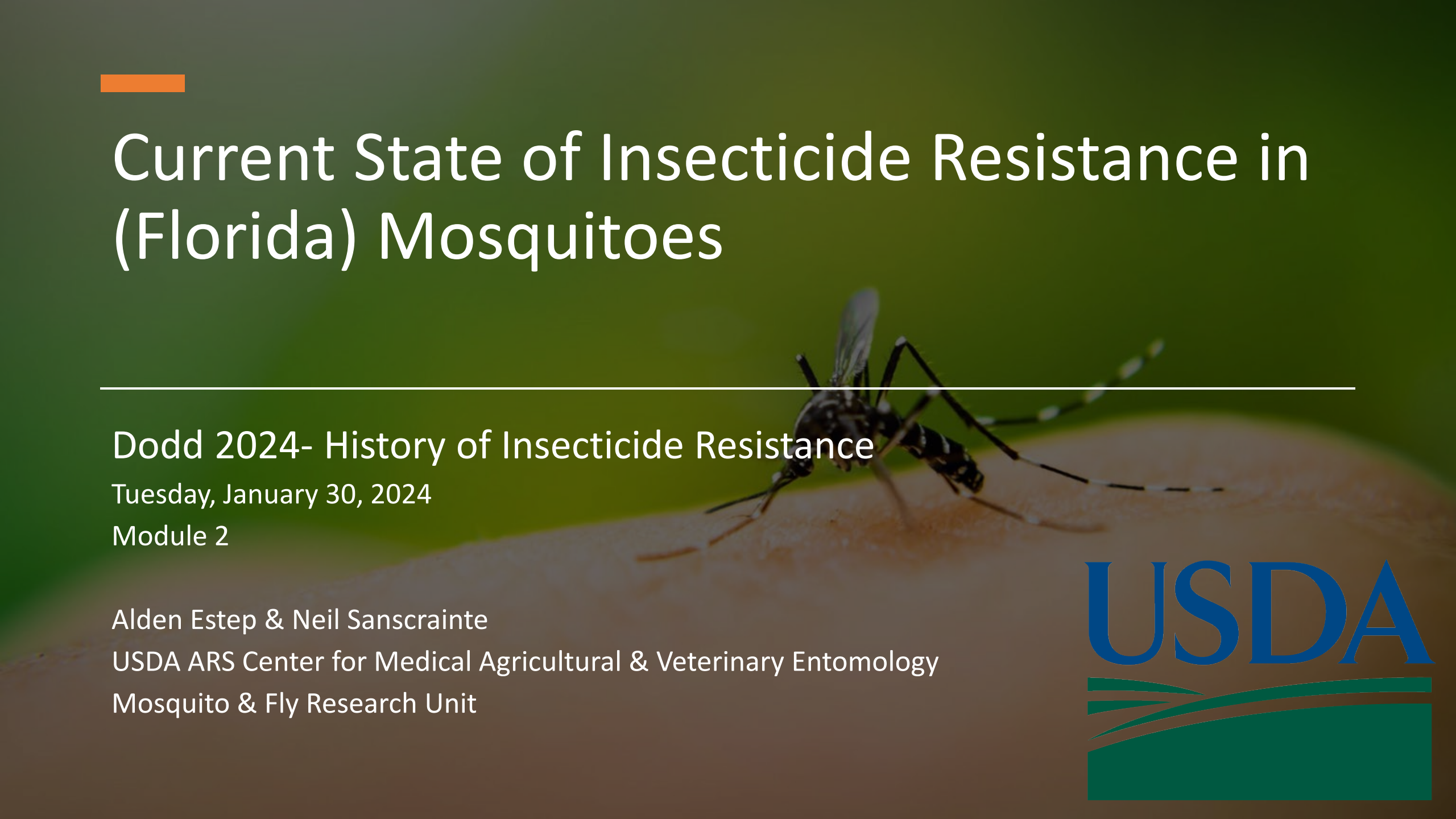
Current State of Insecticide Resistance in (Florida) Mosquitoes



Dodd 2024- History of Insecticide Resistance

Tuesday, January 30, 2024

Module 2

Alden Estep & Neil Sanscrainte

USDA ARS Center for Medical Agricultural & Veterinary Entomology

Mosquito & Fly Research Unit

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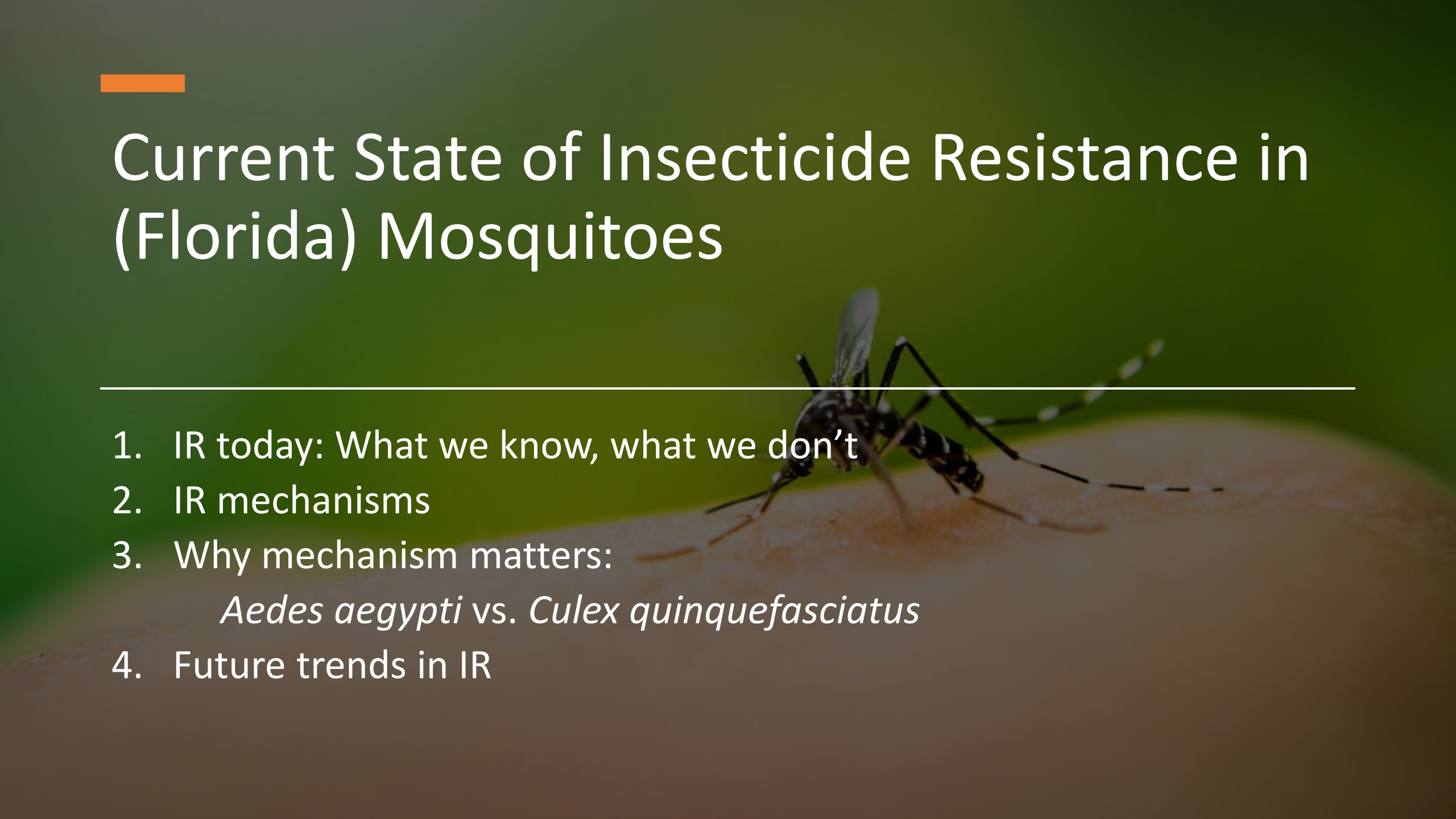

1. IR today: What we know, what we don't
2. IR mechanisms
3. Why mechanism matters:
   Aedes aegypti vs. *Culex quinquefasciatus*
4. Future trends in IR

The Overview:

- Florida leads the country in IR assessment in mosquitoes
- Only state with multiple statewide surveys
- Over 50 published studies have examined IR in Florida
- Resistance studies have been conducted since the 1940s

Most populations of *Aedes aegypti* are resistant to pyrethroids

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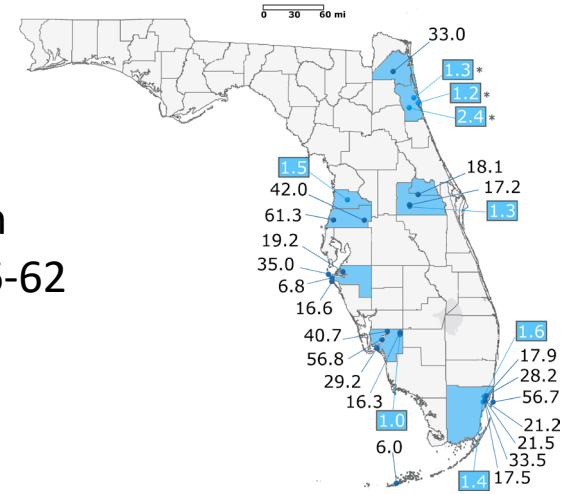
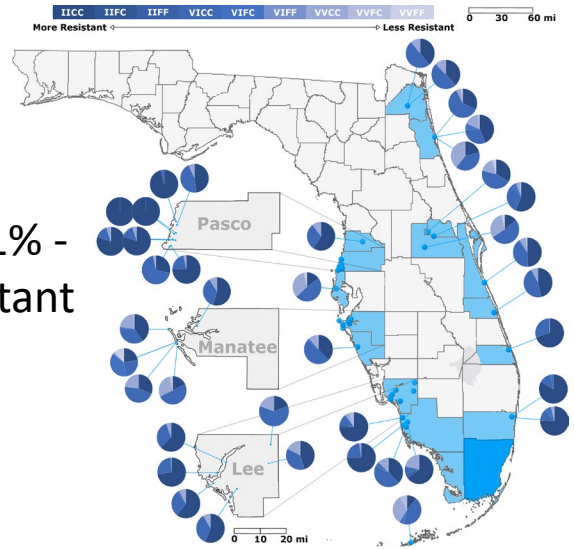
21 populations with permethrin RRs from 6-62



60 populations with 11% - 100% of the most resistant *kdr* genotype



Avg IICC% ~45%

Estep et al. 2018-doi.org/10.1371/journal.pntd.0006544

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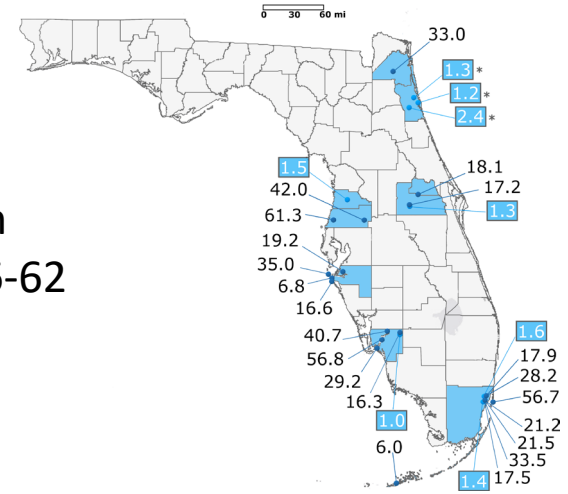

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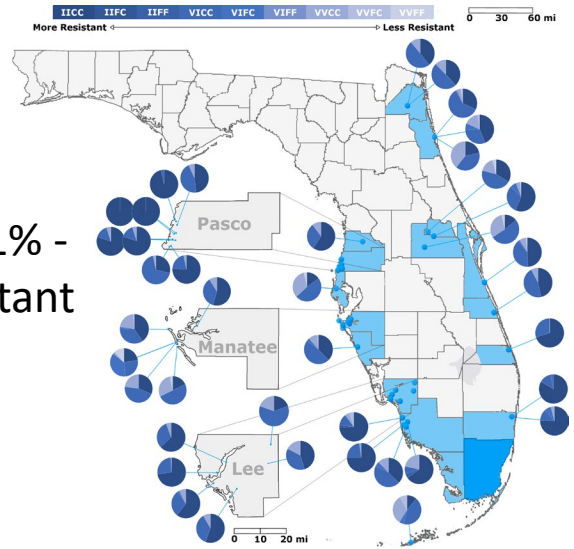

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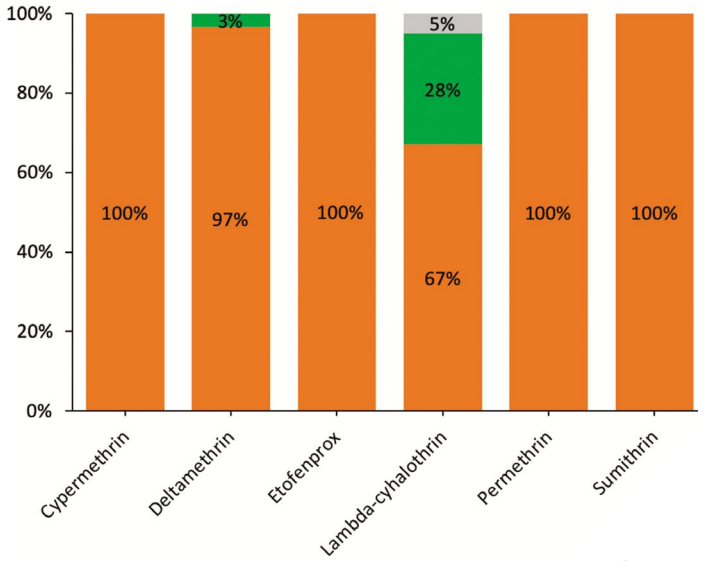
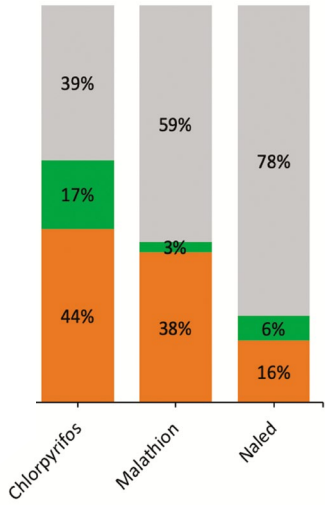


33 populations 100% resistant to permethrin
97% resistant to deltamethrin



Resistance to OPs was variable and tended to be relatively weak

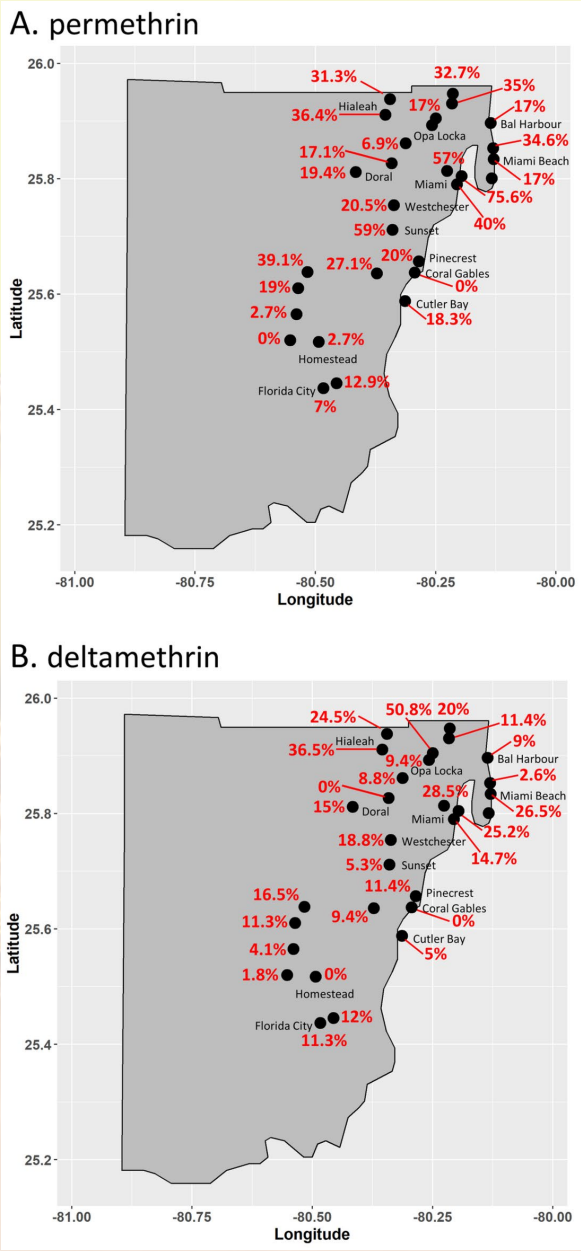
Parker et al. 2020-doi.org/10.1371/journal.pntd.0006544

