A Comprehensive toolbox for the control of *Aedes aegypti* and *Aedes albopictus* in Florida

Purpose:

This publication is intended as a guide of available tools and emerging technologies for mosquito control programs to utilize in response to local transmission of the Zika virus.

This document was created in cooperation with staff from the University of Florida's Florida Medical Entomology Laboratory (FMEL) as well as staff from mosquito control programs in and outside of Florida. Further, the Board of the Florida Mosquito Control Association endorsed this toolbox at their annual meeting in November of 2016.

Introduction:

Distinct natural biology and life history traits of container breeding mosquitoes, *Aedes (Ae.) aegypti* and *Ae. albopictus*, must be considered when formulating a plan for control. Both species have an affinity to oviposit and rest in very cryptic habitats in populated urban areas. Although a limited flight range provides for an increased opportunity to target these species, the infrastructure of cities and roadways add to the unique challenges involved in their control such that the conventional methods of aerial and ground adulticiding may not provide the same efficacy as they do against other species. Controlling *Ae. aegypti* and *Ae. albopictus* requires a fully comprehensive integrated mosquito management (IMM) regime which should include all of the following:

- The initial mosquito control response to occur within 24 hours of notification of a suspected case(s) or positive mosquito pool(s).

- A GIS specialist to map out the case response area that:

    - Is a *minimum* of a 200-yard radius around case patient home for one case or trap collection site for one positive mosquito pool, as described in The Centers for Disease Control and Prevention's (CDC) guidance on control of the species (CDC 2016).
    - Is a minimum of a ½-mile radius around a cluster of case patient homes or positive mosquito collections. According to CDC guidance, an area of 1 mile in diameter would provide a large enough margin of safety around a cluster of cases with a single common location and may vary according to need, possibly even extending well beyond the 1-mile radius of most concern (CDC B, 2016). Include epidemiological investigations that obtain other pertinent information on additional outdoor locations each patient frequents and these locations should be included in the GIS mapping if deemed necessary to contain the origin of infection sites.

- This response to include 1) intensified homeowner education, 2) source reduction, 3) pesticide application at each residence or business property, and 4) surveillance within the defined radius. The Florida Department of Health has created educational materials that can be found at http://www.floridahealth.gov/diseases-and-conditions/zika-virus/.

- The goal is to achieve 90% coverage of the case response area within 5 days.

- Studies that have been performed on the dispersal of *Ae. aegypti* have determined that they generally fly between 100-500 m (McDonald, 1977) though studies in Hawaii and Japan have shown them to fly no more than 200 m (Bonnet *et. al.*, 1946, Mori, 1979). Dispersal of adults is highly dependent upon availability of oviposition sites, carbohydrate sources, and mates (Trpis *et. al.*, 1986). A study conducted in Puerto Rico and Thailand found the majority of *Ae. aegypti* released from study sites were collected from their release house or an adjacent house. Inter-village movement was rarely detected (Harrington *et. al.*, 2005). Although the two target species have a short flight range, other potentially infected humans within the radius are very mobile and therefore, 100% coverage of the case response area must be achieved within 7 days. If personnel resources are limited, the responding agency/program should consider adjusting staff levels to meet case response needs by eliciting additional support to sufficiently cover the case response area.

Pesticide Selection and Application:

It is important to note that some *Ae. aegypti* populations throughout Florida have shown resistance to certain active ingredients currently available for use in mosquito control. The CDC defines insecticide resistance as an overall reduction in the ability of an insecticide product to kill mosquitoes (CDC A, 2016). Due to the short flight range of these species in particular, the results of resistance monitoring may not be valid county-wide, but would reflect pesticide susceptibility within a specific geographic area. A study performed on *Ae. aegypti* in Brazil showed varying levels of insecticide resistance in 9, geographically district, populations throughout the country (Lima, 2011).

The CDC has developed a bottle bioassay to determine whether a particular active ingredient is capable of killing mosquitoes. The technique's manual, referred to as the CDC bottle bioassay, complete with procedures and interpretation of results, can be found at http://www.cdc.gov/malaria/resources/pdf/fsp/ir_manual/ir_cdc_bioassay_en.pdf.

