Practical Bottle Bioassay Techniques

Caroline Efstathion, PhD.

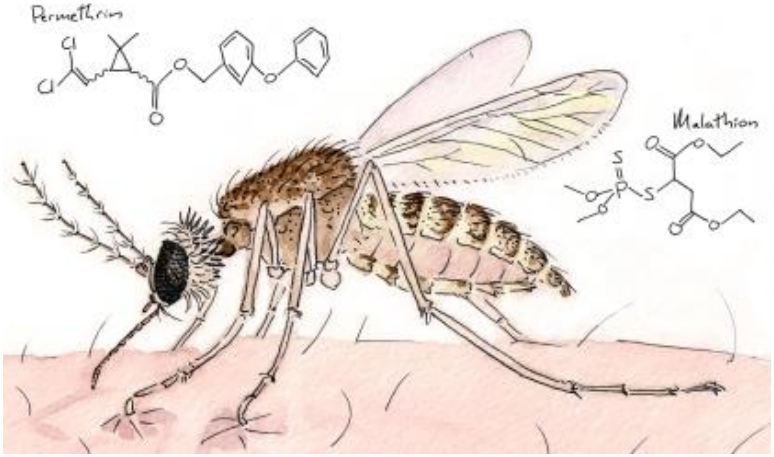
Mosquito Control Services, LLC

Vector Disease Control International

- Microbiology Professor at FAU and St. John's River State College
- FDACS-Florida Department of Agriculture and Consumer Services
- Anastasia Mosquito Control
- Volusia County Mosquito Control
- VDCI-Southeast regional director-FL, GA, AL, MS
- Non-profit for endangered birds

Resistance Monitoring

- WHO Definition of Insecticide Resistance:
  - *The genetic response of a population of mosquitoes that enables some members of that population to survive exposure to a chemical that would prove lethal to a susceptible population*.
- Insecticide resistance is a threat to any mosquito control program
- Ways to mitigate include:
  - IPM
  - Detection and monitoring
  - Management of resistant populations
- Strategies
  - Lowest effective dose
  - Apply less frequently
  - Rotate chemicals
  - Use different classes of insecticides for adulticiding and larviciding
  - Localized treatments vs area wide



Bottle Bioassays

- Provide baseline data for program planning and pesticide selection
- Detect resistance at an early stage
- Continuously monitor the effect of control strategies on insecticide resistance.
- Majority of adult susceptibility testing is done with the CDC bottle bioassay or the WHO tube test
  - Standardized guidelines (diagnostic dose, exposure time, mosquito age)
  - Able to compare over time and space
- How practical is this for operational mosquito control?

WHO Tube Test

CDC Bottle Bioassay

Questions to Ponder

- What are your goals or questions?
- What species are you concerned with?
- What resources do you have?
  - Lab space and equipment, rearing room, rearing supplies, trained personal
- What is your budget?
- How much time can you devote to testing?
- What chemicals to test?

What are your goals or questions

- Determine where in your district there is insecticide resistance to chemicals.
- Are the chemicals you are using still effective?
- If you have disease transmission, what chemical will be the most effective?
- Having treatment failures? Is it resistance?
- Want as a routine part of your IPM or IMM program.

Mosquito Species

Vector

- *Aedes aegypti*
- *Aedes albopictus*
- *Culex quinquefasciatus*
- *Culex nigripalpus*

Pest

- *Aedes taeniorhynchus*
- *Aedes sollicitans*
- *Psorophora species*

Things to consider:

Availability of lab susceptible strains for calibration
Larval or egg collection feasibility
Collect in large enough numbers for testing
Ability to blood feed

Lab Reared

Disadvantages

- Very Time Consuming
- Requires special equipment
- Space for rearing
- Take weeks to months
- Costly

