

An Update on Georgia's Insecticide Resistance Testing Program

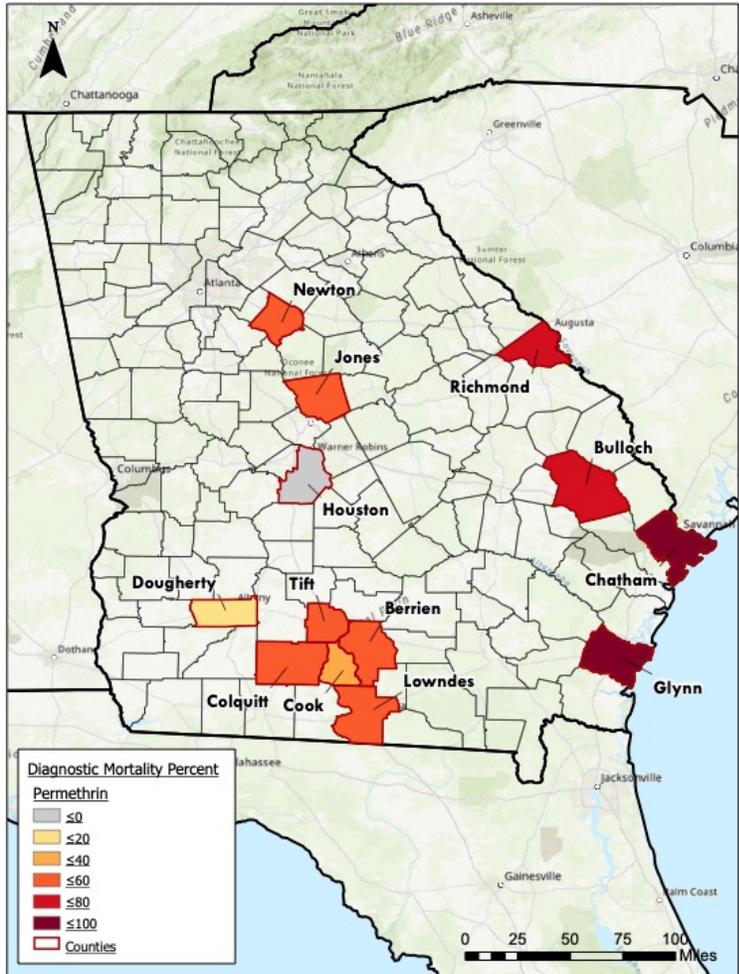


Georgia Mosquito Control Association Conference

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October 20, 2023

Pesticide Resistance Testing

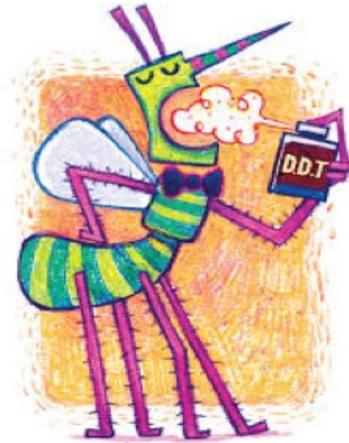


The state entomologists were tasked by the CDC, through the Hurricane Crisis CoAG grant, to conduct insecticide resistance testing in all high-risk urban regions of Georgia.

- Collaborators included Vector Surveillance Coordinators and Environmental Health Specialists around the state who conducted mosquito egg collections, as well as mosquito control technicians from Chatham and Glynn counties who conducted their own resistance testing.

After funding losses in 2020, testing mosquitoes from high-risk counties around the state has continued, but at a much lower level than before due to lack of personnel.

What Is Pesticide Resistance?



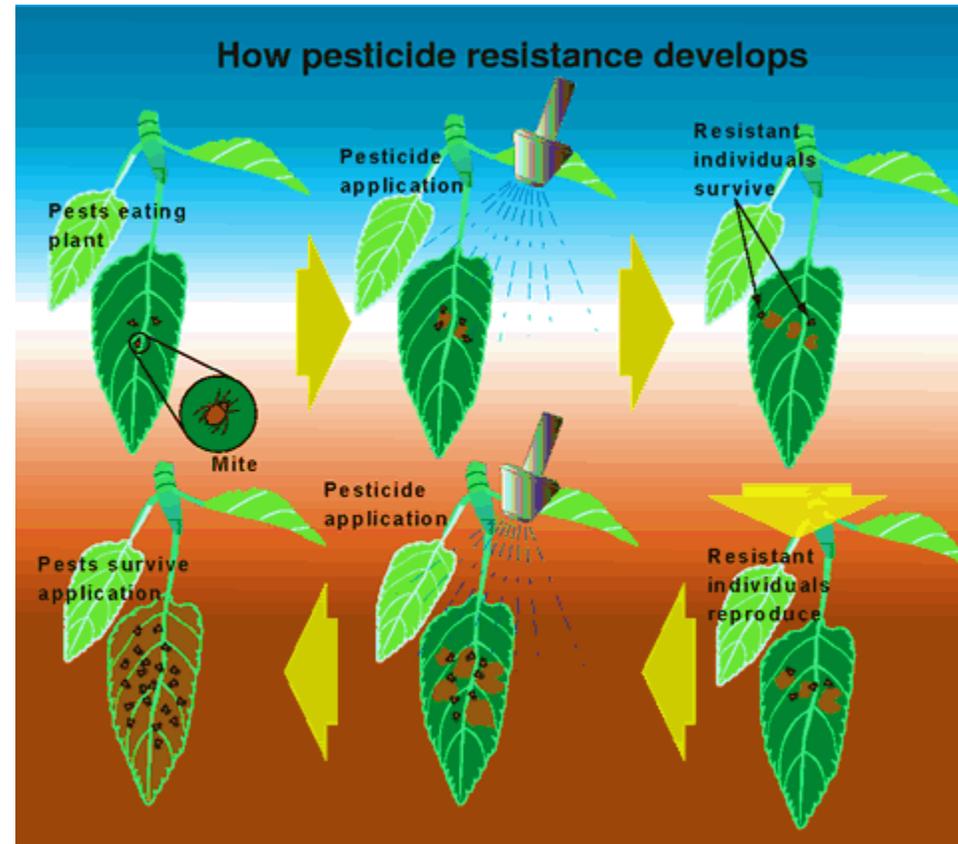
“Insecticide resistance is an overall reduction in the ability of an insecticide to kill mosquitoes. This means that, when used as directed, a product no longer works, or only partially works.” – CDC on Mosquito Resistance

