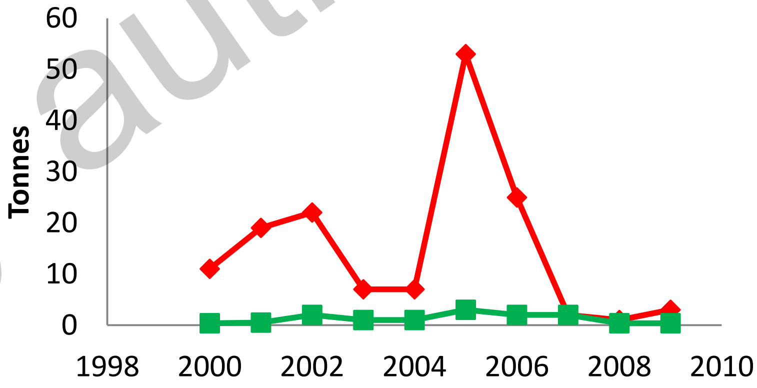
Americas (570 mln)



Western Pacific (1629 mln)



South-East Asia (1737 mln)

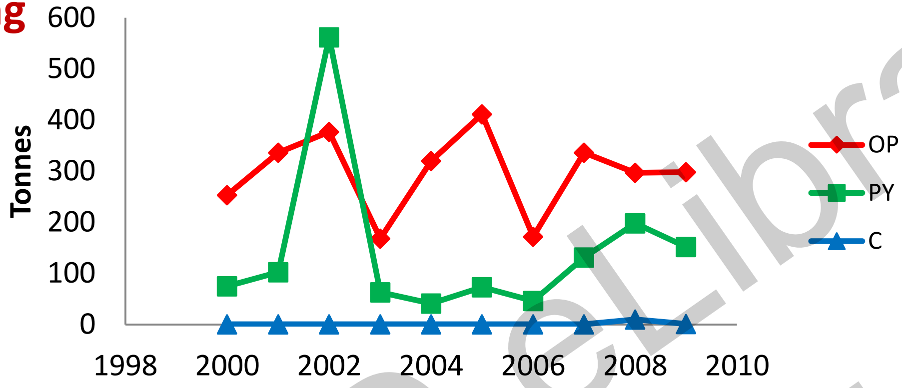


*weighted by proportion of targeted population with available data

Van den Berg et al. 2012 Environ Health Perspectives 120

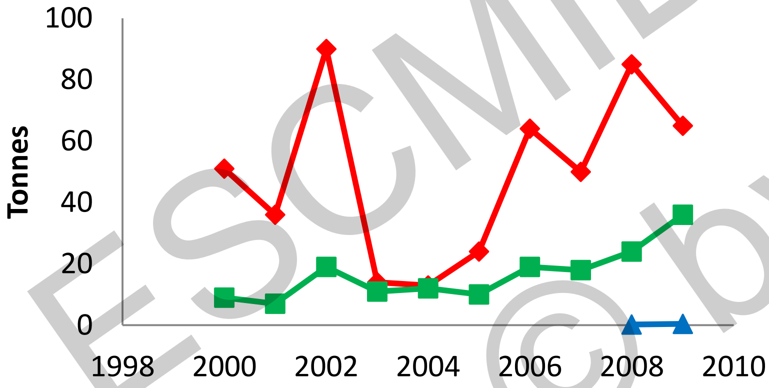
Residual spraying application rate
OP 1.5 g/m²
PY 0.025 g/m²

Americas (570 mln)



Pyrethroids did not constitute a major global share in terms of metric tons applied, but were dominant in terms of area covered by active ingredient (81% of the global spray utility)

Western Pacific (1629 mln)