Most populations of *Culex quinquefasciatus* are resistant to pyrethroids and many are resistant to organophosphates

Insecticide Resistance in Miami *Culex quinquefasciatus*

All Miami populations resistant to permethrin

All Miami populations resistant to deltamethrin



Unlu et al. 2024-doi.org/10.1371/journal.pone.0296046

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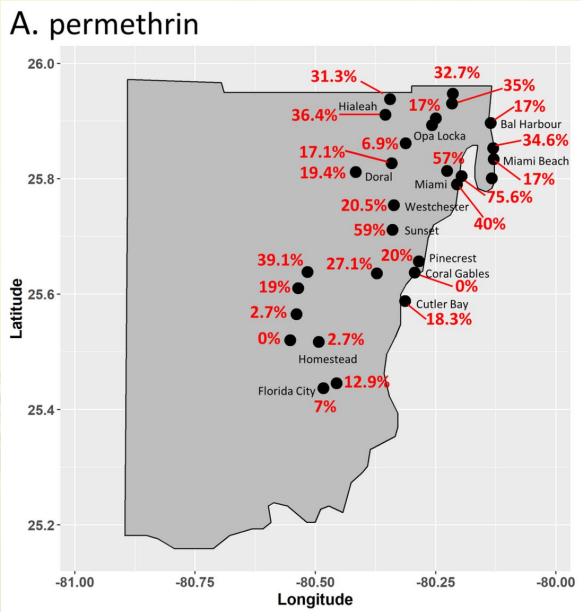
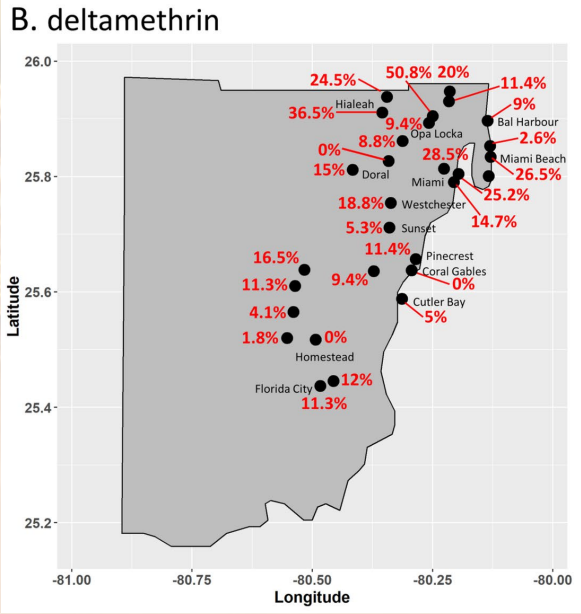
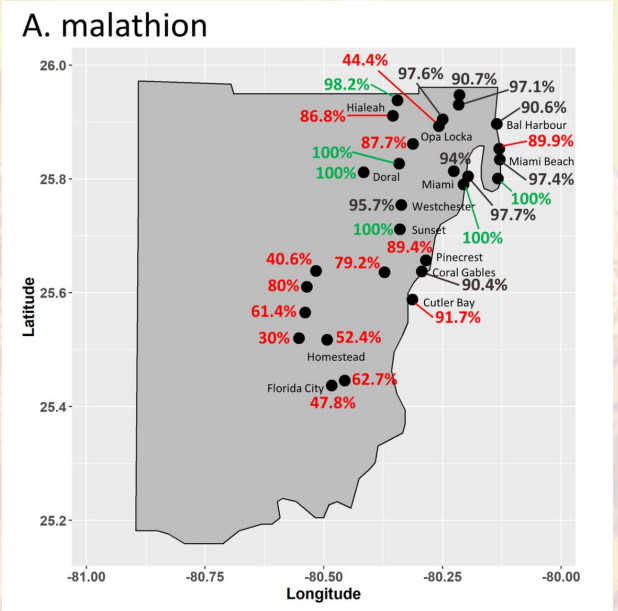
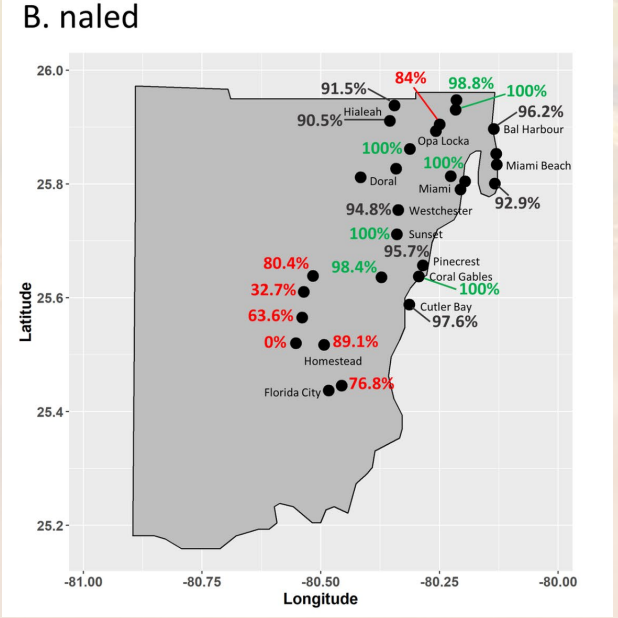




All Miami populations resistant to deltamethrin



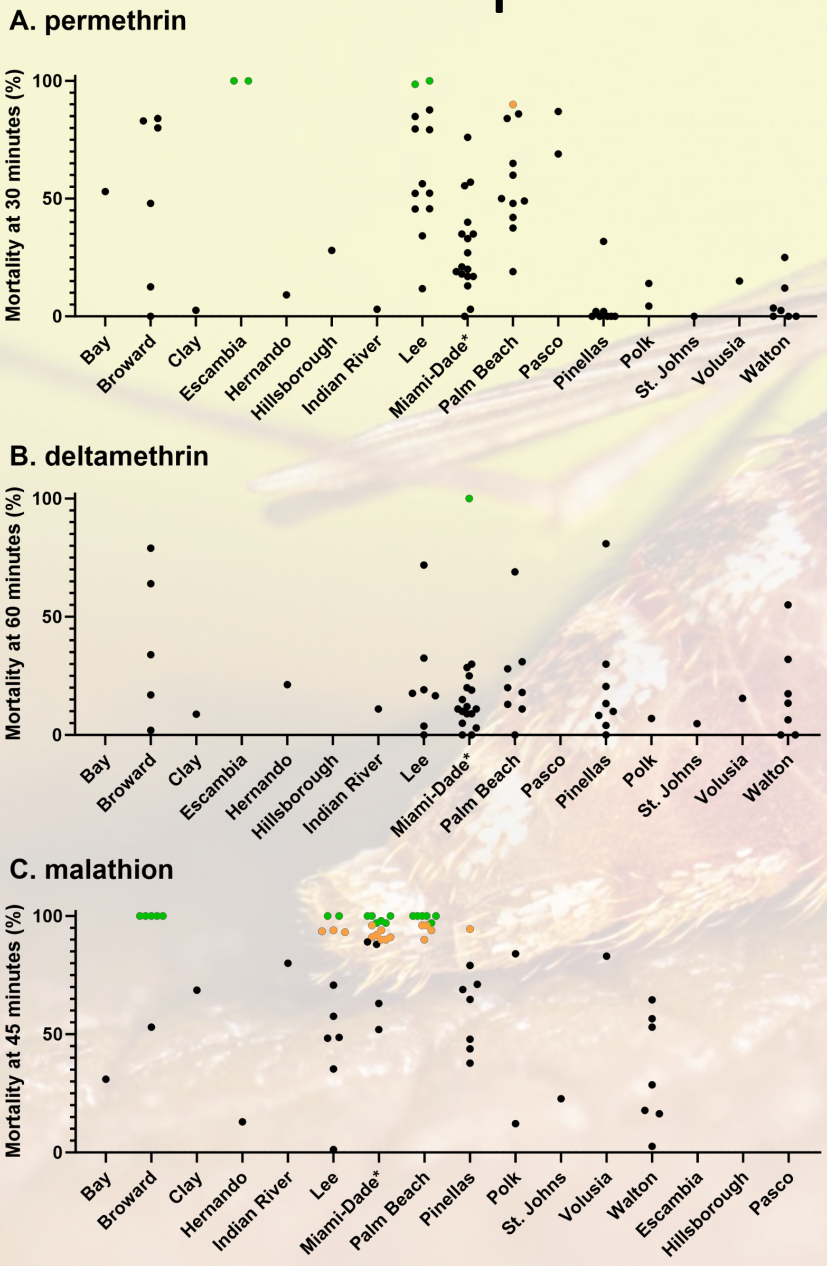
Mixture of IR intensity to malathion



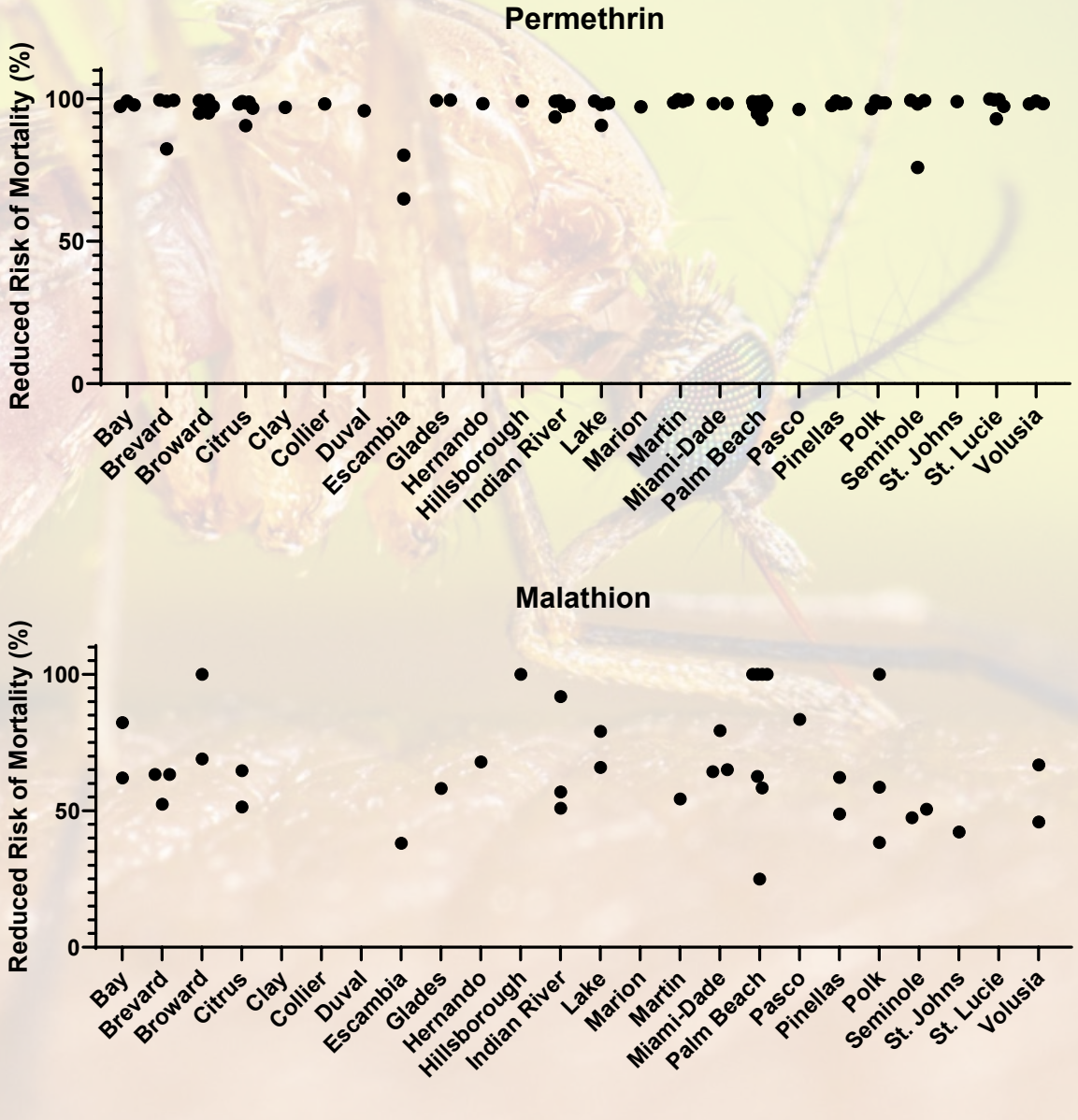
IR can be strong enough to reduce Naled efficacy

Unlu et al. 2024-doi.org/10.1371/journal.pone.0296046

Same pattern observed across the state of Florida



Estep et al (in prep)



Fedirko et al (in prep)

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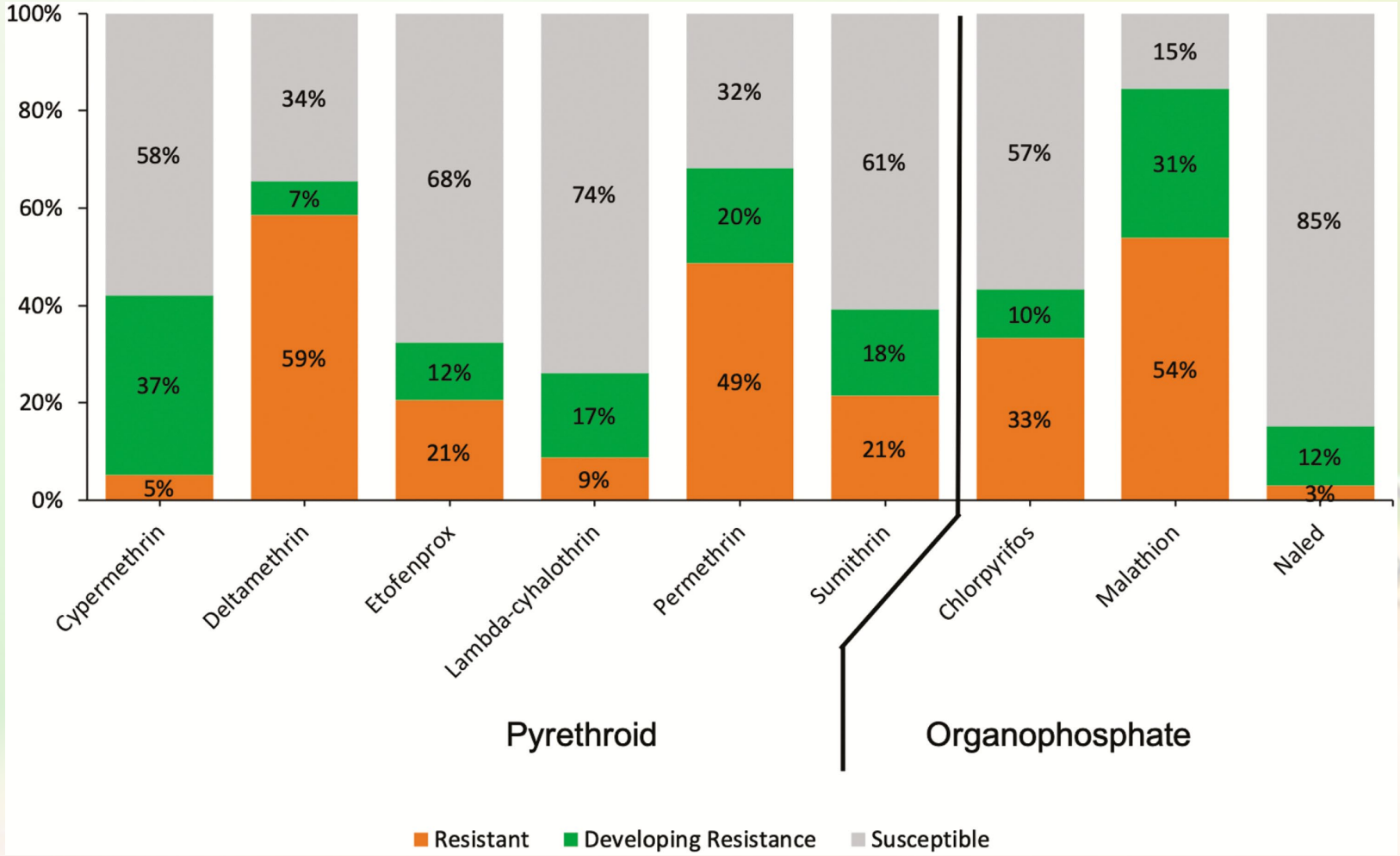

