

Year 2 Quarter 3 (10/01/20 – 12/31/20) 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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13 January 2021

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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 6553 *Aedes* eggs collected, there was \approx a 65% hatch rate, resulting in 4267 *Aedes* adults emerging. The 78 *Culex quinquefasciatus* rafts collected had \approx a 55% hatch rate, resulting in 8534 *Culex quinquefasciatus* adults emerging. The species identification for the resulting *Aedes* adult mosquitoes were all *Aedes aegypti*. The *Aedes* eggs processed this quarter came (county followed by site name):

- a. Broward
 - i. 34th Ave
 - ii. Farragut
 - iii. Shalimar
 - iv. Tarpon
 - v. SR-7 S

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

- b. Brevard
 - i. Satellite High
- c. Broward
 - i. 34th Ave
 - ii. Farragut
 - iii. Shalimar
 - iv. Tarpon
 - v. Pembroke Pines
- d. Indian River
 - i. Water Control District
- e. Miami-Dade
 - i. Allapattah
 - ii. Hialeah
 - iii. Richmond Heights
 - iv. Westchester
 - v. 1480
 - vi. 18541
 - vii. Miami Gardens
 - viii. 870
 - ix. CW71
 - x. Cairo
- f. Palm Beach
 - i. N Street

2. Since the end of the previous quarter, 3 populations of *Aedes aegypti*, 1 population of *Ae. albopictus* and 18 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 (DR), or resistant (R) to 7 active ingredients: malathion, naled, chlorpyrifos, deltamethrin, etofenprox, permethrin, and sumithrin. 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.

			Malathion	Naled	Chlorpyrifos	Deltamethrin	Etofenprox	Permethrin	Sumithrin
<i>Ae. aegypti</i>	Broward	34th Ave	S	R	ND	R	R	R	R
<i>Ae. aegypti</i>	Broward	Farragut	S	DR	ND	R	R	R	R
<i>Ae. aegypti</i>	Broward	Shalimar	S	R	ND	R	R	R	R
<i>Ae. albopictus</i>	Indian River	VLE	R	S	S	R	DR	S	ND
<i>Cx. quinquefasciatus</i>	Brevard	Satellite High	R	R	ND	ND	ND	R	ND
<i>Cx. quinquefasciatus</i>	Broward	34th Ave	DR	R	ND	ND	ND	R	ND
<i>Cx. quinquefasciatus</i>	Broward	Pembroke Pines	S	S	ND	R	R	R	ND
<i>Cx. quinquefasciatus</i>	Indian River	Water Control District	R	R	ND	R	R	R	ND
<i>Cx. quinquefasciatus</i>	Miami-Dade	Miami Beach	R	DR	S	R	R	R	ND
<i>Cx. quinquefasciatus</i>	Miami-Dade	Miami Gardens	R	DR	S	R	R	R	ND
<i>Cx. quinquefasciatus</i>	Miami-Dade	870 NW	DR	S	DR	R	R	R	ND
<i>Cx. quinquefasciatus</i>	Miami-Dade	1480	R	R	R	R	R	R	ND
<i>Cx. quinquefasciatus</i>	Miami-Dade	18541	R	R	R	R	R	R	ND
<i>Cx. quinquefasciatus</i>	Miami-Dade	Allapattah	R	DR	S	R	R	R	ND
<i>Cx. quinquefasciatus</i>	Miami-Dade	Cairo	R	ND	ND	R	ND	ND	ND
<i>Cx. quinquefasciatus</i>	Miami-Dade	Cutler Bay	R	DR	S	R	R	R	ND
<i>Cx. quinquefasciatus</i>	Miami-Dade	CW71	R	R	DR	R	R	R	ND
<i>Cx. quinquefasciatus</i>	Miami-Dade	Hialeah	R	S	S	R	R	R	ND
<i>Cx. quinquefasciatus</i>	Miami-Dade	King's Bay	DR	S	S	R	R	R	ND
<i>Cx. quinquefasciatus</i>	Miami-Dade	Richmond Heights	R	S	R	R	R	R	ND
<i>Cx. quinquefasciatus</i>	Miami-Dade	Westchester	DR	DR	S	R	R	R	ND
<i>Cx. quinquefasciatus</i>	Palm Beach	N Street	R	S	ND	R	R	R	R

3. CDC bottle bioassay testing was performed on mosquitoes from 22 sites against at least one pyrethroid (deltamethrin or permethrin) and one organophosphate (malathion)

