How insecticide exposure can affect “pathogen-blocking” in *Wolbachia*-carrying insecticide-resistant *Ae. aegypti*

March 30, 2023

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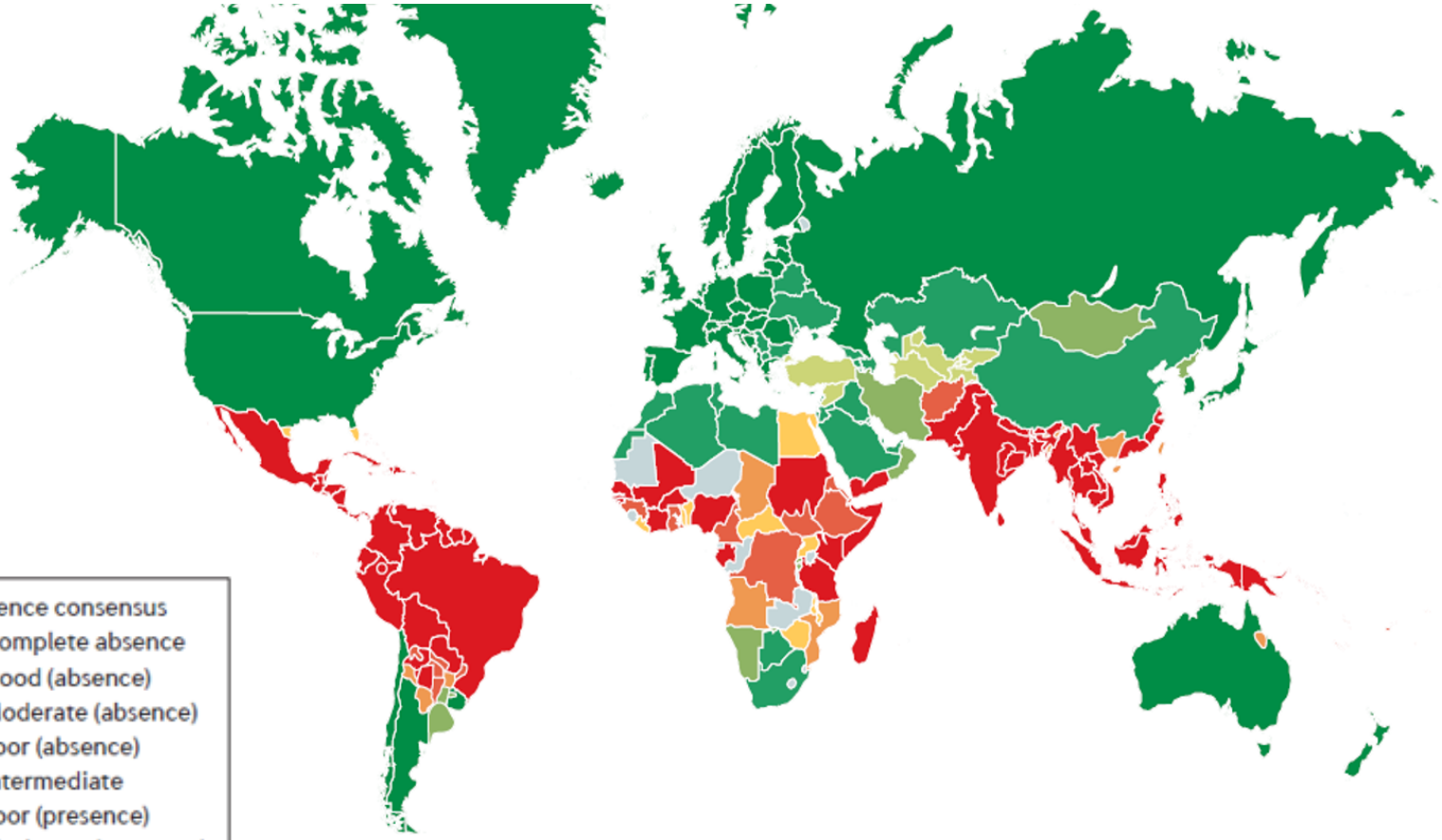
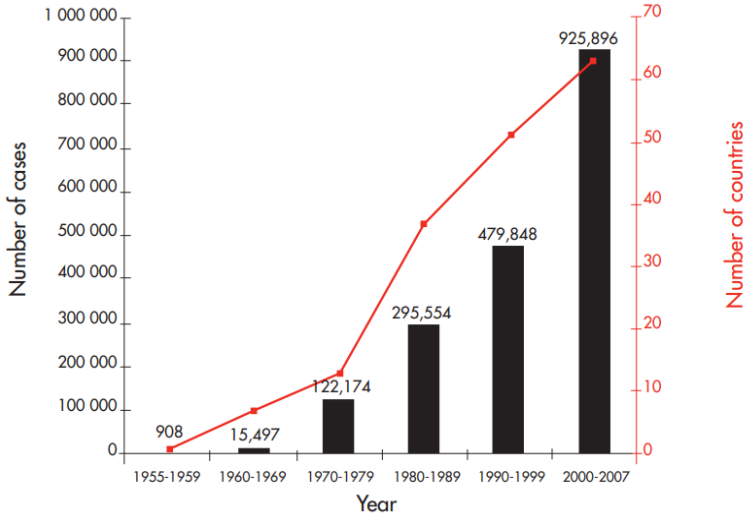
Arboviruses and Insect Vectors (AIV)

Global context

Adapted from Guzman et al. (2016)

OMS, 2009





Distribution of dengue cases in the world (2016)

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Vector control

No specific treatments and vaccines for dengue

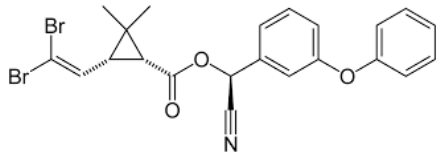
Mechanical control

→ Reduction of breeding sites

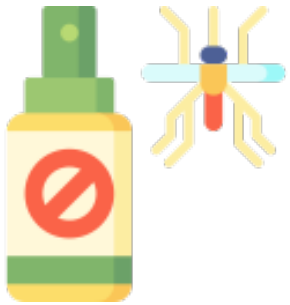
Chemical control *(authorized in France)*

→ Larvicide (*Bti*)

→ Adulticide (Deltamethrin : Pyrethroid type II)



