

# An Update on Georgia's Insecticide Resistance Testing Program

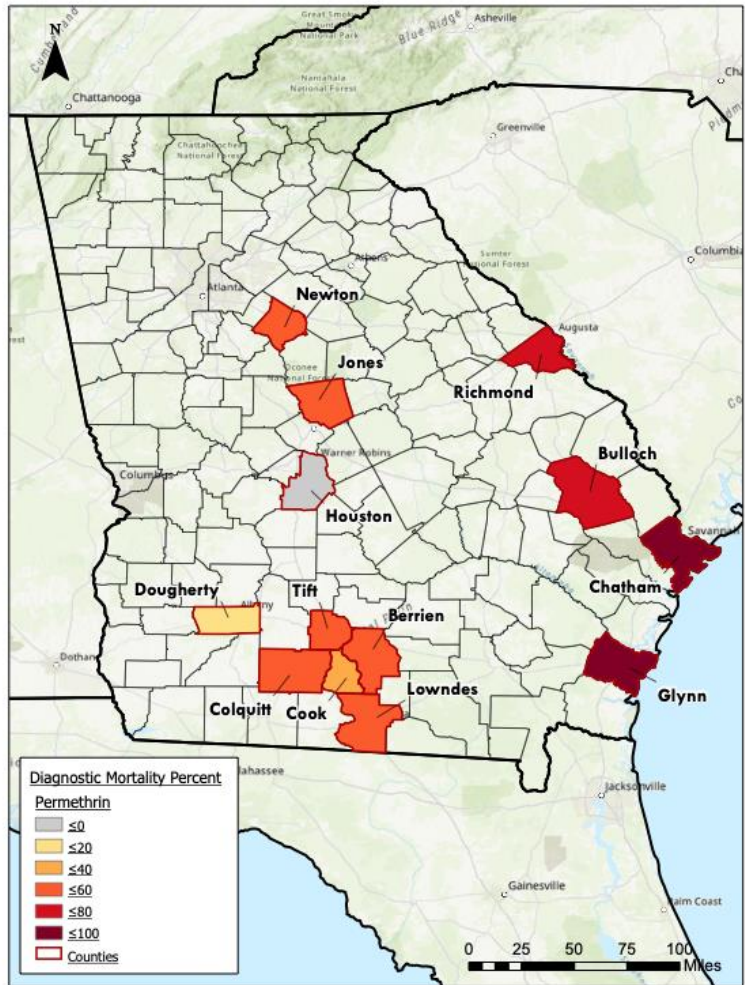


Georgia Mosquito Control Association Conference

Tiffany Thuy-Vi Nguyen, PhD, MPH

October 16, 2024

# 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?

---



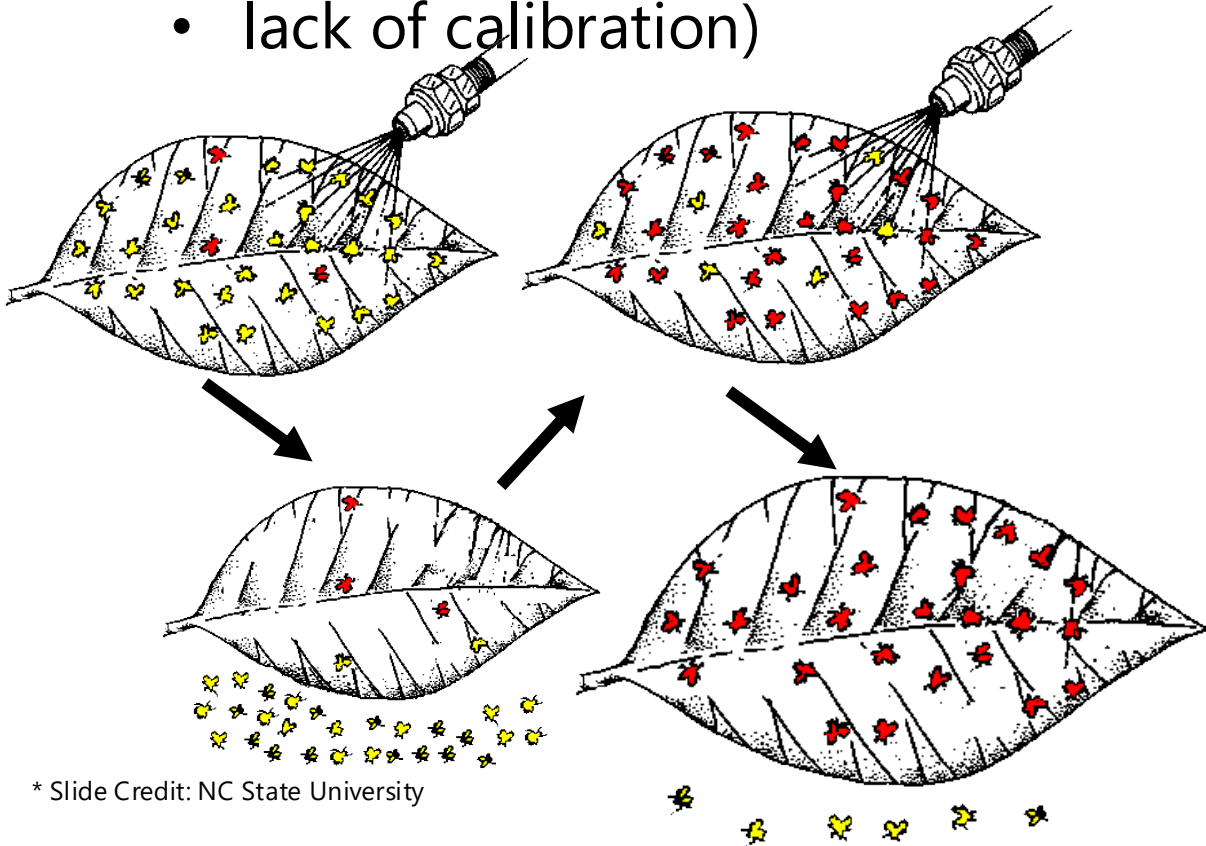
“Insecticide resistance is an overall reduction in the ability of an insecticide to kill mosquitoes. This means that, when used as directed, a product no longer works, or only partially works.” – CDC on Mosquito Resistance

