

Year 2 Quarter 2 (7/01/20 – 9/30/20) Report

Florida Department of Health Contract CODQJ

Improving our understanding of domestic mosquito control of *Aedes aegypti*, *Ae. albopictus*,
and *Culex quinquefasciatus* through assessments of insecticide susceptibility

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Task List

1. Deploy traps each quarter to collect a minimum of 100 eggs from adult *Aedes* and *Culex* mosquitoes or collect 100 *Aedes* and *Culex* larvae from within the identified sites or collect eggs from adult *Aedes* and *Culex* mosquitoes hatched from previously collected eggs from identified sites that were reared to adulthood and allowed to blood feed and lay eggs. Document the number of eggs and larvae collected and the number and the species of adult mosquitoes that hatched from collected eggs in the Quarterly Report.
2. Conduct insecticide resistance testing on mosquitoes collected as eggs (parental generation) or successive generations (within two generations of parental generation) of mosquitoes within 60 days of collection and hatching of eggs. Document the insecticide resistance testing and results in the Quarterly Report.
3. Conduct CDC bottle bioassay testing on mosquitoes from a minimum of three identified sites against one pyrethroid and one organophosphate each quarter. Document the bioassay testing and results in the Quarterly Report and post the CDC bottle bioassay results to Provider's reporting website, <https://fmel.ifas.ufl.edu/>.
4. Map the distribution of where *Aedes* and *Culex* eggs or larvae are collected and used in CDC bottle bioassays each quarter. Document the mapped distribution in the Quarterly Report.
5. Distribute the results of the CDC bottle bioassay testing to the Florida Mosquito Control Program managers in the counties of a minimum of the three identified sites each quarter. Document the distribution of the results in the Quarterly Report.
6. Prepare a Quarterly Report, post it on Provider's reporting website, and submit it to the Contract Manager within 15 days following the end of each quarter, but no later than invoice submission. At a minimum, include the following information in the report:
 - a. The number of eggs and larvae from adult mosquitoes collected and hatched;
 - b. The number of eggs and larvae from adult mosquitoes in which insecticide testing was conducted;
 - c. Documentation of mapping of egg collection results including species identification and location of collection;
 - d. Documentation of results of CDC bottle bioassay testing of mosquitoes for insecticide resistance; and
 - e. Documentation of CDC bottle bioassay testing results distribution to Florida Mosquito Control Program Managers.
7. Identify the methods for distributing information on resistance to tested insecticide active ingredients. Prepare an Annual Report, including the identified methods, and submit it to the Contract Manager within 45 days from the end of the contract term, but no later than submission of the final invoice. At a minimum, include the following in the report:
 - a. The method for informing Florida Mosquito Control Programs, the Department, and the general public on the regions of Florida that are most likely to have

populations of Zika, Dengue, Chikungunya, and West Nile Virus mosquito vectors;

- b. The method for informing Florida Mosquito Control Programs and the Department on the efficacy of the insecticides that are currently being used in their programs (i.e., whether the chemicals are working as they should to reduce the mosquito populations); and
- c. The method for informing Florida Mosquito Control Program managers on the relationship between the CDC bottle bioassay (a lab assay) and the efficacy of spraying mosquitoes at the insecticide label rates.

Tasks Progress

1. Traps were deployed this quarter, and *Aedes* eggs and *Culex quinquefasciatus* rafts were collected from the traps. Of the 1531 *Aedes* eggs collected, there was \approx a 72% hatch rate, resulting in 1102 *Aedes* adults emerging. The 51 *Culex quinquefasciatus* rafts collected had \approx a 59% hatch rate, resulting in 6018 *Culex quinquefasciatus* adults emerging. The species identification for the resulting *Aedes* adult mosquitoes were *Aedes aegypti* and *Aedes albopictus*. The *Aedes* eggs processed this quarter came (county followed by site name):
 - a. Indian River
 - i. Ana's place
 - b. Palm Beach
 - i. N. Main St.
 - ii. Parkside
 - c. St. Lucie
 - i. Betty's House

The *Culex quinquefasciatus* rafts processed this quarter came from (county followed by site name):

