CDC bottle bioassay results show need for rotating organophosphate and pyrethroid-based adulticides against Florida *Aedes aegypti* and *Culex quinquefasciatus*

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UF/IFAS Florida Medical Entomology Laboratory

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DOMESTIC MOSQUITOES IN FLORIDA

Matthew Twombly for NPR

- ***Aedes aegypti, Aedes albopictus, Culex quinquefasciatus***
  - Live in close association with humans
    - Larval habitat
    - Blood meal sources

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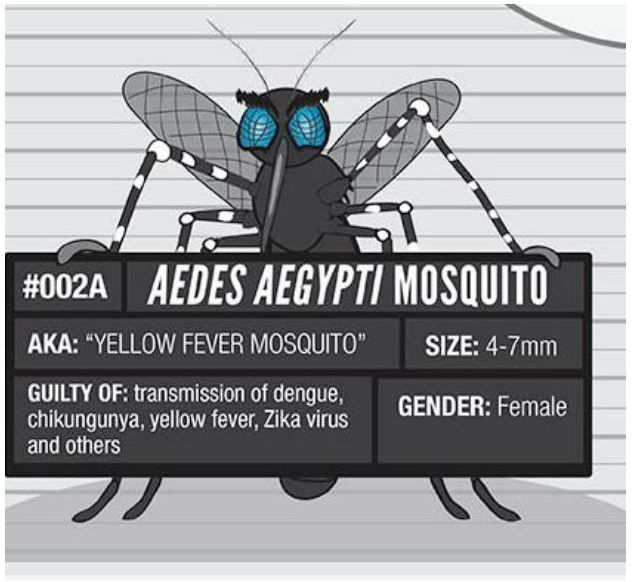
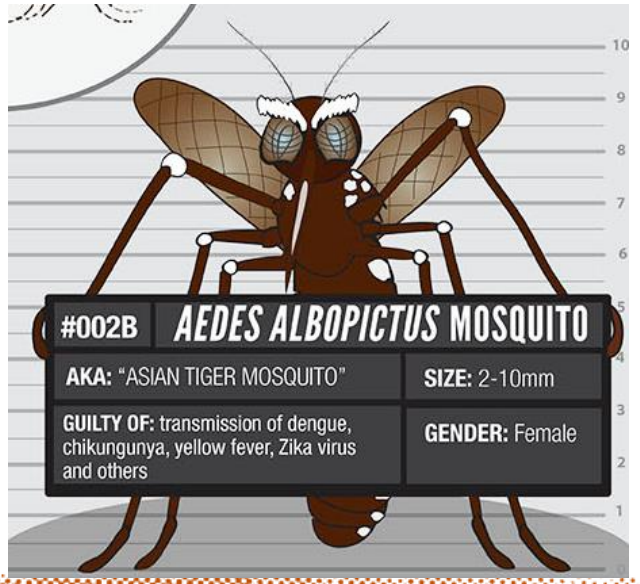
IMPORTANT ARBOVIRUS VECTORS

- Close association with humans
- ***Aedes aegypti* responsible for local transmission of exotics in FL**
  - Outbreaks
    - DENV (2009, 2010, 2013)
    - CHIKV (2014)
    - ZIKV (2016)
  - Sporadic cases
    - So far in 2019: 12 DENV cases
- ***Aedes albopictus* also competent exotic arbovirus vector**





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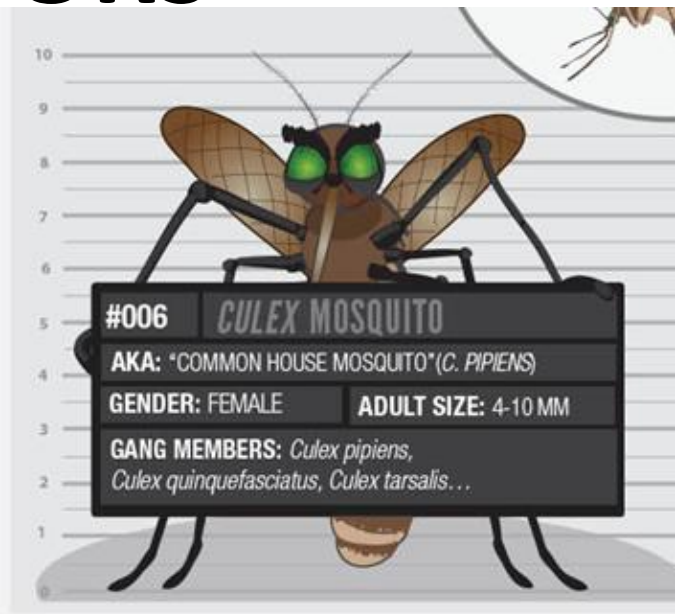
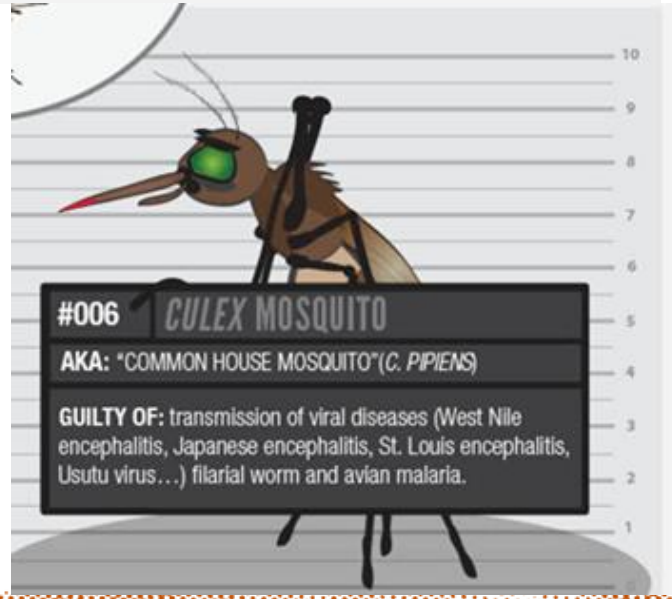
IMPORTANT ARBOVIRUS VECTORS

- ***Culex quinquefasciatus* responsible for transmission of endemics in FL**
  - WNV
    - 2019: 1 human & 7 horse cases
    - 2018: 33 human & 13 horse cases
  - EEEV
    - 2019: 28 horse cases
    - 2018: 3 human & 52 horse cases
  - SLEV
    - 2014: 2 human cases
    - Always chance for resurgence





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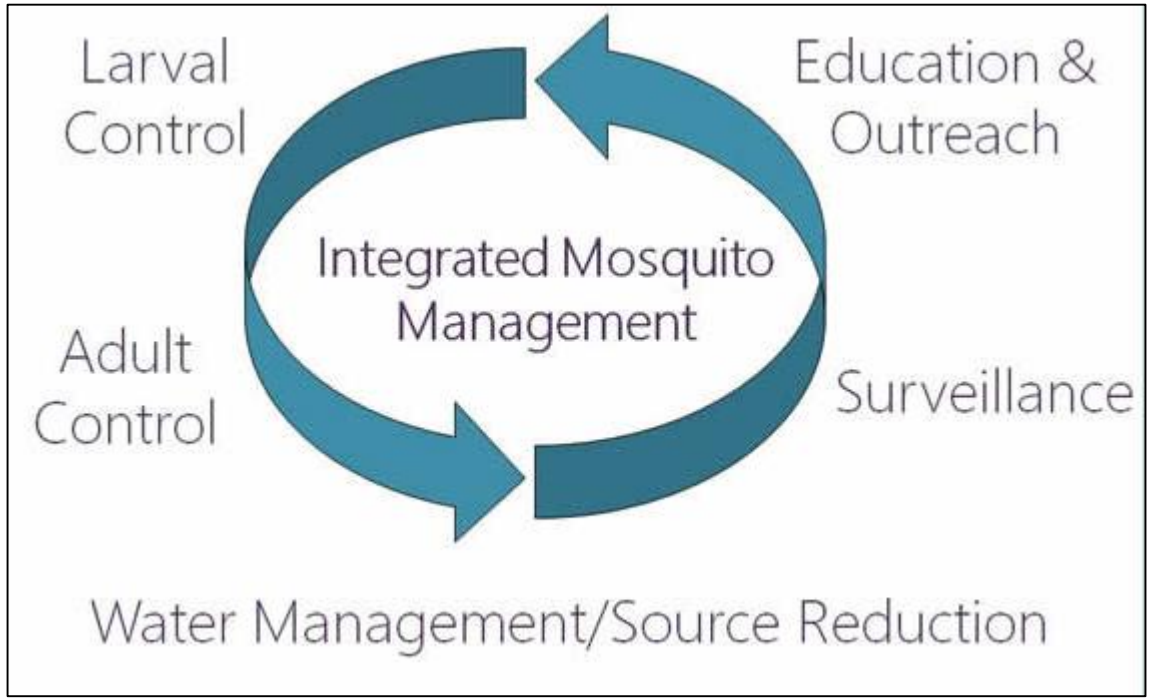
DOMESTIC MOSQUITO CONTROL