Low level IR to permethrin, deltamethrin, malathion & Naled in some populations

Parker et al. 2020-doi.org/10.1371/journal.pntd.0006544

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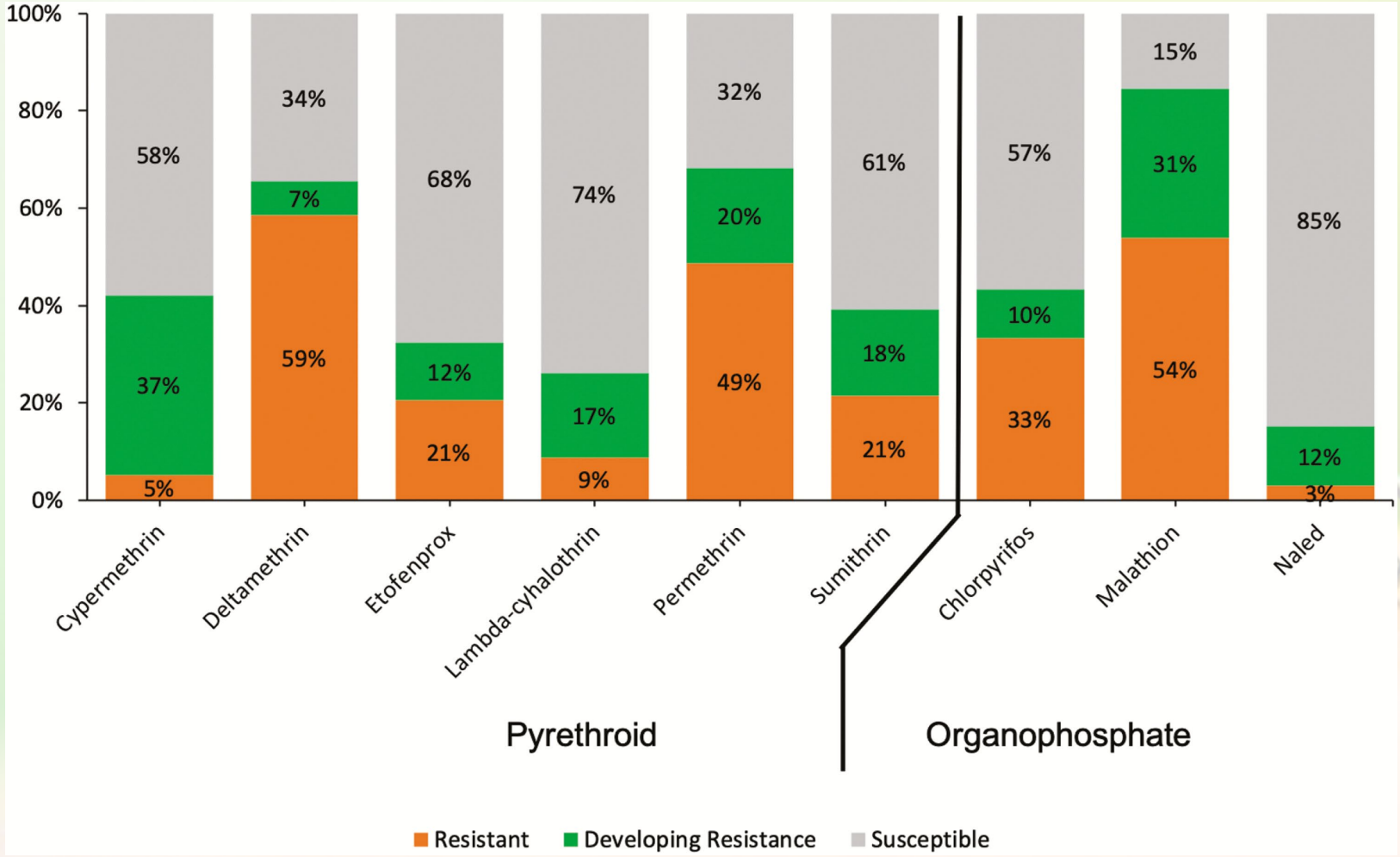
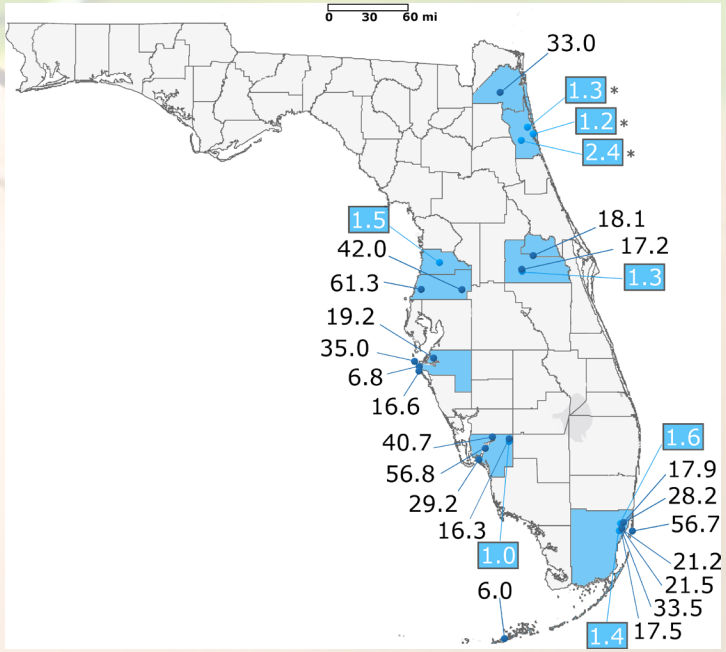


Low level IR to permethrin, deltamethrin, malathion & Naled in some populations

Topical application confirms that the IR to pyrethroids is weak (RR<3)



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IR Status of other species

Cx. nigripalpus

Ae. taeniorhynchus ??

An. crucians

An. quadrimaculatus

IR mechanisms underlying the resistance

- Behavioral resistance
- Penetration (cuticular) resistance
- Metabolic resistance
- Target-site resistance

IR mechanisms underlying the resistance

- Behavioral resistance
  - Alter mobility behavior to avoid contamination
  - Alter reproductive behaviors
  - Fire ants
    - Remove unhealthy materials
    - Grooming of self and others
    - Cover toxic areas

"…behavior that reduces an insect's exposure to toxic compounds or that allows an insect to survive in what would otherwise be a toxic and fatal environment."

Sparks, T. C., Lockwood, J. A., Byford, R. L., Graves, J. B., and Leonard, B. R. (1989). The role of behavior in insecticide resistance. Pestic. Sci. 26, 383–399. doi:10.1002/ps.2780260406

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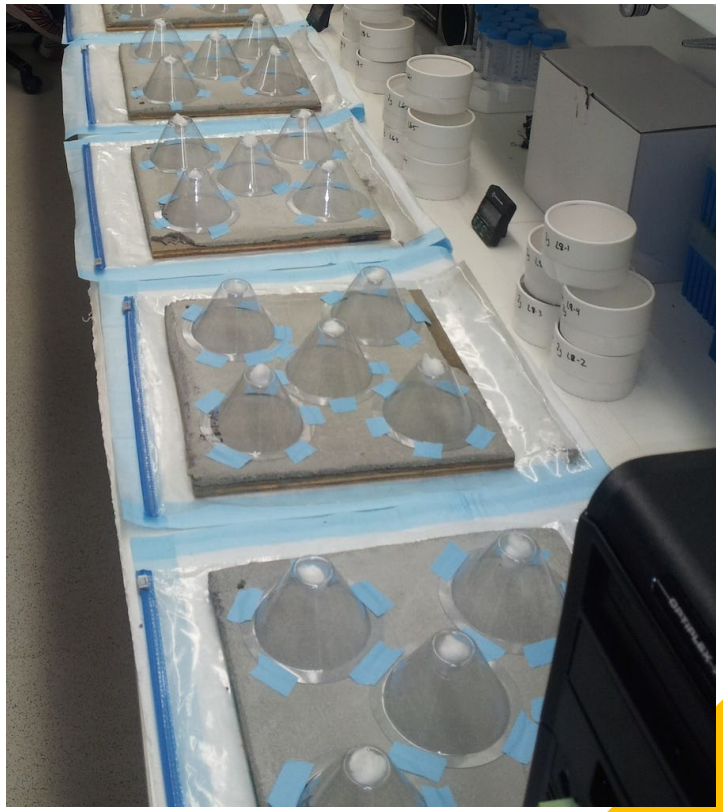

Dunford, J.C., Estep, A.S., Waits, C.M., Richardson, A.G., Hoel, D.F., Horn, K., Walker, T.W., Blersch, J.S., Kerce, J.D. and Wirtz, R.A., 2018. Evaluation of the long-term efficacy of K-Othrine® PolyZone on three surfaces against laboratory reared Anopheles gambiae in semi-field conditions. *Malaria Journal*, *17*(1), pp.1-7.

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Photo Credit: James Gathany Content Providers(s): CDC

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https://www.pctonline.com/news/seven-tips-blow-fly-management/

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IR mechanisms underlying the resistance

- Penetration (cuticular) resistance
  - Resistant *An. gambiae* have thicker cuticles
  - Silencing a cuticle gene in resistant *Cx. pipiens (quinqs)* = decreased cuticle

"Insects create barriers…using their outer cuticle, which protects them against a wide spectrum of insecticides."

Siddiqui JA, Fan R, Naz H, Bamisile BS, Hafeez M, Ghani MI, Wei Y, Xu Y and Chen X (2023), Insights into insecticide resistance mechanisms in invasive species: Challenges and control strategies. Front. Physiol. 13:1112278.

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IR mechanisms underlying the resistance

- Metabolic resistance
  - Resists multiples classes of AIs
  - Multiple classes of enzymes
    - Carboxylesterases
    - Glutathione S-transferases
    - Cytochrome P450
  - Synergists help with
    - Mosquito control
    - Test for metabolic resistance

"Metabolic resistance is a type of resistance inferred by metabolic activities in insects that help them detoxify or break down contaminants or the ability to eliminate toxic compounds from their bodies more quickly."

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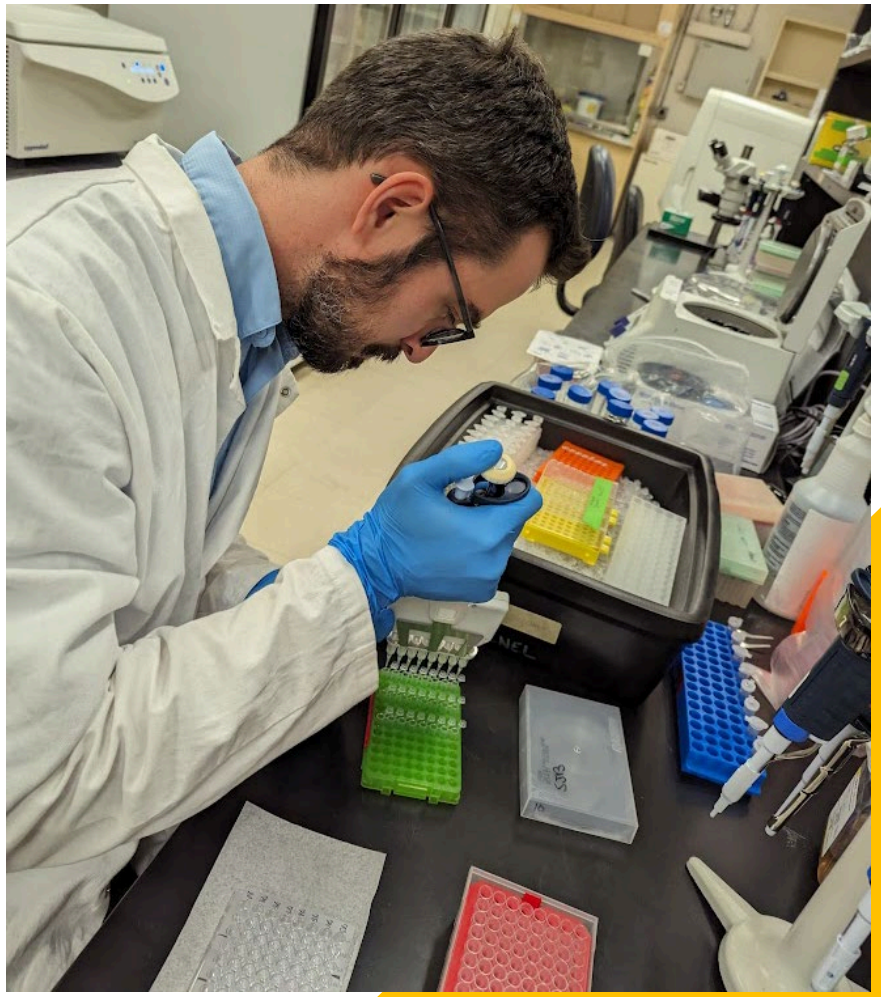

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		CMAVE	LL	LF	FF
Permethrin	LD50	1.08	15.12	21.29	29.46
Permethrin + PBO	LD50	0.12	0.61	1.12	2.01
	Synergist ratio	9.00	24.79	19.01	14.66

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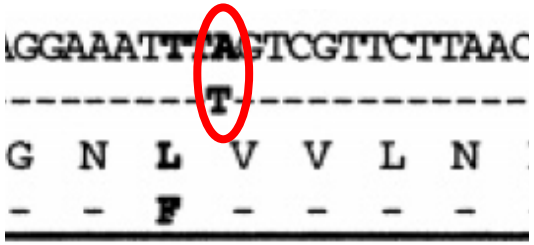
https://lite.lstmed.ac.uk/lite-facilities/lite-laboratories/cdc-bottle-assay

IR mechanisms underlying the resistance

- Target-site resistance



DNA

RNA

protein

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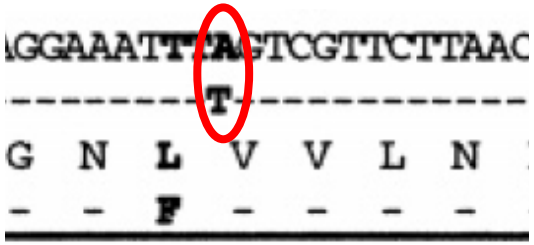

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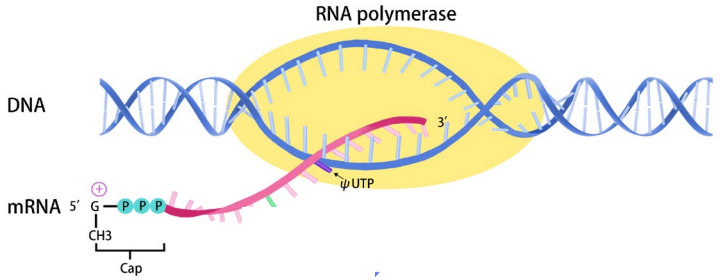