The pesticide no longer effectively kills the mosquitoes.

* Slide Credit: Janet McAllister, Ph.D., CDC

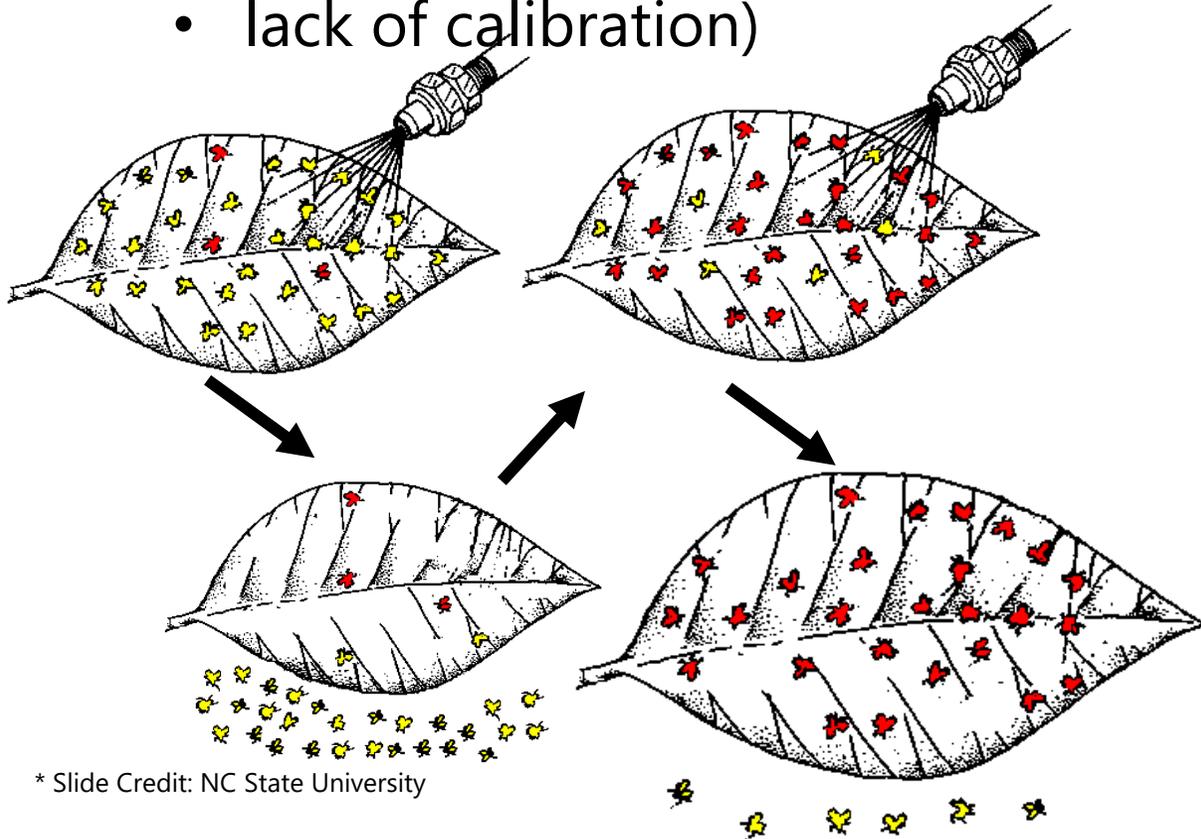
What is Resistance?

Pest species evolve pesticide resistance via natural selection - the most resistant organisms are the ones to survive and pass on their genetic traits to their offspring.