- **Relies on integrated mosquito management**
  - Public education
  - Source reduction
  - Surveillance
  - Larviciding
  - Adulticiding



  - Insecticide resistance
    - Can impact larviciding & adulticiding success

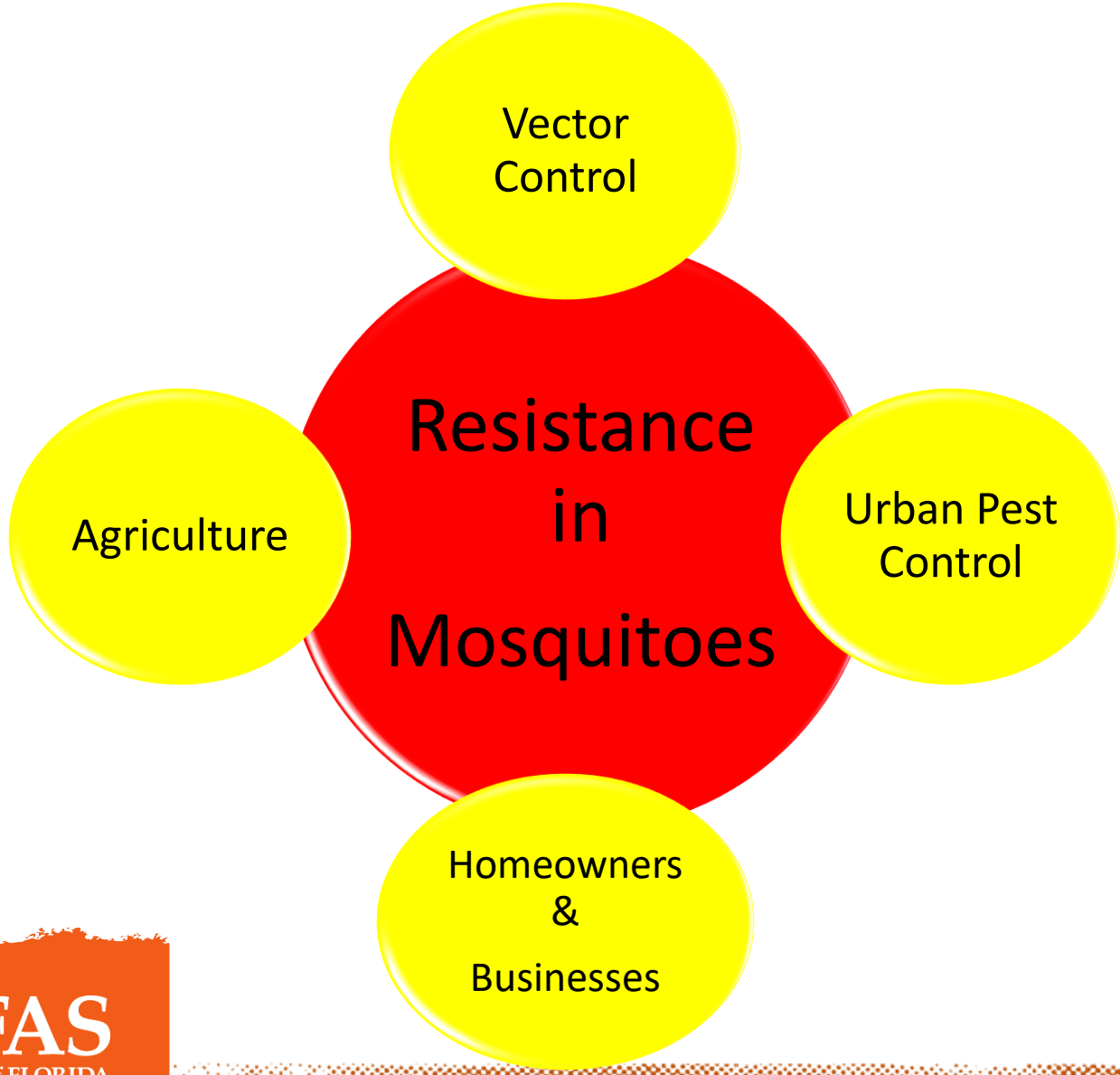
RESISTANCE DEFINITION

Insecticide Resistance is a genetic change in response to selection by toxicants that may impair control in the field.

Slide credit: Janet McAllister, CDC

RESISTANCE IN DOMESTIC MOSQUITOES

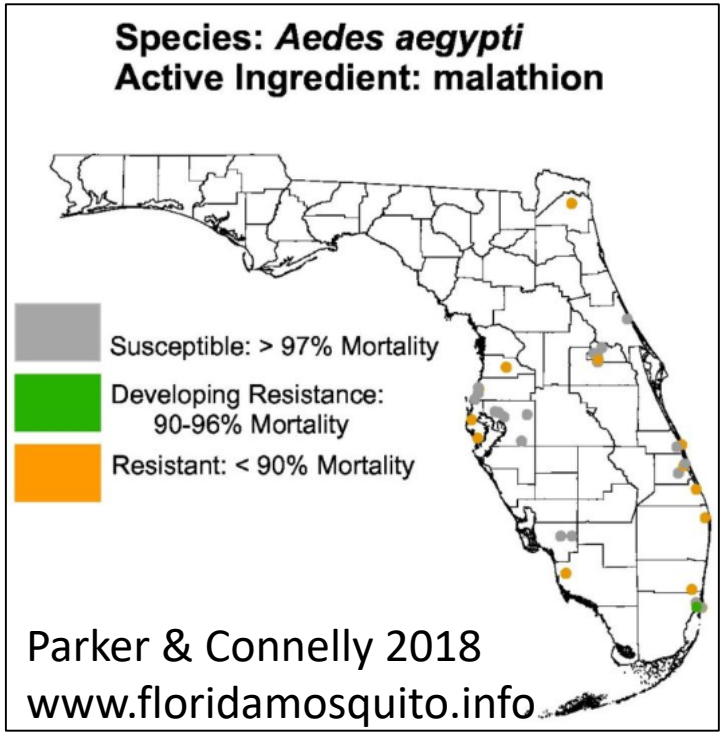
- Influenced by:
  - Insecticides
  - Herbicides
  - Fungicides
  - Fertilizers
  - Non-point source runoff