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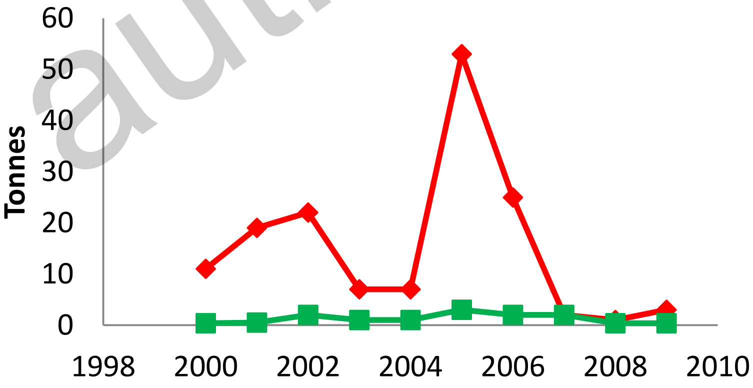
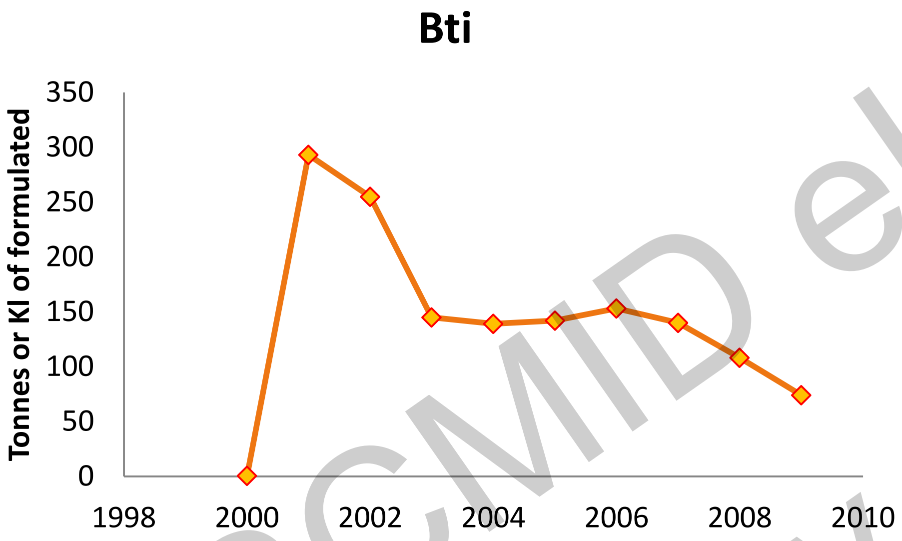
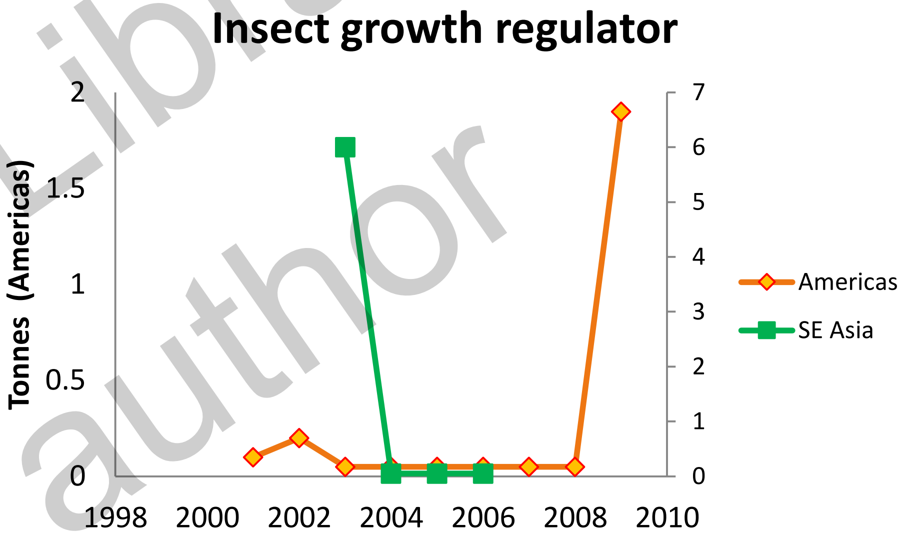


Van den Berg et al. 2012 Environ Health Perspectives 120

Biorational insecticides for dengue control





Commercially available products have different potency (international Toxic Unit).

World Health Organization Pesticide Evaluation Scheme2011

Why do we need more antimosquito agents

- To improve control
- Environmental concerns
- (Human) health concerns
- Insecticide resistance

Search strategies

- Search for toxins in nature
- Nanotechnology
- New synthetic agents («in silico»)
- Insect growth regulators
- Pheromones («lure and kill»)
- «Change» mosquitoes or their infection susceptibility (viral agents, *Wolbachia,* sterile insect release)

Plants as insecticide sources

- Synergisms from mixtures of plant extracts and/or combined with synthetic insecticides