Advantages

- Control for species, age and physiological stage

Wild Caught

Disadvantages

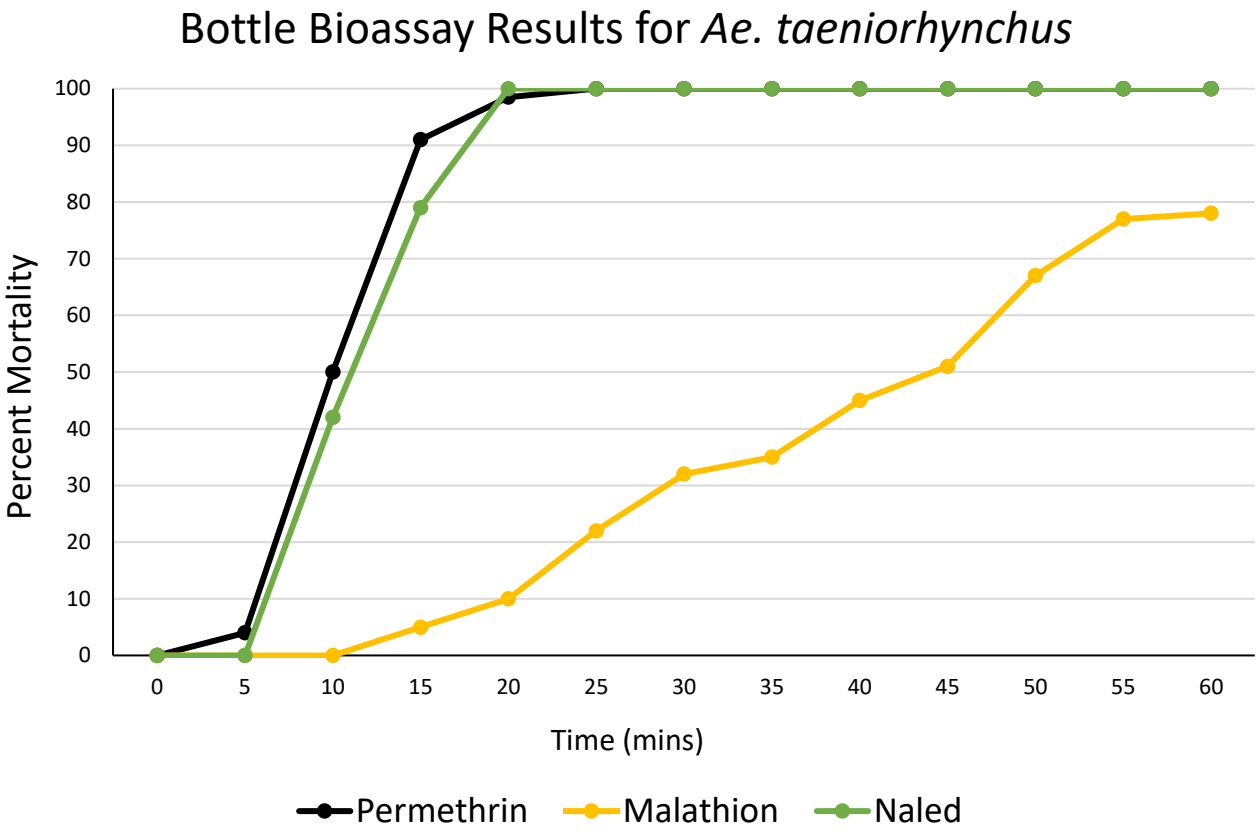
- Mixed species
- Mixed ages
- Mixed physiological states
- No way to calibrate

Advantages

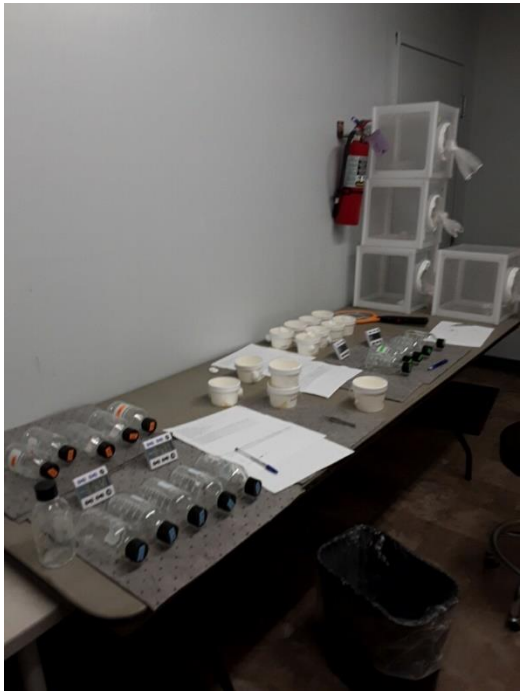
- Not very time consuming
- Can do multiple locations/species fast