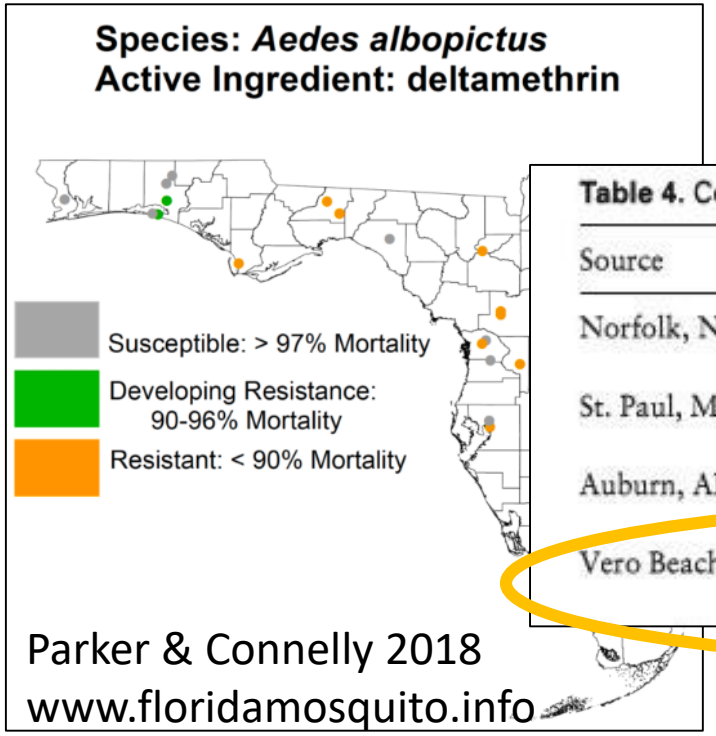
Slide credit: Janet McAllister, CDC

RESISTANCE IN FL DOMESTIC MOSQUITOES

- Pyrethroid and/or organophosphate resistance has been detected in Florida populations of *Ae. aegypti*, *Ae. albopictus*, *Cx. quinquefasciatus*



Parker & Connelly 2018
www.floridamosquito.info



Parker & Connelly 2018
www.floridamosquito.info

Richards et al. 2017

Table 4. Comparative susceptibility and resistance of *Culex* mosquito populations t

Source	Mosquito species (generation)	Bifenthrin
Norfolk, NE	*Culex pipiens* (F_0)	Possible Resistance (92%; $N^a = 24$)
St. Paul, MN	*Culex pipiens* (F_0)	Possible Resistance (95%; $N = 23$)
Auburn, AL	*Culex quinquefasciatus* (F_1)	Resistant (23%; N 40)
Vero Beach, FL	*Culex quinquefasciatus* (F_0)	Resistant (29%; $N = 43$)

- Unknown how widespread resistance is throughout the state

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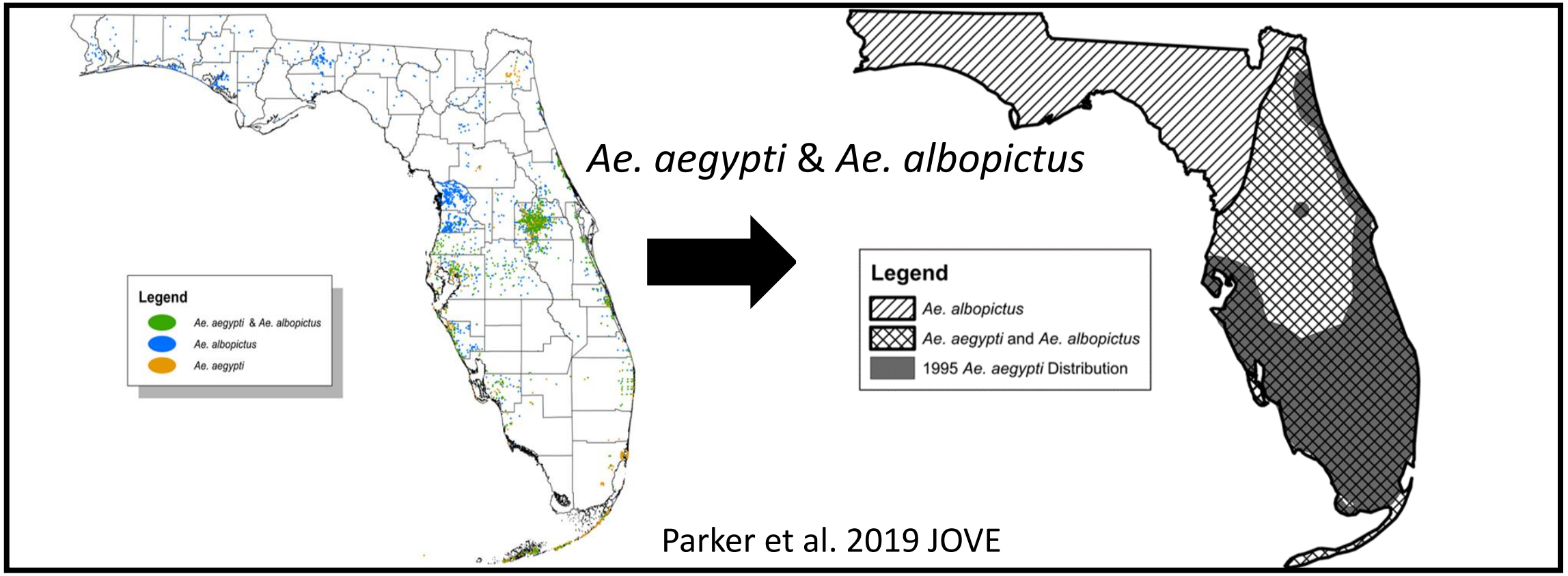
DISTRIBUTION OF FL DOMESTIC MOSQUITOES



- Areas no info on *Ae. aegypti*, *Ae. albopictus*, & *Cx. quinquefasciatus* presence
- All invasive species
  - Distributions can change
    - Routine surveillance

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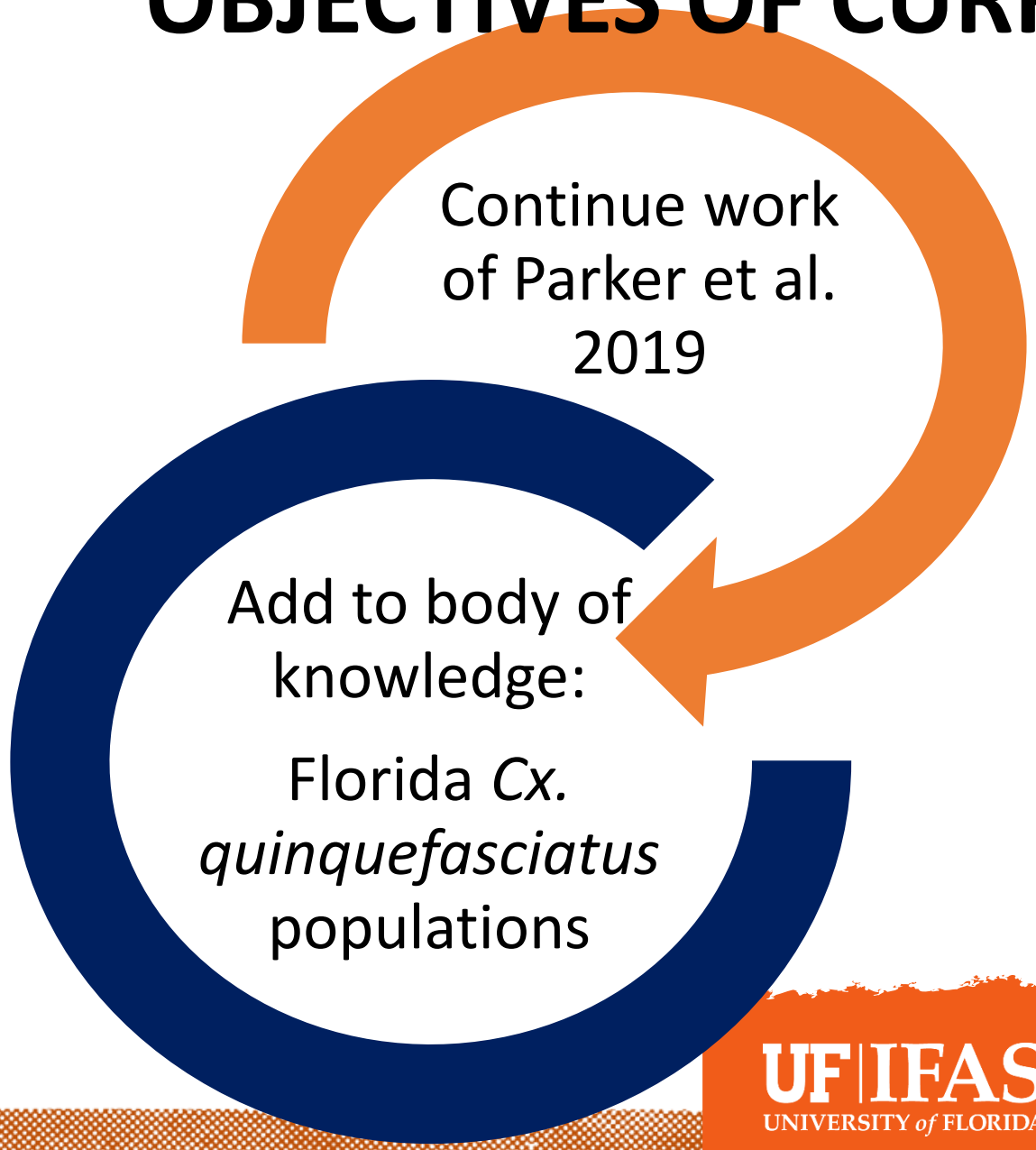
OBJECTIVES OF CURRENT PROJECT