The pesticide no longer effectively kills the mosquitoes.

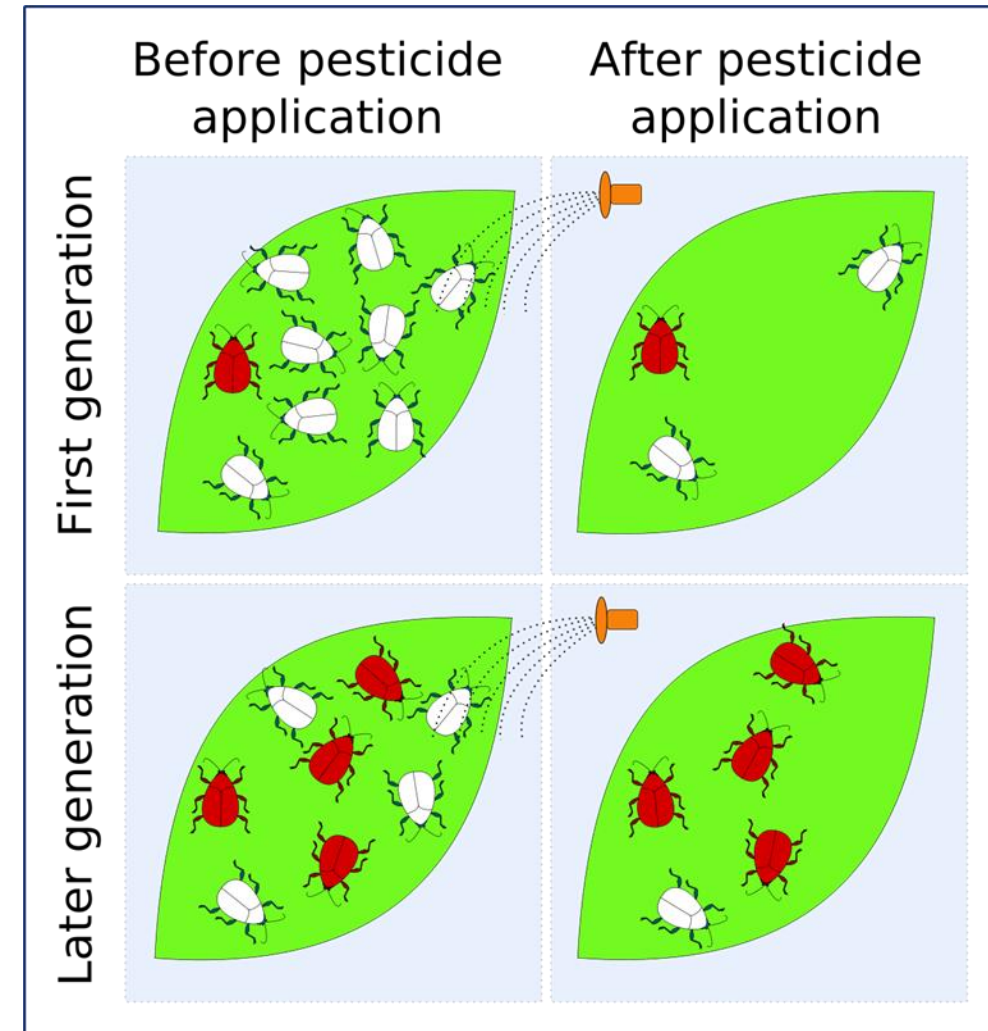
\* Slide Credit: Janet McAllister, Ph.D., CDC