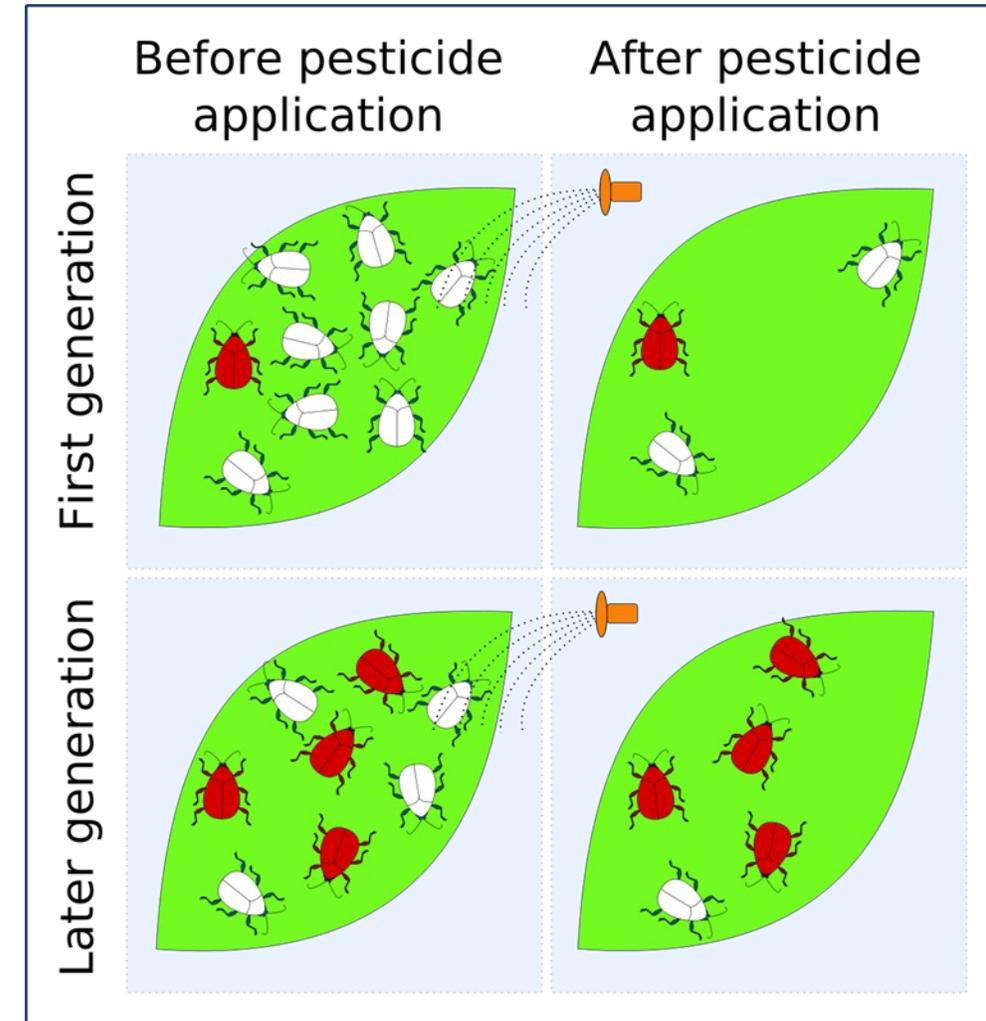


How Do Mosquitoes Become Resistant?

- Using the same pesticide for many years
- Improper application (too weak, too strong, lack of calibration)



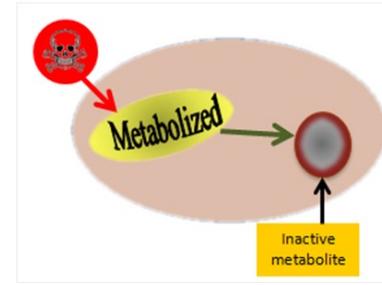
* Slide Credit: NC State University



Types of Resistance

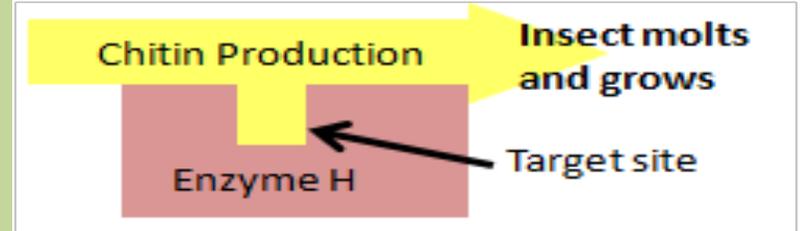
Metabolic

- The insect can clear its body of a toxin, or break a toxin down quicker than other insects



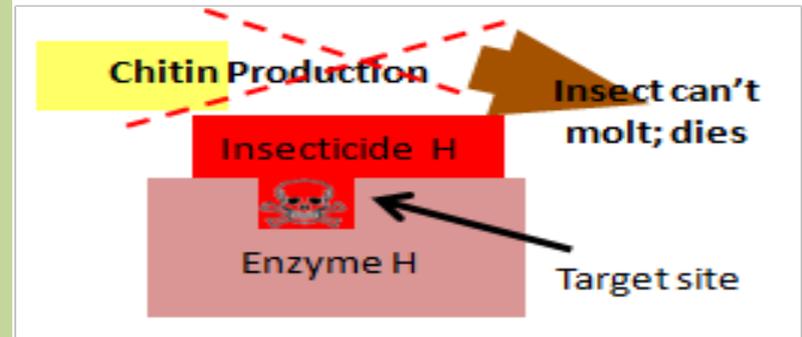
Target-site

- The insecticide can no longer connect at its target-site at a molecular level in the insect



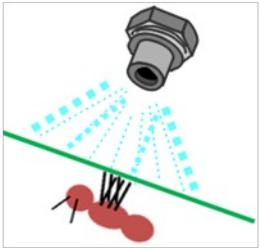
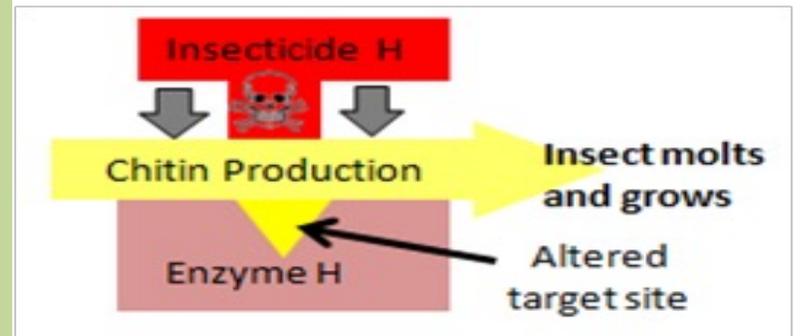
Penetration

- The insects shell more slowly absorbs an insecticide



Behavioral

- Certain insects can sense or steer clear of insecticide dangers



Types of Resistance

Multiple resistance is the phenomenon in which a pest is resistant to more than one class of pesticides.