- Conduct surveillance and insecticide susceptibility evaluations of Florida *Ae. aegypti* & *Ae. albopictus* populations

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Data sheet

Site Selection

Site choice is up to you. Select a site that is reasonably secure so that you don't lose the cups to animals or other interested parties. Make sure to record the GPS coordinates of the site (in decimal degrees). At each site, place 10 – 20 oviposition cups with one egg paper (see photos below) & 2 clips per cup. Make sure that each cup is filled up to the drainage holes with fresh (tap) water. Distribute the cups throughout the site so that egg collections can be maximized. Cups should be placed next to something secure, like the base of a tree, under a bush, etc., and not out in the open or in direct sunlight. If possible, secure the cups to the ground with a tent stake or something similar or put them inside a half cinder block since animals tend to knock them over. Egg papers should be picked up and replaced every 5 - 7 days (any longer than this and the eggs start to hatch).

For those who do not have experience collecting eggs of *Aedes* aegypti and *Aedes albopictus*, the chart below provide some ideas for how to distribute the oviposition cups at a site. It's a flexible protocol, so don't limit yourself to this These are just some examples of where you could put the cups to try to collect *Aedes* eggs.

SITE TYPE	AREA	# CUPS
Neighborhood	5 - 10 houses on the same street	2 - 3 cups per house
metery	Several acres	10 - 20 cups randomly distribute throughout the cemetery at
	Several acres	10 – 20 cups randomly d the property at bas outdoor stair

	n field	paper picked up	coordin	
	6/7/19	6/14/19	27.587340, -80.373463	Ve
2				
3				
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14				
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16				
17				

EGG COLLECTION

- *Aedes* & *Culex* egg collection kits shipped
- *Aedes* kit contains:
  - Ovicups
  - Egg papers
  - Binder clips
  - Plastic bags
  - Mailing envelopes
  - Instructions
  - Data sheets

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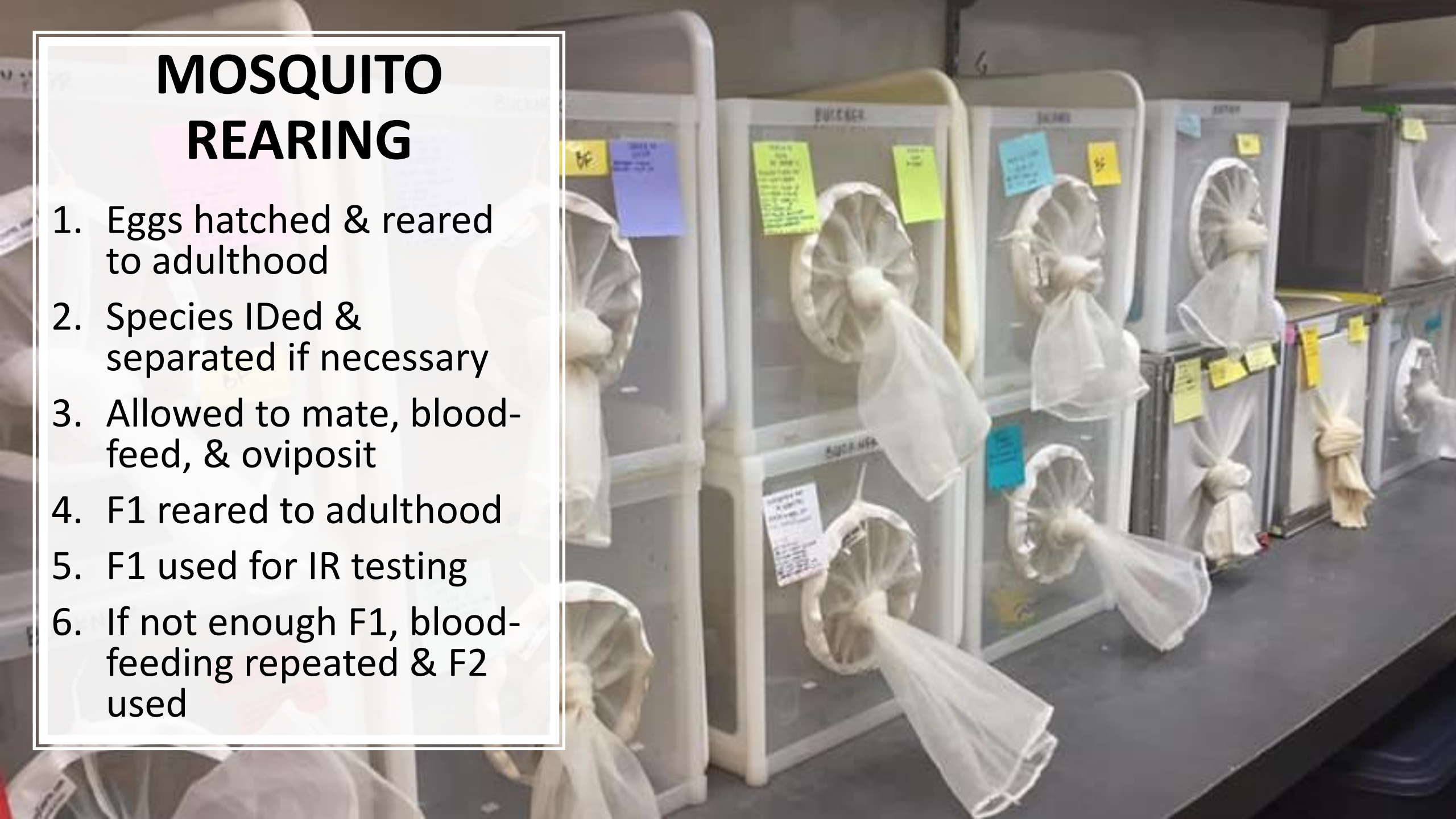
MOSQUITO REARING

1. Eggs hatched & reared to adulthood
2. Species IDed & separated if necessary
3. Allowed to mate, blood-feed, & oviposit
4. F1 reared to adulthood
5. F1 used for IR testing
6. If not enough F1, blood-feeding repeated & F2 used