# 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



# Resistance Influenced by:

---

Insecticides

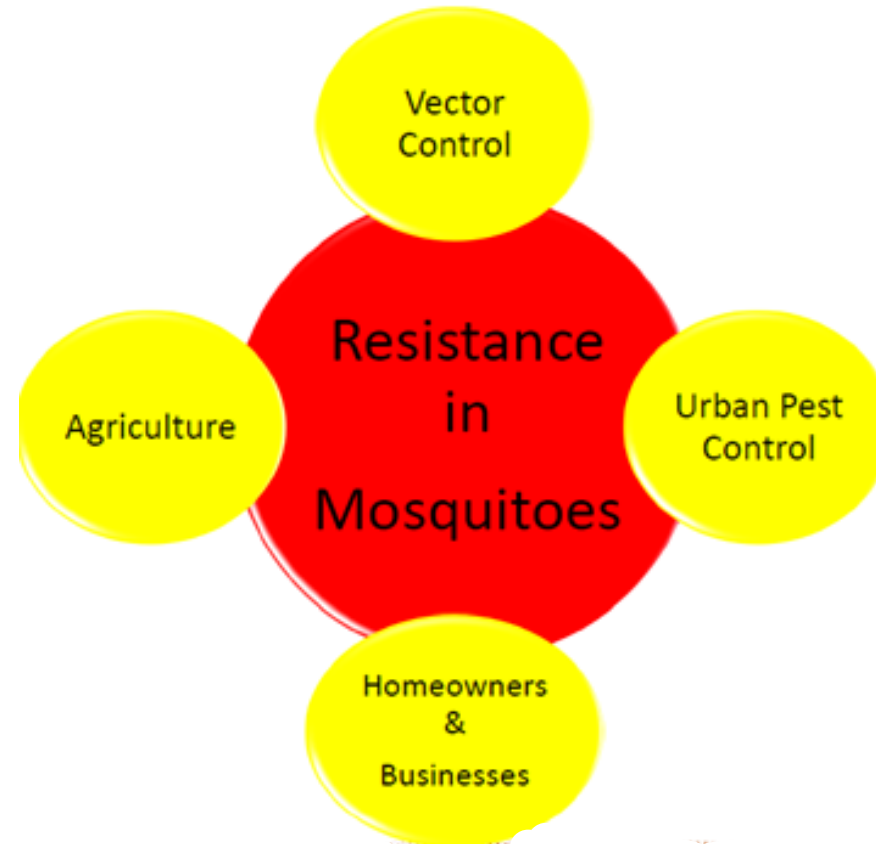
Herbicides

Fungicides

Fertilizers

Non-point source runoff

Slide credit: Janet McAllister, CDC



# Types of Resistance

## Metabolic

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

## Target-site

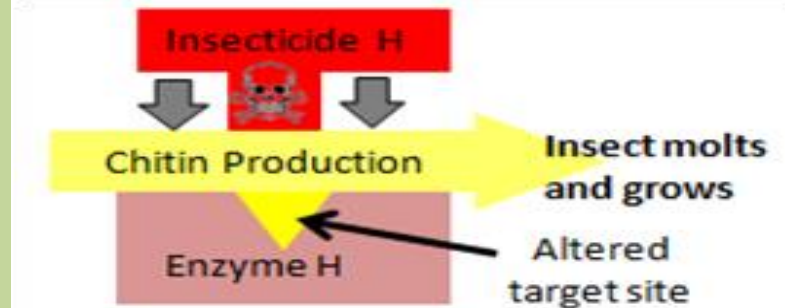
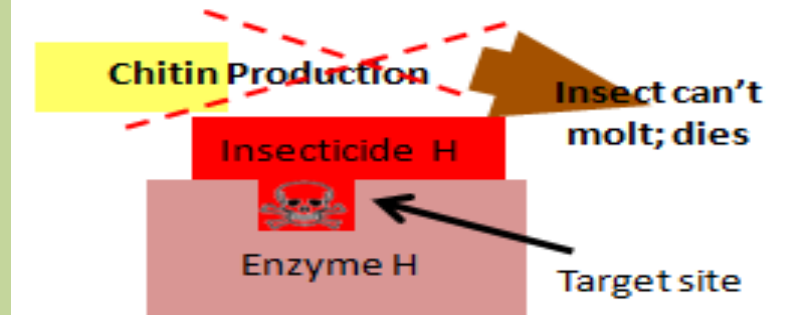
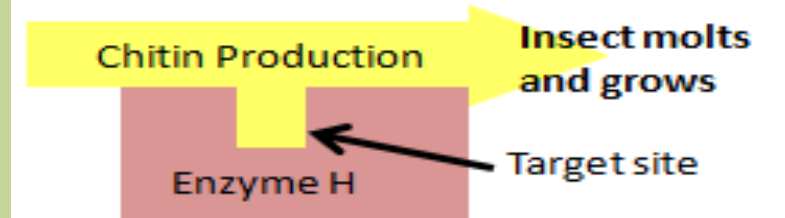
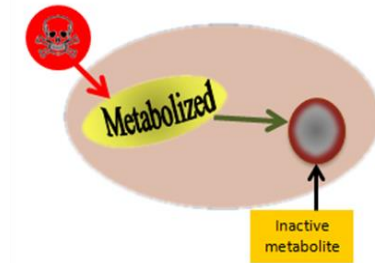
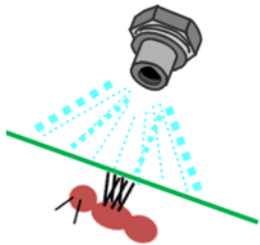
- The insecticide can no longer connect at its target-site at a molecular level in the insect