- Plant-mediated biosynthesis of nanoparticles

Benelli 2016. Parasitol Res 115:23–34

Nanotechnology

Nanoparticle	Target	Non-target	Reference
Ichnocarpus frutescens (Apocynaceae) AgNP	*Ae. albopictus, Culex, Anopheles* lf 185-226 ug/ml NP LC_{50} 14-17 ug/ml	Water bugs, fish *Gambusia affinis* LC_{50} 637-2099 ug/ml	Govindranat et al. 2016 Enzyme & Micro Technol 95:155-163
Hedychium coronarium (Zingiberaceae) AgNP Synergy with copepod	*Ae. aegypti* (l1-pupae) NP LC_{50} 24-72 ug/ml (larva) NP LC_{50} 348 ug/ml (pupae)	Copepod *Megaclycops formosanus* LC_{50} 650 ug/ml	Kalimuthu et al 2017 Process Safety & Environ Protect 109: 82-96
Valeniopsis pachynema algae CdS NP	*Anopheles (A stephensi, A sundaicus)* (l1-pupae) Vp 189-312 ug/ml LC_{50} 14-30 ug/ml (L & P)	Mud crab *Scylla serrata* Cd ion LC50 5 ug/ml LC_{50} 14-17 ug/ml	Sujitha et al 2017 Aquatic Toxicol 188 :100-108

Biorational mosquito larvicides

- Toxins or mixtures from bacteria: Bti (*Bacillus thuringiensis israelensis*), Spinosad (*Saccharopolyspora spinose*)

- Genetically modify organisms (cyanobacteria) to express Bti toxins (Cry4, Cry11) http://www.patentstorm.us/patents/6503500-claims.html

Insect growth regulators

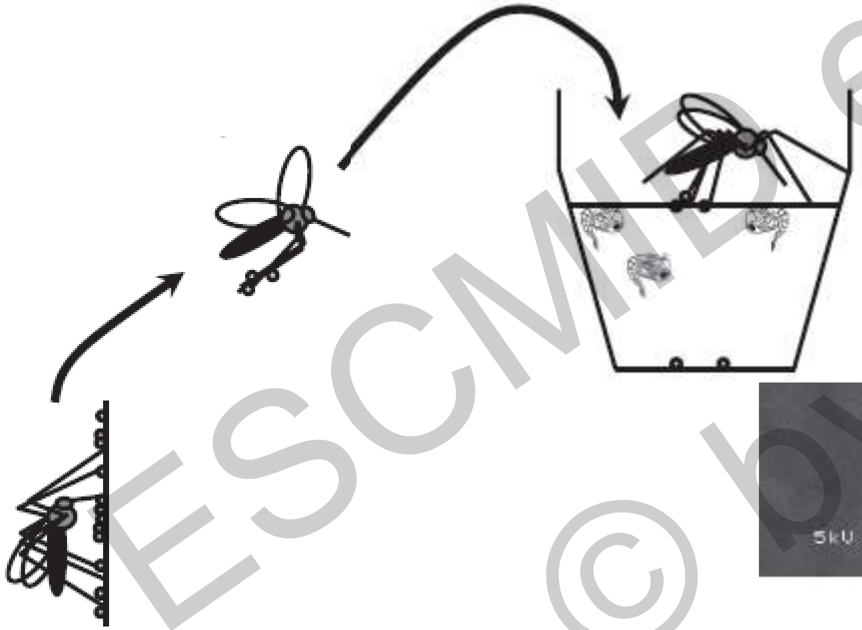
- Juvenile hormone analogues (juvenoids) (eg. methoprene, pyriproxyfen)

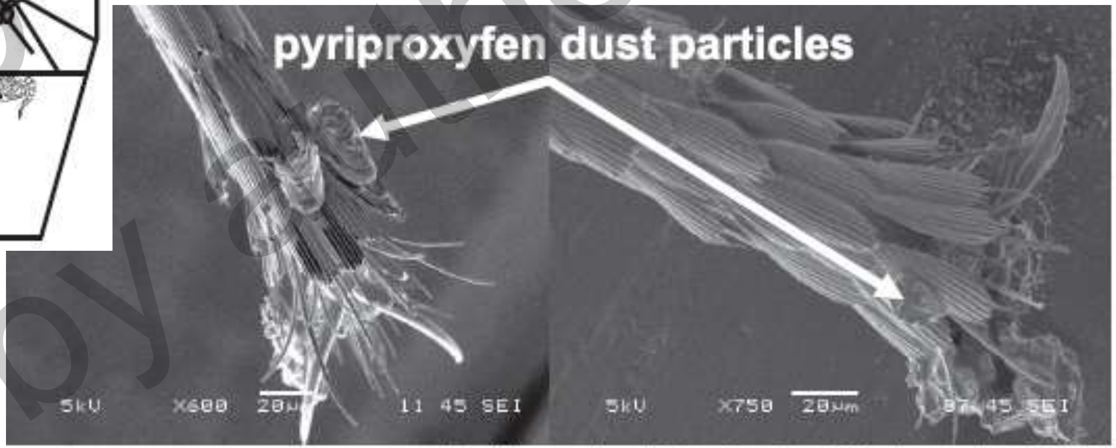
- Chitin synthesis inhibitors (eg. diflubenzuron, triflumuron, novaluron)