Diagnostic Times/Assay Calibration

CDC Diagnostic Dose (per bottle)	Active Ingredient Tested	CDC Diagnostic Time for *Cx. quinq* Seabring colony	MCD 1 assay results on susceptible *Cx. quinq*	MCD 2 Diagnostic Time for *Cx.quinq*
20 µg/bottle	Chlorpyrifos	45 mins	35 mins	
400 µg/bottle	Malathion	45 mins	40 mins	30 mins
2.25 µg/bottle	Naled	45 mins	20 mins	
0.75 µg/bottle	Deltamethrin	60 mins	60 mins	30 mins
12.5 µg/bottle	Etofenprox	30 mins		15 mins
43 µg/bottle	Permethrin	30 mins	20 mins	10 mins
20 µg/bottle	Sumithrin	45 mins		30 mins

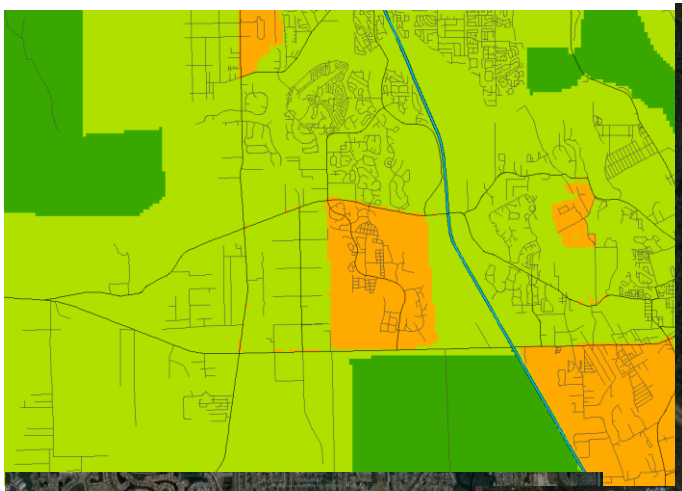

Bottle Bioassay Results for *Ae. taeniorhynchus*
Percent Mortality
100
90
80
70
60
50
40
30
20
10
0
0
5
10
15
20
25
30
35
40
45
50
55
60
Time (mins)
Permethrin
Malathion
Naled

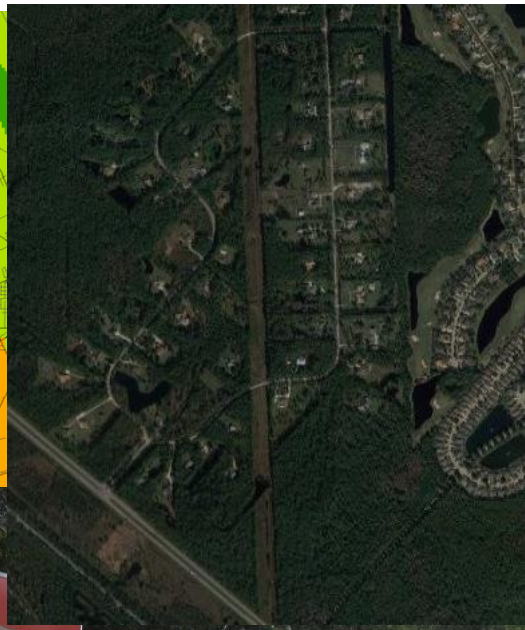
What resources do you have?