RESISTANCE TESTING

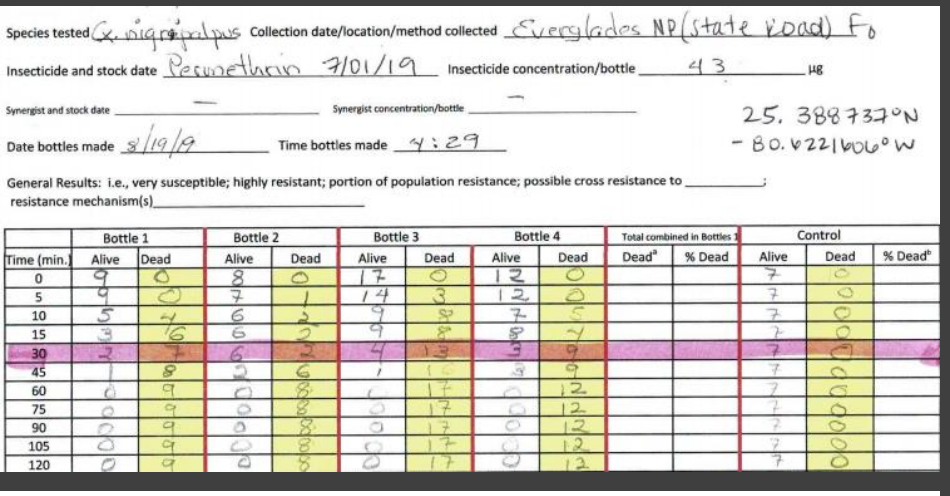
- CDC bottle bioassays conducted to evaluate insecticide susceptibility/resistance
  - 6 AIs tested:
    - Deltamethrin
    - Etofenprox
    - Permethrin
    - Sumethrin
    - Malathion
    - Naled

RESISTANCE TESTING

- Mortality over time recorded for 120 min
- Mortality rates calculated
- Mortality rate at diagnostic time used to characterize population as susceptible, developing resistance, or resistant

Table 1: Sample diagnostic doses and diagnostic times for technical grade insecticides.

Insecticide	Insecticide concentration (µg/bottle)	Diagnostic time per species (minutes)					
		Ae. aegypti	*Ae. albopictus*	*Cx. molestus*	*Cx. pipiens*	*Cx. quinquefasciatus*	*Cx. tarsalis*
hlorpyrifos	20	45	45	45	90	45	60
eltamethrin	0.75	30	30	--	45	60	--
tofenprox	12.5	15	30	105	15	30	60
enthion	800	--	--	30	75	45	45
alathion	400	15	30	30	45	45	45
aled	2.25	30	30	30	45	45	45
ermethrin	43	10	10	30	30	30	30
allethrin	0.05	--	--	--	60	60	--
rethrum	15	15	30	--	45	45	30
methrin	20	10	45	120	30	45	30



Species tested *Cx. nigripalpus* Collection date/location/method collected Everglades NP (state road) F0

Insecticide and stock date Permethrin 7/01/19 Insecticide concentration/bottle 43 µg

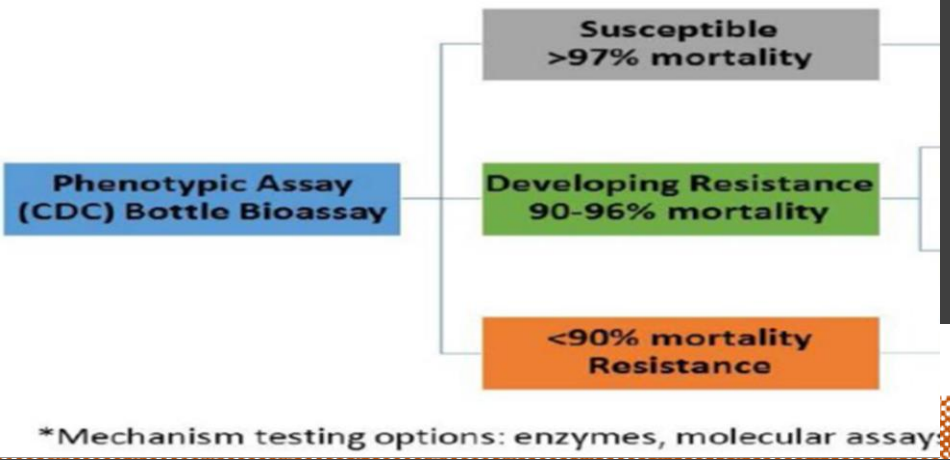
Synergist and stock date — Synergist concentration/bottle —

25.388737°N -80.622166°W

Date bottles made 8/19/19 Time bottles made 4:29

General Results: i.e., very susceptible; highly resistant; portion of population resistance; possible cross resistance to _____; resistance mechanism(s)_____

	Bottle 1		Bottle 2		Bottle 3		Bottle 4		Total combined in Bottles 1		Control		
Time (min.)	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Dead[a]	% Dead	Alive	Dead	% Dead[b]
0	9	0	8	0	17	0	12	0			7	0	
5	9	0	7	1	14	3	12	0			7	0	
10	5	4	6	2	9	8	7	5			7	0	
15	3	6	6	2	9	8	8	4			7	0	
30	2	7	6	2	4	13	3	9			7	0	
45	1	8	2	6	1	16	3	9			7	0	
60	0	9	0	8	0	17	0	12			7	0	
75	0	9	0	8	0	17	0	12			7	0	
90	0	9	0	8	0	17	0	12			7	0	
105	0	9	0	8	0	17	0	12			7	0	
120	0	9	0	8	0	17	0	12			7	0	



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DISTRIBUTION & INSECTICIDE SUSCEPTIBILITY MAPS

- **Maps created using ArcGIS software**
  - Map of collection locations for *Ae. aegypti*, *Ae. albopictus*, *Cx. quinquefasciatus*
  - Separate maps for each species & insecticide active ingredient characterizing each population as either susceptible, developing resistance, or resistant

