A Behind the Scenes Look at Caged Mosquito Field Trials Performed by Manatee County Mosquito Control District

by Katie F Williams

For years mosquito adulticides have been critical in targeting arbovirus vectors and nuisance mosquitoes, ensuring communities receive efficient and effective public health protection (Cornine 2015). In addition to conducting bottle bioassays, it is important to conduct examinations of ground ultra low volume (ULV) applications against caged mosquitoes to gauge the efficacy of adulticide products. Conducting field trials leads to a better understanding of how local environmental factors such as temperature, wind, humidity and time of day contribute to the relative efficacy, feasibility and limitations of different products and technologies (Britch *et al* 2010). Mosquito control districts depend on the data gleaned from these field trials to continually improve their ability to control local mosquito species, and it is imperative to ensure that the trials are successful from start to finish.

At Manatee County Mosquito Control District (MCMCD), located in west central Florida, we prepare weeks ahead of time to ensure every caged mosquito trial goes off without a hitch. Bioassay cages, transfer cages, droplet samplers, and slides are prepared and made by employees in-house. Mosquitoes are collected in the field and reared in the insectary at the district, and trucks are loaded and calibrated for each insecticide. Whether you are experienced in conducting caged mosquito field trials, or you are just getting started, the most crucial element of these field trials happens behind the scenes, both before a trial and during its run. Herein is a behind-the-scenes look at what it takes to pull off each caged mosquito field trial at MCMCD.

GETTING STARTED: BUILDING CAGES

Which comes first, the chicken or the egg? This question still stumps me, but I can say what comes first in our field trials is the bioassay cage; you can't have a caged mosquito field trial without cages to hold mosquitoes! At MCMCD, we use disposable bioassay cages in our field trials originally developed by Gail Stout, a MCMCD employee from 2005 to 2015. Our bioassay cages are semi-rigid, which means that they can be transported easily to and from the field. Plus they are cylindrical, so during the trial, the adulticide is able to reach the mosquitoes from all sides of the cage. Since bioassay cages are disposable, post-trial clean up time is reduced, and we are certain that for every trial, we are starting with clean cages. It certainly takes a village to make these bioassay cages, so it makes for a perfect winter project. Grab your crew and start an assembly line. You will need people to cut mesh and the foam board circles and someone handy with a glue gun.

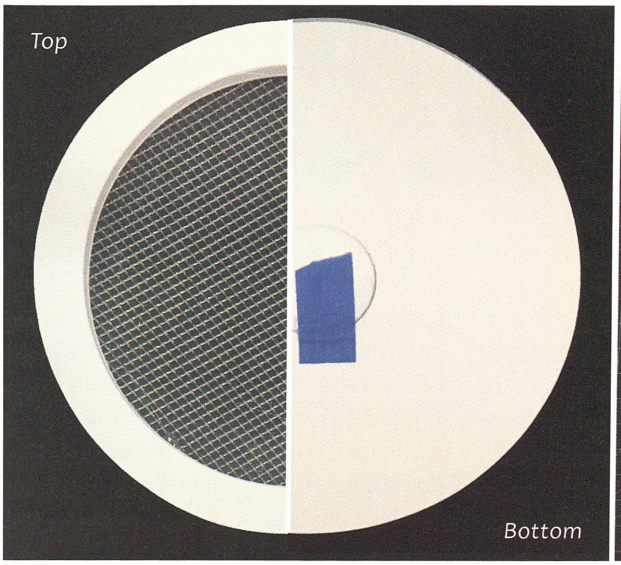
Figure 1: Bioassay cage materials needed include a hot glue gun, glue sticks, circle cutters, tape, monofilament line, stick pins and staples.