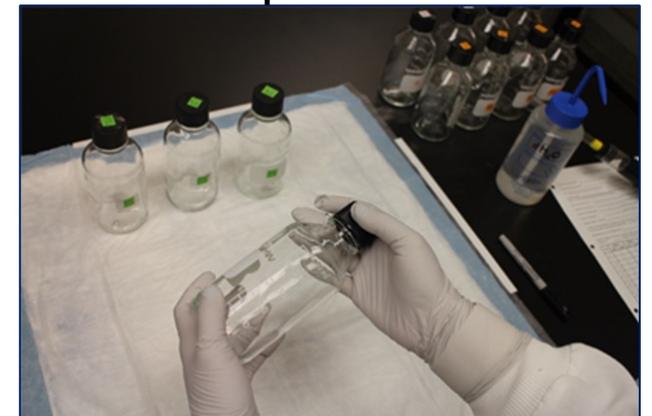
This can happen if one pesticide is used until pests display a resistance and then another is used until they are resistant to that one, and so on.

Cross resistance, a related phenomenon, occurs when the genetic mutation that made the pest resistant to one pesticide also makes it resistant to other pesticides, especially ones with similar mechanisms of action.



How Do We Test for Insecticide Resistance?

1. Collect mosquito eggs in the field.
2. Rear up mosquitoes from eggs to adults in the insectary.
3. Expose mosquitoes to known pesticides (Following CDC Bottle Bioassay protocol-coating the inside of bottles, letting that dry and then introducing the mosquitoes into them)
4. Record the percentage of mosquitoes that die post-exposure (diagnostic times – the time it takes for mortality to reach 100% of susceptible mosquitoes)



Egg Collection

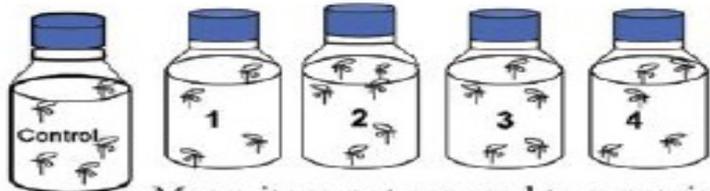




- Plastic shoe box (exterior spray painted black)
- Unbleached brown paper towels (multifold)
- Binder clips
- Water or hay infusion



Synergist-control
bottle: add 1 ml
of ethanol or
acetone



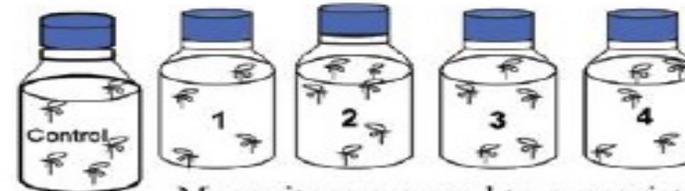
Mosquitoes not exposed to synergist

1. Coat the bottles

2. Introduce mosquitoes,
incubate for 1 hour

3. Transfer to holding
cartons

4. Perform CDC bottle
bioassay with insecticide
coated bottles



Mosquitoes exposed to synergist

Synergist-exposure
bottle: add 1 ml of
synergist



resistance testing
09/04/2018 1:20:47 PM



resistance testing
09/04/2018 1:20:27 PM

- **Organophosphates**
 - Malathion
 - Fenthion
 - Naled
- **Pyrethrin**
- **Synthetic Pyrethroids**
 - Permethrin
 - Resmethrin
 - Sumithrin
 - Prallethrin
 - Etofenprox
 - Bifenthrin
- **Synergist**
 - Piperonyl butoxide

Adulticides



CDC Bottle Bioassay

Location: Harold

Species: *Culex quinquefasciatus*

Diagnostic Dose: 43 µg/bottle

Diagnostic Time: 30 minutes

Insecticide: permethrin

We do not have a susceptible control population, so our diagnostic doses and times are from CDC colonies.

Time (min)	Bottle 1		Bottle 2		Bottle 3		Bottle 4		All test bottles			Control		
	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Total dead	Total	% Dead	Total dead	Total	% Dead
0	10	0	10	0	10	0	10	0	0	40	0.00%	0	10	0.00%
5	10	0	10	0	10	0	10	0	0	40	0.00%	0	10	0.00%
10	10	0	10	0	10	0	10	0	0	40	0.00%	0	10	0.00%
15	10	0	10	0	10	0	10	0	0	40	0.00%	0	10	0.00%
20	10	0	10	0	10	0	10	0	0	40	0.00%	0	10	0.00%
25	10	0	10	0	10	0	10	0	0	40	0.00%	0	10	0.00%
30	10	0	10	0	10	0	10	0	0	40	0.00%	0	10	0.00%
45	10	0	10	0	10	0	10	0	0	40	0.00%	0	10	0.00%
60	10	0	10	0	10	0	10	0	0	40	0.00%	0	10	0.00%
75	8	2	8	2	10	0	8	2	6	40	15.00%	0	10	0.00%
90	6	4	6	4	7	3	6	4	15	40	37.50%	0	10	0.00%
105	6	4	2	8	7	3	6	4	19	40	47.50%	0	10	0.00%
120	6	4	2	8	7	3	3	7	22	40	55.00%	0	10	0.00%