RESULTS

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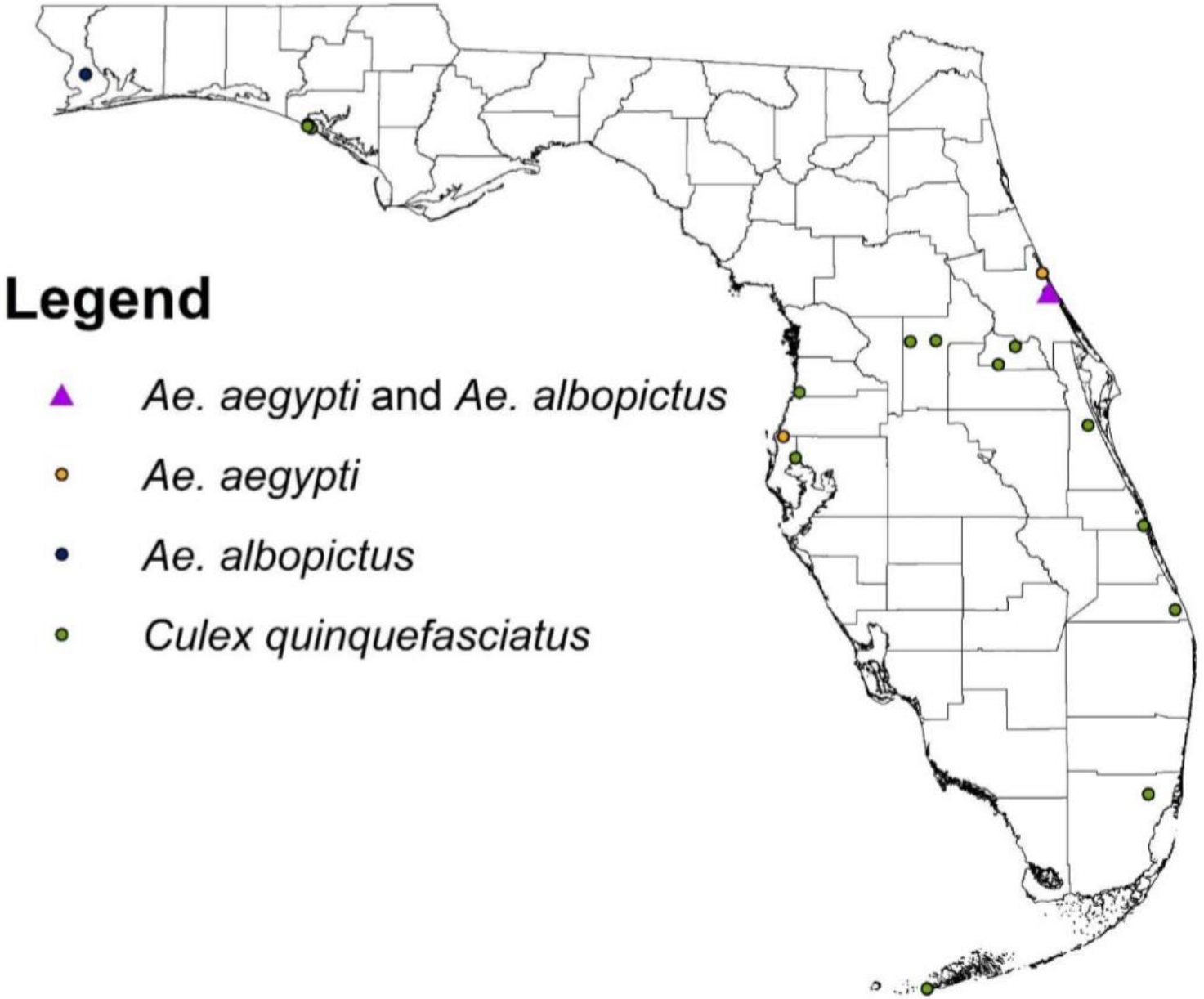
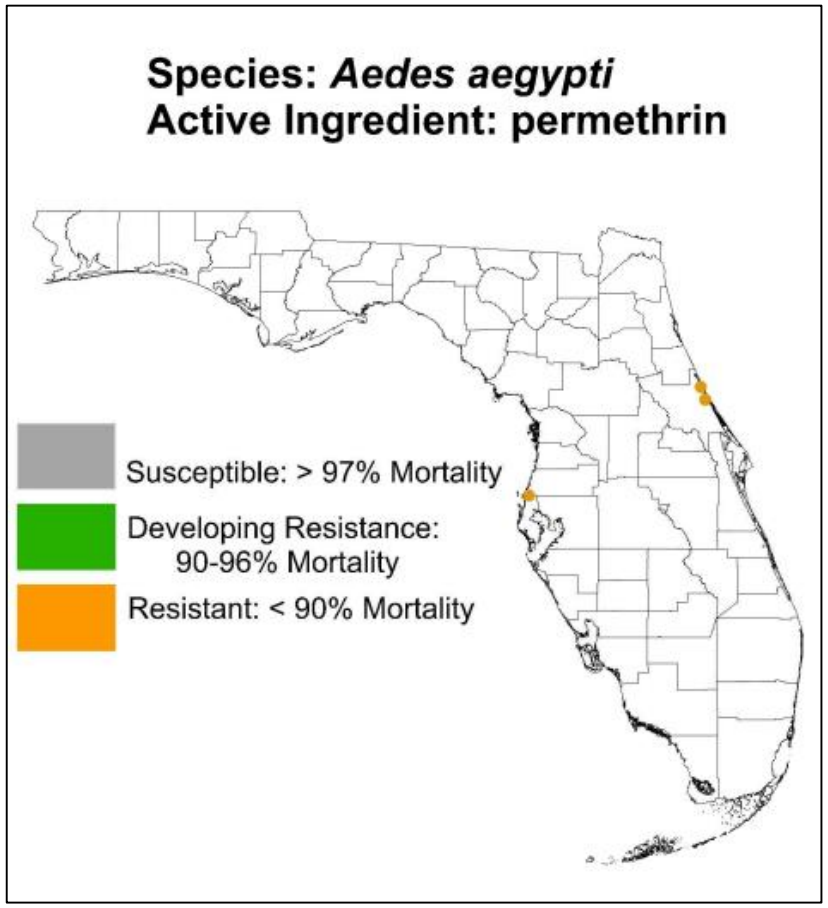
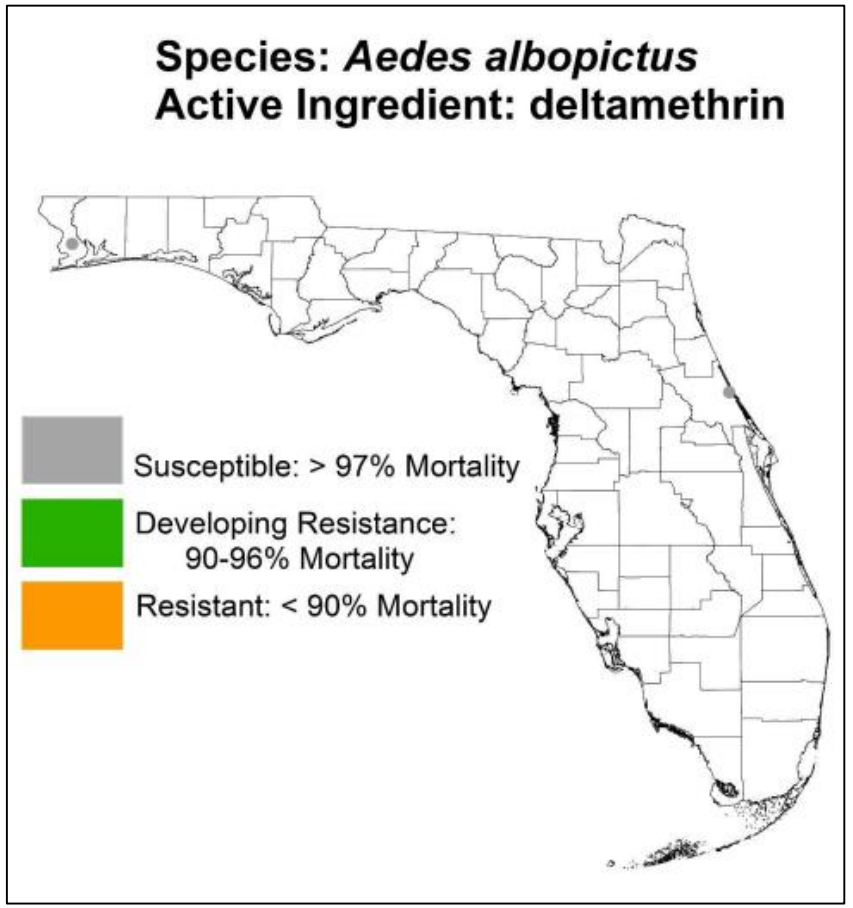
MOSQUITO POPULATIONS COLLECTED FOR TESTING