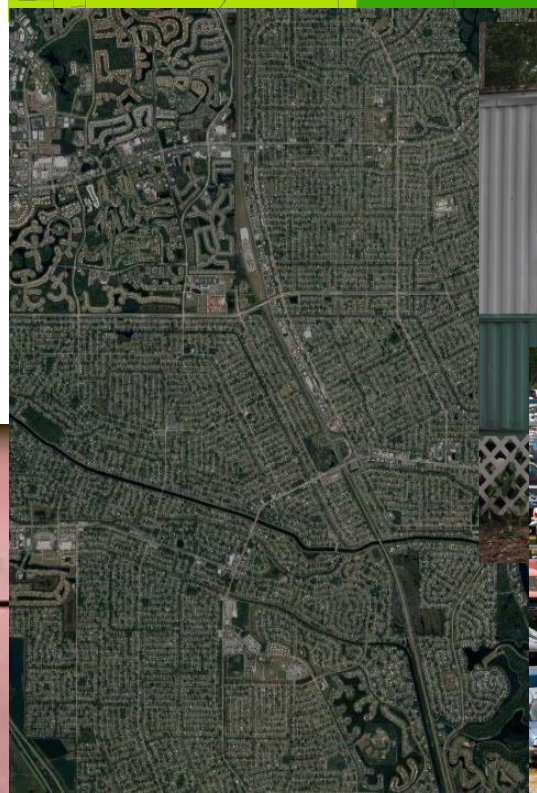


Deciding Where to Test/How Often

- Areas of disease concerns
  - Frequency
  - Habitat
- Spray frequency
  - Once a year
  - Pre and post season
  - Routinely important
- Highest populations
- Flight range







Written Protocols

- Develop your own protocols
  - Allows for comparisons
  - New employees can follow
  - Assay, egg/larva/adult collection, rearing

Bottle Bioassay Protocol

Materials

1) 5 250ml Wheaton Bottles with screw lids per bioassay (1 control and 4 treatment)
2) 1000 µl Micropipette and 1000 µl tips
3) Absorbent pads
4) Aspirator
5) Timers
6) Sharpie marker
7) Labeling tape
8) Disposable gloves
9) Data recording sheet
10) Stock solutions of chemical(s)
11) Acetone or ethanol
12) Mosquitoes

Preparing the bottles

1) Make sure bottles are dry and check each bottle for cracks or chips missing
2) Mark all bottles. Use labeling tape (all same color per assay; eg. blue for Naled, red for permethrin etc.) and label the side and cap of each bottle. Number 1 through 4 for treatment bottles and C for the control bottle. Write the date on each bottle as well. If you are conducting multiple assays with more than one chemical, make sure to also label each bottle with an abbreviation for the chemical (eg. Na for Naled or Pe for permethrin).***It is very important that both the side of the bottle and its cap are labeled the same number and kept together. This insures proper dose of chemical after coating each bottle.**
   ****Steps 3-5 are done inside the fume hood****
3) Add 1ml of acetone to the control bottle.
4) Add the 1ml (1000 µl) of the stock chemical to each bottle labeled 1 through 4. ***Make sure to mix stock solution well before aliquoting*** Replace each cap immediately after adding chemical

***Culex* species Egg Collection Protocol**

Placement of Egg Buckets in the Field

Materials

1) Buckets (5 per site)
2) 2 containers with hay/yeast infusion water
3) Egg cup holders

Procedure for placement of egg buckets

1) Prior to leaving for the field, fill each infusion container with gravid water from barrels. Only fill bucket ¼ to ½ (depending on how old the water is: less than 1 week, fill ½; over 1 week, fill ¾) with gravid water, then fill the rest up with tap water.
2) At site, select a shaded area within shrubs or bushes and out of site to place buckets.
3) Fill bucket with infusion water to approximately 2 inches from the top.
4) Place each bucket so that it is flat and ensure that there is a least one foot of clearance above the bucket from vegetation.

Collection of eggs in the field

Materials

1) Egg cup collection box with:
   a. Petri dishes
   b. filter paper
   c. paint brushes
   d. 1ml pipette
2) Container with hay/yeast infusion water

Procedure for collection of eggs

*****Eggs should be collected no more than **5 days** after placement to prevent eggs from hatching

1) Grab each bucket and move into the sun so you can see egg rafts more clearly
2) Check to see if egg rafts are present, if not, top off water if needed and replace bucket
3) If egg rafts are present, place one piece of filter paper into the bottom of a petri dish

Additional Thoughts

Active Ingredient vs Formulated Product

- CDC recommends using the AI because the formulated product can mask signs of resistance development
- More complex with added potential for error

Field Efficacy Trials

- Additional tool to test formulated product in field situation
  - Semi-field trial
  - Field trial