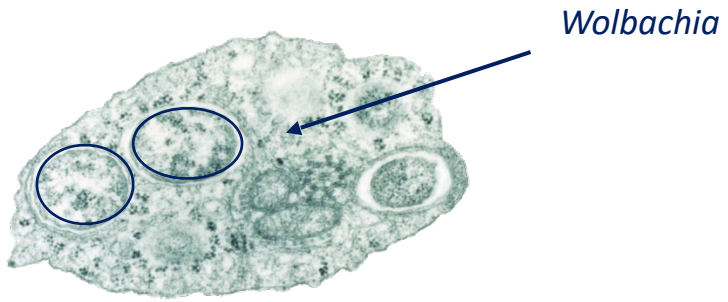
Deltamethrin



Biotechnological alternatives

→ Sterile mosquitoes

→ Use of *Wolbachia*



Crédit : Scott O'Neill

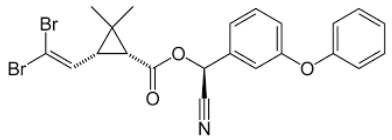
Insecticide resistance

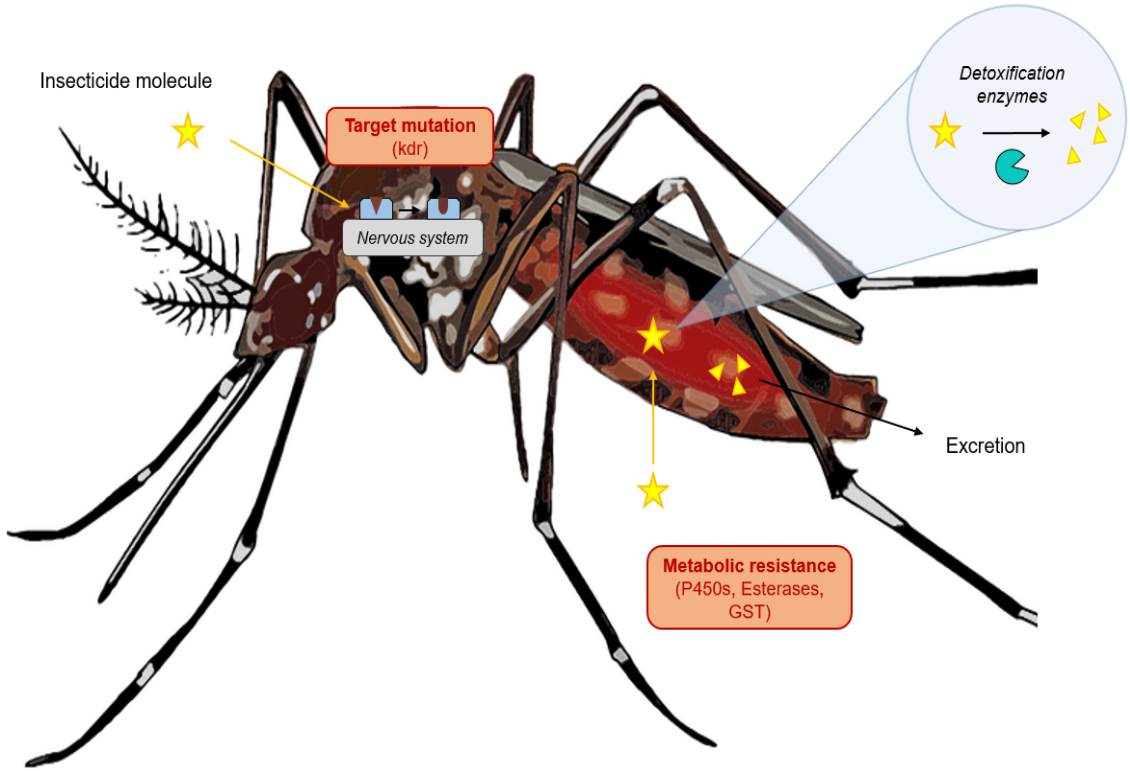
Deltamethrin (pyrethroid type II):

Target: Sodium channels (nervous system)

Action: Kinetic opening modification (alteration of action potentials)

Deltamethrin





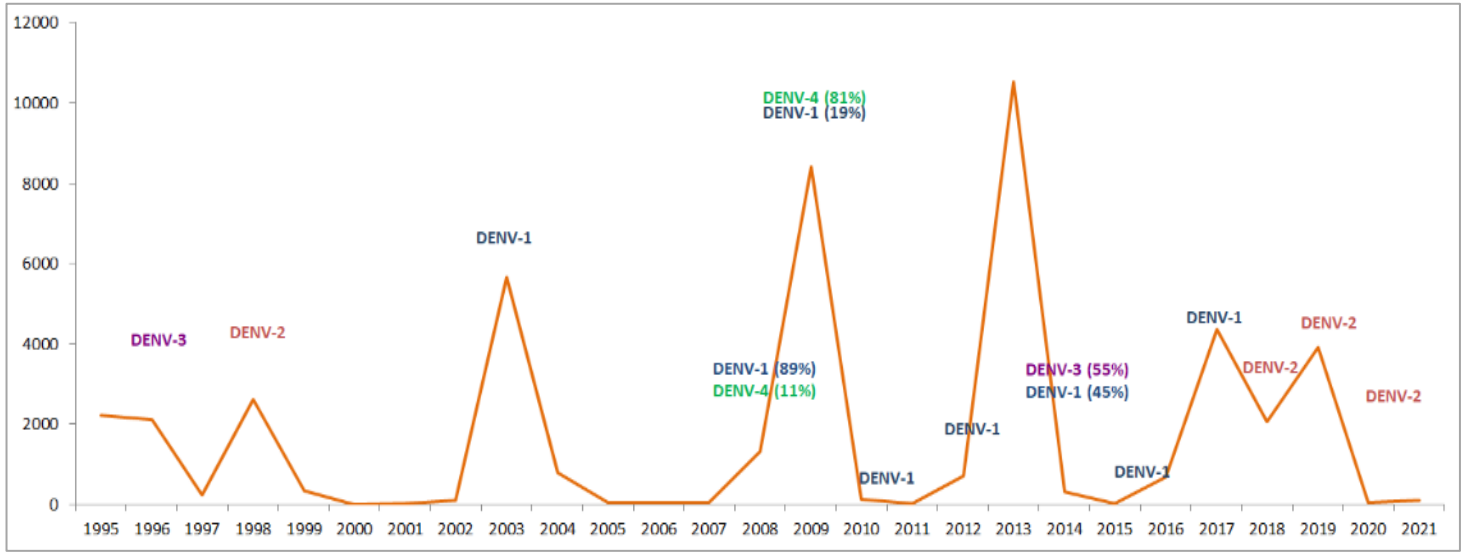
Classical types of resistance in New Caledonia:

- Mutation in sodium channels channels (***kdr* alleles : NaV gene**): (Dusfour et *al*. 2015 ; Cattel et *al*. 2021)
  - **I1011M**: Highly present in NC
  - **F1534C**: High increase in frequency in NC
- Overexpression of detoxification genes (**P450s** (oxydase) **, GST** (glutathion S-transférase)) (Cattel et *al*. 2021)

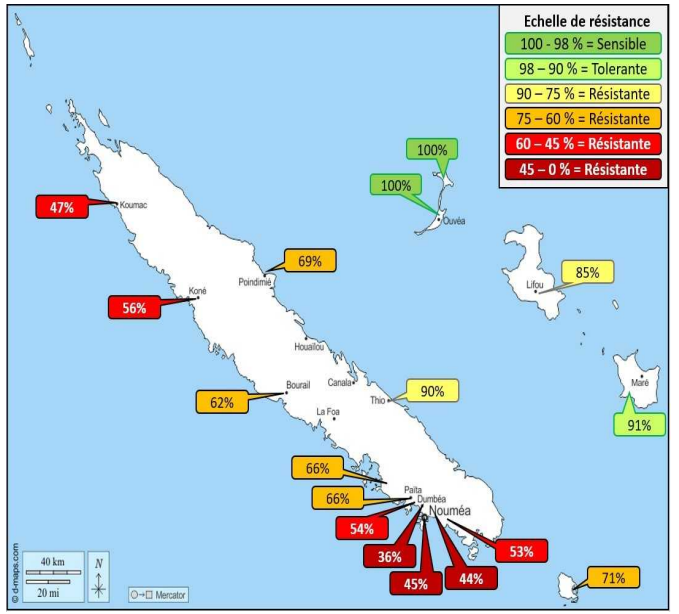
Specific context in New Caledonia

Source: DASS-NC

Confirmed cases of dengue since 1995 in New Caledonia



Atlas of New Caledonia



- Before 2019, **numerous dengue epidemics** (DENV-1, 2, 3, 4) in New Caledonia

  → Use of insecticide (deltamethrin) and selection of resistance

- **Almost all *Ae. aegypti*** populations in New Caledonia are **resistant to deltamethrin**, except on the Ouvéa Island

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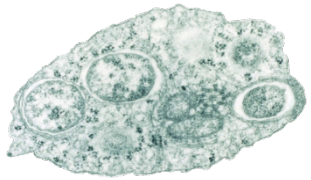
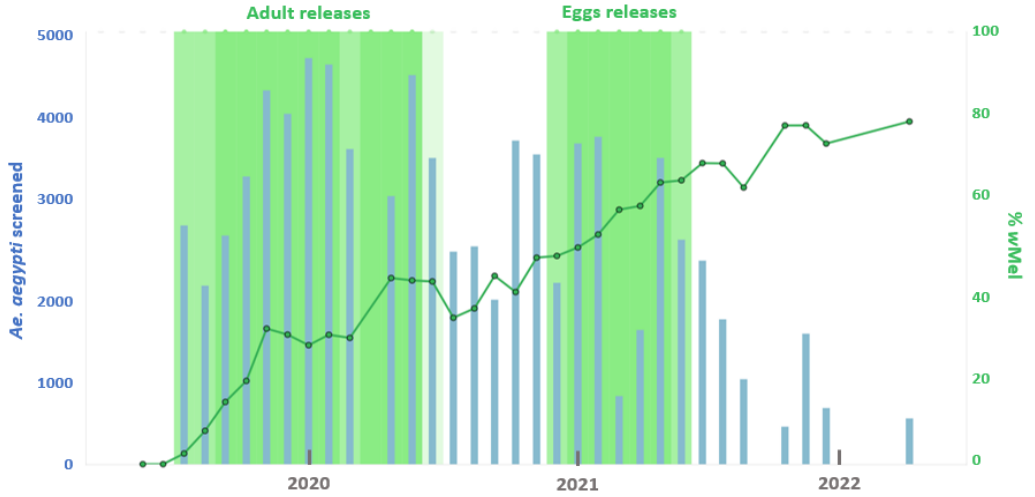
Specific context in New Caledonia