- d. Bay
 - i. Beach Parkway
- e. Citrus
 - i. Suncoast
- f. Miami-Dade
 - i. 41st St
 - ii. 355th St
 - iii. Camillus House
 - iv. Cutler Bay
 - v. Kings Bay
 - vi. Miami Beach
 - vii. Pinecrest
 - viii. Wynwood
- g. Polk
 - i. Tire Center
- h. Pinellas
 - i. Saturn
- i. Volusia
 - i. Museum

2. Since the end of the previous quarter, 1 population of *Aedes aegypti*, 1 population of *Ae. albopictus* and 12 populations of *Culex quinquefasciatus*, respectively, have been tested using the CDC bottle bioassay. Based on the mortality observed at a diagnostic time, the mosquito populations were classified as susceptible (S), developing resistance (D), or resistant (R) to various active ingredients. These results can be found in the table below. If a population was not tested against an active ingredient, no data (ND) is documented in the table.

Species	County	Site	Active Ingredient						
			Malathion	Clorpyrifos	Deltamethrin	Etofenprox	Permethrin	Sumithrin	Bifenthrin
<i>Ae. aegypti</i>	Palm Beach	Mounts	S	ND	R	R	R	R	ND
<i>Ae. albopictus</i>	Palm Beach	Rocky Pines Beach	S	ND	R	R	D	S	ND
<i>Cx. quinquefasciatus</i>	Bay	Parkway	R	ND	ND	ND	R	ND	R
<i>Cx. quinquefasciatus</i>	Bay	Coleman	ND	ND	ND	ND	R	ND	R
<i>Cx. quinquefasciatus</i>	Bay	Pier Park	ND	ND	ND	ND	ND	R	R
<i>Cx. quinquefasciatus</i>	Citrus	Suncoast	ND	ND	ND	ND	R	ND	ND
<i>Cx. quinquefasciatus</i>	Miami-Dade	355th ST	R	R	R	R	R	ND	ND
<i>Cx. quinquefasciatus</i>	Miami-Dade	41st ST Camillus House	S	S	R	R	R	ND	ND
<i>Cx. quinquefasciatus</i>	Miami-Dade	Pinecrest	R	D	R	R	R	ND	ND
<i>Cx. quinquefasciatus</i>	Miami-Dade	Wynwood	D	S	R	R	R	ND	ND
<i>Cx. quinquefasciatus</i>	Polk	Paint Shop	S	ND	ND	ND	R	ND	ND
<i>Cx. quinquefasciatus</i>	St. Lucie	Fairwinds	ND	ND	R	R	R	R	ND
<i>Cx. quinquefasciatus</i>	Volusia	Museum	R	ND	R	R	R	R	ND