Methods: Caged Mosquitoes

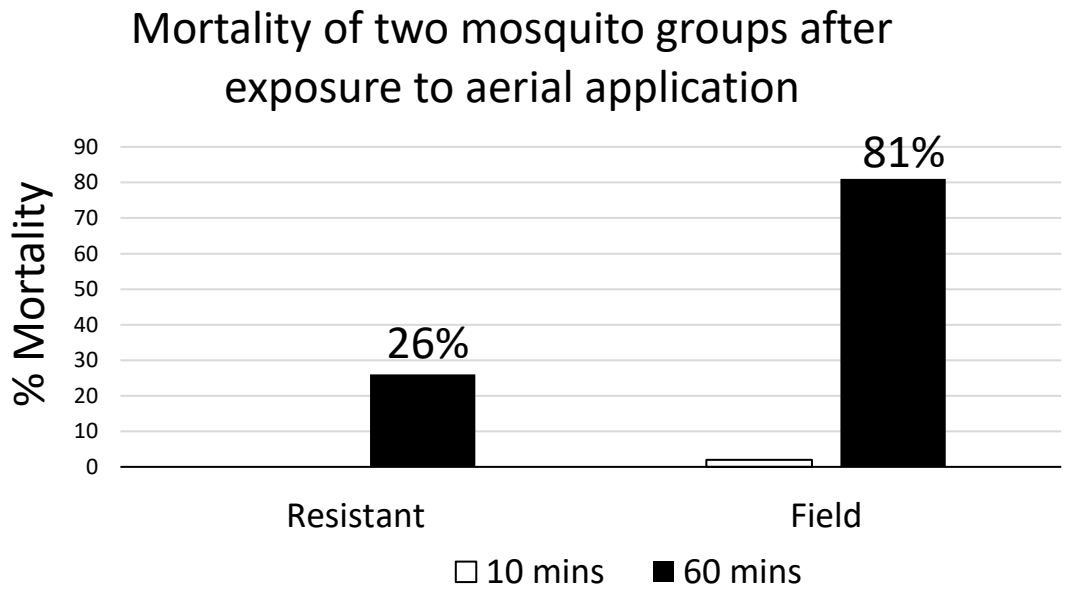
Wild mosquitoes were captured 24 hours prior to the mission.

Brought mosquitoes back to the lab and placed in plastic bug dorms and supplied with sugar water.

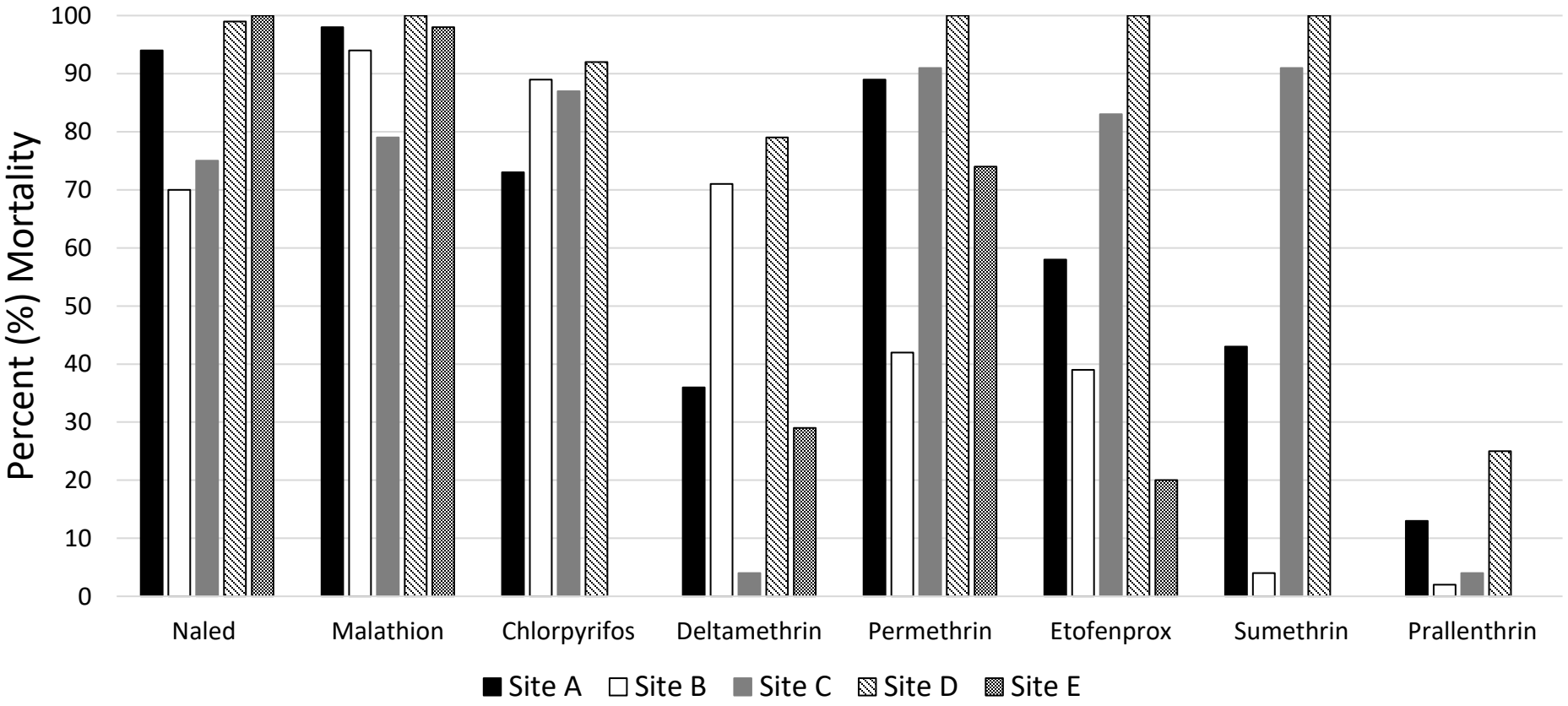
Three hours before spray application, ~25-50 mosquitoes were mechanically aspirated from the bug dorms and placed into the field cages.