- In 2019, **World Mosquito Program**: Release of *Wolbachia*-carrying mosquitoes
  - → *Wolbachia*: **endosymbiotic bacteria**
  - → **Vertical transmission**



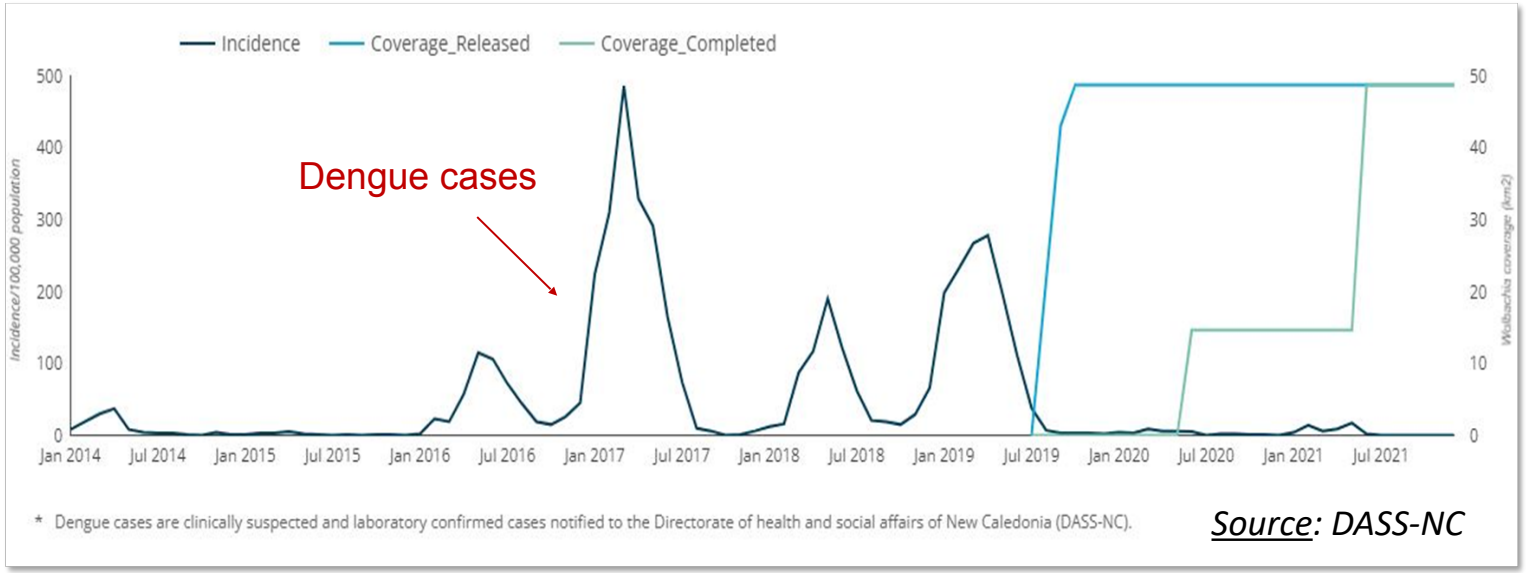
Credit: Scott O'Neill

Source: WMP-NC



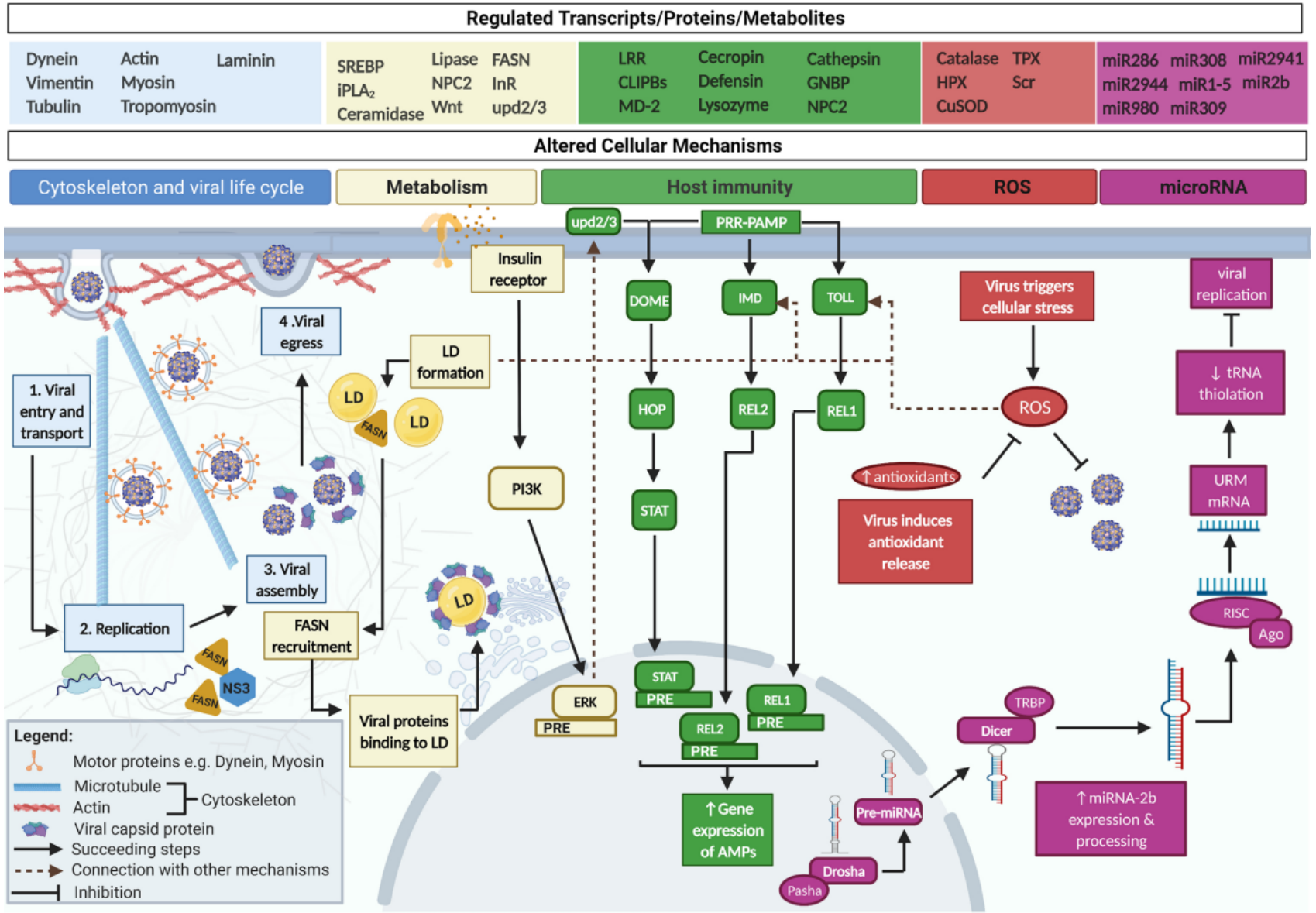
Objective: Spreading *Wolbachia* in *Aedes aegypti* populations in New Caledonia to benefit from the pathogen-blocking effect

- **Decrease in the number of dengue cases** in New Caledonia since the *Wolbachia* strategy was implemented (DASS-NC)
- In 2022, **80% of *Ae. aegypti*** of Nouméa are infected by *Wolbachia*



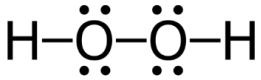
Source: DASS-NC

Wolbachia–Host–Arbovirus relationship



Reyes et al. (2021)

Reactive oxygen species (ROS) and oxidative stress



Exemple of ROS: H_2O_2, O_2^-, HO^-

Origin of ROS:

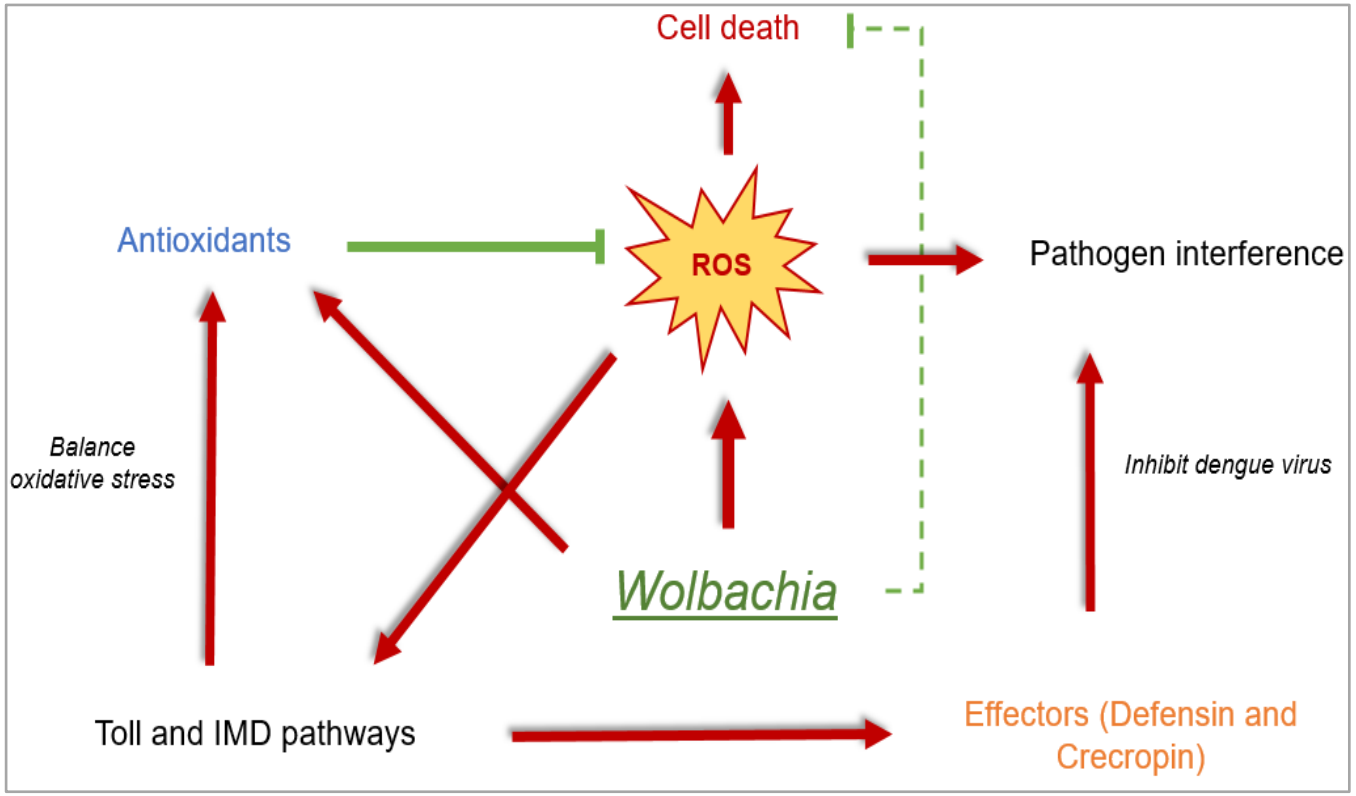
- Endogenous (mitochondria/metabolism)
- Exogenous (radiation, abiotic stress)

Effects of ROS:

- Cell death
- Damaging effects (RNA, DNA …)

Elimination of ROS:

- **Antioxidant enzymes** (Superoxide dismutase)
- **Antioxidant molécules** (vitamin A …)



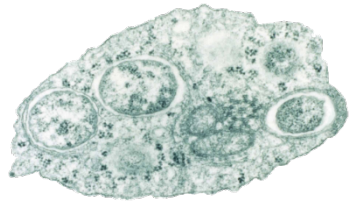
Relationship between Wolbachia and ROS

Hypothesis and objectives of the project

Hypothesis:

- Exposure to an **insecticide treatment** (deltamethrin) may **alter** the **"pathogen-blocking"** effect induced by *Wolbachia* in **insecticide-resistant *Ae. aegypti* carrying *Wolbachia***

  - Presence of *Wolbachia* → Oxidative stress
  - Insecticide resistance → Oxidative stress (P450) or protection against ROS (GST) ???
  - Insecticide exposure → Oxidative stress ???
  - **Is there an accumulation of oxidative stress ? Does this high oxidative stress impact the "pathogen-blocking" effect ?**



Main objectives:

1. Assess the **impact of insecticide resistance** on **oxidative stress (ROS)** in ***Wolbachia*-carrying *Ae. aegypti***
2. Ensure that **insecticide exposure of resistant mosquitoes** does **not alter the blockage of viral replication** by ***Wolbachia***

General method planned

1st year

2nd year

Oct 2023

Sept 2026

Mosquito strains and resistance selection

Bioassays and resistance characterization

Oxidative stress relative to *Wolbachia* and resistance

Oxidative stress and susceptibility to virus infection

Diversity of viral population in mosquitoes

Mosquito strains and resistance selection

Field mission in New Caledonia

W : *Wolbachia*
R : Resistant

→ Collections of mosquitoes in 2 localities
Generation of 4 mosquito strains

W+R+

↳ *Nouméa*

W-R+

↳ *Nouméa*

W-R-

↳ *Ouvéa*

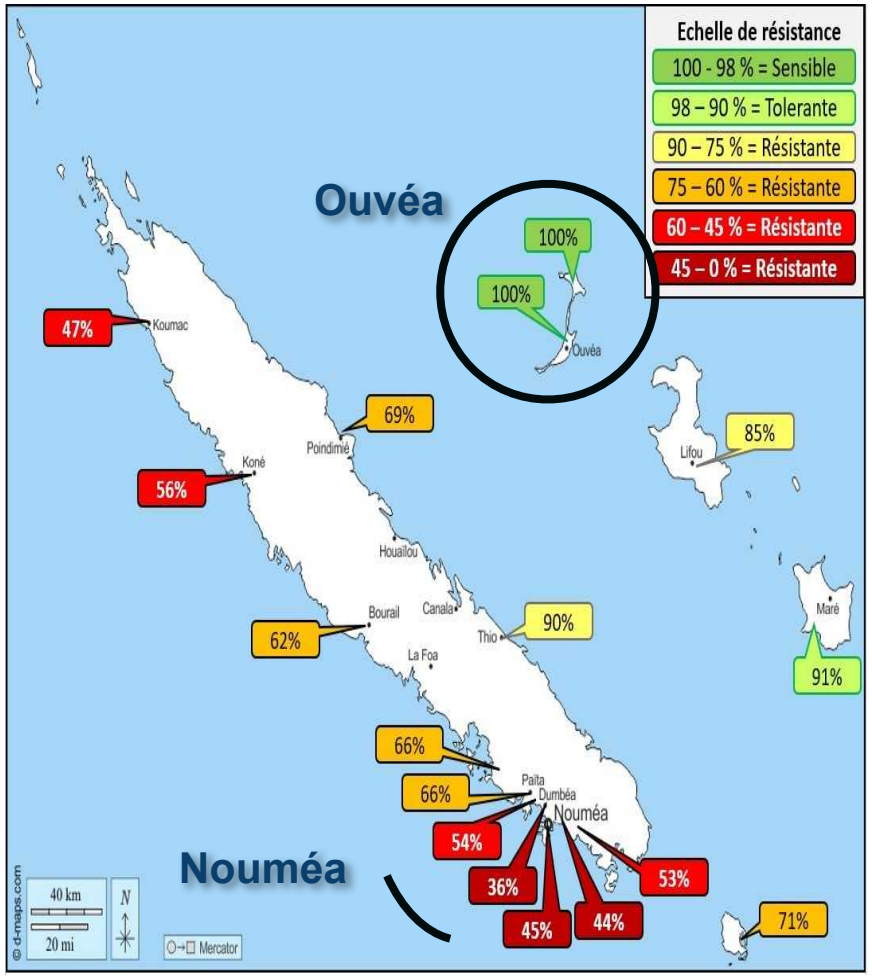
W+R-

↓

Back cross entre ♀ W+R+ et ♂ W-R-
6 generations

Selection by exposure to deltamethrin: increase in the frequency of resistant genes

Tetracycline treatment to eliminate *Wolbachia* = same genetic background as W+R+ but without *Wolbachia*