It is recommended that when choosing an adulticide product to include in the IMM approach, each active ingredient under consideration be tested on area mosquitoes using this method. It is then recommended that the results of resistance monitoring guide decisions on which insecticides should be used for control efforts in any given location. If mosquito populations show resistance to a particular active ingredient, that active ingredient should be taken out of the control response. If local mosquito populations are susceptible to certain active ingredients, it is still wise to rotate pesticide chemistries to delay or prevent resistance development.

Ground Adulticide Treatments

Immediate Knockdown

- Space sprays, ultra low volume (ULV) or thermal fog, will be required for immediate knockdown of potentially infected adult mosquitoes but do not provide residual efficacy. Depending on weather conditions, space sprays may only remain effective for a few hours.

- Permethrin, prallethrin/sumithrin, and deltamethrin are commonly used for ground applications via handheld/backpack ULV units.
- Malathion, in addition to the above products, can be applied using truck mounted ULV sprayers. However, due to the infrastructure of roadways, effective treatment may not be achieved using truck mounted sprayers alone.
    - Repeated truck ULV applications, 1-2 days apart, have been shown to reduce populations of these species by the Lee County Mosquito Control District.

Residual Barrier Applications

- Vegetation likely to serve as resting habitat for adult mosquitoes should be treated with a barrier application, which provides a residual efficacy, using backpack sprayers for residential locations or all terrain vehicle- mounted units for larger recreational areas.

- Common active ingredients used for barrier applications are extended release formulations of bifenthrin and deltamethrin. Barrier applications provide residual efficacy between 1-6 weeks. Some factors that can influence the residual efficacy are product, application method, geographic location in FL, and weather conditions (Cilek, 2008, Qualls *et al*., 2012). Residual barrier applications won't provide immediate knockdown of potentially infected adult mosquitoes which is necessary during a disease situation.

Aerial Adulticide Treatments

- Aerial adulticide applications should be used to treat wide-areas that may be beyond the limitations of truck mounted equipment, inaccessible by foot, or wide-areas that need to be treated immediately.

- Fixed wing or helicopter applications are possible for use, though helicopter applications allow for additional precision to the targeted treatment area.

- More targeted, ground adulticide application as discussed above should be augmented by aerial applications in order to treat large areas if trap counts are above 10 females per trap night (when using BG-Sentinel Trap® deployed around 1 pm and retrieved around 11 am the following day baited with the BG-lure® and CO_2).

- Adult mosquitoes must be in flight in order to contact the space spray and receive a lethal dose. Resting adults may not be exposed to treatment during a single spray event. Lee County Mosquito Control District has found that two consecutive adulticide treatments performed within 24 hours to target a larger portion of the population of potentially infected adults significantly reduced populations of these species.

Ground Larvicide and Pupicide Treatments

- Larval control is essential in stopping the life cycle of these species and performing source reduction in an area-wide fashion is critical. These species also exhibit skip oviposition, in which females will distribute a small number of eggs in multiple sites (Reiter, 2007). Therefore, all natural and manmade containers that are capable of holding water should be drained, dumped, discarded, or covered. Nothing should be overlooked, as these species will lay eggs in any sized container, even bottle caps and plants that hold water in their leaves, leaf axils, trunks, or stumps.
- Any container that cannot be rendered incapable of holding water should be treated with a larvicide such as Bti, methoprene, spinosad, etc. These can be in granular, tabular, or liquid formulations. Residual formulations of these active ingredients are readily available and some are capable of providing efficacy for up to 30 days or more, according to product labels.

- Any pupae present in containers that cannot be rendered incapable of holding water should be treated with a pupicide (monomolecular film).

- Larval indices should trigger additional wide-scale larval control (see **Thresholds** below for indices).

- Liquid formulations of Bti have proven effective at reaching smaller, very cryptic habitats that *Ae. aegypti* and *Ae. albopictus* prefer and are difficult to spot during an on the ground inspection.

- Liquid Bti applications can be performed on the ground using backpack sprayers or truck mounted units such as the Curtis-Dyna Fog LV8, IGEBA U40, or the Buffalo Turbine modified to produce a droplet spectrum between 50-120 microns in size (as show in Miami-Dade County during the 2016 Zika response).

Aerial Larvicide Treatments

- Aerial treatments of liquid Bti formulations are recommended for wide-area treatments in response to a cluster of human cases or positive mosquito pools.

- Fixed wing or helicopter applications are possible, though helicopter applications allow for additional precision to target area.