% Mortality at Diagnostic Time

% Mortality at End of Test (max. 2 hrs)

Statewide Insecticide Resistance Testing of Mosquitoes in GA

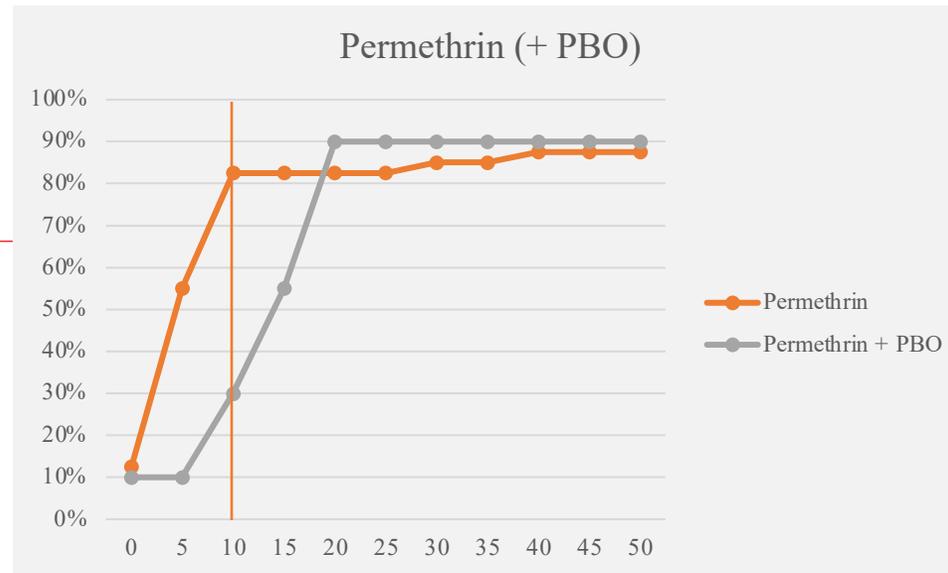
- September 2018
 - Preliminary data from several southern counties showed *Aedes albopictus* to be susceptible to permethrin, but *Culex quinquefasciatus* showing varied levels of resistance to both permethrin and lambda cyhalothrin.
- June 2019
 - Data from several central and southern counties showed *Aedes albopictus* to exhibit varied levels of resistance to permethrin.
 - *Culex quinquefasciatus* showed varied levels of resistance to both permethrin, but were susceptible to malathion.

Statewide Insecticide Resistance Testing of Mosquitoes in GA

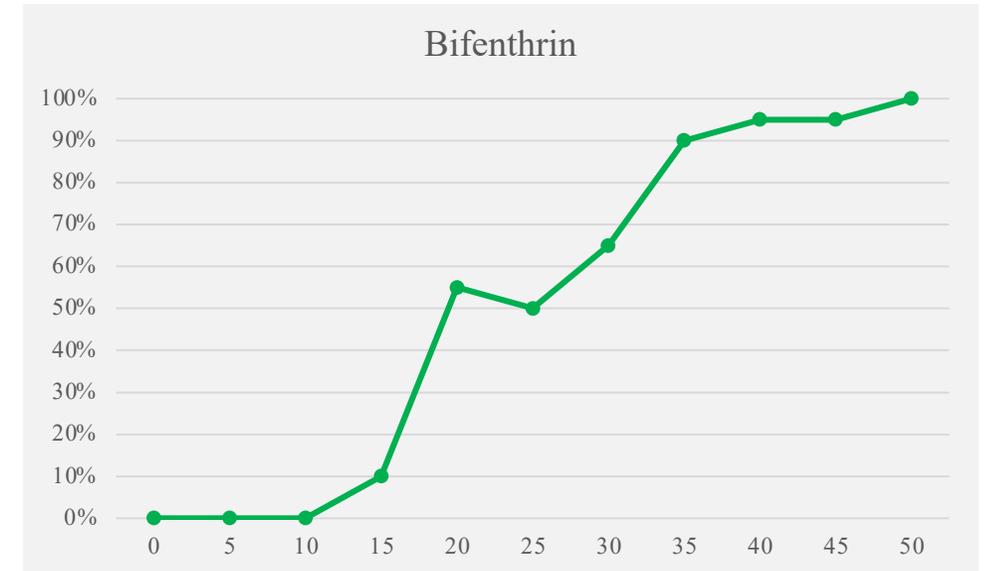
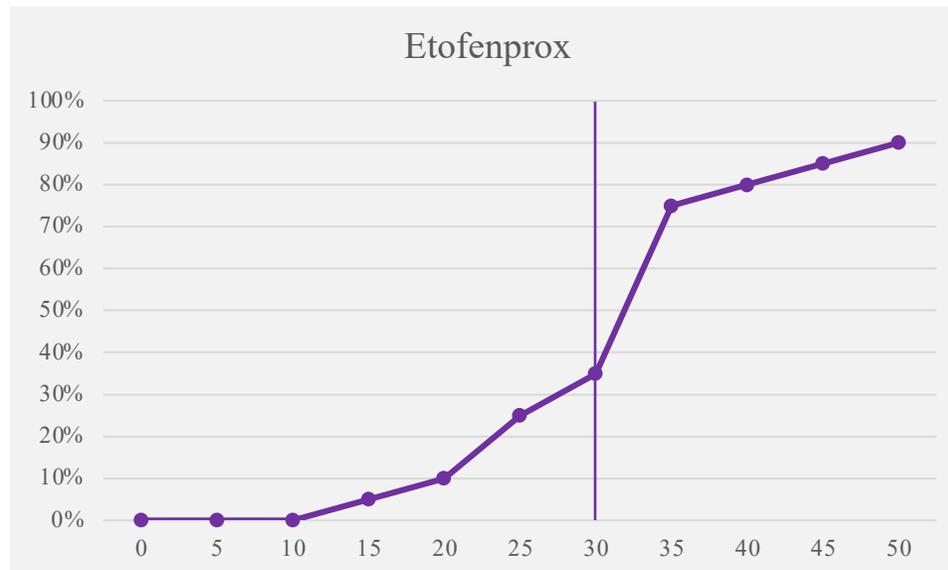
- October 2019
 - Data from several southern and central counties showed *Aedes albopictus* to exhibit varied levels of resistance to permethrin and deltamethrin alone, but were susceptible at varied levels to bifenthrin and deltamethrin used along with the synergist, PBO.
 - *Culex quinquefasciatus* showed varied levels of resistance to, bifenthrin, permethrin and deltamethrin.
 - At one location, *Ae. albopictus* showed high levels of resistance to etofenprox with 30% at the diagnostic time.
 - At another location, *Cx. quinquefasciatus*, showed very high levels of resistance with 7% mortality at diagnostic time and 30% at the end of two hours.