## Penetration

- The insect's 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 mortality over time for up to 120 min or when mortality reached 100%
5. Calculate mortality rates
6. Use mortality rate at (diagnostic time – the time it takes for mortality to reach 100% of susceptible mosquitoes) to characterize the population as susceptible, developing resistance or resistant



# Egg Collection

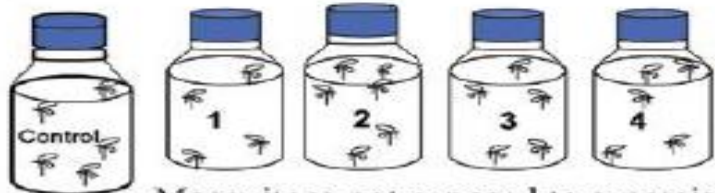




- Plastic shoe box (exterior spray painted black)
- Unbleached brown paper towels (multifold)
- Binder clips
- Water or hay infusion



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



Synergist-exposure  
bottle: add 1 ml of  
synergist



Mosquitoes exposed to synergist



- **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.



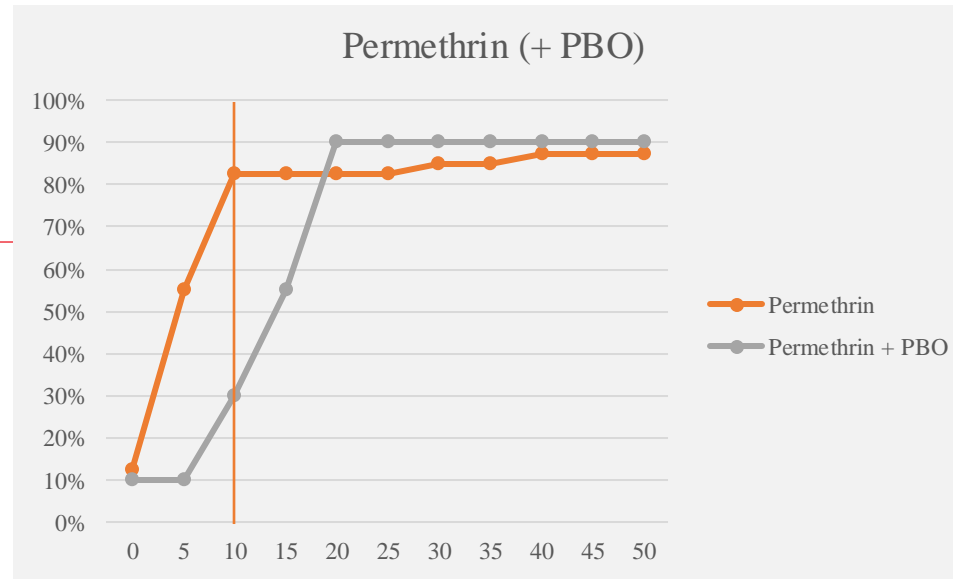
# Statewide Insecticide Resistance Testing of Mosquitoes in GA