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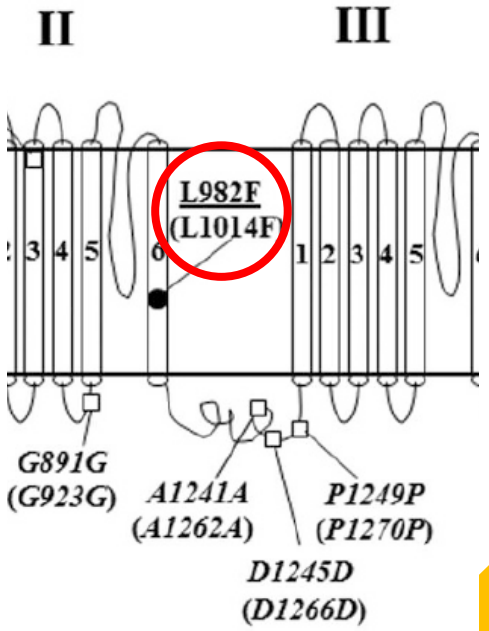
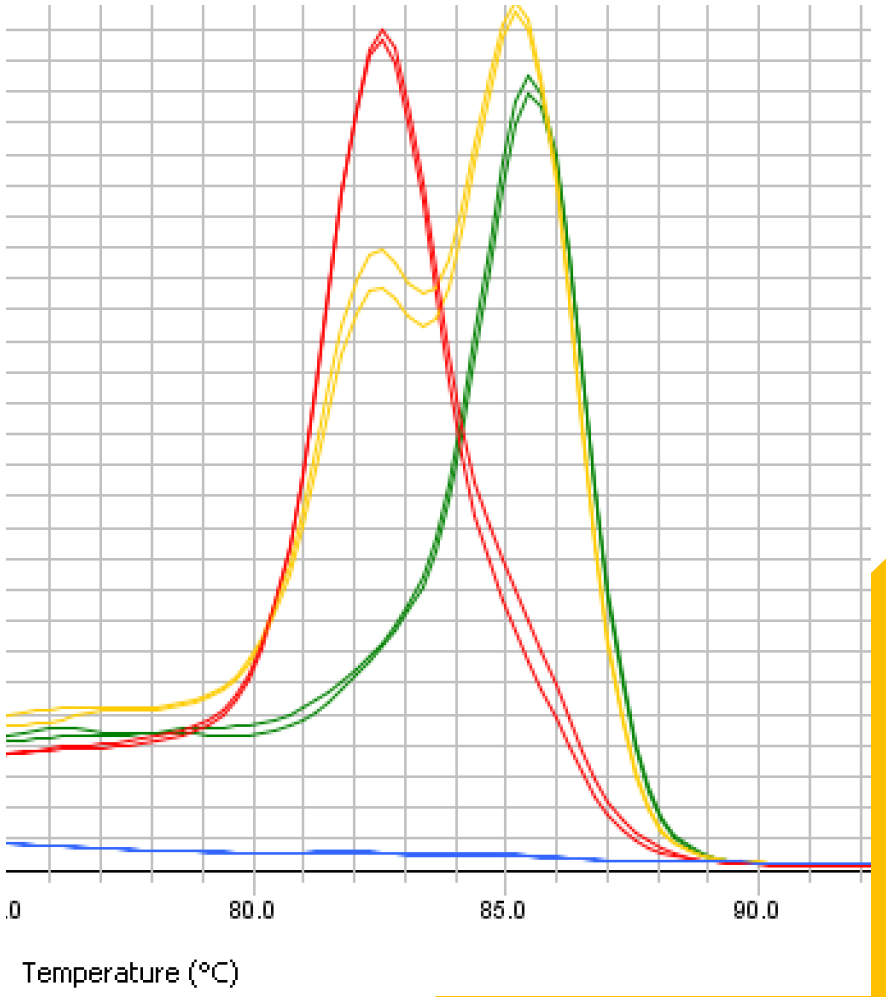


protein

IR mechanisms underlying the resistance

- Target-site resistance
  - *kdr* – knock-down resistance
    - NaV gene
    - *Ae. aegypti*: *kdr* = PYR resistance
  - Many SNPs



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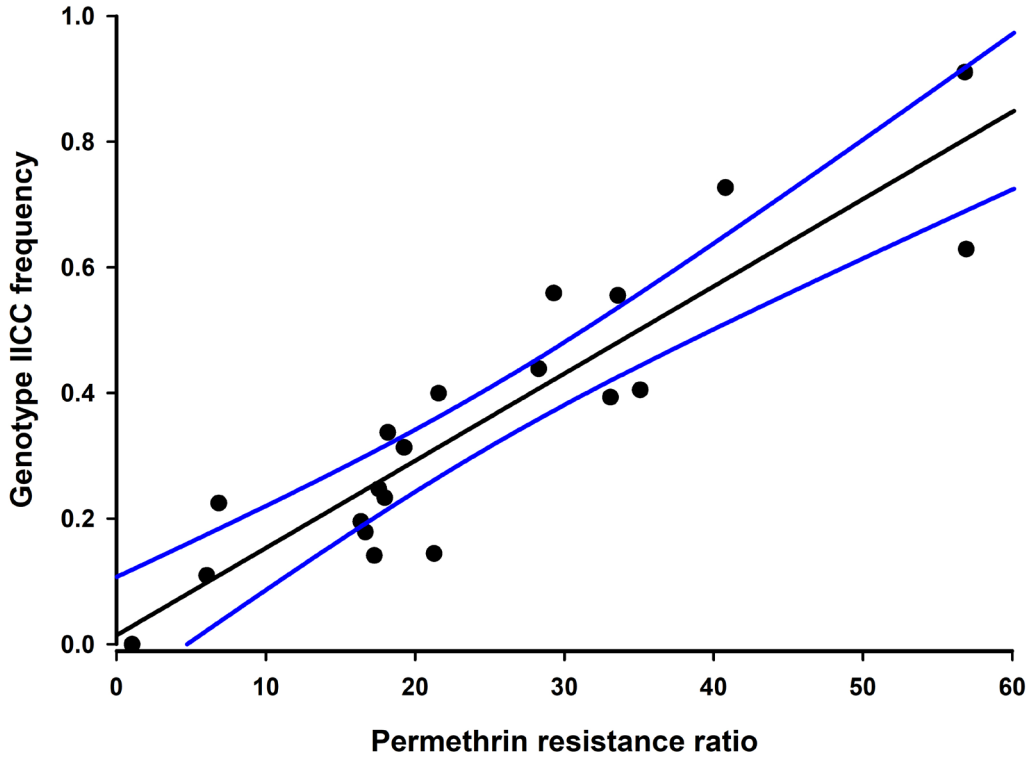

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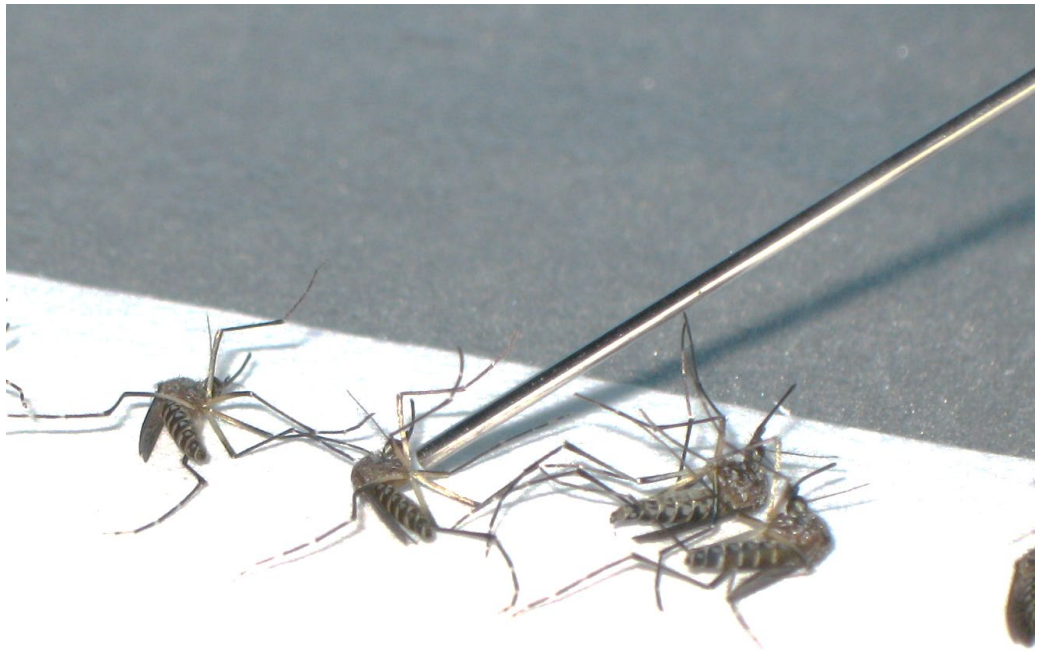
Table 1 | Non-synonymous and synonymous mutations in the sodium channel of *Cx. quinquefasciatus*

Mutation	Strain	n*	Phenotype†	Codons‡ (Frequency [%] ± SE)		
A109S§	S-Lab	60	Susceptible	GCA (65±5.0)	G/TCA (35±5.0)	TCA (0)
	MAmCq^{G0}	60	10-fold	GCA (0)	G/TCA (0)	TCA (100)
	MAmCq^{G6}	60	570-fold	GCA (0)	G/TCA (0)	TCA (100)
L982F§	S-Lab	60	Susceptible	TTA (100)	TTA/T (0)	TTT (0)
	MAmCq^{G0}	60	10-fold	TTA (22± 3.0)	TTA/T (52 ±6.0)	TTT (26 ±7.5)
	MAmCq^{G6}	60	570-fold	TTA (0)	TTA/T (0)	TTT (100)
W1573R§	S-Lab	60	Susceptible	TGG (100)	T/CGG (0)	CGG (0)
	MAmCq^{G0}	60	10-fold	TGG (72± 10.5)	T/CGG (25 ±8.5)	CGG (3.0 ±3.0)
	MAmCq^{G6}	60	570-fold	TGG (0)	T/CGG (8 ±5.5)	CGG (92 ±6.0)
L852L#	S-Lab	60	Susceptible	CTG (100)	CTG/A (0)	CTA (0)
	MAmCq^{G0}	60	10-fold resistance	CTG (27±10)	CTG/A (38±7.5)	CTA (35±5)
	MAmCq^{G6}	60	570-fold resistance	CTG (0)	CTG/A (6.5±2.8)	CTA (93.5±2.9)
G891G#	S-Lab	60	Susceptible	GGC (100)	GGC/A (0)	GGA (0)
	MAmCq^{G0}	60	10-fold resistance	GGC (28±10)	GGC/A (42±7.5)	CTA (30±10)
	MAmCq^{G6}	60	570-fold	GGC (0)	GGC/A (5±5)	CTA (95±5)
A1241A#	S-Lab	60	Susceptible	GCA (100)	GCA/G (0)	GCG (0)
	MAmCq^{G0}	60	10-fold resistance	GCA (2±3)	GCA/G (18±2.9)	GCG (80±5.5)
	MAmCq^{G6}	60	570-fold	GCA (0)	GCA/G (0)	GCG (100)
D1245D#	S-Lab	60	Susceptible	GAC (100)	GAC/T (0)	GAT (0)
	MAmCq^{G0}	60	10-fold resistance	GAC (38±7.5)	GAC/T (45±8.5)	GAT (17±5.5)
	MAmCq^{G6}	60	570-fold	GAC (0)	GAC/T (8±5.5)	GAT (92±5.5)
P1249P#	S-Lab	60	Susceptible	CCG (100)	CCG/A (0)	CCA (0)
	MAmCq^{G0}	60	10-fold resistance	CCG (37±5.5)	CCG/A (42±5.5)	CCA (21±5.5)
	MAmCq^{G6}	60	570-fold	CCG (0)	CCG/A (5.0±5.0)	CCA (95±5.0)
G1733G#	S-Lab	60	Susceptible	GGA (48±12.5)	GGA/G (52±12.5)	GGG (0)
	MAmCq^{G0}	60	10-fold resistance	GGA (0)	GGA/G (5±5.0)	GGG (95±5.0)
	MAmCq^{G6}	60	570-fold resistance	GGA (0)	GGA/G (0)	GGG (100)

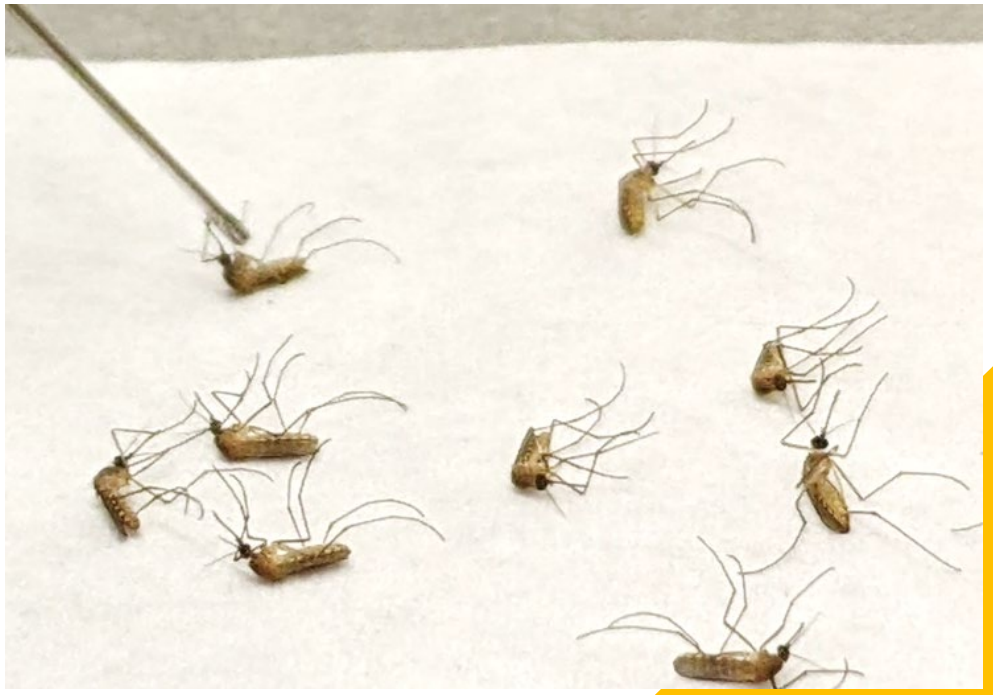
Li, T., Zhang, L., Reid, W.R., Xu, Q., Dong, K. and Liu, N., 2012. Multiple mutations and mutation combinations in the sodium channel of permethrin resistant mosquitoes, Culex quinquefasciatus. *Scientific Reports*, *2*(1), p.781.

Case study – Aedes vs. Culex mechanisms

Ae. aegypti



Cx. quinquefasciatus



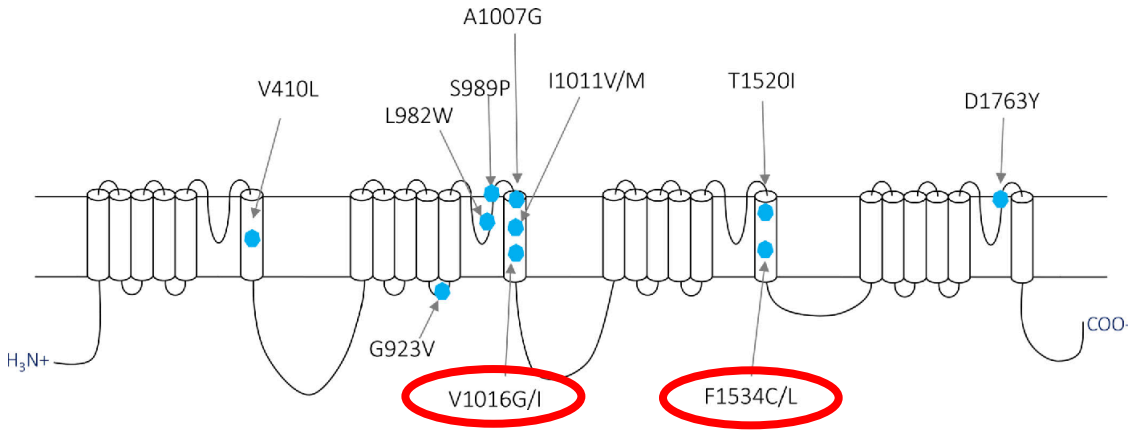
Case study – Aedes vs. Culex mechanisms

- Behavioral resistance
- Penetration (cuticular) resistance
- Metabolic resistance
- Target-site resistance

Case study – Aedes vs. Culex mechanisms

Ae. aegypti

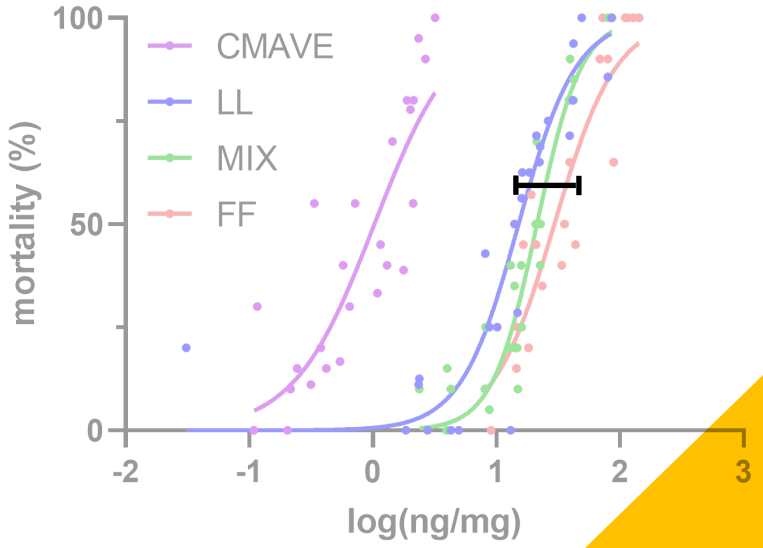
- V1016I & F1534C



Chen, M., Du, Y., Nomura, Y., Zhorov, B.S. and Dong, K., 2020. Chronology of sodium channel mutations associated with pyrethroid resistance in Aedes aegypti. *Archives of insect biochemistry and physiology*, *104*(2), p.e21686.