- This is also a proactive approach and should be considered to reduce populations of *Ae. aegypti* and *Ae. albopictus* to levels below the disease threshold even when local transmission is not suspected (see **Thresholds** below).

Combined Aerial and Ground Adulticide and Larvicide Approach

- When confronted with a wide-area treatment need, combined aerial adulticide and larvicide treatments, along with ground control efforts, are especially effective.

    - Control efforts performed in the Wynwood area of Miami-Dade County in response to the Zika outbreak of 2016 have shown that aerial adulticiding and

aerial liquid larviciding when combined with ground control efforts are effective at reducing *Ae. aegypti* populations below the disease threshold. Similarly, successful suppression of *Ae. aegypti* populations was observed following a combined aerial and ground adulticide and aerial liquid larvicide approach during the Dengue Fever outbreak in the Florida Keys of 2009-2010 and has been used by Lee and Manatee county mosquito control programs.

Novel Control Strategies

Novel control strategies should be considered as potential control options pending confirmed advancement in developmental trials. Many techniques described below have not yet been used for wide scale control of these species. An IMM approach should be used to control *Ae. aegypti* and *Ae. albopictus*, even when considering the use of these novel techniques.

In2Care® Mosquito Trap

In May of 2016, the US Environmental Protection Agency (EPA) granted a public health exemption under the provisions of Section 18 of the Federal Insecticide, Fungicide, and Rodenticide Act, for the CDC to use the auto-dissemination technique of the In2Care® Mosquito Trap in Puerto Rico. In August of 2016, this public health exemption was expanded to include areas of local transmission in the continental United States. This trap combines pyriproxyfen and *Beauveria bassiana* to help manage Zika vector populations. Recommended placement of traps is 10 traps per acre and should be maintained every 4-6 weeks.

The exemption is subject to the following conditions;

- In2Care® Mosquito Traps are not for private use and may only be sold by Univar to state, territory, and/or local officials (including, but not limited to: mosquito control districts, Department of Agriculture, and Department of Health).
- Traps cannot be sold directly to certified pest control operators (PCOs). Traps may be distributed to PCOs by state, territory, and/or local officials if contracted to assist with local control of *Aedes* mosquitoes. The In2Care® systems may only be used by contracted PCOs under the deployment guidance of state, territory, and/or local officials, in conjunction with CDC.
- All state, territory, and local rules and regulations regarding the pesticide uses will apply.
- The In2Care® system may only be deployed where the *Aedes* mosquito is present, and after CDC has confirmed local mosquito-borne transmission of the Zika virus.
- This trap requires FDACS approval prior to procurement and deployment.

SIT/IIT-*Wolbachia* (MosquitoMate)

Sterile Insect Technique (SIT) is an environmentally-friendly, species-specific approach to control insect populations by mass-rearing and releasing sterile males of a target species that compete with wild-type males for female insects. Males may be sterilized by ionizing irradiation. If a female mates with a sterile male, their progeny will die before reaching adulthood, and therefore reduce the next generation's population. Over time, repeated release of sterile males can reduce the insect population to very low levels. Protocols are available for irradiating both *Ae. aegypti* and *Ae. albopictus* to induce complete sterility without significantly reducing the males' competitive fecundity and performance. A critical step for SIT in mosquitoes is the

separation of males from females. Female elimination is crucial to allow for male-release-only, as sterile females are capable of transmitting diseases.
SIT can be combined with the Incompatible Insect Technique (IIT). MosquitoMate ZAP technology uses the naturally-occurring bacterium, *Wolbachia pipientis*. *Wolbachia* infected lines of *Ae. aegypti* and *Ae. albopictus* exhibit cytoplasmic incompatibility, in which treated males that mate with wild-type females will not produce viable offspring due to the incompatibility of the *Wolbachia* strain. *Wolbachia* also provides protection against major human pathogens by preventing transmission, thus eliminating the risk of releasing limited females during the applications of SIT/IIT. On October 4, 2013, an Experimental Use Permit (EUP) for the release of ZAP *Ae. albopictus* was approved for a trial release in the Florida Keys, though a trial never commenced. The ZAP strain for *Ae. aegypti* was approved by the Florida Department of Agriculture and Consumer Services (FDACS) for an additional EUP for a trial release in the Florida Keys in October of 2016. More information on this process can be found at http://mosquitomate.com/science-research/.