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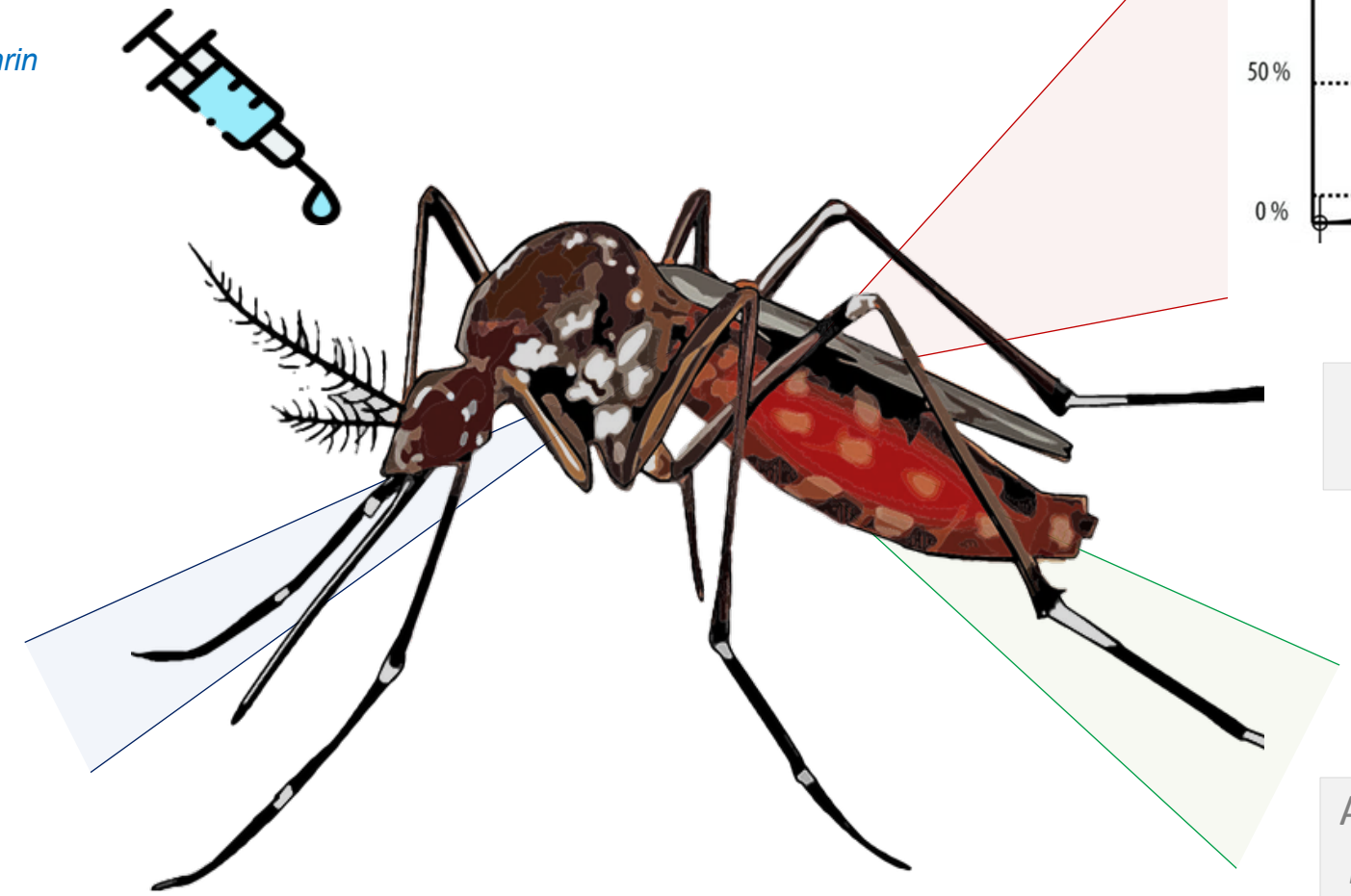
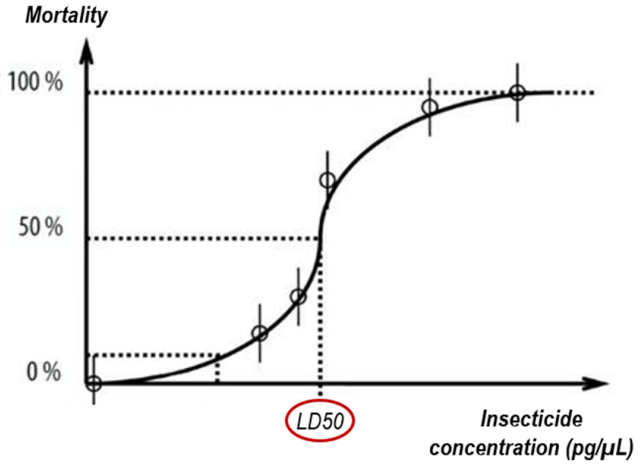
Bioassays and resistance characterization