Cx. quinquefasciatus

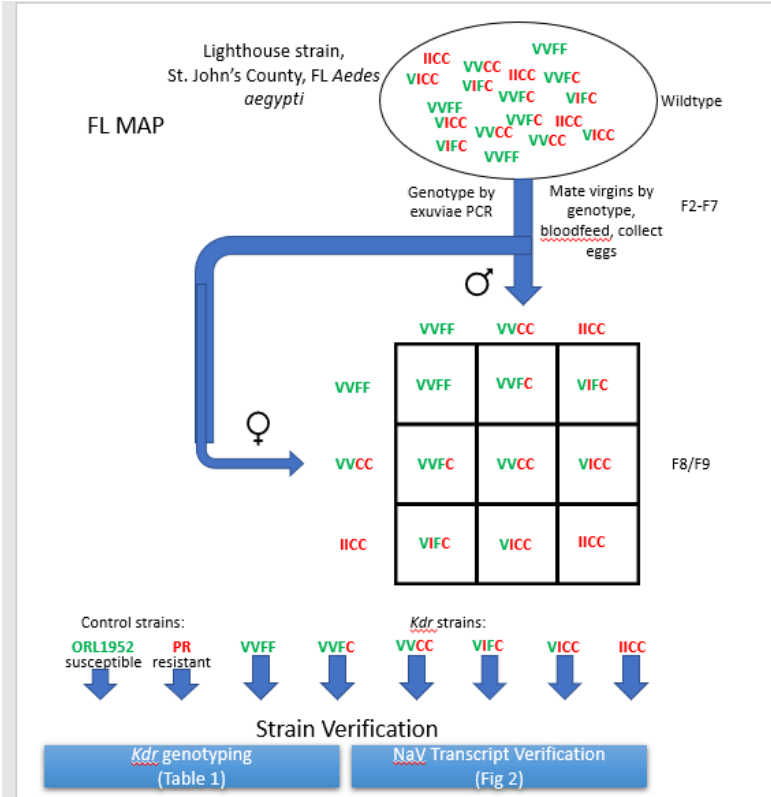
- L1014F



	CMAVE	LL	LF	FF
RR	1	14.68	20.67	28.60

Case study – Aedes vs. Culex mechanisms

Ae. aegypti



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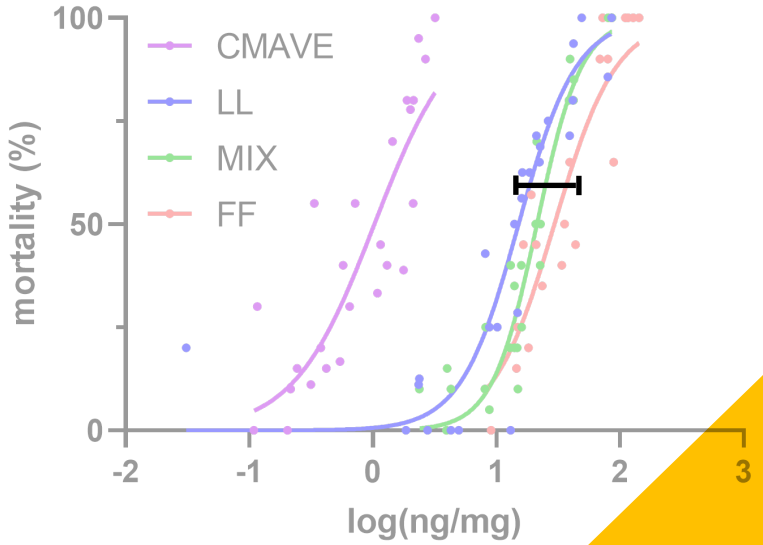

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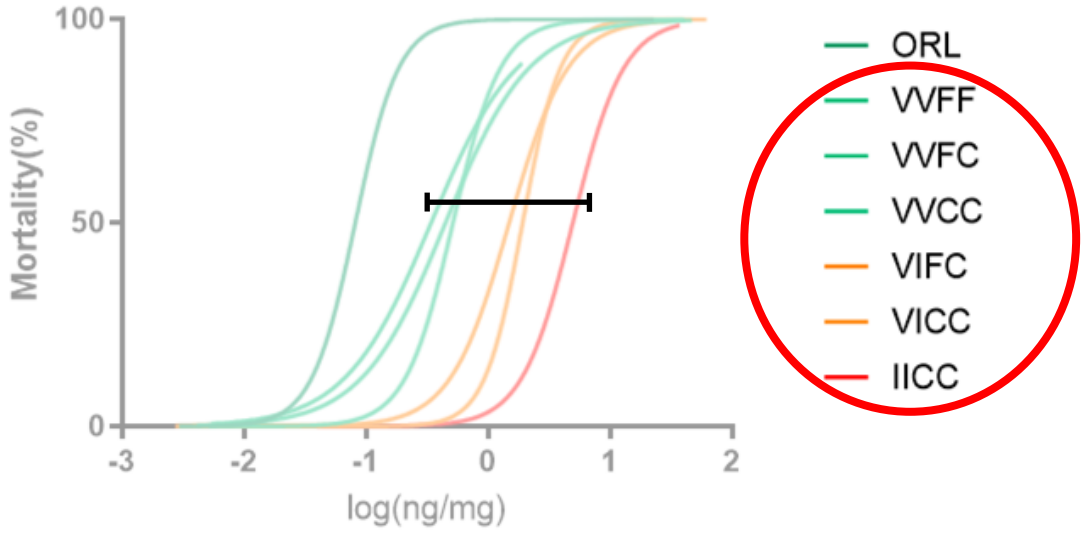

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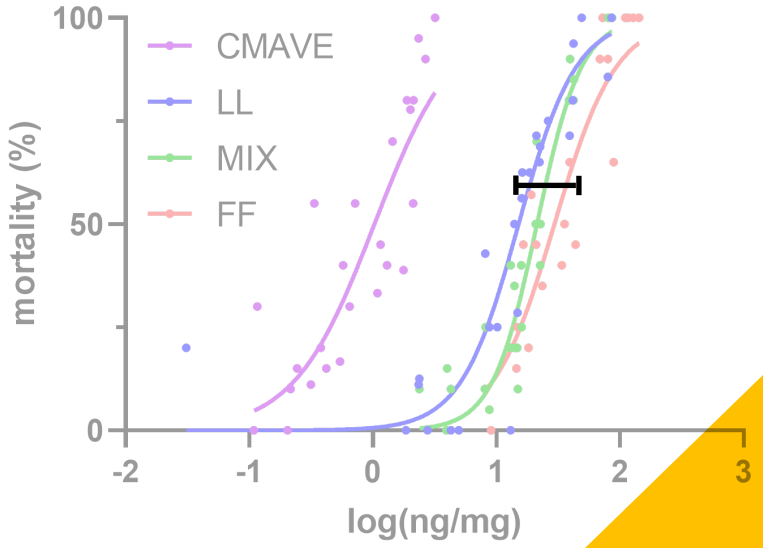
Cx. quinquefasciatus

- L1014F



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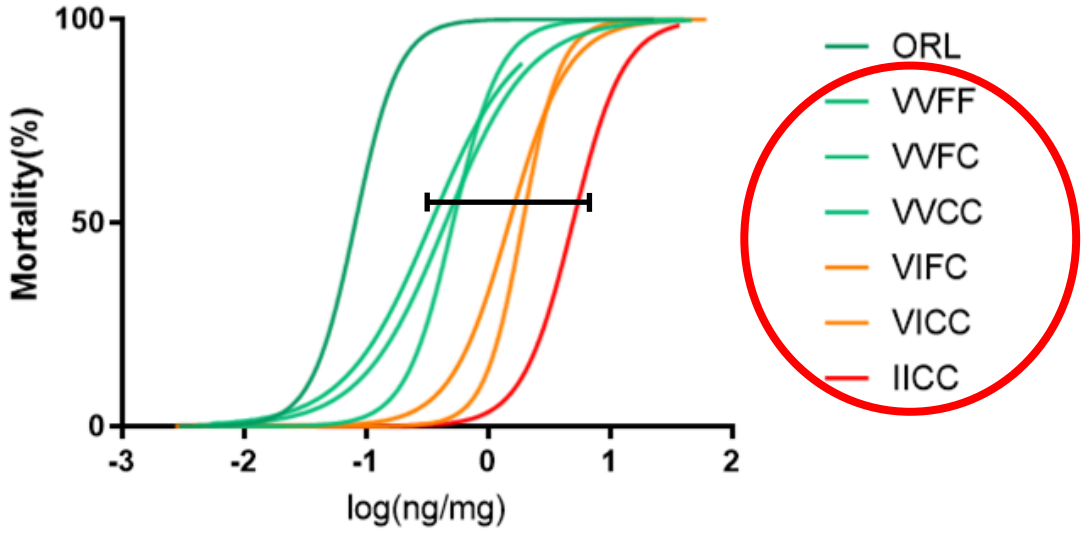

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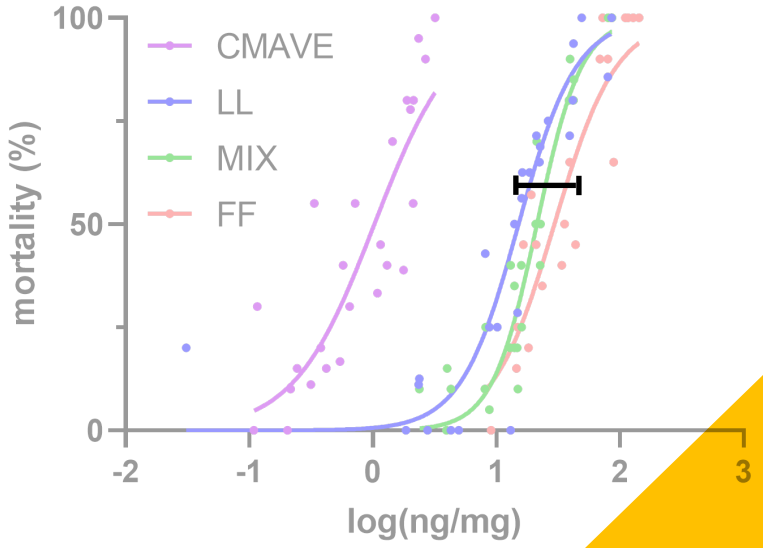

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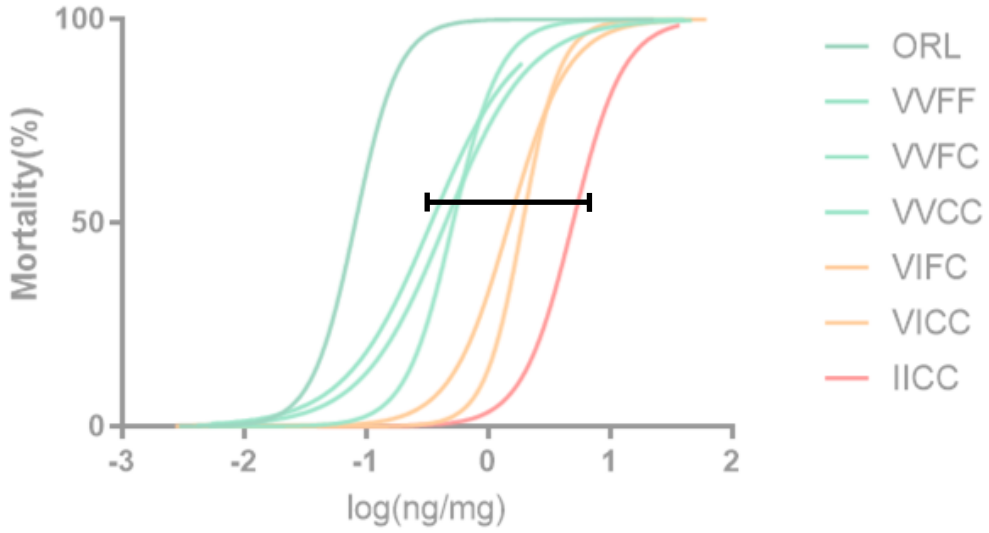

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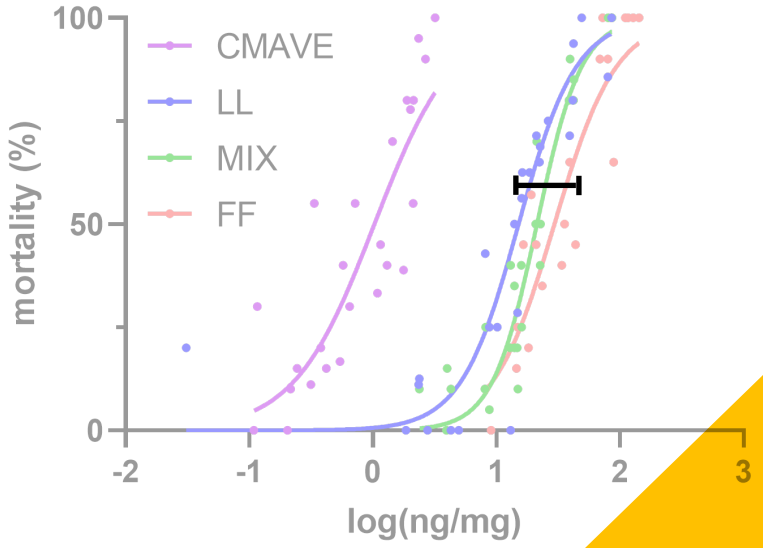

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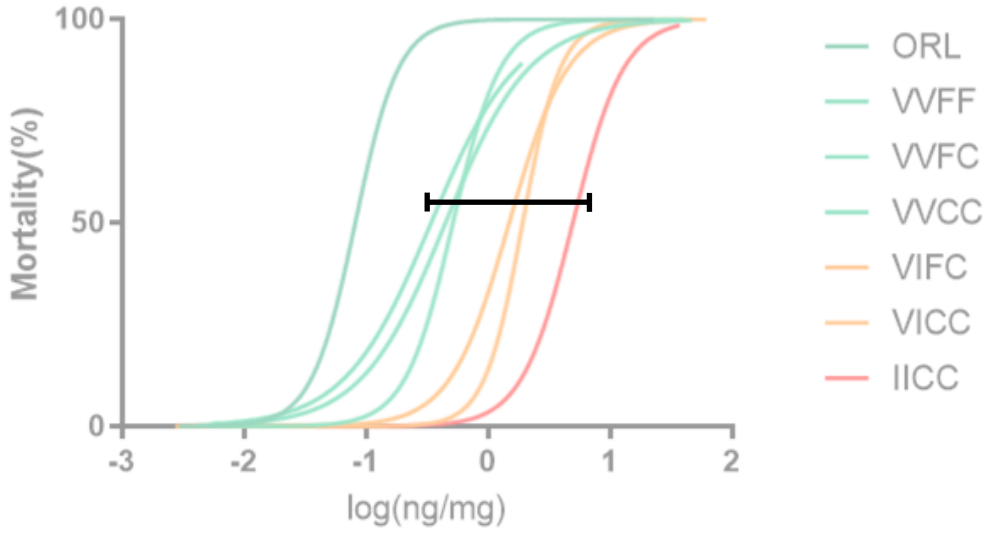

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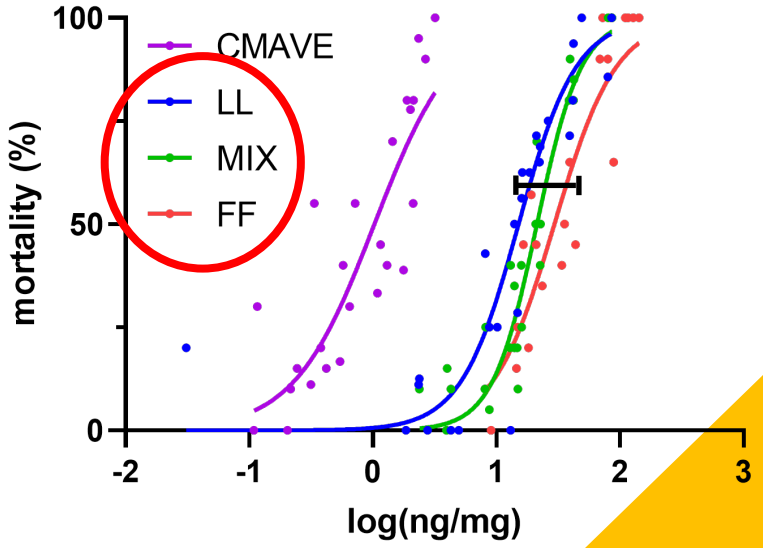