Materials Needed - see Figure 1

- Polypropylene netting: Product #XN-4800-40 from Industrial Netting, Minneapolis, MN (industrialnetting.com)
- Foam board: 3/16" thick 20" x 30" sheets (or larger)
- Foam board circle cutter and drill: FoamWerks Circle Cutter with safety dome for 1 to 6-inch diameter circles and hole drill from Logan Graphic Products Inc, Wauconda, IL (logangraphic.com)
- Stapler and staples
- Scissors or rotary cutter and blade
- Hot glue gun and glue sticks
- Ballpoint stick pins: Dritz® size 17 (27 mm)
- Monofilament line
- Blue painter's tape

Some Assembly Required

The bottom and top of the bioassay cages are made with foam board. Use the circle cutter to cut two 8.5 cm diameter circles per cage. To make the top of the cage, again use the circle cutter and cut out the center of one of the circles, leaving only a 1.0 to 1.5 cm wide rim. Then, to complete the top of the cage, cut an 8 cm diameter circle of netting, and use the hot glue gun to attach it to the foam board rim.



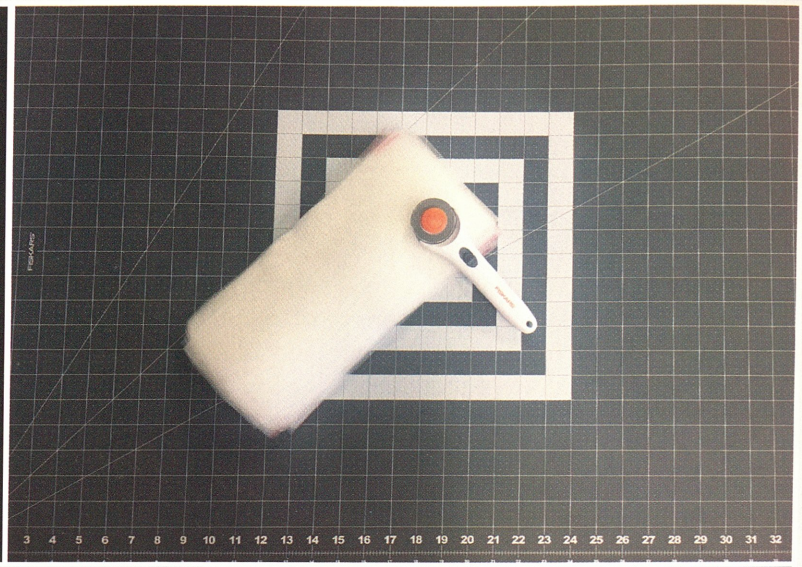


Figure 2: *Left* - Top and bottom of bioassay cages. Figure 3: *Right* - The sides of the cage are constructed by cutting the netting into 5.5 x 11.5-inch pieces using a rotary cutting mat, cutter and grid ruler to measure.

To make the bottom, punch or drill a 2 cm diameter circle of the second foam board circle. This serves as the hole through which mosquitoes are introduced into the cage via an aspirator tube. Tape the 2 cm diameter circle to the bottom of the cage using a small piece of blue painter's tape, to eventually close the hole once the mosquitoes are introduced; see Figure 2.

To make the main body of the bioassay cage, cut a piece of netting that is 14 cm x 29 cm using scissors or a rotary cutter; see Figure 3. Then bring the edges of the long side of netting together and staple the mesh netting, leaving approximately a 1.5 cm edge for future labeling. Now, insert the cage top into the body of the cage so that it is perpendicular to the staples of the cage body. Pin the cage top in place with 4 or 5 stick pins that run parallel to the foam board rim, leaving a 0.5 to 1 cm lip of netting at the top. Next, insert the cage bottom into the cage body. Placing your index finger through the aspiration hole in the cage bottom and pushing it up with your thumb helps orient the cage bottom so it is parallel to the cage top. Again, you want to leave a lip of about 0.5 to 1 cm of netting at the bottom. Once you have the cage bottom in place, secure it with 4 or 5 stick pins. Cut a piece of monofilament line approximately 40 cm long, string it through the netting lip at the top of the bioassay cage, and tie the ends together with a knot. The monofilament line is later used to hang the bioassay cage on a hook attached to a sampling station used in our spray trials. Depending on how many