Cages were placed on impinger poles 45 min before spray application commenced and were collected 30 min after application.

Droplet Characterization for Aerial ULV System

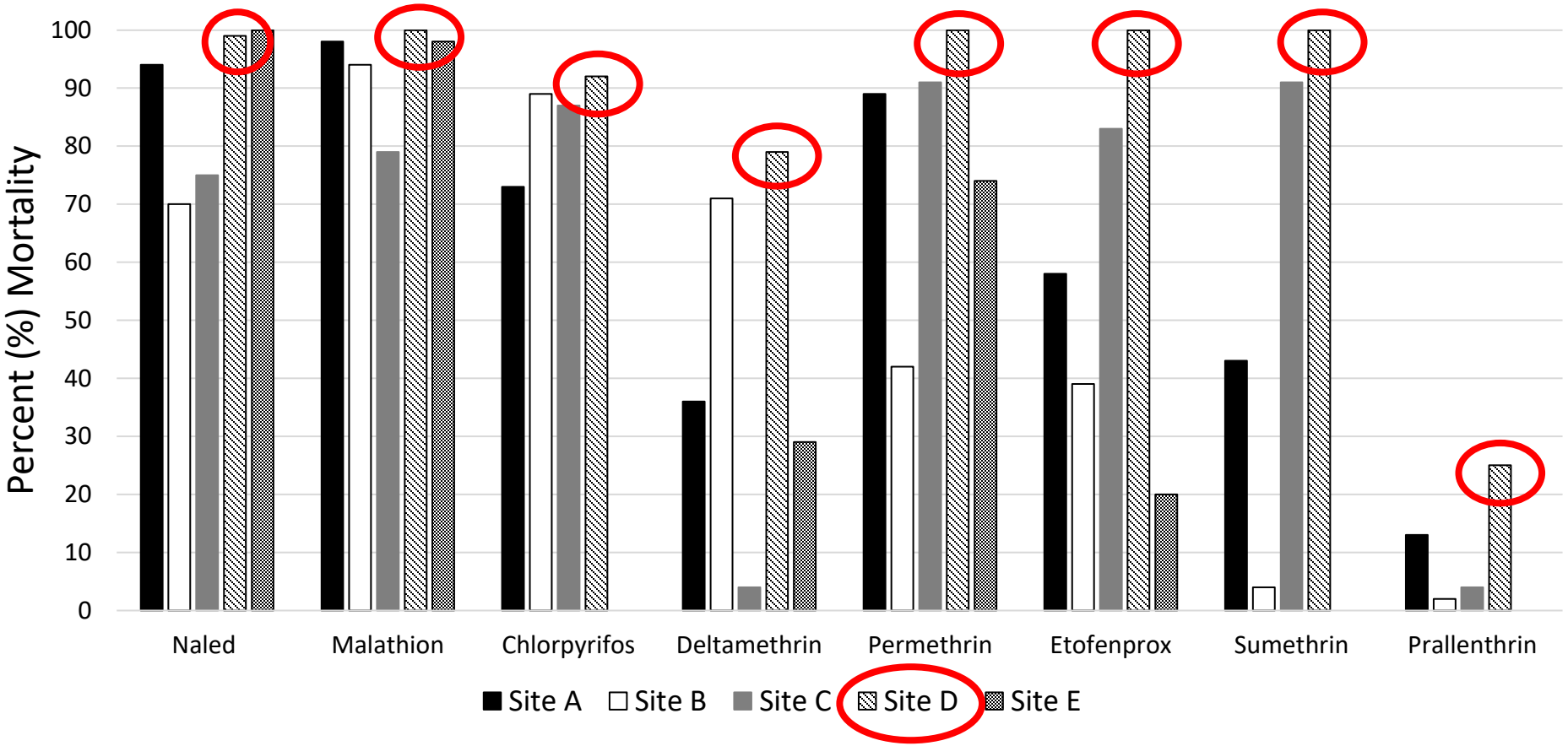


Percent Mortality of *Culex* mosquitoes in the CDC bottle bioassay at 2 hours



Site D is *Culex nigripalpus;* Sites A, B, C and E are *Culex quinquefasciatus*

Percent Mortality of *Culex* mosquitoes in the CDC bottle bioassay at 2 hours



Site D is *Culex nigripalpus;* Sites A, B, C and E are *Culex quinquefasciatus*