Considerations:

- The first field trial of this technology commenced during August of 2016 in California.
- Requires insect rearing capacity, suggested 7:1 ratio of MosquitoMate to wild-type males (therefore used when populations are controlled).
- Adulticides use in the release area may impact released males.
- Requires multiple releases to be effective.
- Requires additional EPA approval to expand the EUP at the federal level as a biopesticide for selected field trial sites. This expansion process is estimated to take approximately 7 months at the EPA via communication with the product registrant.

Oxitec's OX513A Transgenic *Ae. aegypti*

On August 5, 2016, the US Food and Drug Administration's Center for Veterinary Medicine published a final finding of no significant impact and final environmental assessment on the OX513A for an investigational trial on Key Haven in the Florida Keys. This transgenic control method is based on the SIT, but uses genetic control instead of radiation to produce the same sterilization effect. OX513A has two genes, a color marker and the self-limiting control gene which causes offspring to die and any accidentally released females to have a limited life span (FKMCD, 2016). Just like the SIT method, separation of males from females is critical to allow for male-release only. The modified male insects are released to mate with wild-type females. Successful trials have been shown to reduce local *Ae. aegypti* populations by over 90% in Brazil, the Cayman Islands, Panama, and Malaysia (Lacroix, 2012).

Considerations:

- Requires insect rearing capacity, suggested 7:1 ratio of OX513A to wild type males (therefore used when populations are controlled).
- Adulticides use in the release area may impact released males.
- Requires multiple releases and oversight of breeding to be effective.
- Requires additional FDA approval for alternate field trial sites.

Trap-N-Kill Lethal Ovitraps

This trap mimics an ideal larval habitat, attracts gravid mosquitoes for oviposition, and then uses Dichlorovinyl dimethyl phosphate to kill adult females. Traps should be deployed in a 1:10 ratio to existing larval habitats (4-10 traps/home and 20-40 traps/acre). Trials in Brazil, Thailand, and

Puerto Rico have shown significant reduction in populations with the use of this trap (Spring Star, 2016). This pesticide product is currently registered and commercially available.

CDC's Autocidal Gravid Ovitraps (AGO)

Field trials conducted in Puerto Rico have shown that populations can be reduced by capturing gravid *Ae. aegypti* females with sticky gravid traps that attract older females ready to oviposit and prevents successful oviposition (Barrera *et al.*, 2013). The AGO is only as effective as its ability to compete with natural breeding habitats, so source reduction must be conducted. This trap does not employ an active ingredient and therefore does not require registration as a pesticide.

Surveillance

A minimum of 1 BG-Sentinel Trap® should be deployed at each case patient home or directly adjacent properties. Using a higher number of traps increases the likelihood of collecting the target species. Additional traps can be deployed at locations that serve as prime resting habitat for these species (near ample oviposition sites) or any area where there is significant adult abundance within case response area, as resources permit. Traps should be deployed with dry ice or CO^2 to enhance trapping in addition to the proprietary BG-lure®.

Laboratory real-time Polymerase Chain Reaction (PCR) virus testing of all *Ae. aegypti* and *Ae. albopictus* collected during adult surveillance activities can be performed at FDACS's Bronson Animal Disease Diagnostic Laboratory (BADDL) in Kissimmee. A cold chain should be maintained from the time specimens are collected from the field to the time they arrive at the laboratory for virus detection. The BADDL protocol for submission can be found in appendix A.

Ovitraps should be utilized to collect *Ae. aegypti* and *Ae. albopictus* eggs which, in addition to monitoring population density, can also be reared to adulthood and tested for resistance to commonly used adulticide active ingredients. If resistance testing cannot be done locally, the University of Florida's Florida Medical Entomology Laboratory may be available to test eggs for resistance. This will help inform control decisions. If local mosquito populations are found to have resistance to a certain active ingredient, use of that ingredient should be discontinued and an alternative be considered.

Follow up surveillance/inspection of the response area shall be conducted weekly for 45 days after the initial identification of each locally-acquired human case or positive mosquito pool. The 45-day period equates approximately to three mosquito incubation periods based on CDC guidance (CDC, 2016). The area should be retreated as needed based on larval and adult surveillance. If adult abundance remains high or increases, additional adulticide treatments should be performed as needed and within label requirements to decrease population numbers. If larval indices remain high or increase, additional source reduction and larvicide treatments should be performed.