---

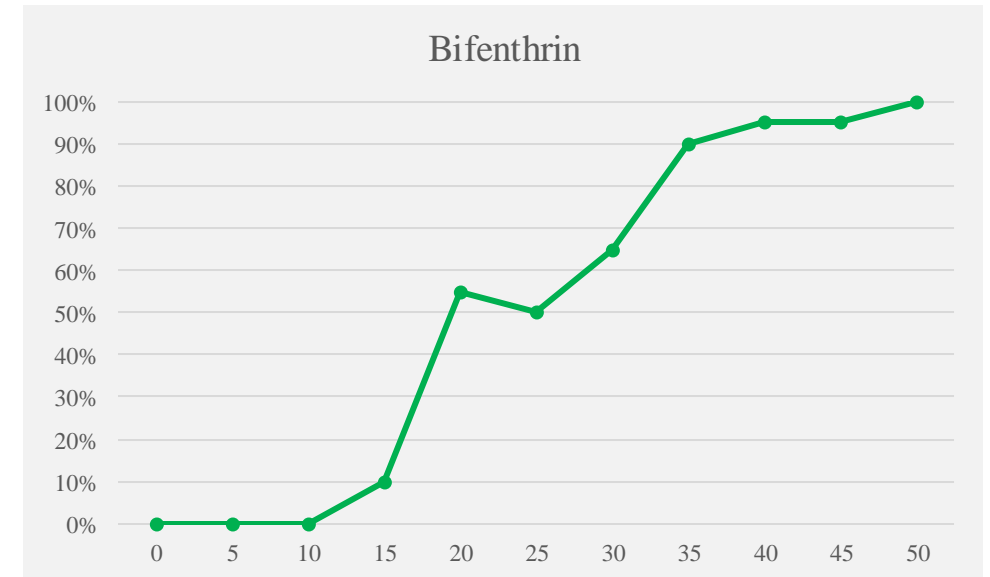
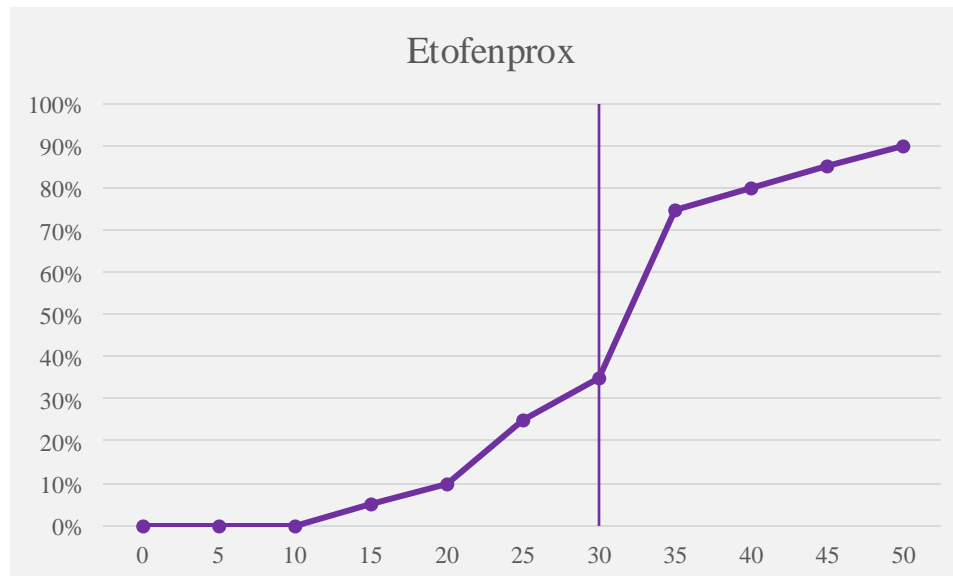
- October 2019
  - Data from several southern and central counties showed *Aedes albopictus* to exhibit varied levels of resistance to permethrin and deltamethrin alone, but were susceptible at varied levels to bifenthrin and deltamethrin used along with the synergist, PBO.
  - *Culex quinquefasciatus* 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