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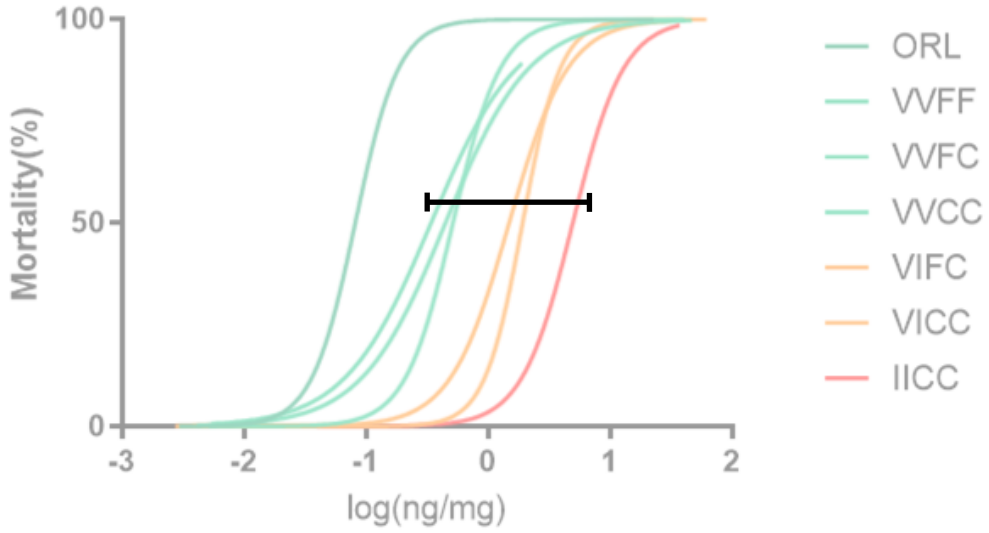

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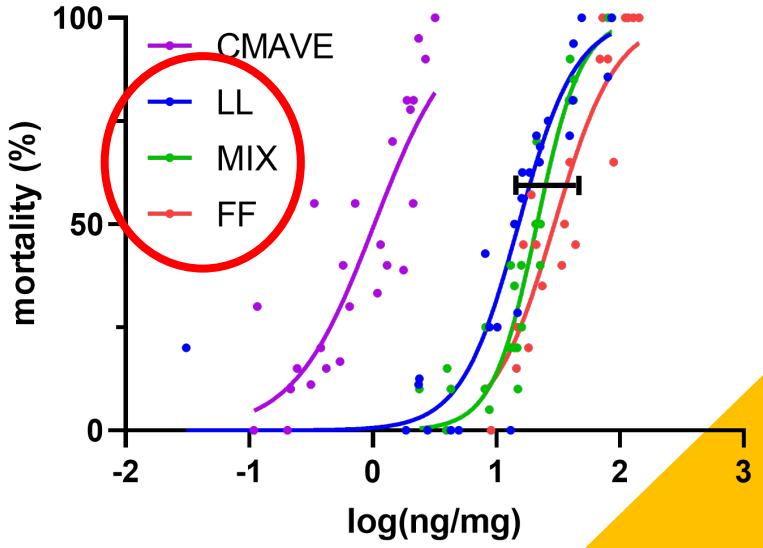

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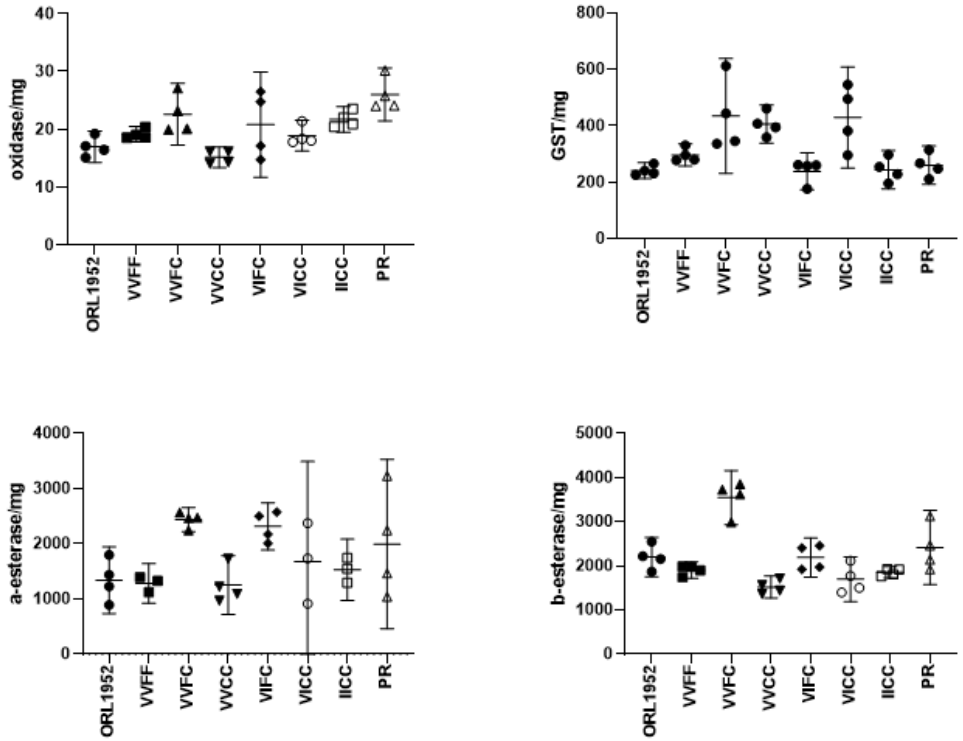

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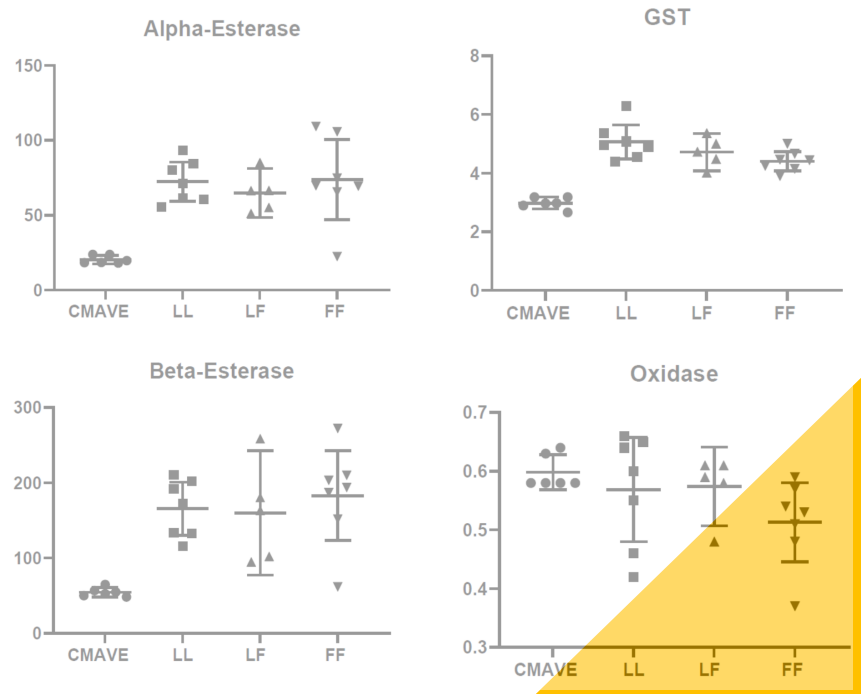
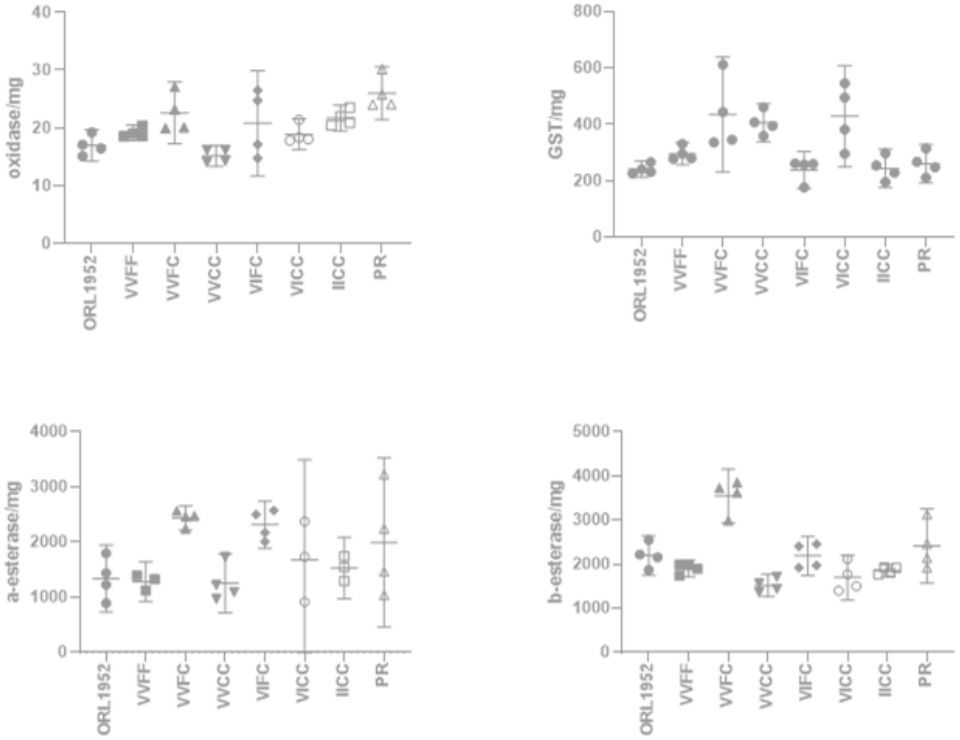


Case study – Aedes vs. Culex mechanisms

Ae. aegypti



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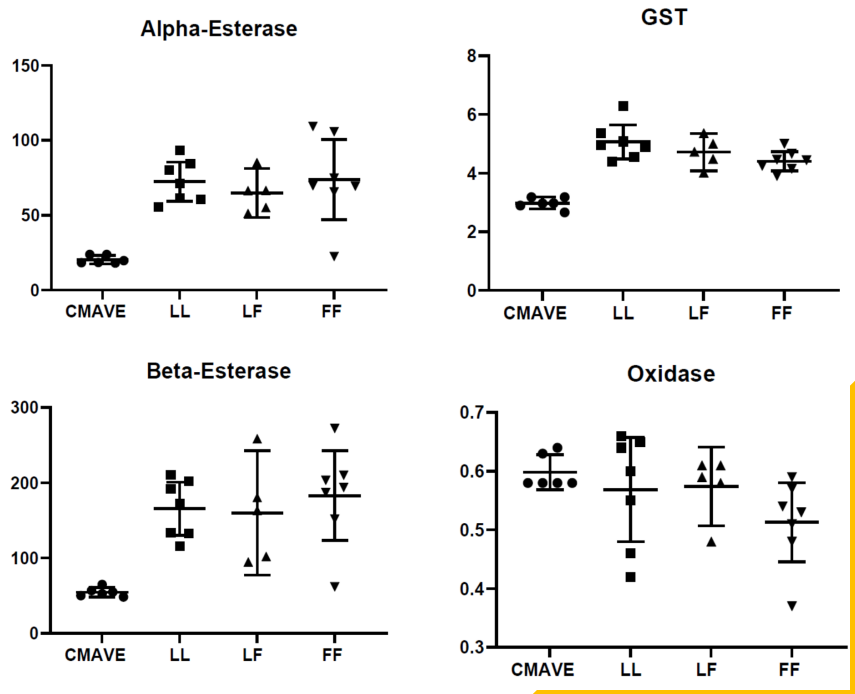

Case study – Aedes vs. Culex mechanisms

Ae. aegypti

- Topically applied permethrin + PBO = low SR

		ORL	VVFF	VIFC	IICC
Permethrin	LD50	0.081	0.44	1.91	4.92
Permethrin + PBO	LD50	0.12	0.37	1.61	3.28
	Synergist ratio	0.7	1.2	1.2	1.5

Cx. quinquefasciatus

- Topically applied permethrin + PBO = high SR

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Permethrin	LD50	1.08	15.12	21.29	29.46
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Case study – Aedes vs. Culex mechanisms

Ae. aegypti

- Target site resistance = major mechanism
  - pyrethroid IR increases with # of IC alleles (>10X difference)
- Metabolic resistance ≠ major mechanism
  - No enzyme upregulation
  - Low SPs

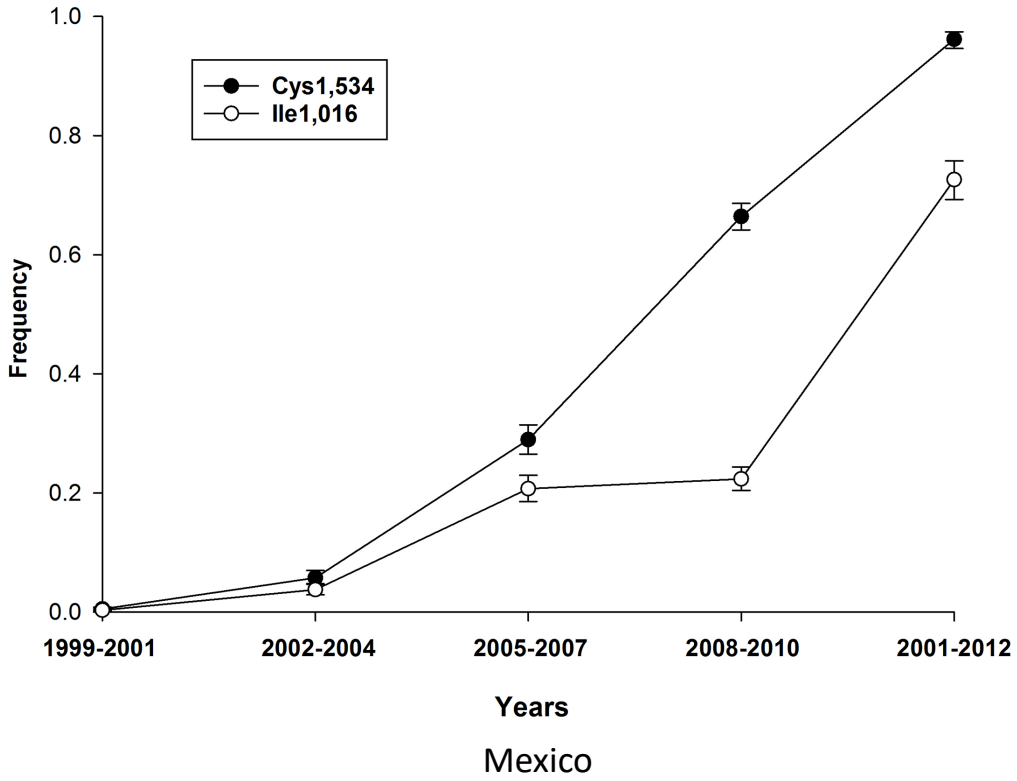
Cx. quinquefasciatus

- Target site resistance ≠ major mechanism
  - *kdr* accounts for only <2X difference in pyrethroid IR
- Metabolic resistance = major mechanism
  - Increased enzyme upregulation
  - High SPs

What is the future of insecticide resistance in mosquitoes?