- Control of arbovirus transmission through larval habitat treatments depend on maximizing their impacts on adult mosquito density
- Uncertainty over the relative productivity of specific habitats, hence need to identify and treat all potential sites

Insect growth regulators

- Female mosquitoes transport pyriproxifen particles to small or cryptic larval habitats, increasing control coverage

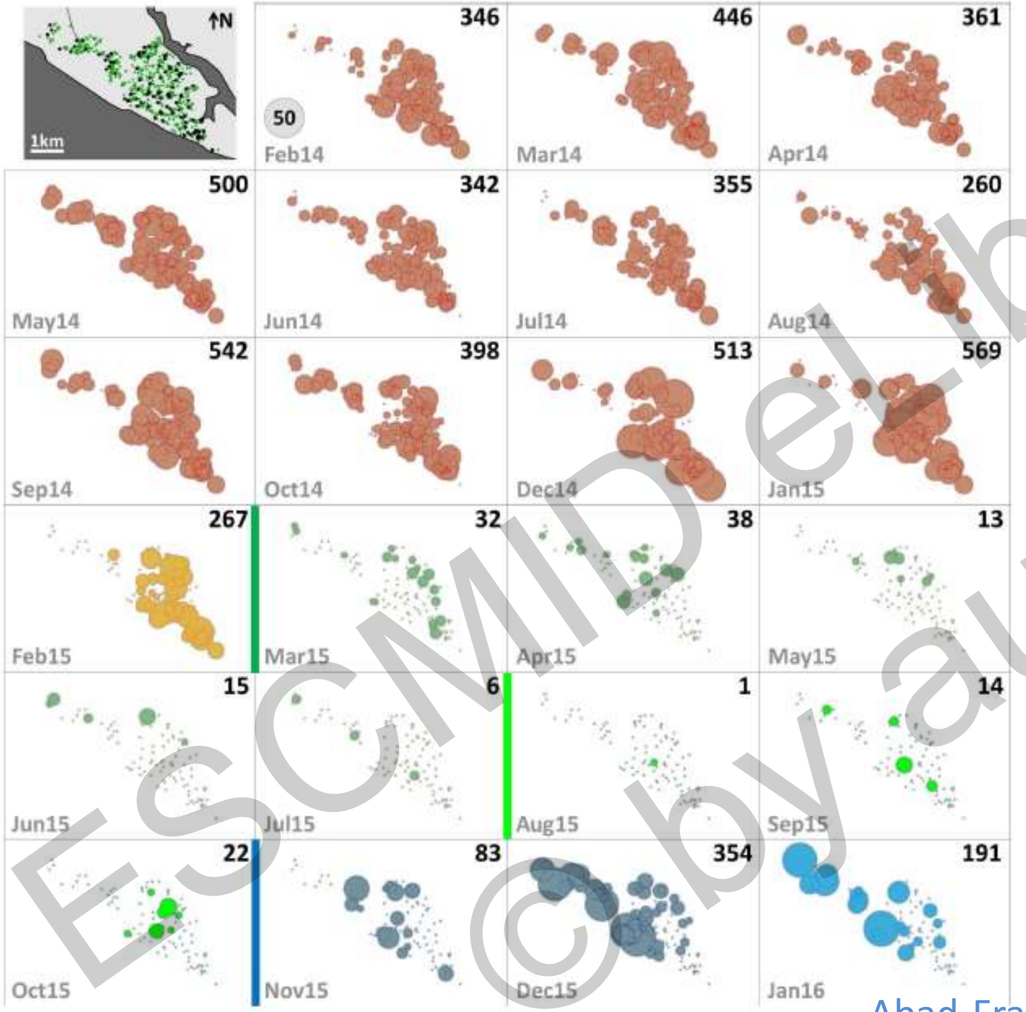


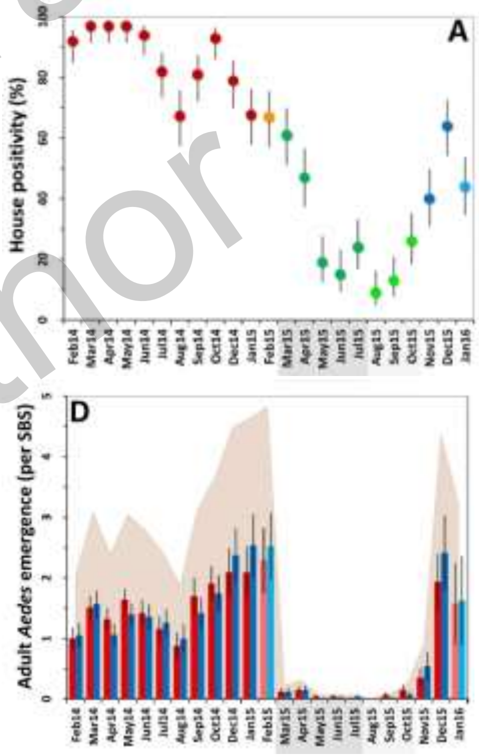


Rothamsted Centre for Bio Imaging 2008

Gregor J. Devine et al. PNAS 2009;106:11530-11534

Mosquito-disseminated insecticide for citywide VC & to block arbovirus. Manacapuru, Amazonian Brazil





Abad-Franch et al 2017. PLoS Med 14(1): e1002213

Pheromones («lure and kill»)

- **Lethal ovitraps:** leading mosquitoes to insecticide source instead of treating sources

Michaelakis et al 2018 Pest Manag Sci 74:392-397

Pheromones («lure and kill»)

- **Lethal ovitraps:** leading mosquitoes to insecticide source instead of treating sources
- Mosquito Oviposition Pheromone (MOP) 6-acetoxy-5-hexadecanolide
  - increased oviposition in pots treated with temephos (OP),
  - did not increase oviposition on pots treated with diflubenzuron (IGR)
  - first increased but later (>7d of ageing) decreased oviposition in Spinosad treated pots

Michaelakis et al 2018 Pest Manag Sci 74:392-397

Alter behaviour

- Prallethrin is a relatively volatile sublethal pyrethroid that causes excitation and may flush mosquitoes from cryptic habitats, exposing them to ULV droplets

Fonseca et al 2013. Pest Manag Sci 69: 1351–1361

«Change» mosquitoes or their infection susceptibility

- Sterile insect technique (SIT) - Female killing (FK) technologies
- New transgenic strategies allow conditional lethal mutations to be inserted into mosquito genomes, which allow for adjustment of the age of mortality, female-specific lethality, bisexual lethality and manipulation of germline-specific gene expression.

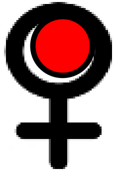
Endosymbionts: *Wolbachia pipientis*

- *Wolbachia* is an endoparasite of insects, transmitted from female to egg. Depending on host & bacterial strain may kill males, cause parthenogenesis and cytoplasmic incompatibility (CI), reduce fitness