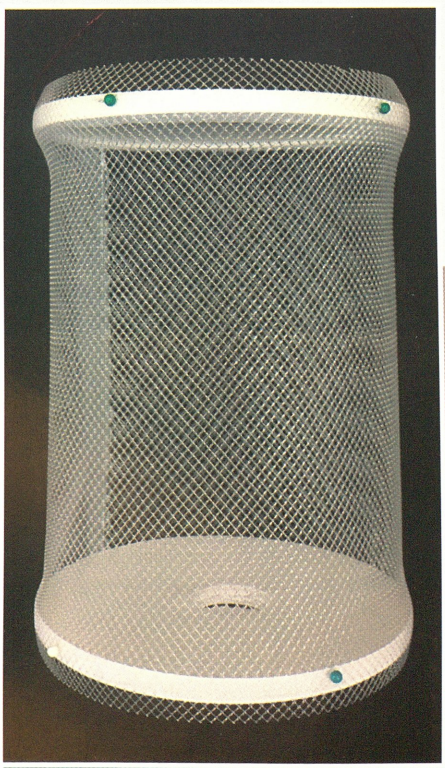


Figure 4: *Left* - Completed bioassay cage. Figure 5: *Right* - Bioassay cage assembly is a team effort.

employees are working on these, it takes approximately 12 to 15 minutes to complete one cage see Figure 4. To make construction more efficient, we usually have two people cutting circles, a person on the glue gun, a person cutting mesh and stapling, and a few people building the final product all at the same time; see Figure 5. Once the cages are built, store them in sturdy boxes. On the day of your spray trial, all you have to do is load the mosquitoes into your bioassay cages and label them with a sticker that includes replicate number, sampling station location and mosquito species.

TRANSFER CONTAINERS

One hour post-spray application, we transfer our mosquitoes to clean transfer containers to prevent any secondary exposure to adulticide residue on the bioassay cages. We use 8 oz paper soup/hot food cups with vented flat lids purchased from Webstaurant store.com to make the transfer containers. To prepare these for trials, we use a single edge razor blade to cut the inside of the lid off. After the mosquitoes are transferred into these cups, a piece of black mesh – also called tulle fabric – is placed over the cup, and held securely by the cut rim of the lid; see Figure 6. The tulle can be purchased anywhere fabric is sold; we purchase ours at Walmart.

SAMPLING STATIONS

Next, it's time to get the sampling stations built and ready to go; see Figure 7. Each sampling station consists of a 5 foot long metal stake, PVC T-bar, hooks to hold the bioassay cages, a droplet sampler, a rod holder, and Teflon™-coated 3 x 75 mm rods.

We make our own Florida Latham Bonds (FLB) droplet samplers in-house to determine droplet size and density, in order to characterize the distribution of the ULV spray cloud throughout our test grid (Bonds 2012). Each sampler contains a rotary switch to turn on/off, battery holders and straps to hold four internal, replaceable alkaline AA batteries with a body made of PVC components. The slide holder atop the FLB sampler is made by cutting a nylon-threaded rod (3/8" – 16 thread, 6-foot length; Product # 98831A590 from McMaster-Carr Supply Company, Elmhurst, IL, www.mcmaster.com) into 8-inch sections. A hole is drilled through each end of the rod holder for the rods to go into, and 3/8" hex nuts are used to hold the rods in place. Prefabricated FLB droplet samplers can be purchased from John W Hock Company, Gainesville, FL (johnwhock.com).