Objective: Characterize the resistance to deltamethrin of *Ae. aegypti*

Topical application of deltamethrin



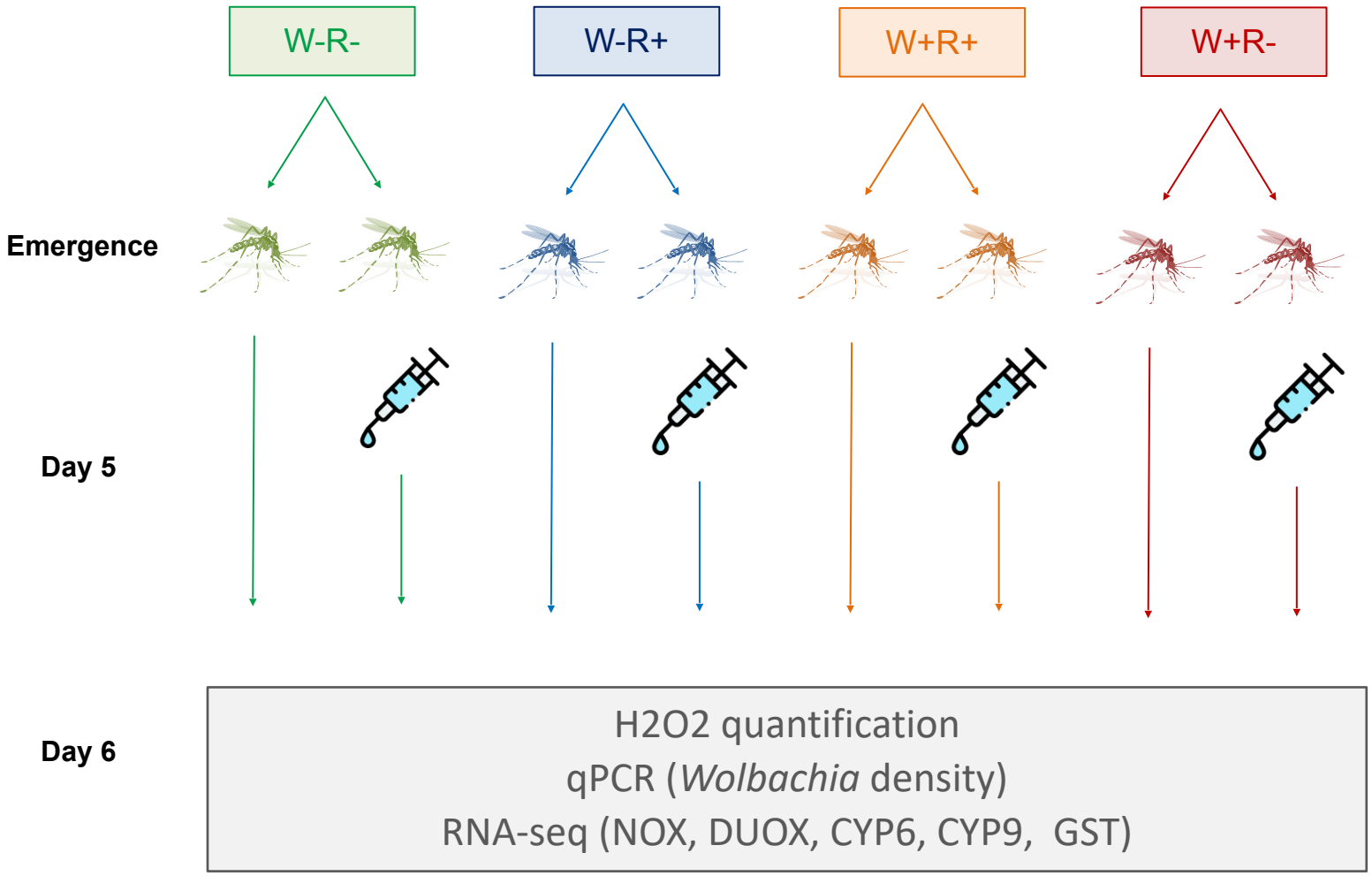


Calculation of LD50 and RR50 by dose-response

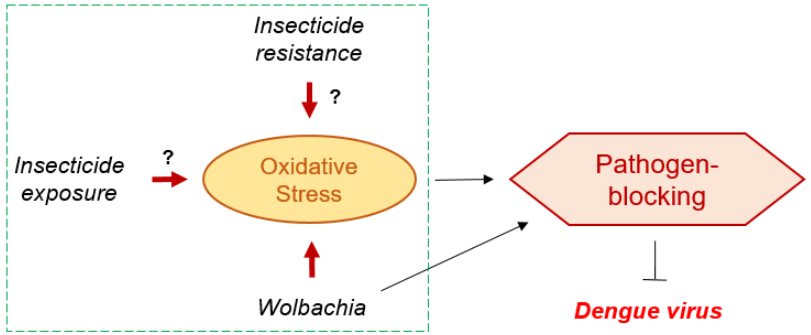
Determine sub-lethal doses of deltamethrin

Assessing the frequency of *kdr* mutations by RT-qPCR

Oxidative stress relative to *Wolbachia* and resistance

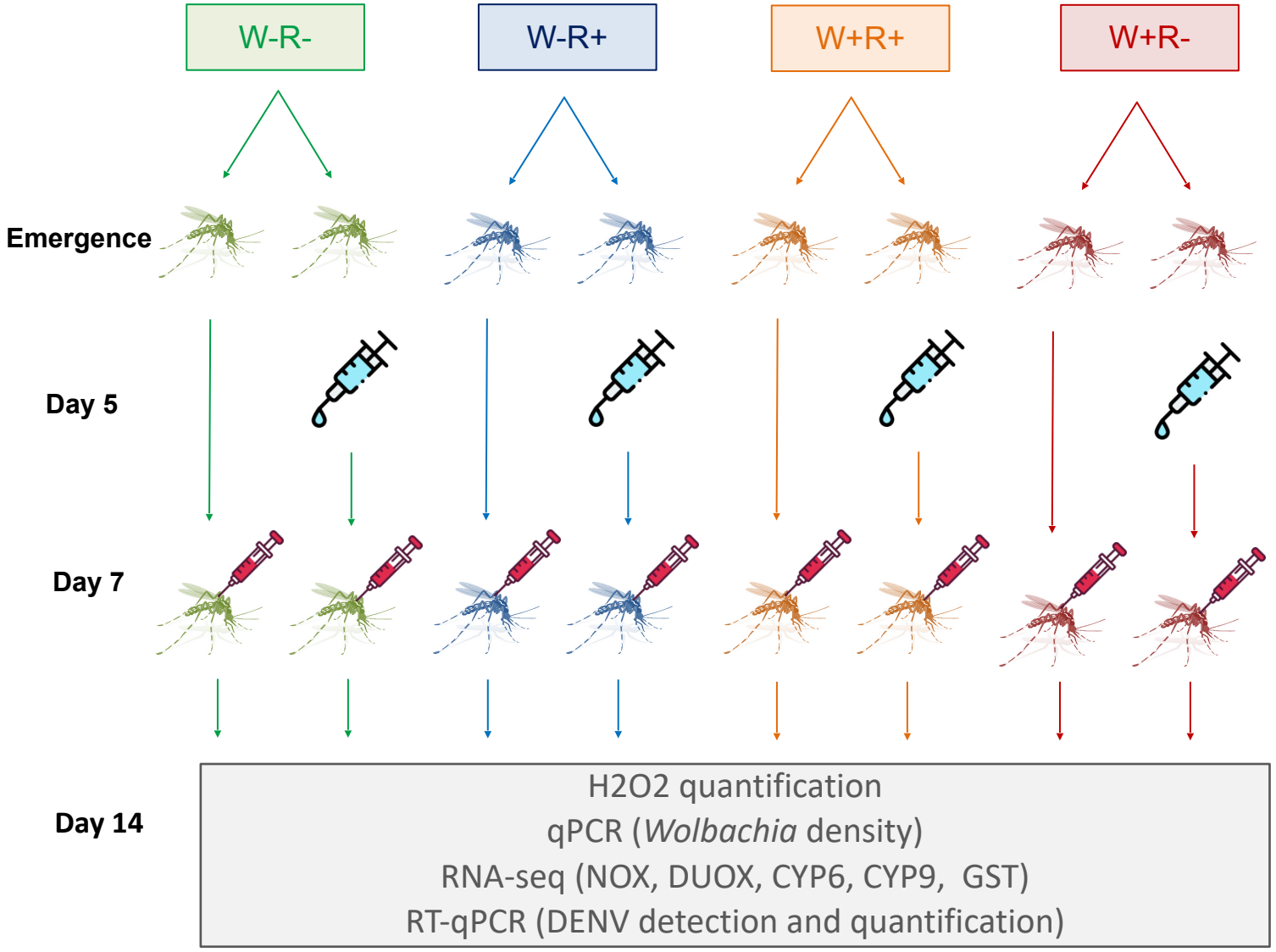


Objective

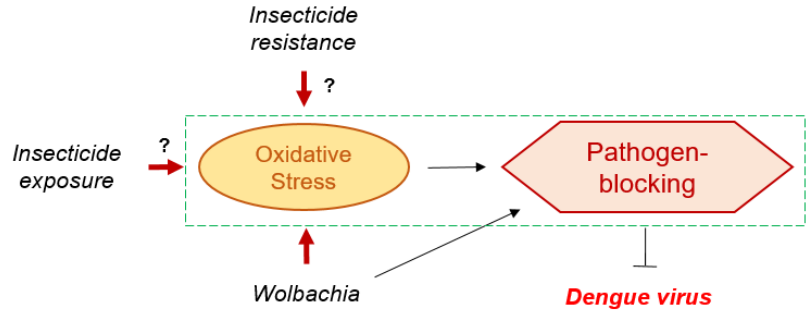


- **H2O2 quantification**: direct indicator of oxidative stress
- ***Wolbachia* density**: to understand if there is an impact of insecticide exposure on *Wolbachia* density and hence potentially "pathogen-blocking" effect
- **Genes expression by RNAseq:**
  - Oxidative stress markers (NADPH oxydase, dual oxydase, P450s)
  - Enzymes found in NC in 2015
  - Other gene expression modulation

Oxidative stress and susceptibility to virus infection



Objective



- **Intrathoracic injection** of the virus
- Study of the **same parameters** (H2O2, *Wolbachia* density et genes expression) as the previous part to study the **impact of the presence of the virus**
- **Detection** and **quantification** of DENV

Deltamethrin

Intrathoracic injection DENV

Conclusion

The objective is to ensure that the efficiency of the "**pathogen-blocking**" effect is **not affected by exposure** to the insecticide

Measure the **impact** (positive or negative) **of insecticide exposure on viral infection**, and verify that ***Wolbachia* densities** in insecticide-resistant mosquitoes are not affected by exposure to the insecticide.

Assessing the existence of cumulative **oxidative stress (ROS)** due to *Wolbachia* presence, insecticide resistance and exposure to the insecticide which may increases this stress.