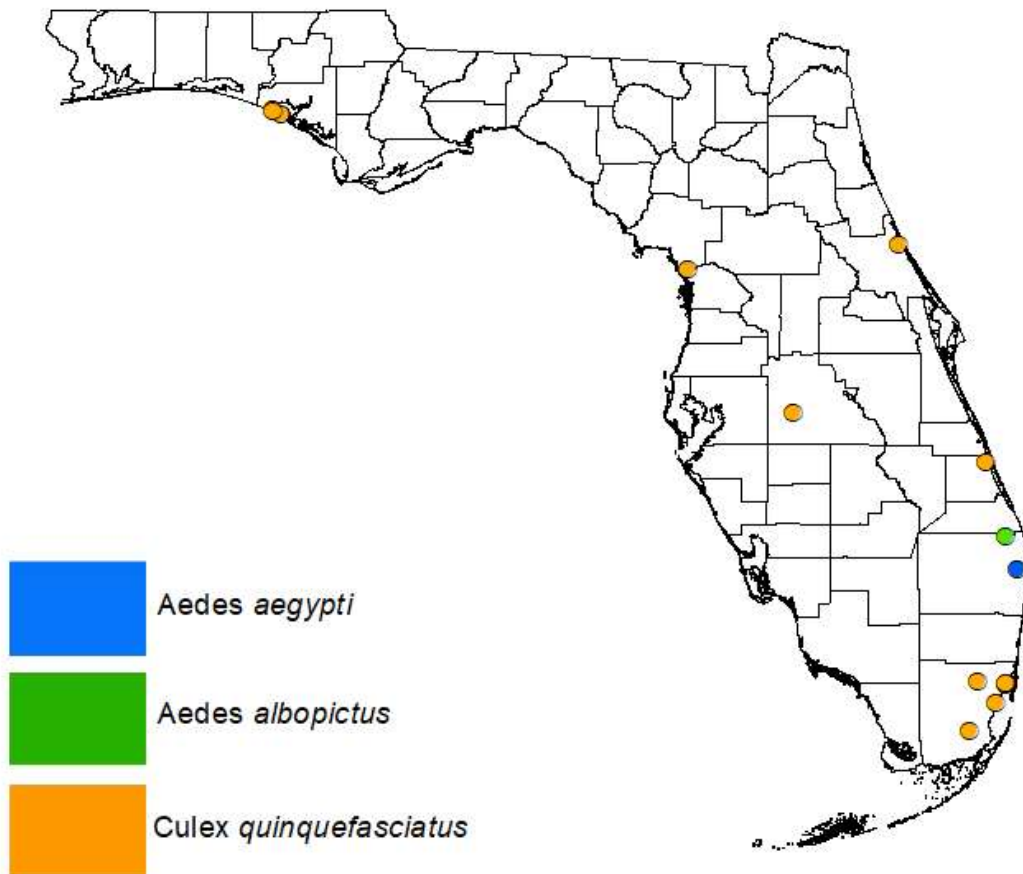
3. CDC bottle bioassay testing was performed on mosquitoes from 10 sites against at least one pyrethroid (permethrin) and one organophosphate (malathion) active ingredient (AI). The total number of active ingredients that each mosquito population was tested against can be found in the table below. The CDC bottle bioassay results for each active ingredient can be found at <https://fmel.ifas.ufl.edu/>.

Species Tested	County	Site	# of AIs Tested
<i>Aedes aegypti</i>	Palm Beach	Mounts	5
<i>Aedes albopictus</i>	Palm Beach	Rocky Pines	5
<i>Culex quinquefasciatus</i>	Bay	Beach Parkway	3
<i>Culex quinquefasciatus</i>	Bay	Coleman	2
<i>Culex quinquefasciatus</i>	Bay	Pier Park	2
<i>Culex quinquefasciatus</i>	Citrus	Suncoast	1
<i>Culex quinquefasciatus</i>	Miami-Dade	355 th St	5
<i>Culex quinquefasciatus</i>	Miami-Dade	41 st St	5

<i>Culex quinquefasciatus</i>	Miami-Dade	Camillus House	5
<i>Culex quinquefasciatus</i>	Miami-Dade	Pinecrest	6
<i>Culex quinquefasciatus</i>	Miami-Dade	Wynwood	5
<i>Culex quinquefasciatus</i>	Polk	Tire Center	2
<i>Culex quinquefasciatus</i>	St. Lucie	Fairwinds	4
<i>Culex quinquefasciatus</i>	Volusia	Museum	5

4. The distribution of where *Aedes* eggs and *Culex* egg rafts or larvae were collected from and used in CDC bottle bioassays was mapped and can be found on page 8 of this report. The distribution map can also be found at <https://fmeal.ifas.ufl.edu/>.
5. Results of the CDC bottle bioassay testing were distributed as reports by email to Florida mosquito control program managers. Specifically, the results sent to program managers for the Museum site in Volusia County, Tire Center site in Polk County, and Fairwinds site in St. Lucie County were forwarded to the FDOH Contract Manager to meet the requirement of documenting the distribution of results for 3 sites. An example report can be found on pages 9-10.
6. This Quarterly Report in combination with the information posted on the Reporting website at <https://fmeal.ifas.ufl.edu/> satisfies this task.
7. All necessary information will be provided in the Final Annual Report.