Additionally, Teflon-coated 3 x 75 mm slides can also be purchased from John W Hock, but if you want to try making slides in-house like us, this is how we do it: We order clear extruded acrylic

rectangular rods (1/8" thick x 1/8" wide x 6' length; Product # 8728K11) from McMaster-Carr. First, because fingerprints can look like ULV drops under the microscope, always wear latex gloves while handling the rods to prevent getting fingerprints on them. We start by cleaning the 6-foot rods with warm soapy water. We recommend using Decon™ Contrex soap (Decon Labs, King of Prussia, PA, deconlabs.com) because it does not leave a residue. Once the rods are clean and dry, we place 8 rods together lengthwise and cover one side of the 6-foot rods with 1-inch wide clear tape with Teflon® (McMaster-Carr Product # 7562A13). Then, the rods are cut down to the proper length of 75 mm using a hammer and a single edge razor blade.

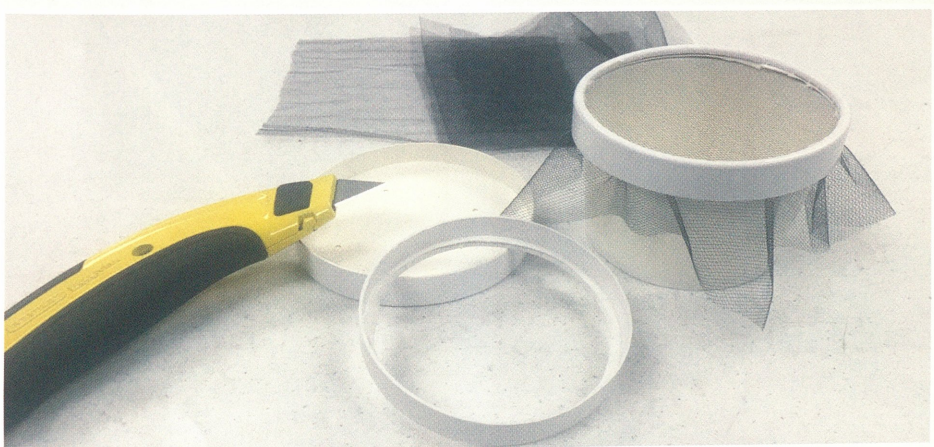


Figure 6: Transfer containers for holding mosquitoes post-spray application.

We find it helpful to draw an arrow using an ultra fine point Sharpie on the bottom of each cut group of slides pointing to the side with the tape and then, using the single edge razor blade, separate them into individual slides; see Figure 8.

MOSQUITOES

The mosquitoes used in our ground ULV field trials are collected as eggs or larvae in Manatee County and reared to adulthood in our insectary. Many things go into to ensuring we have healthy and happy mosquitoes on the day of the trial. Our insectary temperature and relative humidity are usually 28°C and 75%, respectively. We raise a variety of mosquito species in our insectary. *Aedes aegypti* eggs are collected from little black jars (LBJ) in Cortez, FL; see Figure 9. The LBJs contain a piece of germination paper lining the rim of the jar, approximately 300 ml water, and 5 grams of a 3:2 mixture