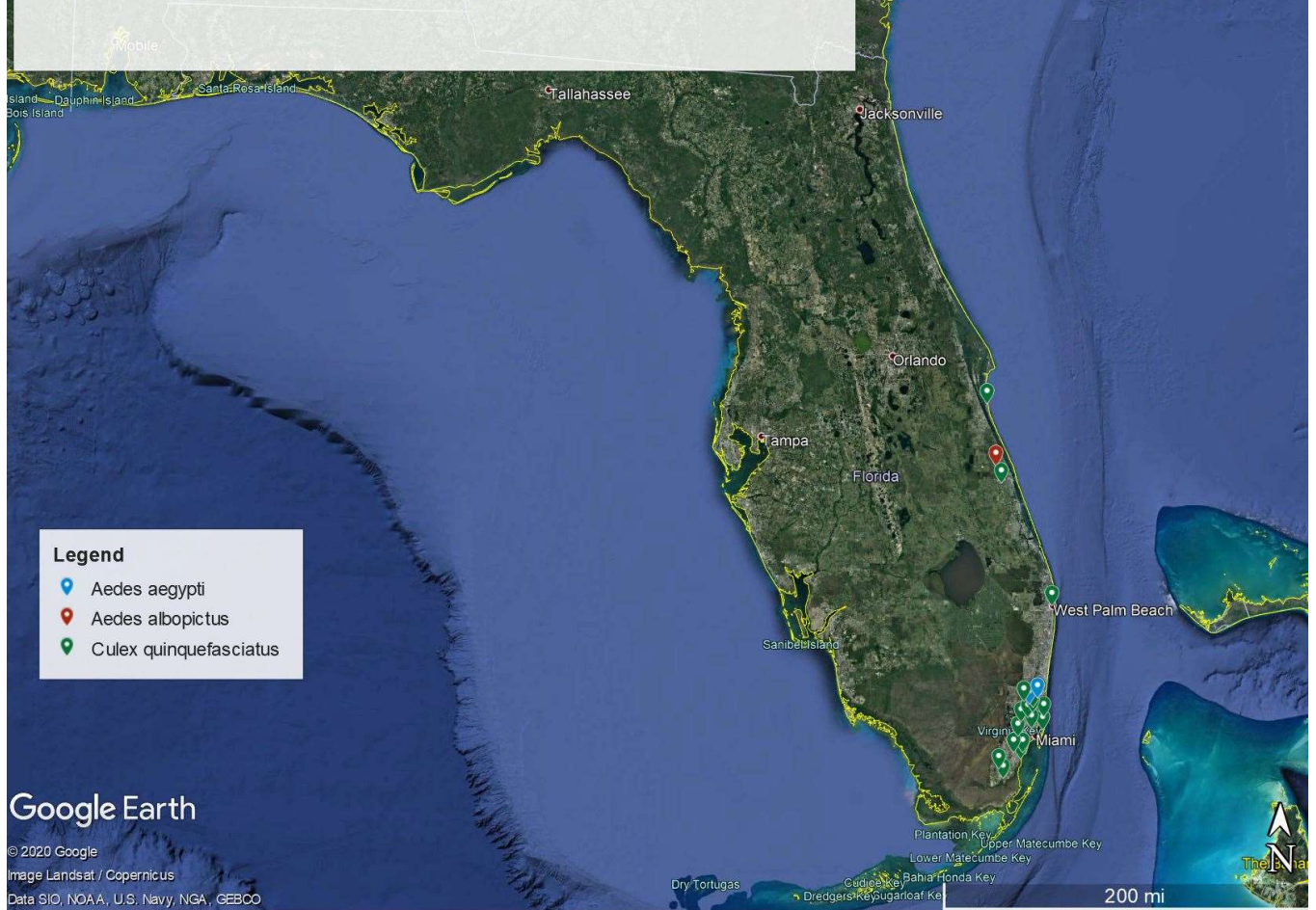
active ingredient (AI). The total number of active ingredients that each mosquito population was tested against can be found in the table below. The CDC bottle bioassay results for each active ingredient can be found at <https://fme1.ifas.ufl.edu/>.

Species	County	Site	# of AIs Tested
<i>Aedes aegypti</i>	Broward	34th Ave	6
<i>Aedes aegypti</i>	Broward	Farragut	6
<i>Aedes aegypti</i>	Broward	Shalimar	6
<i>Aedes albopictus</i>	Indian River	VLE	6
<i>Culex quinquefasciatus</i>	Brevard	Satellite High	3
<i>Culex quinquefasciatus</i>	Broward	34th Ave	3
<i>Culex quinquefasciatus</i>	Broward	Pembroke Pines	5
<i>Culex quinquefasciatus</i>	Indian River	Water Control District	5
<i>Culex quinquefasciatus</i>	Miami-Dade	Miami Beach	5
<i>Culex quinquefasciatus</i>	Miami-Dade	Miami Gardens	6
<i>Culex quinquefasciatus</i>	Miami-Dade	870 NW	6
<i>Culex quinquefasciatus</i>	Miami-Dade	1480	6
<i>Culex quinquefasciatus</i>	Miami-Dade	18541	6
<i>Culex quinquefasciatus</i>	Miami-Dade	Allapattah	6
<i>Culex quinquefasciatus</i>	Miami-Dade	Cairo	2
<i>Culex quinquefasciatus</i>	Miami-Dade	Cutler Bay	6
<i>Culex quinquefasciatus</i>	Miami-Dade	CW71	6
<i>Culex quinquefasciatus</i>	Miami-Dade	Hialeah	6
<i>Culex quinquefasciatus</i>	Miami-Dade	King's Bay	6
<i>Culex quinquefasciatus</i>	Miami-Dade	Richmond Heights	6
<i>Culex quinquefasciatus</i>	Miami-Dade	Westchester	6
<i>Culex quinquefasciatus</i>	Palm Beach	N Street	6

