
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

Prepared by:

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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 a minimum of three 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 4,561 Aedes eggs and 25 Culex quinquefasciatus egg rafts were collected from the traps. Of the 4,561 Aedes eggs collected, there was ≈ 79% hatch rate, resulting in 3,603 Aedes adults emerging. The Culex quinquefasciatus egg rafts collected had a ≈ 77% hatch rate, resulting in 2,495 Culex quinquefasciatus adults emerging. The resulting Aedes adult mosquitoes were identified as Aedes aegypti and Ae. albopictus. The Aedes eggs processed this quarter came from (county followed by site name):
   a. Miami-Dade
      i. Brickell
      ii. King’s Bay
      iii. Little Havana
   b. Palm Beach
      i. Mounts
      ii. Rocky Pines
   c. Seminole
      i. Halsey

The Culex quinquefasciatus egg rafts processed this quarter came from (county followed by site name):
   a. Citrus
      i. 10th St
   b. Seminole
      i. Palm
      ii. Winding Pines

2. Since the end of the previous quarter, Aedes aegypti from 7 sites, Ae. albopictus from 1 site, and Culex quinquefasciatus from 2 sites, respectively, have been tested for resistance using the CDC bottle bioassay. Based on the mortality observed at a diagnostic time, the mosquito populations were classified as susceptible, developing resistance, or resistant to the various active ingredients. These results were incorporated into maps and can be found on pages 7 - 24 of this report.

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 the mosquitoes from each site were tested against can be found in the table below. The CDC bottle bioassay results for each active ingredient can also be found at https://fmel.ifas.ufl.edu/.
<table>
<thead>
<tr>
<th>Species Tested</th>
<th>County</th>
<th>Site</th>
<th># of AIs Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aedes aegypti</em></td>
<td>Brevard</td>
<td>Viera</td>
<td>6</td>
</tr>
<tr>
<td><em>Aedes aegypti</em></td>
<td>Miami-Dade</td>
<td>Homestead</td>
<td>6</td>
</tr>
<tr>
<td><em>Aedes aegypti</em></td>
<td>Miami-Dade</td>
<td>Little River</td>
<td>6</td>
</tr>
<tr>
<td><em>Aedes aegypti</em></td>
<td>Miami-Dade</td>
<td>Miami Beach</td>
<td>6</td>
</tr>
<tr>
<td><em>Aedes aegypti</em></td>
<td>Miami-Dade</td>
<td>Wynwood</td>
<td>6</td>
</tr>
<tr>
<td><em>Aedes aegypti</em></td>
<td>Volusia</td>
<td>Holly Hill</td>
<td>6</td>
</tr>
<tr>
<td><em>Aedes aegypti</em></td>
<td>Volusia</td>
<td>South Daytona</td>
<td>4</td>
</tr>
<tr>
<td><em>Aedes albopictus</em></td>
<td>Brevard</td>
<td>EFSC</td>
<td>6</td>
</tr>
<tr>
<td><em>Culex quinquefasciatus</em></td>
<td>Citrus</td>
<td>10th St</td>
<td>6</td>
</tr>
<tr>
<td><em>Culex quinquefasciatus</em></td>
<td>Seminole</td>
<td>Winding Pine</td>
<td>6</td>
</tr>
</tbody>
</table>

4. The map of sites where *Aedes* eggs and *Culex* egg rafts were collected from and used in CDC bottle bioassay testing this quarter can be found on page 25 of this report. The distribution map can also be found at [https://fmel.ifas.ufl.edu/](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 Little River, Miami Beach, and Wynwood sites in Miami-Dade County, Viera and EFSC sites in Brevard County, and 10th Street site in Citrus County were forwarded to the Contract Manager. An example report can be found on pages 26-27.

6. This Quarterly Report in combination with the information posted on the Reporting website at [https://fmel.ifas.ufl.edu/](https://fmel.ifas.ufl.edu/) satisfies this task.

7. All necessary information will be provided in the Final Annual Report.
Species: Aedes aegypti
Active Ingredient: permethrin

Susceptible: >97% mortality
Developing Resistance: 90-96% mortality
Resistant: <90% mortality

Credit: E. Buckner & D. Ramirez,
UF/IFAS Florida Medical Entomology Laboratory
Species: Aedes *aegypti*
Active Ingredient: malathion

Susceptible: >97% mortality
Developing Resistance: 90-96% mortality
Resistant: <90% mortality

Credit: E. Buckner & D. Ramirez,
UF/IFAS Florida Medical Entomology Laboratory
Species: Aedes aegypti
Active Ingredient: naled

Susceptible: >97% mortality
Developing Resistance: 90-96% mortality
Resistant: <90% mortality

Credit: E. Buckner & D. Ramirez,
UF/IFAS Florida Medical Entomology Laboratory
Species: Aedes aegypti
Active Ingredient: deltamethrin

Susceptible: >97% mortality
Developing Resistance: 90-96% mortality
Resistant: <90% mortality

Credit: E. Buckner & D. Ramirez, UF/IFAS Florida Medical Entomology Laboratory
Species: Aedes aegypti
Active Ingredient: etofenprox

Susceptible: >97% mortality
Developing Resistance: 90-96% mortality
Resistant: <90% mortality

Credit: E. Buckner & D. Ramirez,
UF/IFAS Florida Medical Entomology Laboratory
Species: Aedes aegypti
Active Ingredient: sumithrin

Susceptible: >97% mortality
Developing Resistance: 90-96% mortality
Resistant: <90% mortality

Credit: E. Buckner & D. Ramirez,
UF/IFAS Florida Medical Entomology Laboratory
Species: Aedes *albopictus*
Active Ingredient: permethrin