Examples indicate that IR will continue to build to pyrethroids

- The increase of the resistant genotypes is the standard in *Aedes aegypti*



Mexico

Vera-Maloof et al. 2015-doi.org/10.1371/journal.pntd.0004263

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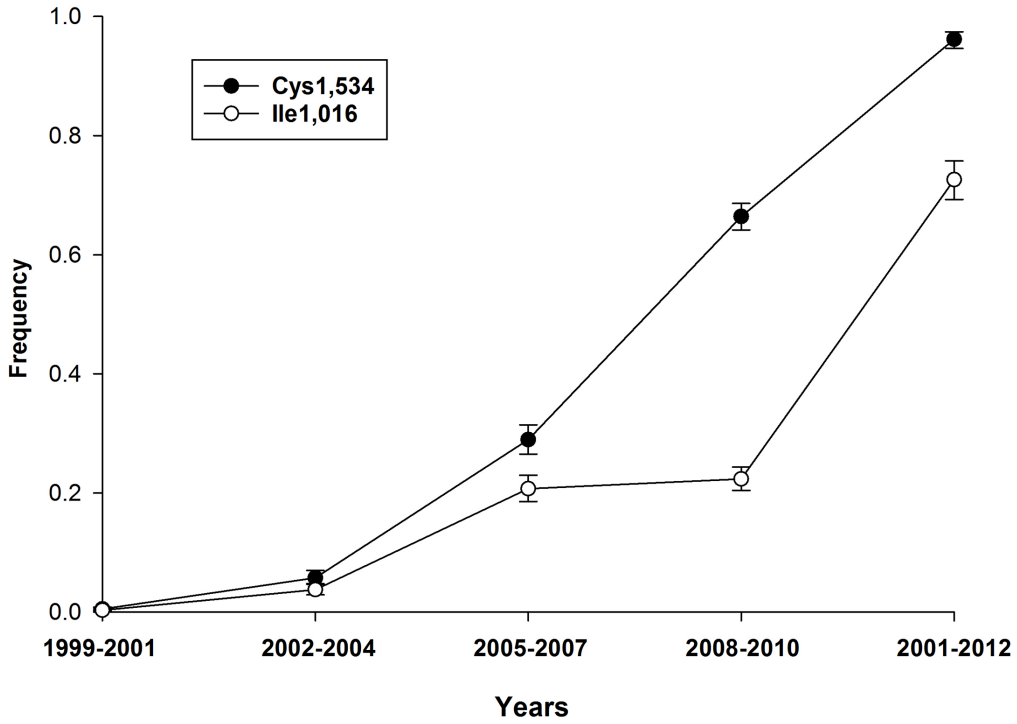

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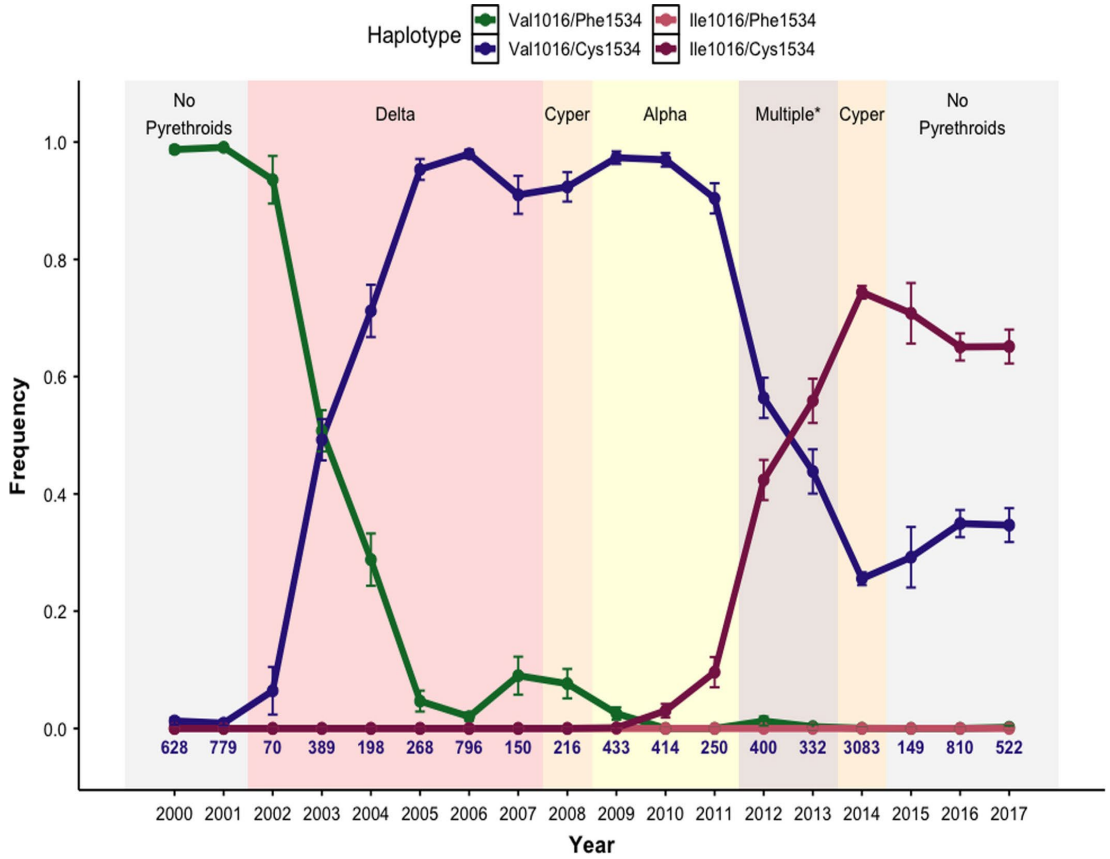

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Baltzegar et al- doi.org/10.1111/eva.13269

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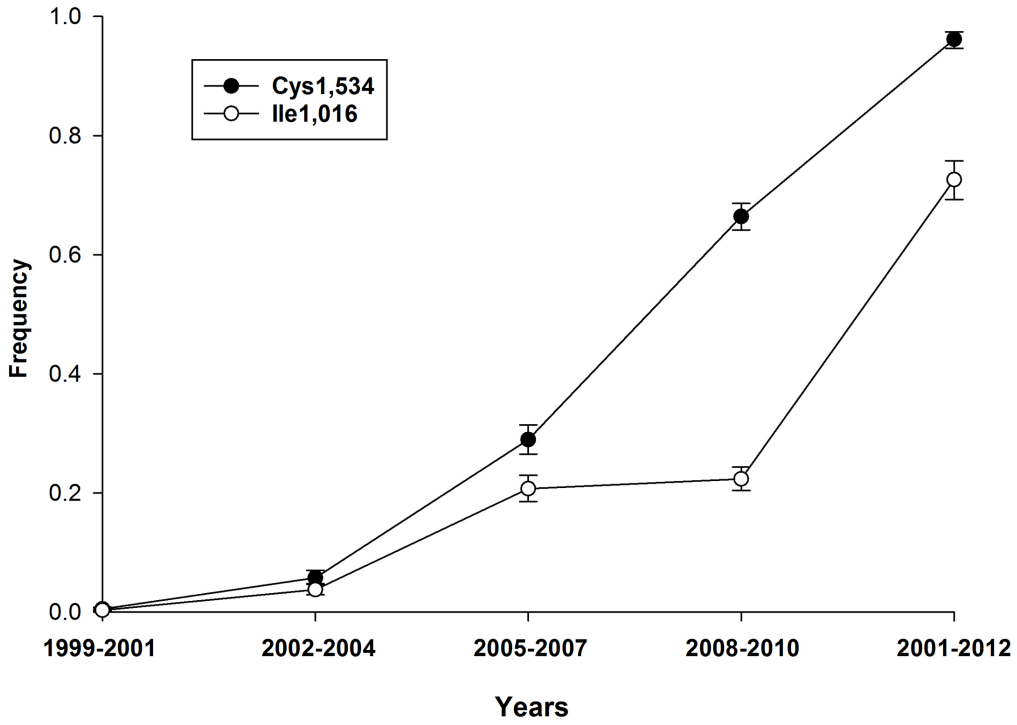

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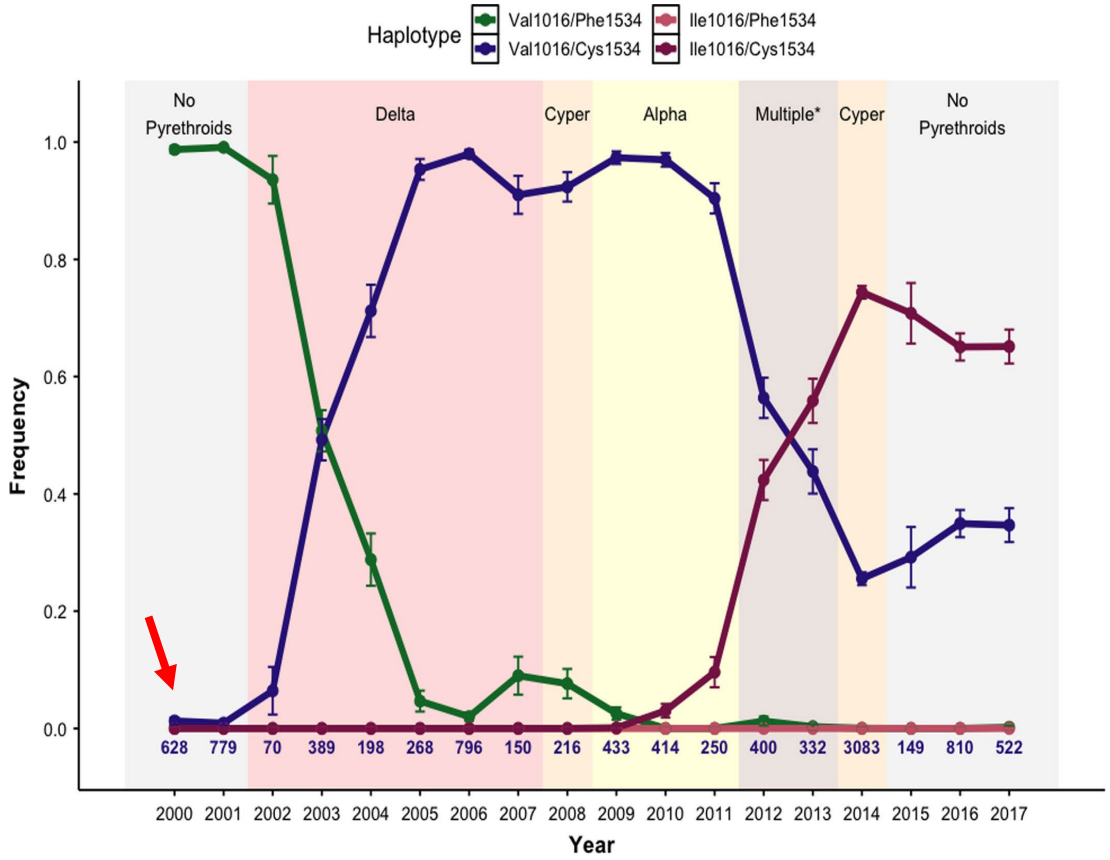

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- The same appears to be occurring in US populations
  - Houston populations appear to be holding steady or increasing in IR
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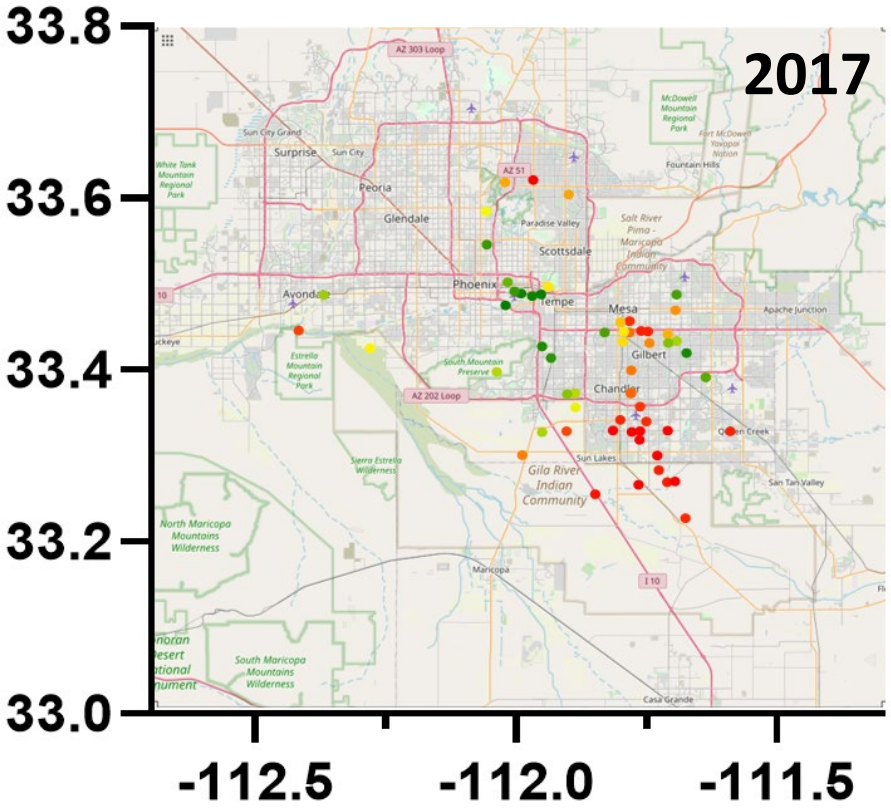

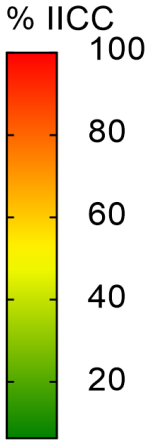

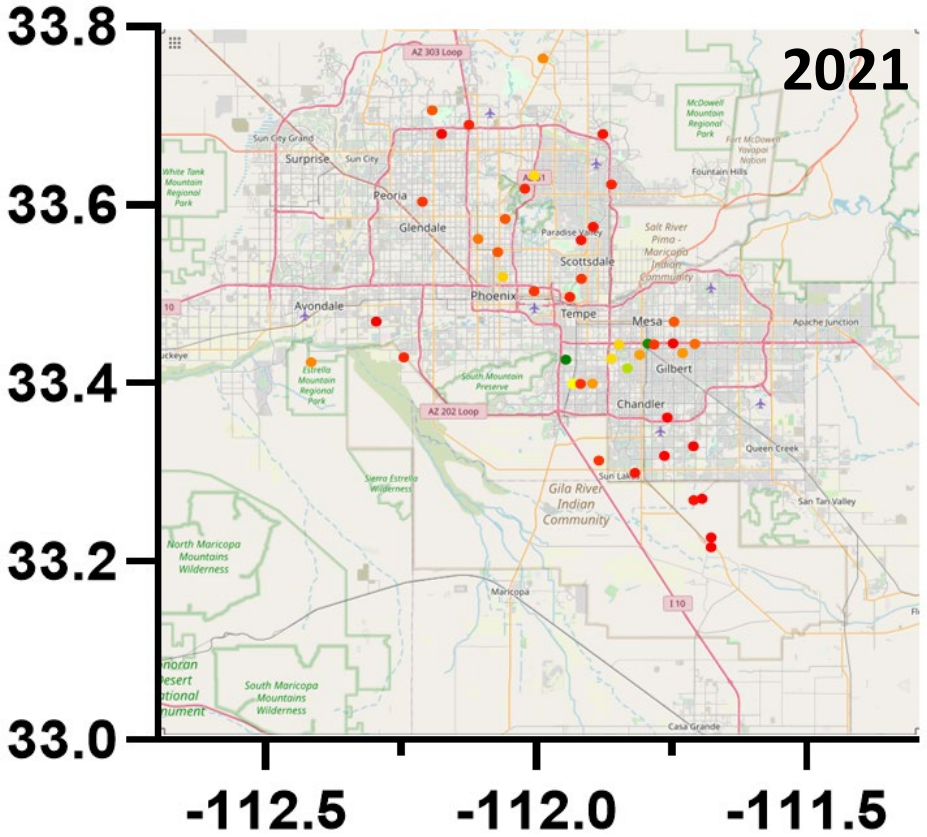

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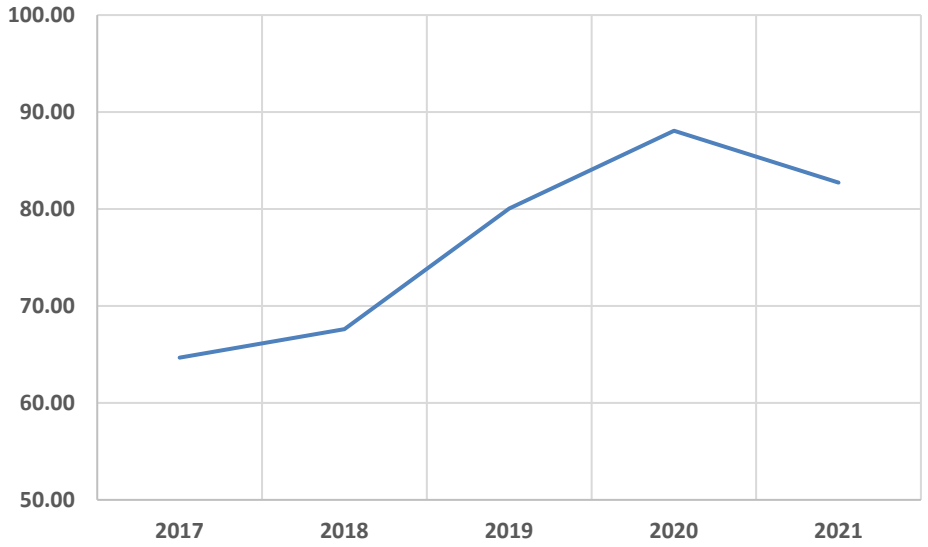