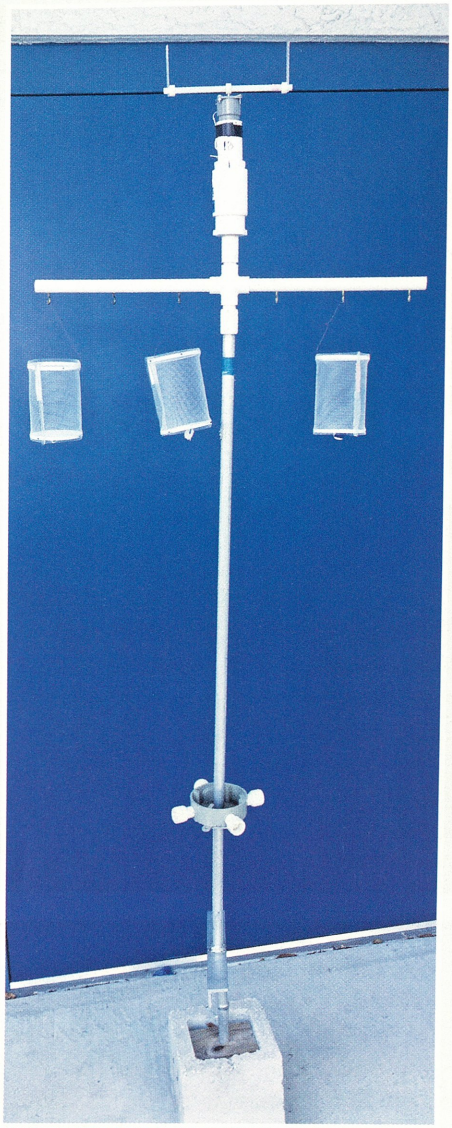


Figure 7: Sampling station.

of liver powder and brewer's yeast. Inspectors collect *Ae taeniorhynchus* larvae throughout the county and collect *Culex quinquefasciatus* rafts from a local wastewater treatment plant. Larvae are reared in 58.4-cm-long x 41.3-cm-wide x 15.2-cm high plastic containers containing 3 liters of reverse-osmosis filtered water and fed daily with 50 ml of the liver-yeast slurry solution until pupation. Pupae are transferred to water-filled cups and placed into mesh cages with constant access to a 10% sucrose solution on filter paper. For the purposes of our trials, we use adult female mosquitoes between the ages of 2 to 10 days old; see Figure 10.

FIELD TRIAL PREPARATION

Once we have all of the major components of a field trial prepared and ready to go, we can begin to prepare for the actual trial day. So that you aren't running around like a chicken with your head cut off on the day of the trial, it's best to start preparing as soon as you know the date of your trial. Here is how a typical week goes for us if our trial is on a Wednesday.

Monday / Tuesday

1. Place the slides in the spinner rods, label each rod, and place them in a plastic holding container. Depending on the number of trials we plan on conducting in an evening, we use colored tape (Fisherbrand™ Labeling Tape Rainbow Pack 13 mm x 13 m, Product # 15-901-R, Thermo Fisher Scientific, fishersci.com) to label each spinner rod corresponding with its placement in the test grid.

2. Labels are made for the bioassay cages and correspond with grid placement using the labeling tape.

3. Cut squares of tulle to be used to cover the transfer containers.

Wednesday: Day of Trial

4. The droplet samplers are loaded with four AA alkaline batteries and placed in buckets that correspond with grid placement along with extra batteries and droplet samplers. The T-bars for each sampling station are also included in the bucket to make for easy set up at the test site.

5. The mosquitoes are added to bioassay cages using a battery-powered hand-held aspirator (Hausherr's Machine Works, Toms River, NJ) about 5-6 hours before testing. Bioassay cages containing 15-20 female mosquitoes are labeled and placed in plastic containers that correspond with their