- Susceptible: >97% mortality
- Developing Resistance: 90-96% mortality
- Resistant: <90% mortality

Credit: E. Buckner & D. Ramirez,
UF/IFAS Florida Medical Entomology Laboratory
Species: Aedes *albopictus*
Active Ingredient: malathion

Susceptible: >97% mortality
Developing Resistance: 90-96% mortality
Resistant: <90% mortality

Credit: E. Buckner & D. Ramirez,
UF/IFAS Florida Medical Entomology Laboratory
Species: Aedes *albopictus*
Active Ingredient: naled

- Susceptible: >97% mortality
- Developing Resistance: 90-96% mortality
- Resistant: <90% mortality

Credit: E. Buckner & D. Ramirez,
UF/IFAS Florida Medical Entomology Laboratory
Species: Aedes albopictus
Active Ingredient: deltamethrin

Susceptible: >97% mortality
Developing Resistance: 90-96% mortality
Resistant: <90% mortality

Credit: E. Buckner & D. Ramirez, UF/IFAS Florida Medical Entomology Laboratory
Species: Aedes *albopictus*
Active Ingredient: etofenprox

Susceptible: >97% mortality
Developing Resistance: 90-96% mortality
Resistant: <90% mortality

Credit: E. Buckner & D. Ramirez,
UF/IFAS Florida Medical Entomology Laboratory
Species: Aedes *albopictus*
Active Ingredient: sumithrin

Susceptible: >97% mortality
Developing Resistance: 90-96% mortality
Resistant: <90% mortality

Credit: E. Buckner & D. Ramirez,
UF/IFAS Florida Medical Entomology Laboratory
Species: *Culex quinquefasciatus*
Active Ingredient: permethrin

Susceptible: >97% mortality
Developing Resistance: 90-96% mortality
Resistant: <90% mortality

Credit: E. Buckner & D. Ramirez,
UF/IFAS Florida Medical Entomology Laboratory
Species: Culex quinquefasciatus
Active Ingredient: malathion

Susceptible: >97% mortality
Developing Resistance: 90-96% mortality
Resistant: <90% mortality

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

Active Ingredient: naled

- **Susceptible:** >97% mortality
- **Developing Resistance:** 90-96% mortality
- **Resistant:** <90% mortality

Credit: E. Buckner & D. Ramirez,
UF/IFAS Florida Medical Entomology Laboratory
Species: Culex *quinquefasciatus*
Active Ingredient: deltamethrin

Susceptible: >97% mortality
Developing Resistance: 90-96% mortality
Resistant: <90% mortality

Credit: E. Buckner & D. Ramirez,
UF/IFAS Florida Medical Entomology Laboratory
Species: Culex quinquefasciatus
Active Ingredient: etofenprox

Credit: E. Buckner & D. Ramirez,
UF/IFAS Florida Medical Entomology Laboratory
Species: Culex *quinquefasciatus*
Active Ingredient: sumithrin

Susceptible: >97% mortality
Developing Resistance: 90-96% mortality
Resistant: <90% mortality

Credit: E. Buckner & D. Ramirez,
UF/IFAS Florida Medical Entomology Laboratory
Distribution of domestic mosquito populations tested for insecticide resistance
January 1 - March 31, 2020

Credit: E. Buckner & D. Ramirez,
UF/IFAS Florida Medical Entomology Laboratory
Assay: CDC bottle bioassay
Conducted by: UF/IFAS/FMEL – Buckner Lab
Species: Aedes albopictus
Source: Reared from eggs
Site: Brevard County (EFSC)
Address: 250 Community College Parkway Southeast, Palm Bay, Florida 32909
Coordinates: 27.993386, -80.6302709

Date of assay: 1/21/20 – 1/23/20


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 a strain of susceptible Aedes albopictus
Column 4: Site specific Aedes albopictus; % mortality at the CDC diagnostic time

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDC diagnostic dose (per bottle)</td>
<td>Active ingredient tested</td>
<td>CDC diagnostic time; 100% mortality expected at given time using strain of susceptible Aedes albopictus</td>
<td>EFSC Aedes albopictus; % mortality at the CDC diagnostic time</td>
</tr>
<tr>
<td>43 ug/bottle</td>
<td>Permethrin</td>
<td>5 min</td>
<td>96 %</td>
</tr>
<tr>
<td>400 ug/bottle</td>
<td>Malathion</td>
<td>30 min</td>
<td>99 %</td>
</tr>
<tr>
<td>2.25 ug/bottle</td>
<td>Naled</td>
<td>15 min</td>
<td>98 %</td>
</tr>
<tr>
<td>12.5 ug/bottle</td>
<td>Etofenprox</td>
<td>30 min</td>
<td>100 %</td>
</tr>
<tr>
<td>0.75 ug/bottle</td>
<td>Deltamethrin</td>
<td>15 min</td>
<td>75 %</td>
</tr>
<tr>
<td>20 ug/bottle</td>
<td>Sumithrin</td>
<td>15 min</td>
<td>95 %</td>
</tr>
</tbody>
</table>


The EFSC population of Aedes albopictus is resistant to Deltamethrin; developing resistance to Permethrin and Sumithrin; susceptible to Malathion, Naled and Etofenprox.

Date: February 6th, 2020

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UF/IFAS
Florida Medical Entomology Laboratory
Overview of Insecticide Resistance Testing Algorithm

- Phenotypic Assay (CDC) Bottle Bioassay
  - Susceptible >97% mortality
  - Developing Resistance 90-96% mortality
  - <90% mortality Resistance

- Consider baseline enzyme testing
  - Mechanism testing*
  - Field testing
  - Intensity and mechanism testing
  - Alternative control methods

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