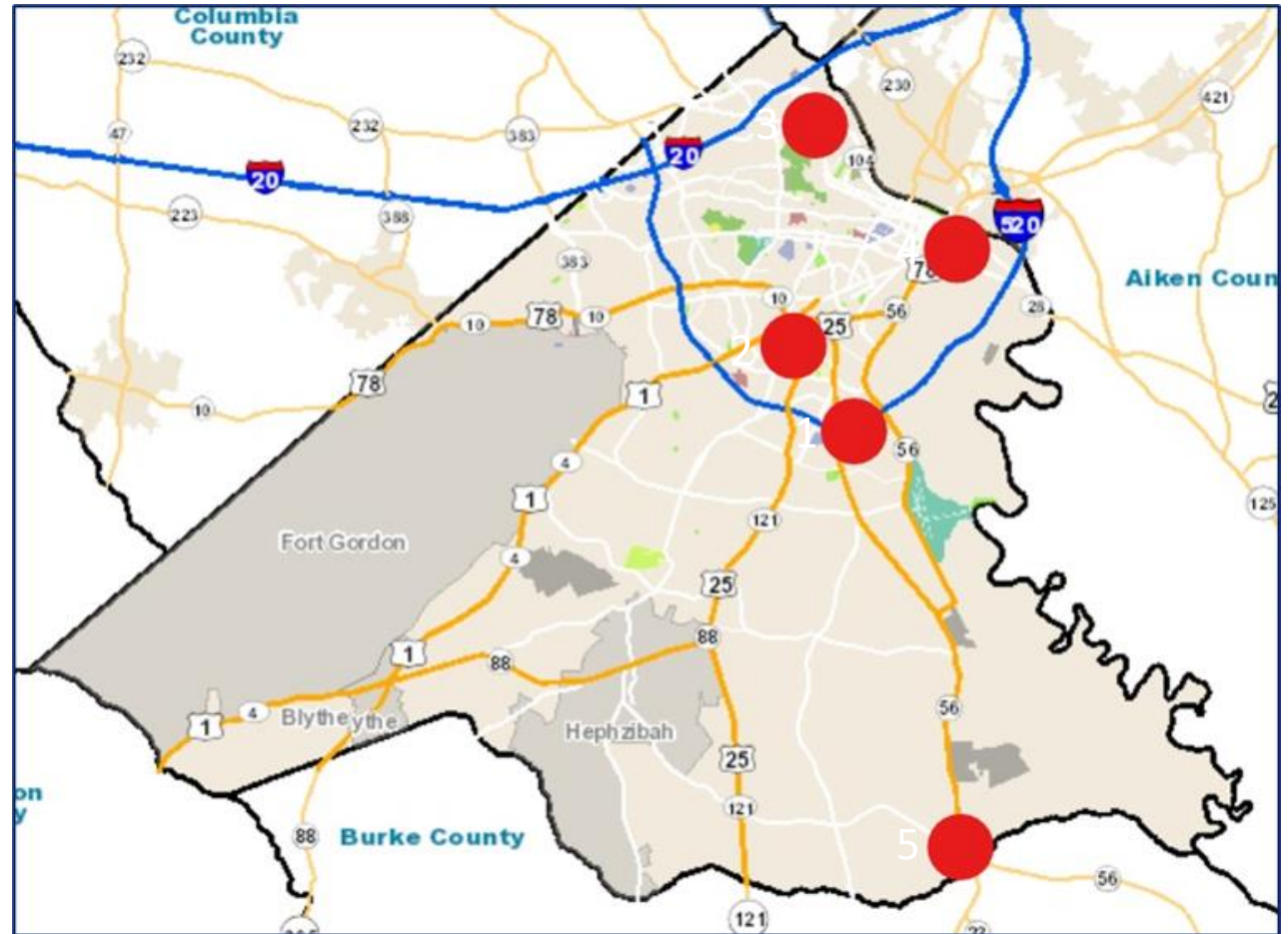


\*All reached 100% mortality by 2 hours.