Fall 2019

- *Aedes albopictus*
 - Permethrin
 - + PBO
 - Etofenprox
 - Bifenthrin

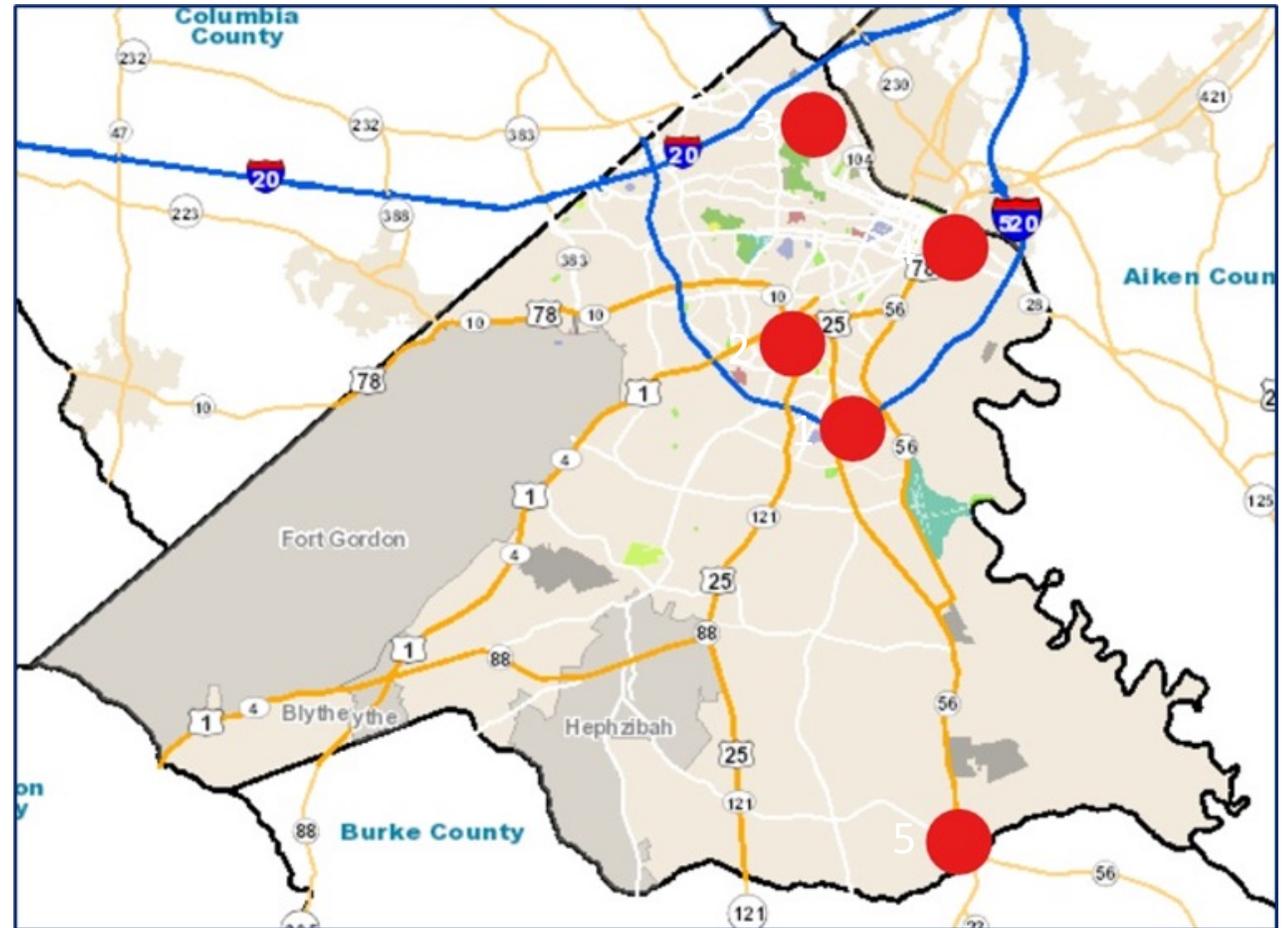


*All reached 100% mortality by 2 hours.