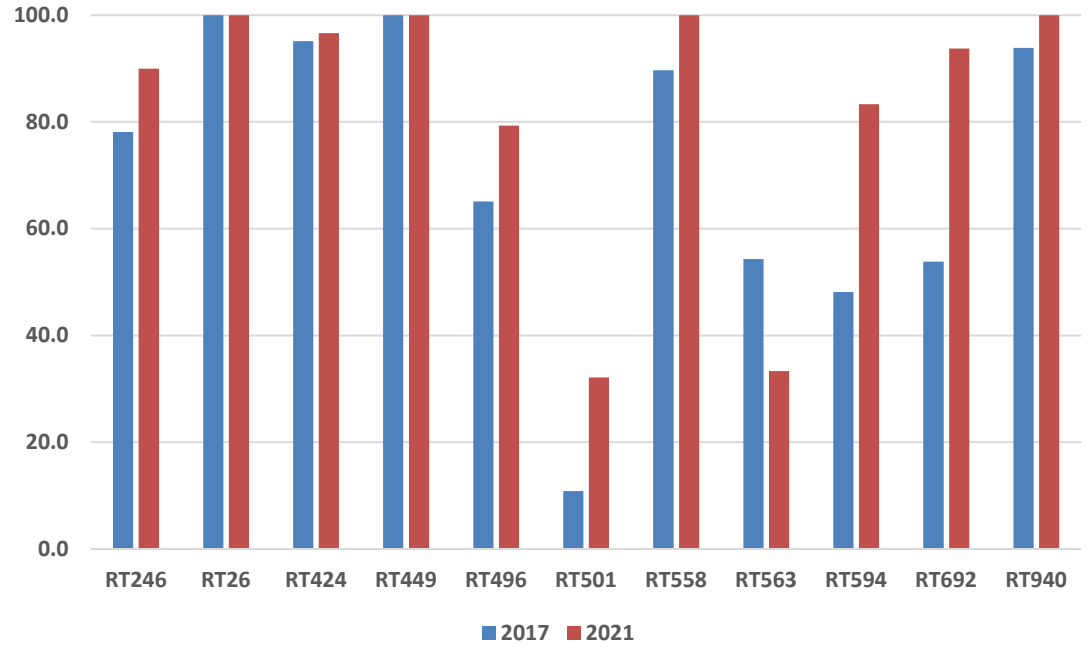

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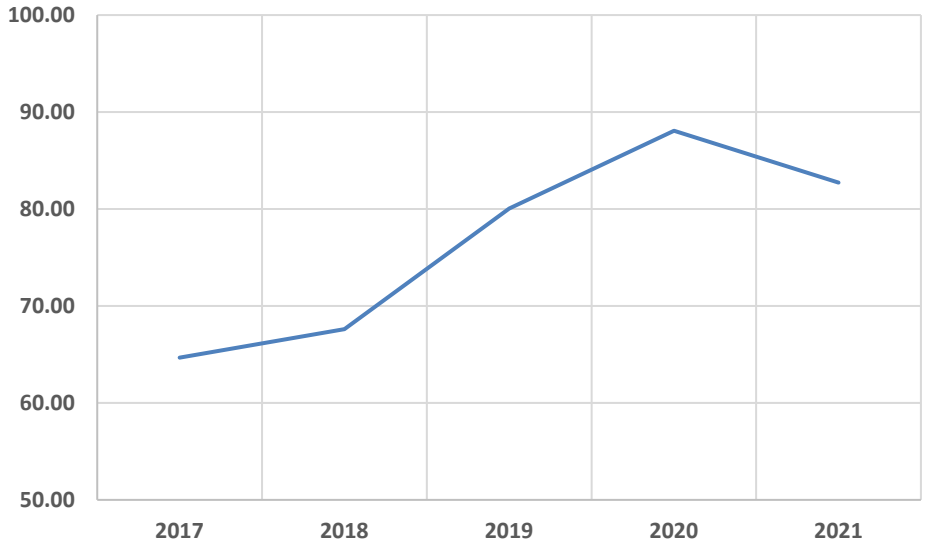
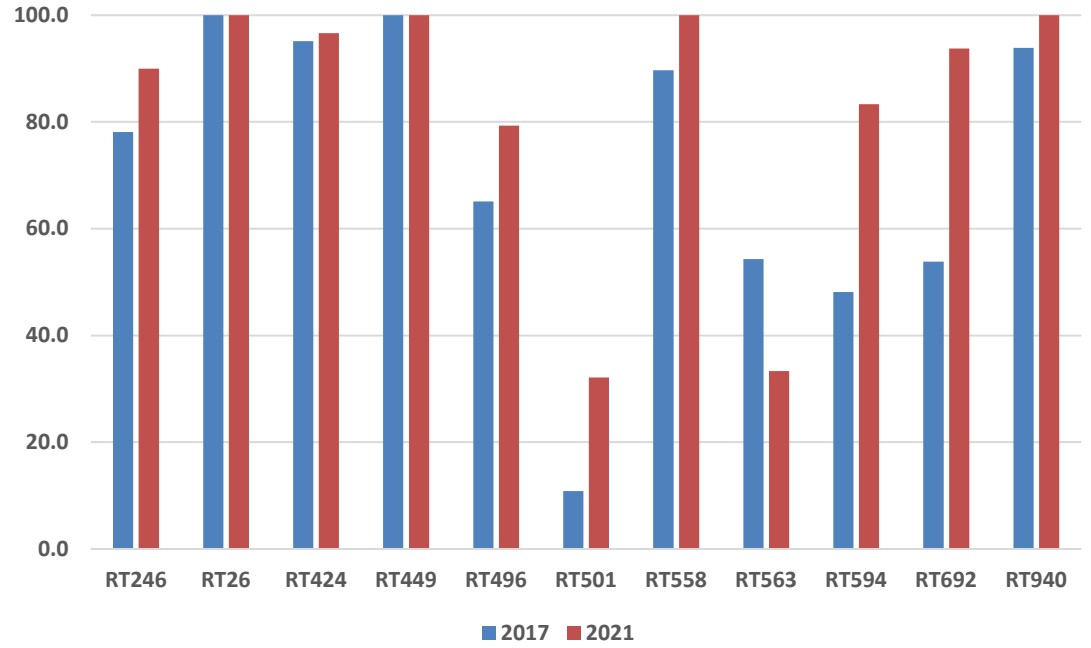


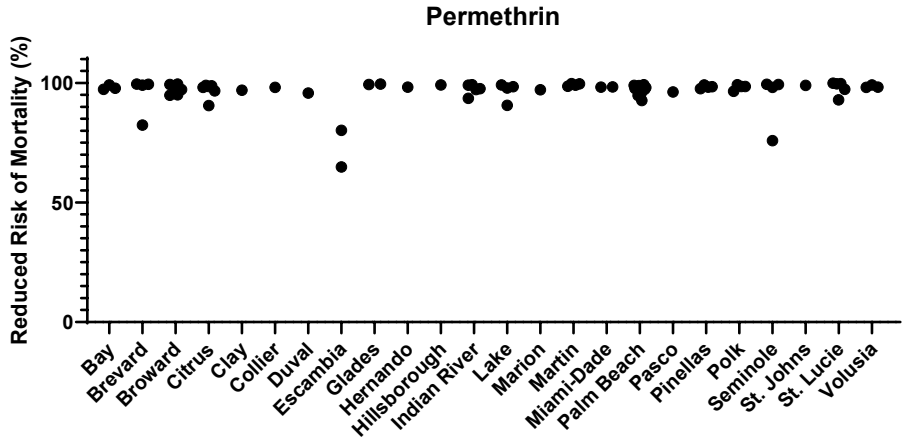


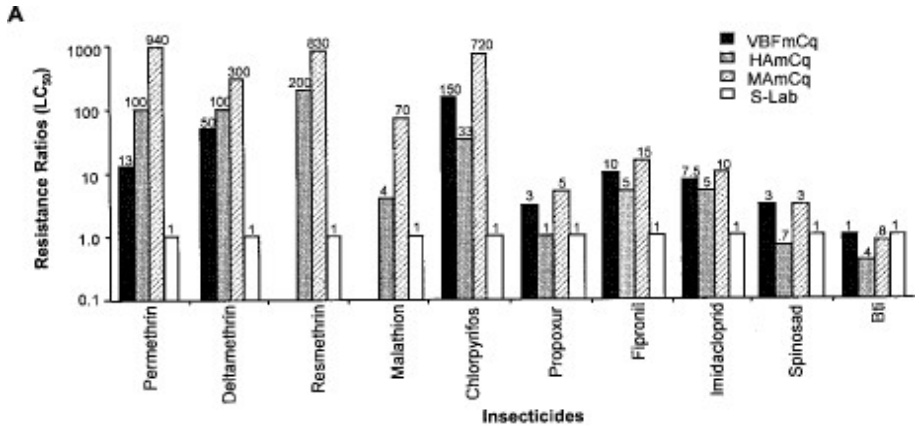
- Currently, IR to OPs is low but there is history in *Ae. aegypti*

What is the future of insecticide resistance in mosquitoes?

Historical examples indicate that IR will continue to build to pyrethroids & OPs

- *Culex quinquefasciatus* are resistant but it can get much worse with more pressure





Unlu et al. 2024-doi.org/10.1371/journal.pone.0296046

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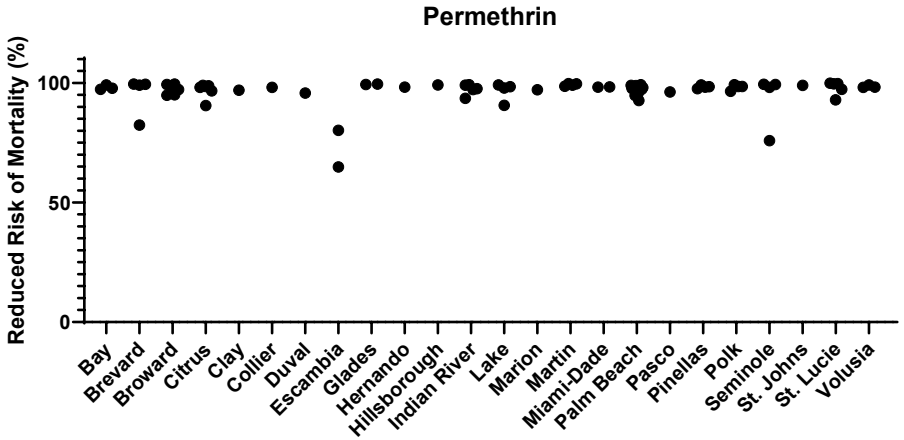
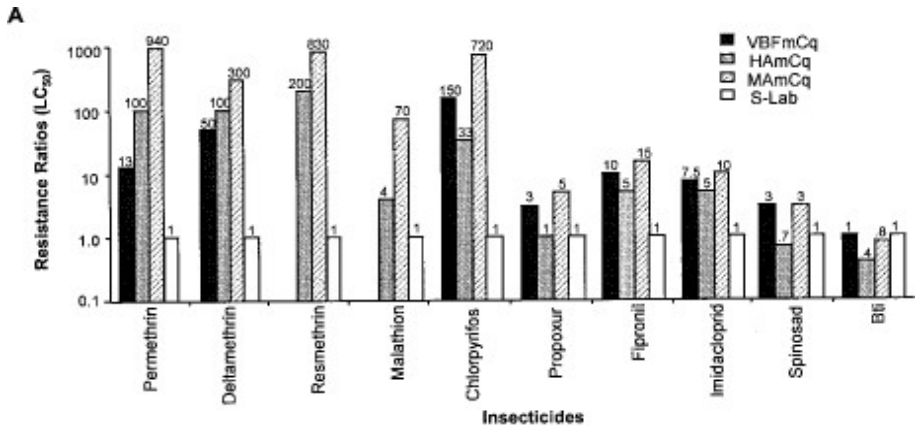
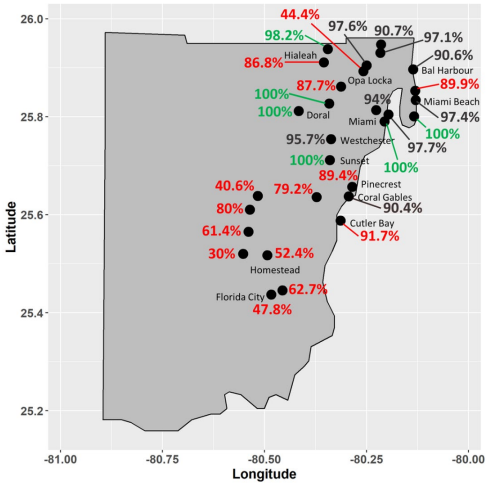


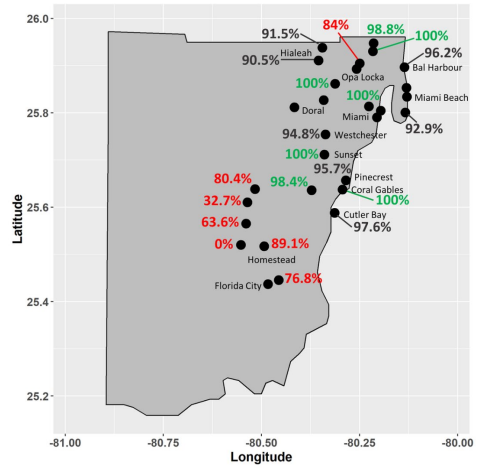


- OPs may or may not be effective
  →Mechanism unknown

A. malathion



B. naled



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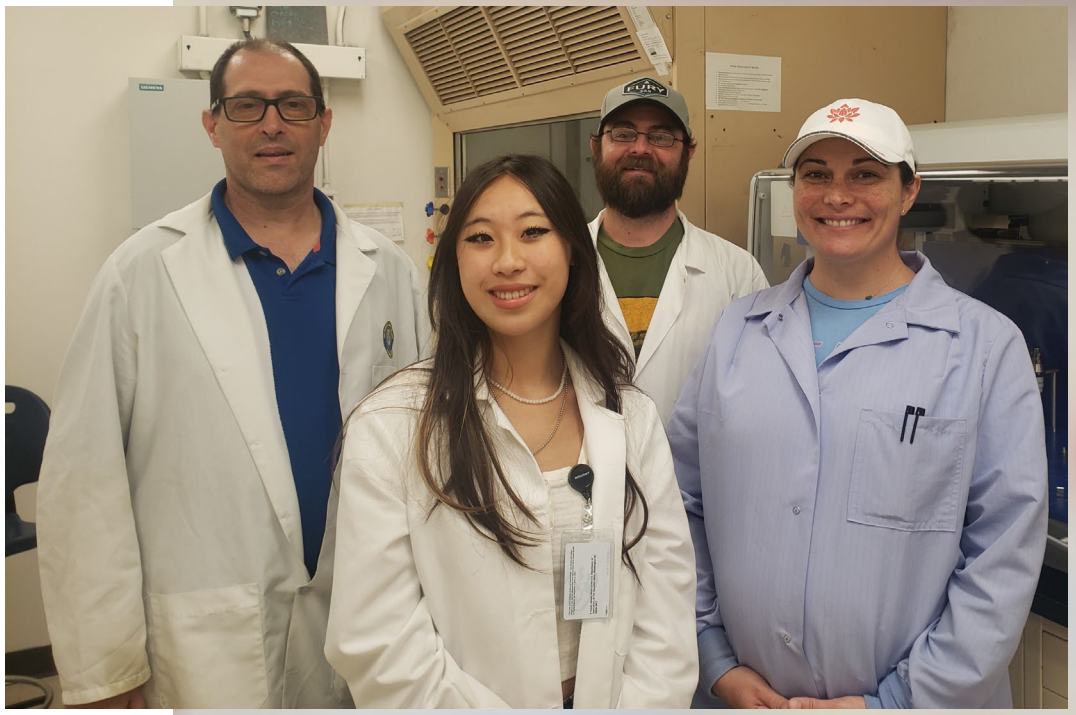
Current State of Insecticide Resistance in (Florida) Mosquitoes

1. IR today: What we know, what we don't
2. IR mechanisms
3. Why mechanism matters:
   Aedes aegypti vs. *Culex quinquefasciatus*
4. Future trends in IR



Thank you!
Questions?

Just reach out…

alden.estep@usda.gov

neil.sanscrainte@usda.gov

Alden Estep – Research Entomologist
Neil Sanscrainte – Molecular Biologist

USDA ARS Center for Medical Agricultural & Veterinary Entomology

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