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 9 of this report. The distribution map can also be found at <https://fmel.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 Water Control District site in Indian River County, N Street site in Palm Beach County, and 34th Street and Shalimar sites in Broward 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 10-11.
6. This Quarterly Report in combination with the information posted on the Reporting website at <https://fmel.ifas.ufl.edu/> satisfies this task.
7. All necessary information will be provided in the Final Annual Report.

Mosquito populations tested for insecticide resistance

Distribution of domestic mosquito populations tested for insecticide resistance during quarter of October 1 - December 31, 2020



Assay: CDC bottle bioassay

Conducted by: UF/IFAS/FMEL – Buckner Lab

Species: *Culex quinquefasciatus*

Source: Reared from egg rafts

Site: Palm Beach (N Street)

Address: N Street. West Palm Beach, FL 33401

Coordinates:26.70278, -80.05977

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 susceptible *Culex quinquefasciatus*

Column 4: Site specific *Culex 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
CDC diagnostic dose (per bottle)	Active ingredient tested	CDC diagnostic time; 100% mortality expected at given time using strain of susceptible <i>Culex quinquefasciatus</i>	N Street <i>Culex quinquefasciatus</i> % mortality at the CDC diagnostic time	N Street <i>Cx. quinquefasciatus</i> % mortality at 2 hours	N Street <i>Culex quinquefasciatus</i> % mortality at 24 hours
43 ug/bottle	Permethrin	30 min	38%	68%	37 %
400 ug/bottle	Malathion	30 min	87%	100 %	N/A
2.25 ug/bottle	Naled	45 min	98%	100 %	N/A
12.5 ug/bottle	Etofenprox	30 min	4 %	9 %	6 %
0.75 ug/ bottle	Deltamethrin	30 min	7 %	24 %	15 %
20 ug/bottle	Sumithrin	30 min	2 %	14 %	8 %

Using the CDC guidelines (<http://www.cdc.gov/zika/vector/insecticide-resistance.html>) on interpreting the data for management purposes (see page 2 of this document for reference):

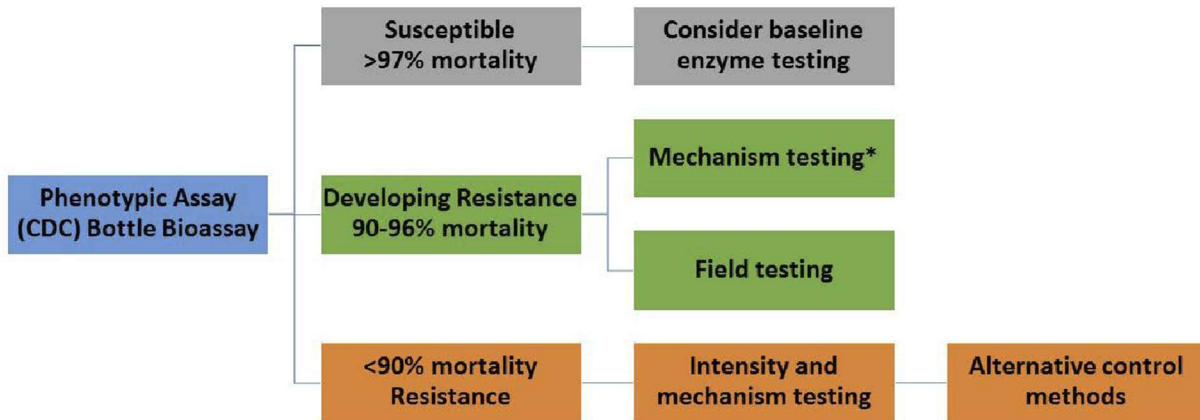
The N Street population of *Culex quinquefasciatus* is resistant to Permethrin, Malathion, Etofenprox, and Deltamethrin and Sumithrin; and susceptible to Naled.

24 hours after the CDC Bottle Bioassay was started, the N Street population of *Culex quinquefasciatus* exhibited knocked down resistance to Permethrin, Etofenprox, Deltamethrin and Sumithrin.

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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