Insecticide Resistance Bottle Bioassay Evaluation of *Culex quinquefasciatus* Mosquitoes From Coachella Valley, 2019

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Southern house mosquito | *Culex quinquefasciatus* (Say)

The objective of this study was to determine whether locally collected mosquitoes were resistant to adulticide products using the CDC bottle bioassay. The Coachella Valley Mosquito and Vector Control District (hereafter, the District) evaluates adulticide resistance of field-collected mosquitoes to a susceptible colony strain on an annual basis. The results of these evaluations determined whether the products were appropriate for quickly bringing down the adult mosquito abundance and disrupting virus transmission.

Wild *Culex quinquefasciatus* mosquitoes were collected by placing gravid water bait (water infused with Brewer's yeast and ground alfalfa pellets) in a plastic tub overnight and eggs were collected the next morning. Mosquitoes were collected from the cities of Indio, La Quinta, and Palm Desert. Three egg rafts were placed per larval tray and larvae were fed daily a mixture of liver powder, ground fish flakes, brewer's yeast, and ground alfalfa pellets. Larvae were kept in rearing chambers at 55% relative humidity, 28°C, and 16:8 (L:D) h cycle. During the pupal stage, preliminary pupae were removed to increase the female: male ratio and subsequent pupae were placed in a cage for emergence. Adults were 3–5 d old with mixed sex (60F:40M ratio) and fed 10% sugar water daily. The susceptible CQ1 colony strain (originally collected as adults from Merced, CA in the 1950s) is kept in colony under similar rearing conditions.

Two hundred fifty (250) milliliter glass Wheaton bottles were coated with 1 ml of the pesticide product diluted in acetone. Formulations of these products were calculated using the concentration of the active ingredient (a.i.) as indicated in the table. For the control, 1 ml of acetone solvent was added to the bottles. The inside of the bottles were fully coated before rolling in a fume hood to allow the solvent to evaporate. Once the solvent evaporated, the assays were conducted within 24 h.

For the assays, an average of 28 mosquitoes ranging from 13 to 41 mosquitoes were aspirated into each bottle. For each adulticide tested, four replicate bottles were used for each city's sample population and the susceptible colony mosquitoes. Mosquito mortality was assessed at 15 min after initial exposure and every 15 min thereafter until all mosquitoes in a bottle were dead or the 2 h count was completed. Mosquitoes were observed as knocked down or 'dead' if they could no longer have controlled flight or stand. Results of mosquito mortality were analyzed using WHO guidelines for mosquito mortality for bottle bioassays. We observed the mosquito mortality of the wild strain when the colony strain reached 100% mortality. Resistance was evident when fewer than 80% of the mosquitoes died at this diagnostic time.

This study documented evidence of resistance of local wild mosquitoes to adulticide products. Scourge 18 + 34 was used at the District in 2016 and 2017. This product registration was discontinued in 2015 but we had some remaining in storage purchased prior to its discontinuation. Merus 3.0 was registered in CA in 2019 and is labeled for use in organic areas, providing an advantage over other adulticide products. This was our first evaluation of Merus 3.0. DeltaGard was registered in CA in 2018 and has been used at the District for less than 1 yr. Aqua-Reslin has been in use at the District since 2015.

The percent mortality at diagnostic dose are italicized in Table 1. All three mosquito strains demonstrated resistance to all adulticides tested.1

1This research was supported by the Coachella Valley Mosquito and Vector Control District as part of the routine resistance management work. No special research funds were allocated for this work.
| Product (a.i.) (a.i. dose µg/ml) | Strain         | Mean % mortality at time (min) after exposure |
|-------------------------------|---------------|---------------------------------------------|
|                               |               | 15  | 30  | 45  | 60  | 75  | 90  | 105 | 120 |
| **Aqua-Reslin (Permethrin)** (22) | Colony        | 42.7 | 98.3 | 100 | 100 | 100 | 100 | 100 | 100 |
|                               | Indio         | 0.8  | 1.5  | 3.61| 10.0| 25.3| 35.9| 48.2| 55.7|
|                               | La Quinta     | 0    | 10.1 | 25.3| 74.2| 84.3| 91.5| 93.5| 97.2|
|                               | Palm Desert   | 0    | 0    | 13.3| 68.3| 88.6| 97.2| 100 | 98.9|
| **DeltaGard (Deltamethrin)** (7) | Colony        | 6.8  | 74.1 | 95.0| 96.7| 100.0| 100.0| 100.0| 100.0|
|                               | Indio         | 0.0  | 2.6  | 8.1 | 27.0| 36.1| 46.0| 56.7| 65.7|
|                               | La Quinta     | 0.0  | 16.7 | 23.2| 61.4| 75.4| 85.5| 87.6| 90.0|
|                               | Palm Desert   | 0.0  | 4.0  | 12.0| 44.6| 66.1| 81.8| 76.5| 82.5|
| **Merus 3.0 (Pyrethrins)** (10) | Colony        | 51.5 | 59.9 | 66.9| 91.9| 96.1| 97.5| 98.4| 100.0|
|                               | Indio         | 0.0  | 0.0  | 0.0 | 0.8 | 0.8 | 1.4 | 3.2 | 3.9 |
|                               | La Quinta     | 0.0  | 0.0  | 0.0 | 0.0 | 0.0 | 0.0 | 4.9 | 4.9 |
|                               | Palm Desert   | 0.0  | 2.0  | 2.8 | 5.1 | 8.7 | 18.1| 23.7| 23.0|
| **Scourge 18 + 54 (Resmethrin)** (10) | Colony        | 37.1 | 78.1 | 92.5| 97.4| 99.1| 99.1| 99.1| 100.0|
|                               | Indio         | 0.0  | 0.0  | 1.0 | 1.7 | 2.4 | 4.8 | 9.1 | 22.6|
|                               | La Quinta     | 0.0  | 0.0  | 0.9 | 5.9 | 11.8| 15.3| 32.4| 35.2|
|                               | Palm Desert   | 0.6  | 0.6  | 0.6 | 6.9 | 9.4 | 20.2| 44.3| 47.5|