# 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

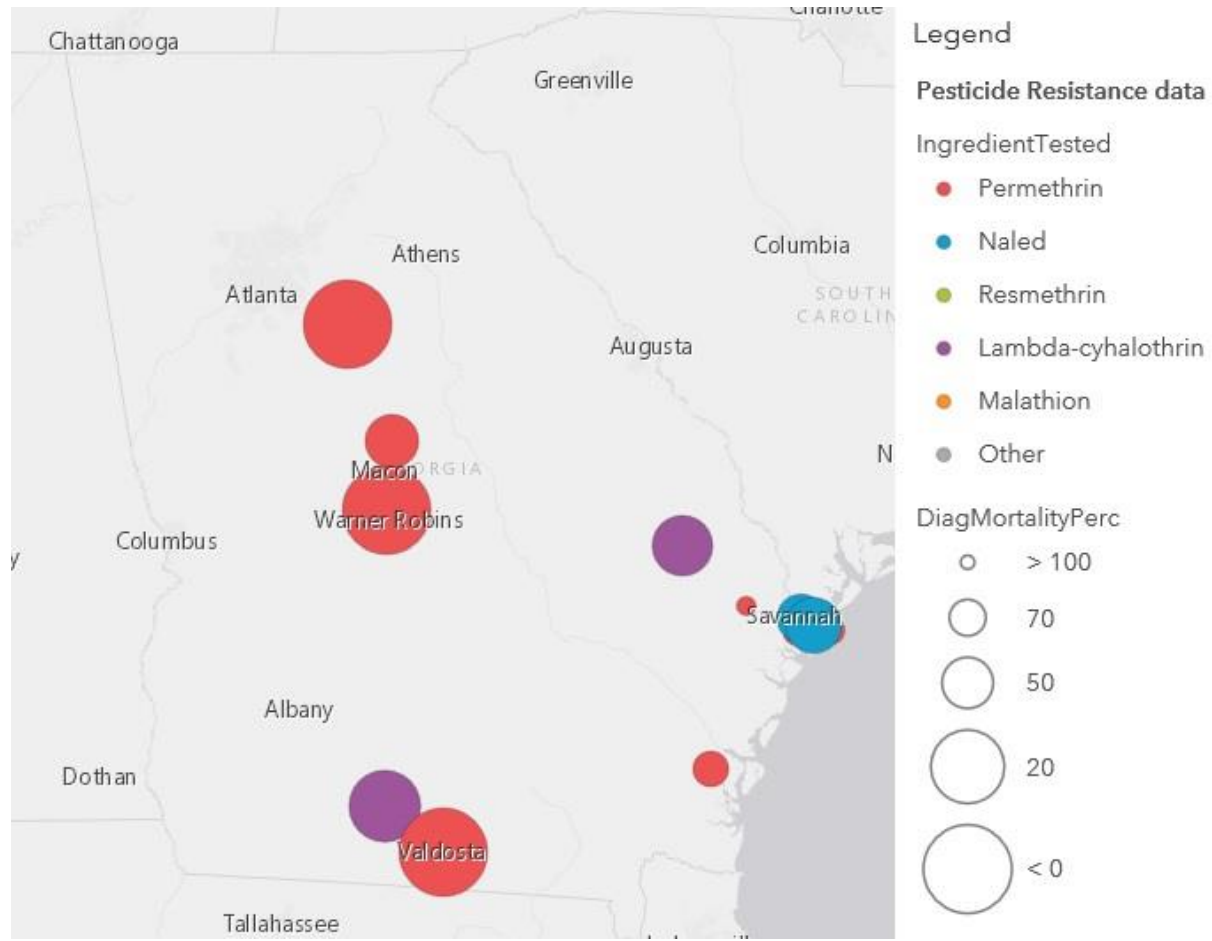


SPECIES	LOCATIONS	INSECTICIDE	% MORTALITY AT DT 2020	% MORTALITY AT DT 2021	% MORTALITY AT 2 HRS. 2020	% MORTALITY AT 2 HRS. 2021
<i>Culex coronator</i>	Garrett / <b>Beattie</b>	Permethrin	87.5	90	100	100
	<b>Beattie</b>	Malathion		42		96
<i>Culex territans</i>	<b>Garrett</b>	Permethrin		100		
<i>Culex quinquefasciatus</i>	Harold	Permethrin	0	65	55	100
		Permethrin + PBO	50	61	100	100
		Malathion	100	3	100	65
	Garrett / <b>Beattie</b>	Permethrin	37.5	17	100	95
	<b>Beattie</b>	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

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

<b>SPECIES</b>	<b>LOCATIONS</b>	<b>INSECTICIDE</b>	<b>% MORTALITY AT DT 2021</b>	<b>% MORTALITY AT 2 HRS. 2021</b>
<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: 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

	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%



**CDC Bottle Bioassay Data Recording Form**

Date: **9/24/2024**

Species: *Culex quinquefasciatus*

Insecticide: Permethrin

Diagnostic Dose: 43 µg/bottle

Diagnostic Time: 30 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	10	0	10	0	10	0	10	0	0	40	0%	0%	10	0
5	10	0	10	0	10	0	10	0	0	40	0%	0%	10	0
10	9	1	7	3	9	1	9	1	6	40	15%	0%	10	0
15	6	4	5	5	6	4	6	4	17	40	43%	0%	10	0
20	4	6	2	8	5	5	3	7	26	40	65%	0%	10	0
25	4	6	2	8	5	5	3	7	26	40	65%	0%	10	0
30	4	6	2	8	5	5	3	7	26	40	65%	0%	10	0
45	3	7	1	9	2	8	3	7	31	40	78%	0%	10	0
60	2	8	1	9	2	8	2	8	33	40	83%	0%	10	0
75	1	9	1	9	1	9	1	9	36	40	90%	0%	10	0
90	0	10	0	10	1	9	0	10	39	40	98%	0%	10	0
105	0	10	0	10	0	10	0	10	40	40	100%	0%	10	0
120														

**CDC Bottle Bioassay Data Recording Form**

**Date: 9/24/2024**

**Species: *Culex quinquefasciatus***

**Insecticide: Fenthion**

**Diagnostic Dose: 800 µg/bottle**

**Diagnostic Time: 45 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	10	0	11	0	10	0	20	0	0	51	0%	0%	10	0
5	10	0	11	0	10	0	20	0	0	51	0%	0%	10	0
10	10	0	11	0	10	0	20	0	0	51	0%	0%	10	0
15	9	1	10	1	9	1	20	0	3	51	6%	0%	10	0
20	9	1	7	4	8	2	15	5	12	51	24%	0%	10	0
25	7	3	6	5	5	5	12	8	21	51	41%	0%	10	0
30	2	8	3	8	2	8	9	11	35	51	69%	0%	10	0
45	0	10	0	11	0	10	0	20	51	51	100%	0%	10	0