CDC Diagnostic Dose (per bottle)	Active Ingredient Tested	Site 4 (Mixed) % mortality at 60 mins	Site 4 (Mixed) % mortality at Calibrated Diagnostic Time
20 µg/bottle	Chlorpyrifos	76%	
400 µg/bottle	Malathion	100%	
2.25 µg/bottle	Naled	100%	100%
0.75 µg/bottle	Deltamethrin	N/A	N/A
12.5 µg/bottle	Etofenprox	N/A	N/A
43 µg/bottle	Permethrin	91%	20%
0.05 µg/bottle	Prallethrin	59%	
20 µg/bottle	Sumithrin	100%	

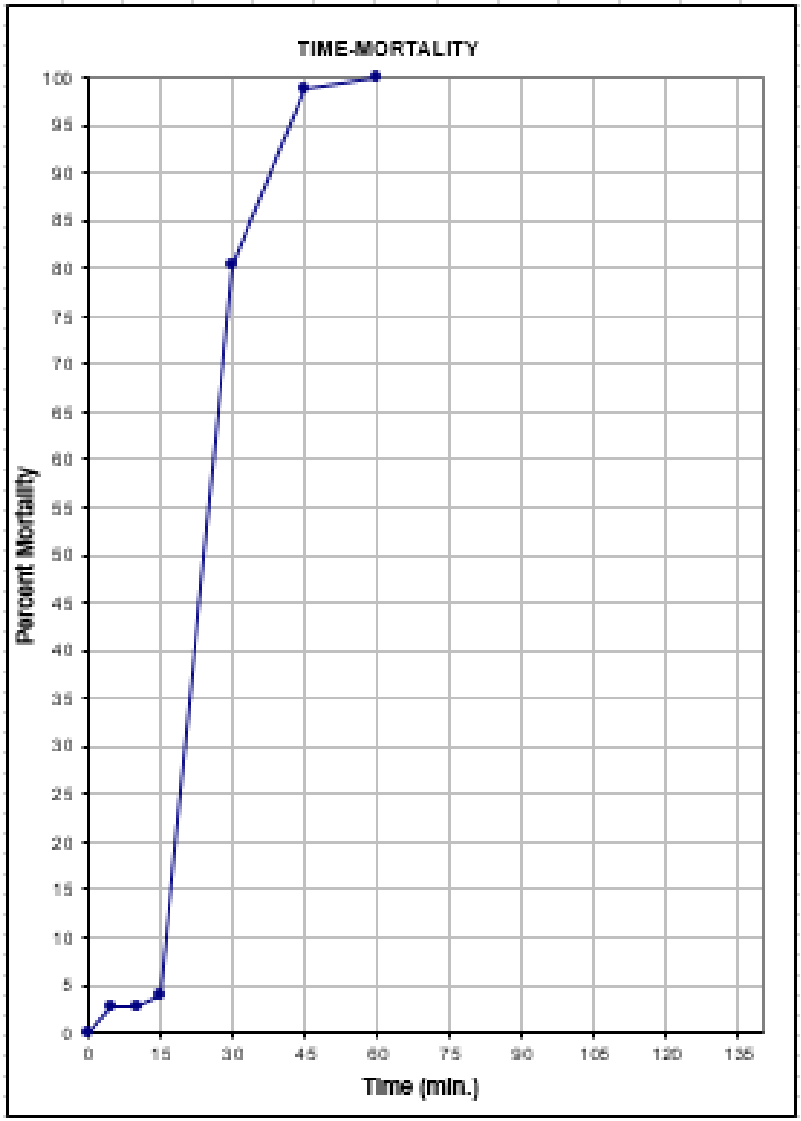
A mortality in the range of 98-100% indicates susceptibility per WHO