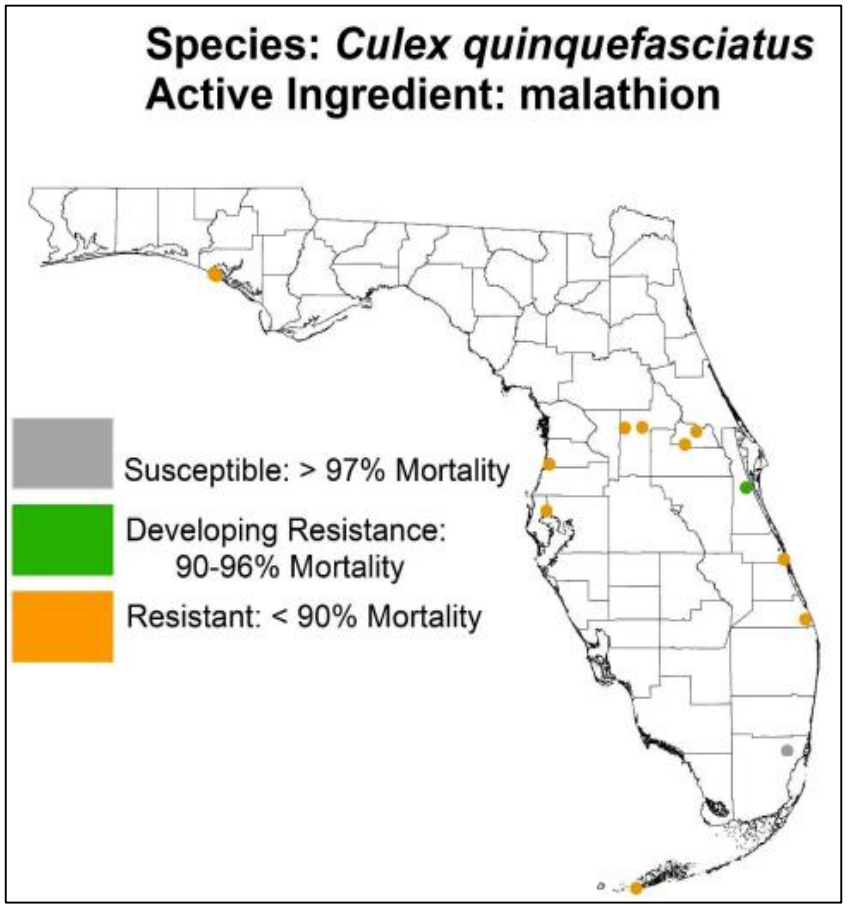


County	Species	Populations
Volusia	*Ae. aegypti*	2
Pinellas	*Ae. aegypti*	1
Escambia	*Ae. albopictus*	1
Volusia	*Ae. albopictus*	1
Seminole	*Cx. quinquefasciatus*	3
Lake	*Cx. quinquefasciatus*	2
Hernando	*Cx. quinquefasciatus*	1
Pinellas	*Cx. quinquefasciatus*	1
Brevard	*Cx. quinquefasciatus*	1
Polk	*Cx. quinquefasciatus*	1
Martin	*Cx. quinquefasciatus*	1
Miami-Dade	*Cx. quinquefasciatus*	1
Monroe	*Cx. quinquefasciatus*	1

INSECTICIDE SUSCEPTIBILITY MAPS







Comparative % mortality of *Aedes aegypti* mosquitoes to 6 AIs

County	Population	Malathion 400 ug/mL	Naled 2.25 ug/mL	Deltamethrin 0.75 ug/mL	Etofenprox 12.5 ug/mL	Permethrin 43 ug/mL	Sumethrin 20 ug/mL
Pinellas	Tarpon Springs	100	67	57	3	7	12
Seminole	Casselberry	99	100	45	12	10	37
Volusia	Holly Hill	100	84	41	13	9	17
Volusia	Port Orange	100	65	10	8	10	5

- **IR detected in all pops**
- **All pops susceptible to malathion, least resistant to naled**
- **Deltamethrin & sumethrin: Pyrethroids highest mortality**

Comparative % mortality of *Aedes albopictus* mosquitoes to 6 AIs

County	Population	Malathion 400 ug/mL	Naled 2.25 ug/mL	Deltamethrin 0.75 ug/mL	Etofenprox 12.5 ug/mL	Permethrin 43 ug/mL	Sumethrin 20 ug/mL
Escambia	Cantonment	98	100	100	98	100	100
Volusia	Port Orange	100	99	100	100	100	100

- **Susceptible to 6 AIs**

Comparative % mortality of *Cx. quinquefasciatus* mosquitoes to 6 AIs

County	Population	Malathion 400 ug/mL	Naled 2.25 ug/mL	Deltamethrin 0.75 ug/mL	Etofenprox 12.5 ug/mL	Permethrin 43 ug/mL	Sumethrin 20 ug/mL
Brevard	Viera	91	96	7	46	0	8
Hernando	Spring Hill	43	6	38	4	15	18
Lake	Tavares	14	1	44	11	39	3
Lake	Leesburg	36	70	16	2	12	0
Martin	Hobe Sound	60	23	9	3	0	1
Miami-Dade	Miami	100	100	0	36	17	26
Monroe	Key West	64	0	4	7	10	-
Pinellas	Oldsmar	66	22	13	4	4	0
Polk	Bartow	79	22	52	9	21	16
Seminole	Sanford	61	9	10	6	3	11
Seminole	Casselberry	70	51	16	0	15	14
Seminole	Forest City	75	60	1	2	2	2

- **Resistance to > 1 AI in all pops**
- **AIs susceptible or least resistant varied- malathion or naled**

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EXTENSION PROGRAM: USING RESISTANCE TESTING RESULTS TO MAKE MANAGEMENT RECOMMENDATIONS

MOSQUITO CONTROL PROGRAM MANAGERS RECEIVE:

- **Report with results for all AIs tested**
- **Results-driven management recommendations**

Assay: CDC bottle bioassay
Conducted by: UF/IFAS/FMEL – Buckner Lab
Species: *Aedes aegypti*
Source: Reared from eggs
Site: Pinellas County (Sawgrass)
Address: 1100 Sawgrass Dr., Tarpon Springs, FL 34689
Coordinates: 28.172633, -82.735169

Date of assay: 7/16/19 – 7/17/19

Following the CDC Guidelines for insecticide resistance monitoring http://www.cdc.gov/zika/vector/insecticide-resistance.html, resistance is determined by the percentage of mosquitoes that die (mortality rate) in the diagnostic time.

The data shown below provides:

Column 1: CDC recommended diagnostic dose (per bottle)
Column 2: Active ingredient tested
Column 3: Diagnostic time from FMEL assays; 100% mortality expected at given time using ORL strain of susceptible *Aedes aegypti;*
Column 4: Site specific *Aedes aegypti;* % mortality at the CDC diagnostic time

1	2	3	4
CDC diagnostic dose (per bottle)	Active ingredient tested	Diagnostic time; 100% mortality expected at given time using ORL strain of **susceptible** *Aedes aegypti*	Sawgrass *Aedes aegypti;* % mortality at the CDC diagnostic time
43 ug/bottle	Permethrin	15 min	7%
400 ug/bottle	Malathion	30 min	100%
2.25 ug/bottle	Naled	30 min	67%
12.5 ug/bottle	Etofenprox	30 min	3%
20 ug/bottle	Sumethrin	30 min	12%
0.75 ug/bottle	Deltamethrin	15 min	57%

Using the CDC guidelines (http://www.cdc.gov/zika/vector/insecticide-resistance.html on interpreting the data for management purposes:

The Sawgrass population of *Aedes aegypti* is resistant to Permethrin, Naled, Etofenprox, Sumethrin and Deltamethrin; Susceptible to Malathion.

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772-226-6606
eva.buckner@ufl.edu

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From: Buckner,Eva
Sent: Tuesday, July 30, 2019 5:56 PM
To: blawton@pinellascounty.org <blawton@pinellascounty.org>; Berro, Alissa M <amberro@co.pinellas.fl.us>; Stuck, Jason L <jstuck@co.pinellas.fl.us>
Subject: Insectide resistance testing results

Hi Brian,
My lab has finished testing your *Ae. aegypti* population for insecticide resistance against 6 active ingredients. The results are attached in a Word document. Based on these results, here are my management recommendations:
If you need a quick kill option for adult *Ae. aegypti* (ex. DENV local transmission), consider using malathion.