**Distribution of domestic mosquito
populations tested for insecticide resistance
July 1 - September 30, 2020**



Credit: E. Buckner & D. Ramirez,
UF/IFAS Florida Medical Entomology Laboratory

Assay: CDC bottle bioassay
 Species: *Culex quinquefasciatus*
 Source: Reared from egg rafts
 Site: Volusia County (Museum)
 Address: 352 S. Nova Rd, Daytona Beach, FL 32114
 Coordinates: 29.1921, -81.0365
 Dates of assay: 8/05 – 8/06/20

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) at the diagnostic time. The knockdown resistance (kdr) mechanism is being phenotypically expressed if % mortality at 24 hours is less than % mortality at the end of the 2-hour long bioassay.

The data shown below provides:

Column 1: CDC recommended diagnostic dose (DD) (per bottle)

Column 2: Active ingredient (AI) tested

Column 3: Diagnostic time (DT) from FMEL assays; 100% mortality at DD using susceptible *Cx. quinquefasciatus*

Column 4: Site-specific *Cx. quinquefasciatus* % mortality at the CDC diagnostic time

Column 5: Site-specific *Cx. quinquefasciatus* % mortality at end of 2-hour bioassay

Column 6: Site-specific *Cx. quinquefasciatus* % mortality 24 hours after bioassay started (Pyrethroids only)

1	2	3	4	5	6
DD	AI	DT	Museum <i>Cx. quinquefasciatus</i> % mortality at DT	Museum <i>Cx. quinquefasciatus</i> % mortality at 2 hours	Museum <i>Cx. quinquefasciatus</i> % mortality at 24 hours
43 ug	Permethrin	10 minutes	0%	87%	63%
400 ug	Malathion	30 minutes	77%	100%	N/A
12.5 ug	Etofenprox	15 minutes	14%	38%	14%
20 ug	Sumithrin	30 minutes	45%	77%	20%
0.75 ug	Deltamethrin	30 minutes	7%	49%	25%

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

The Museum population of *Culex quinquefasciatus* is resistant to Permethrin, Etofenprox, Sumithrin and Deltamethrin; susceptible to Malathion.

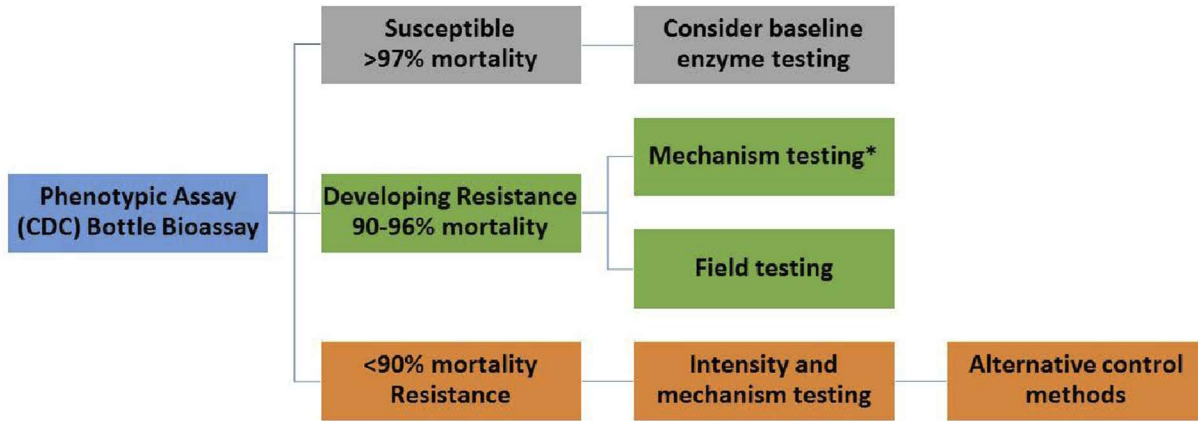
The Museum population of *Culex quinquefasciatus* exhibited knockdown resistance to Permethrin, Etofenprox, Sumithrin, and Deltamethrin.

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Overview of Insecticide Resistance Testing Algorithm

From: <http://www.cdc.gov/zika/pdfs/guidelines-for-aedes-surveillance-and-insecticide-resistance-testing.pdf>



*Mechanism testing options: enzymes, molecular assays, bottle bioassay with inhibitors