A mortality of less than 98% is suggestive of the existence of

Susceptible population

Resistant population

CDC Bottle Bioassay Mixed Species



- Done just prior to an aerial spray mission
- Disease Case
- Wanted to make sure chemical would work

Time	Bottle 1		Bottle 2		Bottle 3		Bottle 4		Total		Control	
	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Dead	%	Alive	Dead
0	15	0	28	0	14	0	20	0	0	0	27	0
5	15	0	28	0	13	1	19	1	2	2.5974	27	0
10	15	0	28	0	13	1	19	1	2	2.5974	27	0
15	15	0	28	0	13	1	18	2	3	3.8961	27	0
30	9	6	3	25	0	13	3	17	61	80.263	27	0
45	0	15	0	28	0	13	1	19	75	98.684	24	3
60	0	15	0	28	0	13	0	20	76	100	24	3
75	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	#####	#####	N/A	0
90	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	#####	#####	N/A	0
105	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	#####	#####	N/A	0
120	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	#####	#####	N/A	0

Field Efficacy Trial Aerial ULV Application

Cage	Total # mosquitoes	% Mortality at 60 mins
1A	21	76
1B	23	78
2	18	11
3	37	95
4	14	86
5A	28	82
5B	22	95
6	33	91
7	36	67
8A	26	88
8B	14	57
	272	**75**

In Conclusion

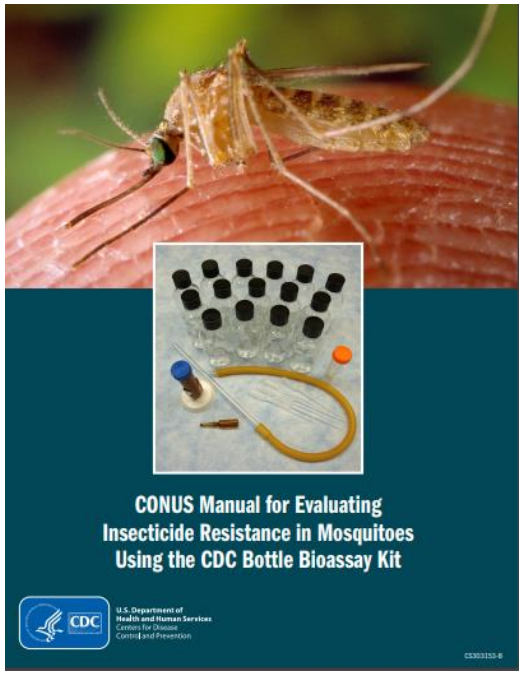
- Whichever method or process you choose, you NEED to be monitoring for resistance!
- Be consistent, write a protocol
  - Allows for comparison and monitoring trends
- Decide on a method that addresses your concerns or questions
- Your results can help guide the choice of insecticide used for spraying
- Have a plan for utilizing for results and adjusting your IPM program

References

- CDC Bottle Bioassay

  https://www.cdc.gov/mosquitoes/mosquito-control/professionals/cdc-bottle-bioassay.html

- Collection and Rearing of Container Mosquitoes and a 24-h Addition to the CDC Bottle Bioassay Casey Parker Journal of Insect Science, Volume 20, Issue 6, November 2020, 13, https://doi.org/10.1093/jisesa/ieaa059
- Florida Mosquito Control White Paper 2018
  - https://fmel.ifas.ufl.edu/media/fmelifasufledu/7-15-2018-white-paper.pdf
- Great video by Eva Buckner-email her Eva.Buckner@ufl.edu



Questions?

Email: cefstathion@vdci.net