Provide **recommendations** on the **use of insecticides** when implementing new strategies based on ***Wolbachia***

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Department of Virology

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Thank you for your attention

March 30, 2023

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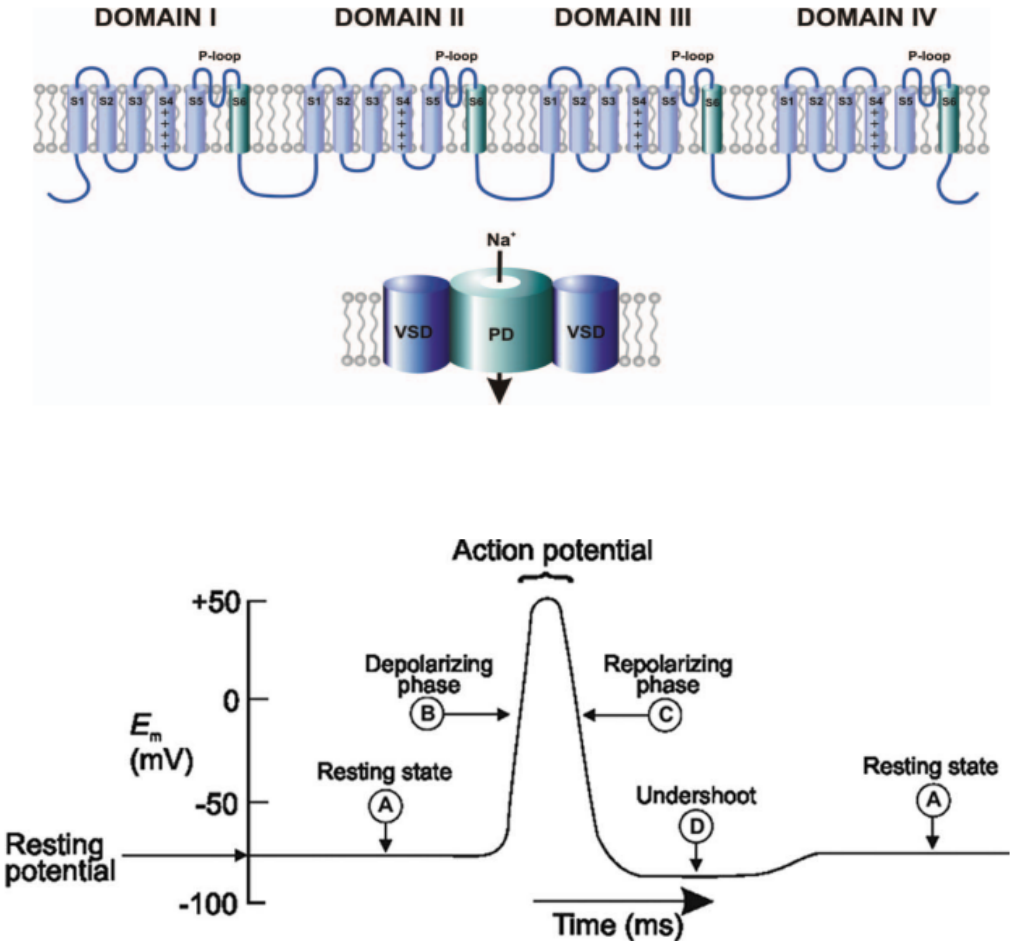
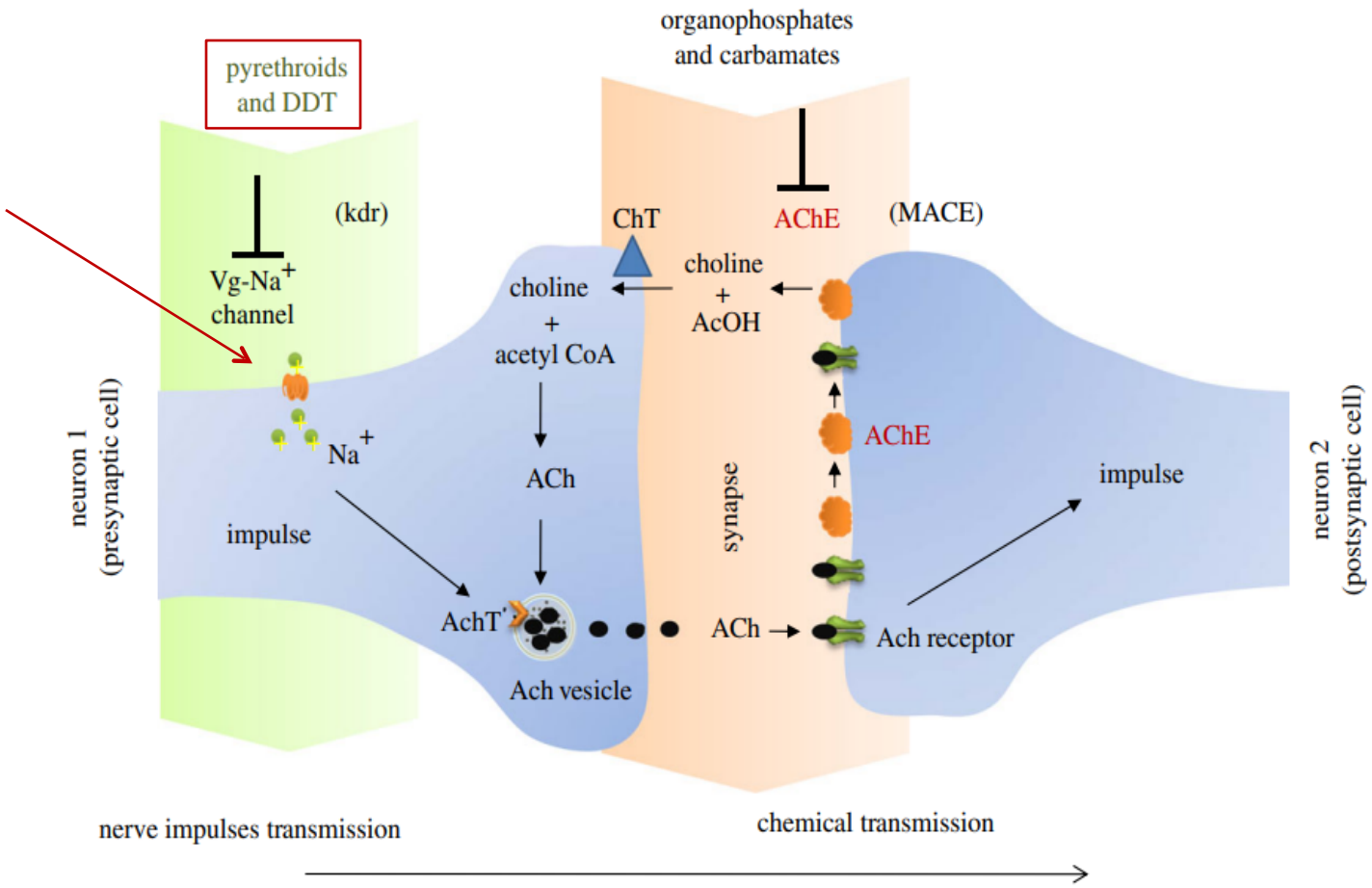
Insecticide mechanisms