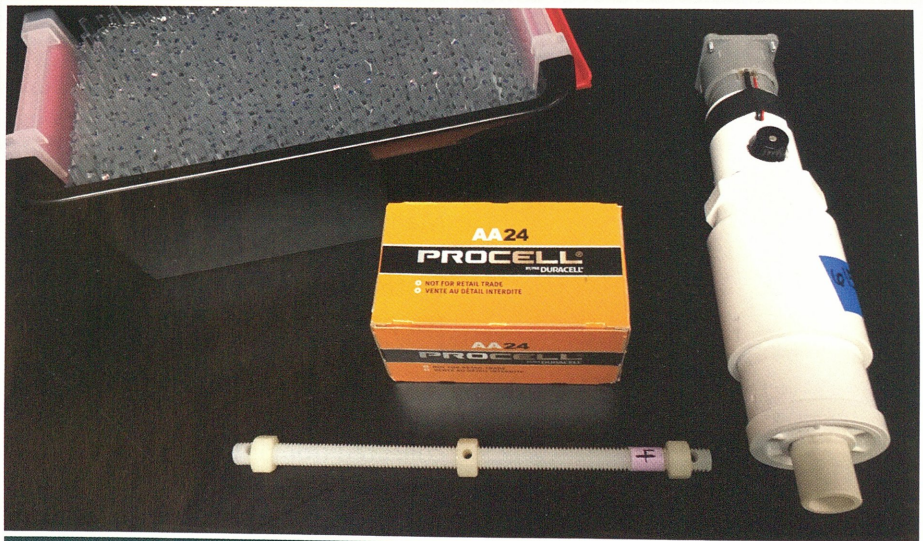


Figure 8: 3 x 75 mm rods, droplet sampler, and rod holder.

Figure 9: Little Black Jar.

Figure 10: Larvae in plastic containers (*left*) and adults in laboratory mesh cages in insectary (*right*).

placement in the test grid. Note: Make sure you label everything. Blue painter's tape and the labeling tape are a must!

6. Make up enough cotton balls soaked in 10% sucrose solution for each bioassay cage and transfer container. Pre- and post-trial mosquitoes are given constant access to the soaked cotton balls.

7. Prior to our spray trials, a 25 mm glass slide with Teflon tape is waved approximately 15 feet behind the spray truck for each product and then analyzed using DropVision® (Leading Edge Associates LLC, Fletcher, NC, www.leateam.com) to determine if the $DV_{0.5}$ (volume median diameter) and $DV_{0.9}$ of droplets fall within the product label's recommended range.

8. Make sure weather station computers are charged and set up for the date of trial.

9. Place AAA batteries in the Kestrel Weather Tracker (kestrelmeters.com) and set correct date and time. Calibrate the compass by stepping outside and turning in three slow circles until calibration is complete.

10. Make sure you have everything loaded in the trucks before leaving for the test site. We use a checklist to make sure we are ready to go!

Let's Head Out!

The time of each trial depends on sunset. We usually head to Sanctuary Cove, an unfinished neighborhood development in Palmetto, FL, approximately one hour before sunset, which gives us plenty of time to set up our weather station and test grid.

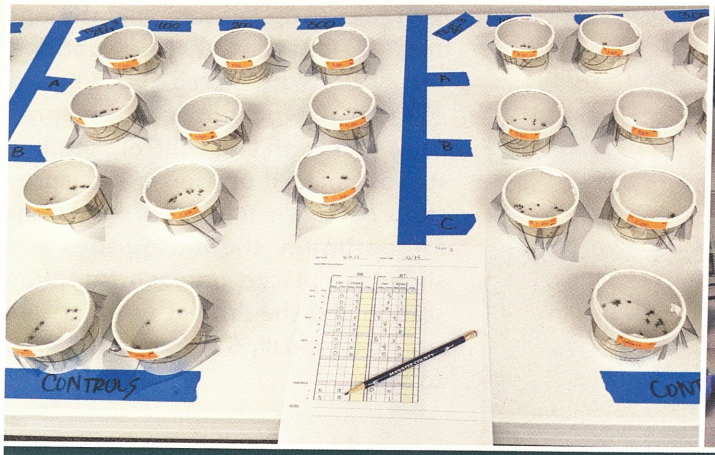
We start by setting up the weather station to determine if the weather is conducive to spraying. Our weather station consists of a Kestrel 4500 NV Model Pocket Weather Tracker used to record temperature, wind direction, wind speed, and relative humidity at ground level as well as two DirecTemp® temperature probes placed on a PVC mast at 1.2 m and 10 m above ground. This allows us to know when the temperature at both heights is suitable for keeping our ULV spray at ground-level. Additionally, two Model NM100 Weather Stations (New Mountain Innovations Inc, Old Lyme, CT, newmountain.com) are placed at 1.7 m and 10 m above ground to record wind speed and direction; see Figure 11. Optimum conditions consist of an inversion – when ground temperature is cooler than aloft – and wind speeds of less than 10 miles per hour.