Egg Collection Sites, Richmond County

- 5 locations
 1. Apple Valley
 - Suburban, industrial
 - Most *Culex* overall
 2. Harold
 - Suburban
 - Most *Culex pipiens* complex
 3. Garrett
 - Suburban, commercial
 - Zone with most adulticide treatments
 4. Greene
 - Urban, commercial
 - Historical *Culex* problems
 5. McBean
 - Rural, agricultural, industrial

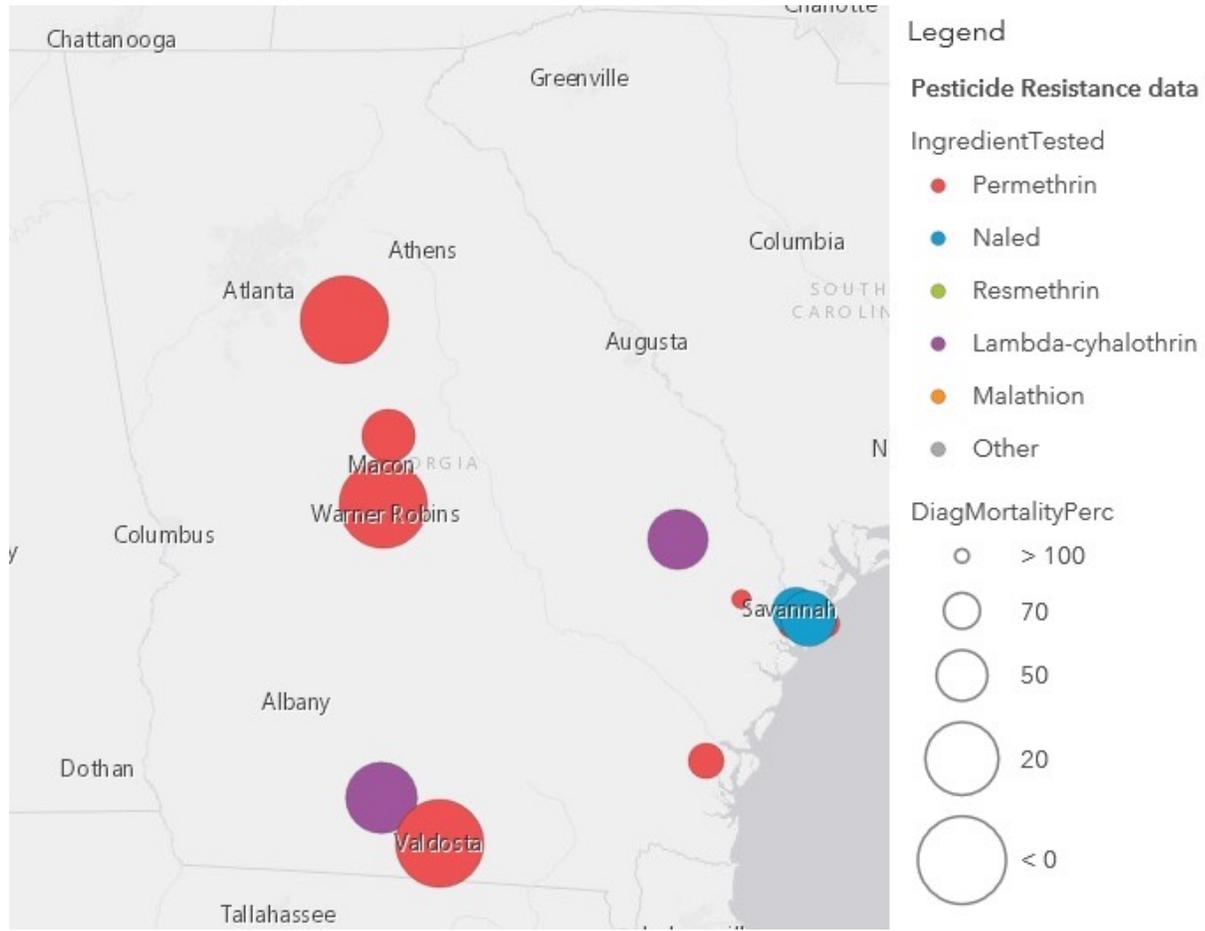


SPECIES	LOCATIONS	INSECTICIDE	% MORTALITY AT DT 2020	% MORTALITY AT DT 2021	% MORTALITY AT 2 HRS. 2020	% MORTALITY AT 2 HRS. 2021
<i>Culex coronator</i>	Garrett / Beattie	Permethrin	87.5	90	100	100
	Beattie	Malathion		42		96
<i>Culex territans</i>	Garrett	Permethrin		100		
<i>Culex quinquefasciatus</i>	Harold	Permethrin	0	65	55	100
		Permethrin + PBO	50	61	100	100
		Malathion	100	3	100	65
	Garrett / Beattie	Permethrin	37.5	17	100	95
	Beattie	Malathion		62		100
<i>Culex erraticus</i>	Apple Valley	Permethrin		65		98
		Permethrin + PBO		48		100
		Malathion		54		100
	McBean	Permethrin	92.5		100	
			45		100	
<i>Culex restuans</i>	Apple Valley	Permethrin	81.7		100	
	Garrett	Permethrin	92.5	95	100	100
		Permethrin + PBO	100	93	100	100
		Malathion		20		100

% Dead at Diagnostic Time	IR Level
> 97%	susceptible
90-96%	developing resistance
< 90%	resistant