T. G. E. Davies et al., 2007



Jean-Philippe DAVID et al., 2013



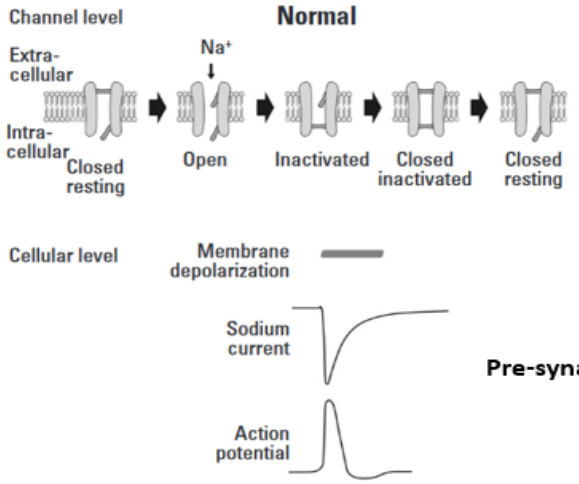
VSD: Voltage Sensing Domains

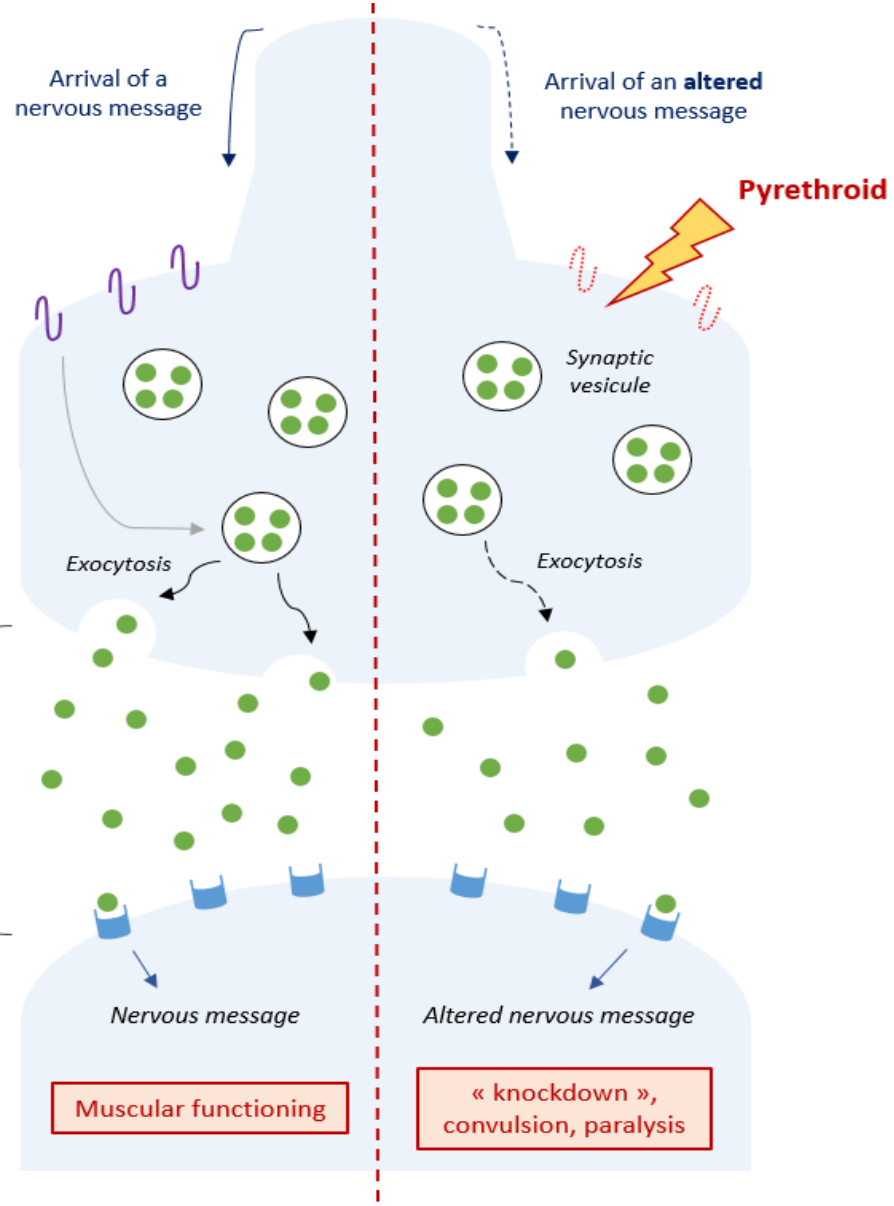
Insecticide mechanisms

X

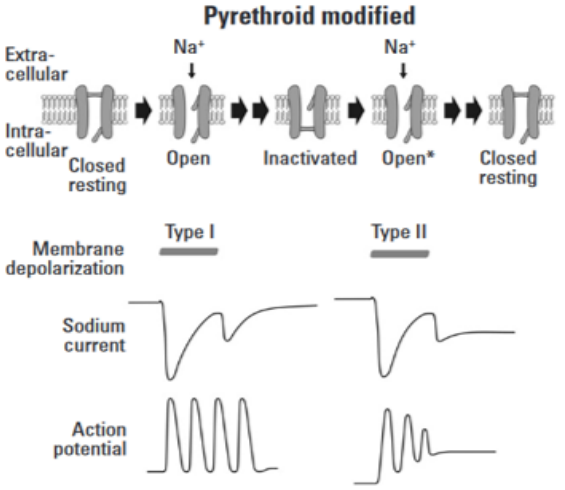
30.03.2023

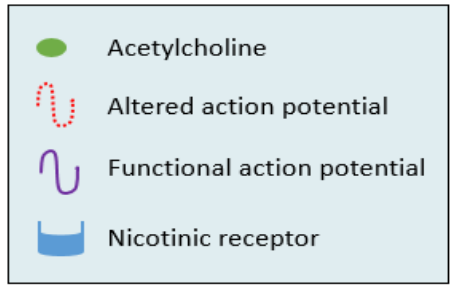
Timothy J. Shafer et al., 2005





Timothy J. Shafer et al., 2005



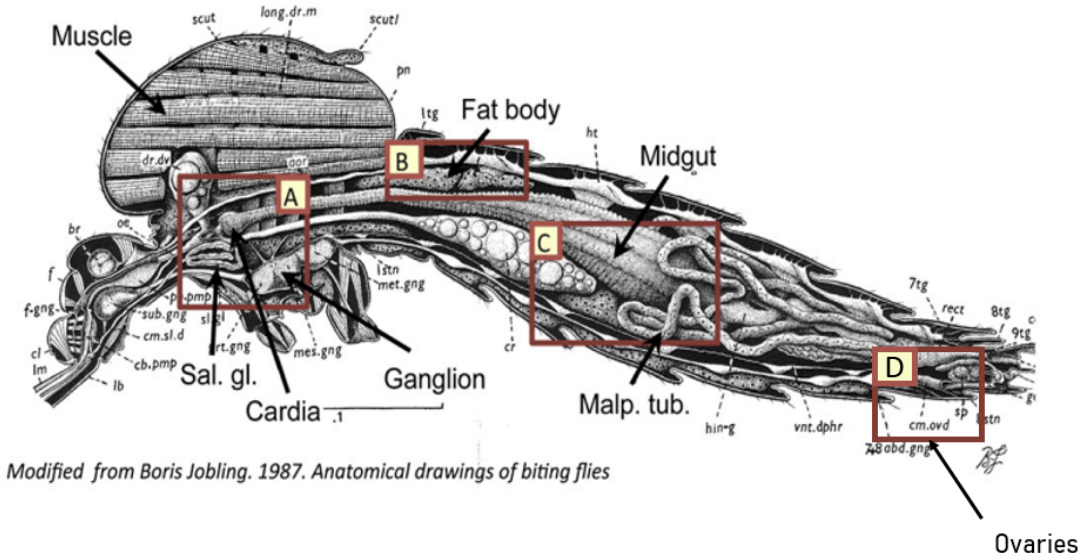


No Pyrethroid

With Pyrethroid

Effects of *Wolbachia* on the pathogen-blocking in *Ae. aegypti*

Adapted from Moreira et al., 2009



Modified from Boris Jobling. 1987. Anatomical drawings of biting flies

- **Localisation** of *Wolbachia* (**reproductive and somatic tissues**)
- Different **original hosts**
- **Different mechanistic effects** on *Ae. Aegypti*
- Affects **different viruses** depending on the **strain**

Reyes et al., 2021

Wolbachia strain	Natural host	Mechanistic effect on *Ae. aegypti*	Virus	References
Supergroup A				
*w*AlbA	*Ae. albopictus*	Did not reduce oral/intrathoracic viral infection	DENV (oral and intrathoracic), ZIKV (intrathoracic)	Chouin-Carneiro et al., 2020[b]
		Reduced oral infection	ZIKV only	
*w*MelPop	*D. melanogaster*	Increase in cholesterol cellular content	DENV	Geoghegan et al., 2017[a]
		Immunity	DENV	Fraser et al., 2020 [b]
*w*MelPop-CLA	*D. melanogaster*	Immunity	DENV, CHIKV	Moreira et al., 2009[b] Asad et al., 2018[a,b]
*w*Mel	*D. melanogaster*	Increase in cholesterol cellular content	DENV	Geoghegan et al., 2017[a,b]
		Decrease selected lipids necessary for viral infection	DENV, ZIKV	Koh et al., 2020[b] Manokaran et al., 2020[a]
		Reduced activity of insulin receptor	ZIKV	Haqshenas et al., 2019[a,b]
		Little expression of defensin and cecropin. Not comparable with *w*MelPop	DENV	Fraser et al., 2020[b]
		Cellular regeneration	DENV	Ford et al., 2020[b]
Supergroup B				
*w*AlbB	*Ae. albopictus*	Direct inhibition of viral binding and entry	DENV, ZIKV	Lu et al., 2020[a]
		ROS-mediated toll activation	DENV	Pan et al., 2012[b]
*w*Pip	*Cx. quinquefasciatus*	Did not confer protective immunity	DENV	Fraser et al., 2020[b]

[a] *in vitro.*
[b] *in vivo.*

Multiple Wolbachia strains under supergroups A and B have been found to induce varying pathogen blocking effects in Ae. aegypti. These strains are tested either in vitro, in vivo, or both.