- No naturally occurring *Wolbachia* infections of *Ae. aegypti*
- Pathogens (virus, parasites) were not able to replicate in mosquito species trans-infected with *Wolbachia* from a different host

Hoffmann *et al.* 2015

Endosymbionts: *Wolbachia pipientis*

- Releasing infected males = sterile insect technology



- Releasing infected females
  - Reduces population through CI
  - Decreases / stops vectorial capacity for other pathogens

Viral agents requirements for mosquito control

- High capacity to infect target mosquito
- Lethal or fitness altering
- Limited host range
- No phylogenetic relations with virus that infect –replicate in vertebrates
- Stable genome, non-recombinant nor integrates to host (or other virus) genome
- Technically & economically feasible production

Viral agents

	Baculoviridae Deltabaculovirus CuniNPV (DNAdc)	Parvoviridae Brevidensovirus AaeDNV (RNA)
Mosquito host	*Culex (Culex)*	*Aedes* spp.
Effects on mosquitoes	Lethal to larvae	Dose dependent larval lethality; adult longevity, fertility & fecundity. Paratransgenic agent?
Environment	Effective in eutrophic aquatic environments	Mortality influenced by larval density & age, and water temperature
Production	*in vivo* amplification. Product may be stored (suspension or powder)	*In vivo, in vitro* Viroden (USSR)
Limitations	Requires magnesium salts in larval habitat	Requires relatively high doses for lethal effects

Closing thoughts

- Need to better assess efficiency of current products on disease transmission
- Continue the development of alternative target specific products/low toxicity for non target
- Importance of sustainable management

Images

- CDC
- Janet McAllister-CDC http://slideplayer.com/slide/4607444/
- Wikipedia
- Berón C, RE Campos, **RM Gleiser**, L.M. Díaz Nieto, O.D Salomón, N Schweigmann. 2016. Investigaciones sobre mosquitos de Argentina. Universidad Nacional de Mar del Plata, Mar del Plata. ISBN: 978-987-544-721-9 http://archivo.inbiotec-conicet.gob.ar/publicaciones/libros/2016/Investigaciones-sobre-mosquitos-de-Argentina.pdf