SPECIES	LOCATIONS	INSECTICIDE	% MORTALITY AT DT 2021	% MORTALITY AT 2 HRS. 2021
<i>Aedes albopictus</i>	Harold	Permethrin	50	100
		Malathion	71	100

Pesticide Resistance Map



CDC Bottle Bioassay Data Recording Form

Date: 9/22/2023

Species: *Aedes albopictus*

Insecticide: Permethrin

Diagnostic Dose: 43 µg/bottle

Diagnostic Time: 10 minutes

Location of Mosquito Collection: Burning Tree

Date of Mosquito Collection: 9/7/2023

Time (min)	Bottle 1		Bottle 2		Bottle 3		Bottle 4		All test bottles			Control		
	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Total dead	Total	% Dead	Total dead	Total	% Dead
0	10	0	10	0	10	0	10	0	0	40	0%	0%	10	0
5	2	8	2	8	2	8	3	7	31	40	78%	0%	10	0
10	0	10	0	10	0	10	0	10	40	40	100%	0%	10	0

CDC Bottle Bioassay Data Recording Form

Date: 9/22/2023

Species: *Aedes albopictus*

Insecticide: Permethrin

Diagnostic Dose: 43 µg/bottle

Diagnostic Time: 10 minutes

Location of Mosquito Collection: Apple Valley

Time (min)	Bottle 1		Bottle 2		Bottle 3		Bottle 4		All test bottles			Control		
	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Total dead	Total	% Dead	Total dead	Total	% Dead
0	20	0	20	0	20	0	20	0	0	80	0%	0	20	0%
5	17	3	15	5	13	7	6	14	29	80	36%	0	20	0%
10	0	20	0	20	0	20	0	20	80	80	100%	0	20	0%

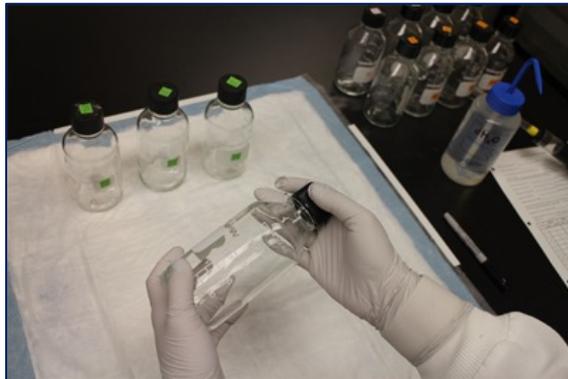
CDC Bottle Bioassay Data Recording Form**Date: 9/19/2023****Species: Culex quinquefasciatus****Insecticide: Permethrin****Diagnostic Dose: 43 µg/bottle****Diagnostic Time: 30 minutes****Location of Mosquito Collection: Apple Valley****Date of Mosquito Collection: 9/6/2023**

	Time (min)	Bottle 1		Bottle 2		Bottle 3		Bottle 4		All test bottles			Control		
		Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Total dead	Total	% Dead	Total dead	Total	% Dead
	0	20	0	20	0	20	0	20	0	0	80	0%	0	20	0%
	5	15	5	15	5	13	7	17	3	20	80	25%	0	20	0%
	10	13	7	14	6	13	7	14	6	26	80	33%	0	20	0%
	15	5	15	8	12	4	16	5	15	58	80	73%	0	20	0%
	20	0	20	2	18	1	19	2	18	75	80	94%	0	20	0%
	25	0	20	1	19	0	20	1	19	78	80	98%	0	20	0%
	30	0	20	0	20	0	20	0	20	80	80	100%	0	20	0%

Notes about interpreting results...

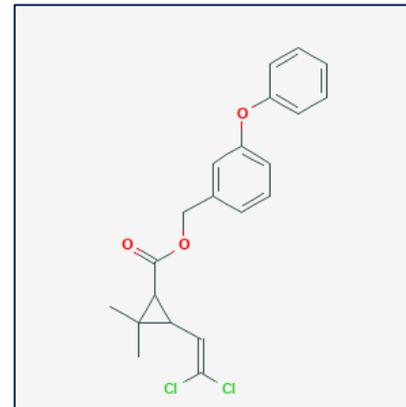
Types of Studies

- Controlled laboratory studies vs. field studies



Test Formulations

- Technical grade active ingredients vs. commercial products
- Developing resistance does not mean that a product will not work.



The primary goal of resistance surveillance is the measurement of resistance:

- As it exists...
- At a particular place...
- At a particular time.



Bottle Bioassay

http://www.cdc.gov/parasites/education_training/lab/bottlebioassay.html

Managing Resistance

Pest resistance to a pesticide can be managed by reducing selection pressure by this pesticide on the pest population.

This can be achieved by:

- Avoiding unnecessary pesticide applications
 - Following the label closely
 - Calibrating equipment properly on a schedule
- Rotating chemical and/or using non-chemical control techniques when possible
- leaving untreated refuges where susceptible pests can survive

Adopting the integrated pest management (IPM) approach usually helps with resistance management.

- Species-specific treatments
- Determining thresholds for levels of treatment

Insecticide Resistance Management

Plan: IRM should be considered an integral part of any vector control program

Monitor:

- The susceptibility status of the target mosquito population should be monitored during the planning phase to guide choice of intervention.
- Monitoring should be continued to identify changes in susceptibility profile.

Rotation:

- Where possible, guided by susceptibility monitoring data, plan to rotate insecticides by Mode of Action class, either temporally or spatially.
- In the absence of susceptibility data, the rotation of products between MoA classes will reduce selection pressure for resistance development. Include mosquito larvicides with alternative MoA where appropriate.

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