**CDC Bottle Bioassay Data Recording Form**

**Date: 9/24/2024**

**Species: *Culex quinquefasciatus***

**Insecticide: Permethrin**

**Diagnostic Dose: 43 µg/bottle**

**Diagnostic Time: 30 minutes**

**Location of Mosquito Collection: Marvin Griffin**

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	8	2	8	2	6	4	5	5	5	13	40	33%	0%	10	0
10	6	4	4	6	3	7	3	7	24	40	60%	0%	10	0	
15	3	7	4	6	3	7	3	7	27	40	68%	0%	10	0	
20	3	7	4	6	2	8	2	8	29	40	73%	0%	10	0	
25	3	7	3	7	2	8	1	9	31	40	78%	0%	10	0	
30	2	8	2	8	2	8	1	9	33	40	83%	0%	10	0	
45	1	9	2	8	1	9	0	10	36	40	90%	0%	10	0	
60	0	10	1	9	1	9	0	10	38	40	95%	0%	10	0	
75	0	10	1	9	1	9	0	10	38	40	95%	0%	10	0	
90	0	10	0	10	0	10	0	10	40	40	100%	0%	10	0	

**CDC Bottle Bioassay Data Recording Form**

**Date: 9/24/2024**

**Species: *Culex quinquefasciatus***

**Insecticide: Naled**

**Diagnostic Dose: 2.25 µg/bottle**

**Diagnostic Time: 45 minutes**

**Location of Mosquito Collection: Marvin Griffin**

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	5	5	8	2	10	0	7	3	10	40	25%	0%	10	0
10	4	6	4	6	10	0	7	3	15	40	38%	0%	10	0
15	0	10	2	8	5	5	4	6	29	40	73%	0%	10	0
20	0	10	2	8	5	5	2	8	31	40	78%	0%	10	0
25	0	10	2	8	5	5	2	8	31	40	78%	0%	10	0
30	0	10	1	9	5	5	1	9	33	40	83%	0%	10	0
45	0	10	0	10	5	5	1	9	34	40	85%	0%	10	0
60	0	10	0	10	1	9	1	9	38	40	95%	0%	10	0
75	0	10	0	10	0	10	0	10	40	40	100%	0%	10	0

**CDC Bottle Bioassay Data Recording Form**

**Date: 9/24/2024**

**Species: *Culex quinquefasciatus***

**Insecticide: Malathion**

**Diagnostic Dose: 400 µg/bottle**

**Diagnostic Time: 45 minutes**

**Location of Mosquito Collection: Marvin Griffin**

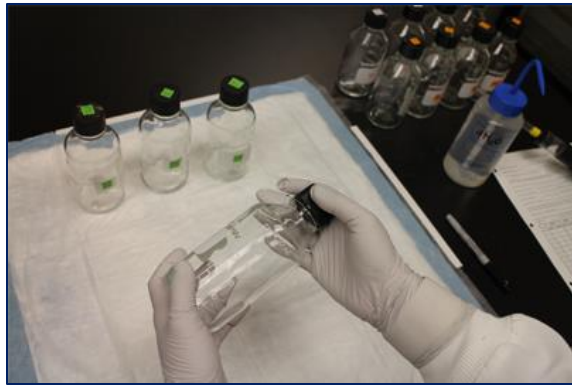
Time (min)	Bottle 1		Bottle 2		Bottle 3		Bottle 4		All test bottles			Control		
	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Total dead	Total	% Dead	Total dead	Total	% Dead
0	10	0	10	0	10	0	10	0	0	40	0%	0%	10	0
5	10	0	7	3	10	0	10	0	0	40	0%	0%	10	0
10	9	1	7	3	10	0	10	0	4	40	10%	0%	10	0
15	7	3	7	3	8	2	8	2	10	40	25%	0%	10	0
20	5	5	5	5	5	5	6	4	19	40	48%	0%	10	0
25	4	6	5	5	4	6	6	4	21	40	53%	0%	10	0
30	3	7	2	8	3	7	3	7	29	40	73%	0%	10	0
45	2	8	0	10	0	10	2	8	36	40	90%	0%	10	0
60	0	10	0	10	0	10	0	10	40	40	100%	0%	10	0

# Notes about interpreting results...

---

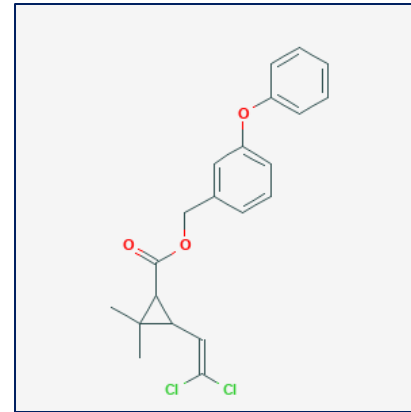
## Types of Studies

- 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](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.

Tiffany Thuy-Vi Nguyen, PhD, MPH  
678-832-8820  
[thuy-vithi.nguyen@dph.ga.gov](mailto:thuy-vithi.nguyen@dph.ga.gov)

Rosmarie Kelly, PhD, MPH  
404-408-1207  
[rosmarie.kelly@dph.ga.gov](mailto:rosmarie.kelly@dph.ga.gov)

[www.GAmosquito.org](http://www.GAmosquito.org)