Next, we set up our sampling stations in a 3 x 3 grid with 100 ft separations. The stations are placed 100, 200 and 300 feet downwind from the spray truck path with two control sampling stations placed upwind of the spray truck path. When weather conditions are optimum, the mosquitoes and slides are placed on each sampling station and the droplet samplers are turned on usually 5 to 10 minutes prior to spray.

Ground ULV applications are made using a truck-mounted London Fogger at 10-20 mph depending on the desired application rate for each trial. Approximately 10 minutes post-spray, depending on wind speed, the bioassay cages and slides are collected from each sampling station and immediately brought back to the district for transfer, while some of the crew remains behind to conduct further replicates.

Mosquitoes from both the control and treatment sampling stations are

Figure 11: New Mountain weather stations are used in the field.



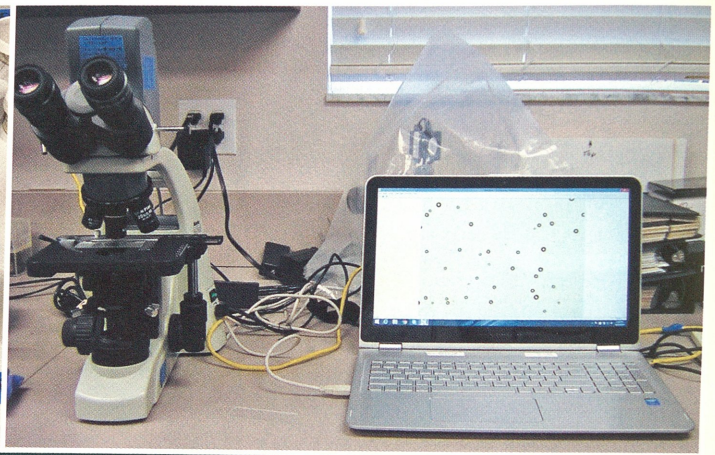


Figure 12: Transfer cups with mosquitoes. Figure 13: Droplet analysis using a compound microscope and DropVision.

knocked down for 28 seconds with CO_2, transferred to the holding containers, covered with mesh netting, and given access to cotton balls soaked with 10% sucrose solution. The number of mosquitoes knocked down is recorded one hour post-application and the number of mosquitoes dead in each holding container is recorded 12 hours post-application; see Figure 12. To determine droplet density and size, a slide from each sampling station is analyzed using a compound microscope and DropVision; see Figure 13.

TAKE HOME MESSAGE

The goal of mosquito control districts when performing adulticide applications, a key component of an integrated pest management program, is to achieve the most effective control in order to prevent arbovirus transmission and reduce mosquito populations before they become a nuisance (Faraji *et al* 2016). Caged mosquito field trials play a pivotal role in providing evidence that ground ULV applications of adulticides against local populations of mosquitoes can be efficacious depending on the species being targeted, the product, and the application rate. MCMCD will continually monitor the efficacy of our adulticide program to ensure successful mosquito control through yearly ground ULV field trials and resistance testing in Manatee County, FL.

ACKNOWLEDGMENTS

A successful trial depends on the dedicated employees who do all the hard work from start to finish. Throughout the years we have developed quite the routine and can complete a trial in two hours when all goes well! Of course, this would not be possible without the help of our wonderful ground crew including Dwight Andress, Chris Bustle, Pat Conrad, Jeffrey Davis, Wes Thompson and Joshua Jackson. A special thank you also goes out to entomology technician Mary Geesey for all of her help. And finally, thank you to my supervisor in the biology department, Dr Eva Buckner, who plans and helps prepare and execute each trial, as well as my Director Mark Latham and Assistant Director Christopher Lesser for their guidance and support throughout the trial season. With everyone's help we ensure quality data is gathered for the district.

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