To prevent resistance rates from increasing in your *Ae. aegypti* population:
1. If not already, consider using a product containing malathion or naled.
2. Rotate the organophosphate product containing malathion or naled with a pyethroid product containing deltamethrin, permethrin, or sumethrin, or both sumethrin and permethrin.
3. The product containing deltamethrin, permethrin, or sumethrin should be applied at the highest label rate.
4. If you choose to use a permethrin product, I recommend using a permethrin 30-PBO 30 product, because a high concentration of permethrin should kill those individuals resistant to permethrin in your population.

Please let me know if you have any questions about the results or my recommendations.

Best,
Eva

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IMPACT OF RESEARCH & EXTENSION IN FL

- Helpful to programs do not perform surveillance and/or resistance testing
- Shows which AIs effectively kill adult mosquitoes
  - Important especially after natural disaster or during arbovirus transmission event
- If implemented, management strategies provided should prevent insecticide resistance rates from increasing
  - In general, *Ae. aegypti* & *Cx. quinquefasciatus*: Rotate organophosphate w/ pyrethroid
  - Will make recommendations for *Ae. albopictus* as needed
- **E**xisting insecticides continue to be effective against adult mosquitoes

FOLLOW OUR PROGRESS @ WWW.FMEL.IFAS.UFL.EDU!

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Updated every 3 months

ACKNOWLEDGEMENTS

- Dr. Barry Alto, Co-PI
- Dr. Roxanne Connelly
- Casey Parker
- Funding provided by the Florida Department of Health grant CODQJ and CDC
- Mosquito control programs provided mosquito eggs
- Current & former lab members assisted project: Daviela Ramirez, Ana Romero-Weaver, Natalie Kendziorski, Sierra Schluep, Amy Hallock, Ashley Page, Shawna Bellamy, and Rebecca Zimler

Florida HEALTH

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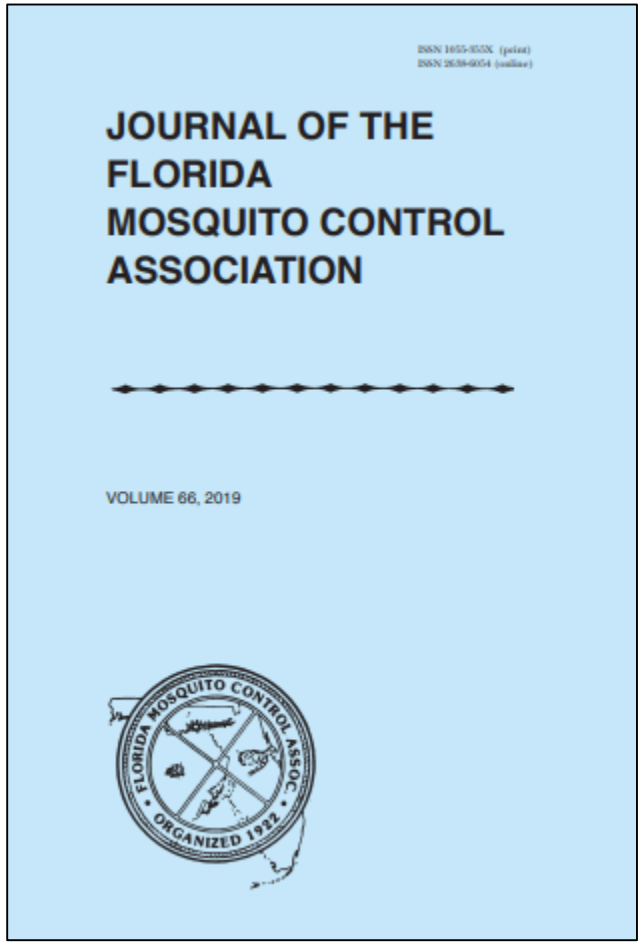
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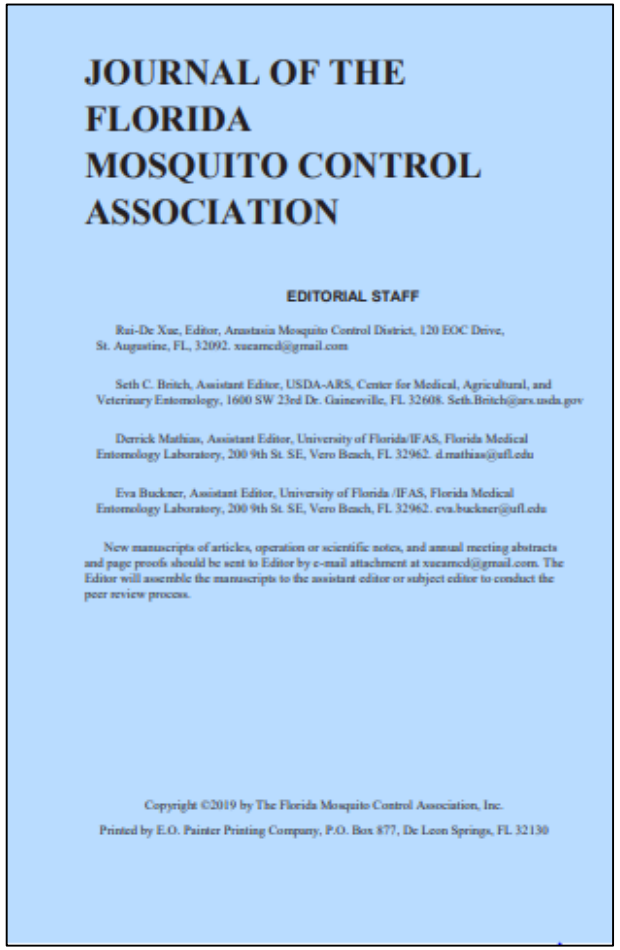
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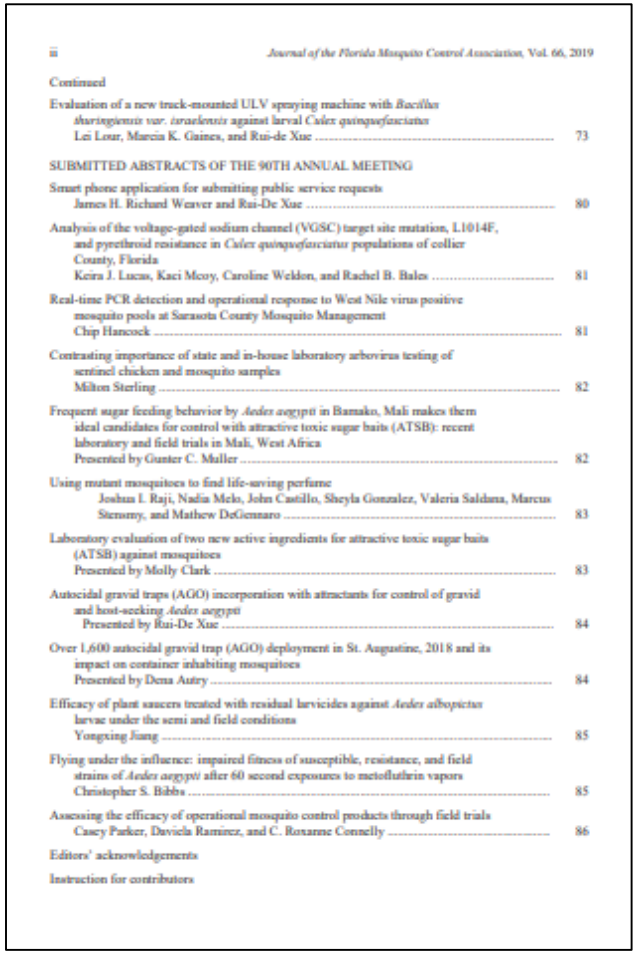

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- **JFMCA resuscitated after 25 years**
- **Accepts all submissions**
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