Thresholds

Mosquito disease thresholds for similar arboviruses transmitted by the same mosquito species (Dengue, Chikungunya, and Yellow Fever viruses) have been identified from previous outbreaks. Various studies have shown that a House Index of 5% (Soper, 1967), a Container Index of 10% (Conner *et al*., 1923), or a Breteau Index of 5 (Brown, 1977) prevented Yellow Fever Virus transmission. Additional studies have shown that a House Index of 1% (Pontes *et*

al., 2000), a Container Index of 1.8%, and a Breteau Index of 1.2 was needed to suppress Dengue transmission in Taiwan (Chang *et al.*, 2015).

- House Index (percent (%) of houses with at least one positive container)
- Container Index (% of all containers with water that are larva/pupa positive)
- Breteau Index (number of positive containers per 100 houses)

Establishing disease infection rates for human infections and outbreaks for Dengue virus has been difficult using adult mosquito surveillance in the past, as it was not until the development of the BG-Sentinel Trap® and some additional gravid traps that made it possible to accurately estimate adult abundance of these mosquito species and therefore track infection rates. During previous outbreaks of Dengue virus, a density of two or more female mosquitoes per gravid sticky trap, per week were associated with human cases, whereas less than one female per trap, per week, was considered a safe level of adult abundance (CDC, 2016). A recent study performed by the CDC in Puerto Rico using gravid sticky traps found a lack of Chikungunya transmission when the density of *Ae. aegypti* was less than 2 females per trap, per week (Ritchie *et al*., 2004). Recent surveillance of virus in mosquito pools performed in Miami during a Zika outbreak have isolated Zika virus in 1 female *Ae. aegypti* collected in one BG Sentinel Trap® per night (baited with BG-lure® and CO_2).

Innovative Techniques

BG Sentinel traps are expensive and some mosquito control programs in Florida have found creative means of adapting some of the character traits of this trap, making them more attractive to *Ae. aegypti* and *Ae. albopictus*. Trap comparisons have shown negligible differences when compared to the original BG Sentinel Trap®, according to the respective program. Below are a few innovative adult surveillance techniques deployed by local mosquito control programs throughout Florida in recent years.

- Pasco County Mosquito Control District has found the use of CDC light traps combined with the BG-lure® and CO_2 to be effective at collecting *Ae. aegypti* and *Ae. albopictus*.
- Broward and Orange county mosquito control programs have fabricated their own version of the BG Sentinel Trap® using laundry baskets and 5 gallon buckets baited with the BG-lure® and CO_2.

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Appendix A: Arboviral testing for Zika, Dengue, and Chikungunya by Real-Time Polymerase Chain Reaction (PCR) from mosquito samples. Bronson Animal Disease Diagnostic Laboratory (BADDL). April 2016.

Arboviral Testing for Zika, Dengue, and Chikungunya by Real-Time Polymerase Chain Reaction (PCR) from Mosquito Samples

Bronson Animal Disease Diagnostic Laboratory (BADDL)

April 2016

The BADDL offers a multiplex real-time PCR assay for rapid detection of Zika, Dengue, and Chikungunya viruses in Mosquito pools.

- **Sample type**
  - Arboviral real-time PCR detection will be done from pools of up to 50 mosquitoes. Since Zika, Dengue, and Chikungunya viruses are spread through the bite of certain *Aedes* species of mosquitoes, the preferred species to sample for testing will be *Aedes aegypti* and *Aedes albopictus*.
  - Other species may be submitted for testing with prior arrangement with the Laboratory Director.

- **Sample Collection Information**

Submissions in 2 ml tube with Species Identification

  - Ideal**y,** Mosquitoes should be separated by species and submitted in pyrogen-free (DNAse/RNase free) 2.0 ml conical shaped tube (see Figure 1). These tubes can be provided by the laboratory upon request at an additional cost. Tubes can be ordered from a number of suppliers including Fisher, VWR, and USA Scientific.
  - Pooled single species of Mosquitoes should be placed in tubes.
  - Pool no more than 50 mosquitoes per tube.
  - Label the tube with a bar code number or specific sample identification number.
  - List the submitter, sample ID number, mosquito species, number pooled, collection location, premises ID (if available), and date collected on the sample submission form.
  - Note: Do not pool mosquitoes of different species, collection dates, or collection locations in the same tube.

Figure 1

Submissions in Containers without Species Identification

  - If speciation is not possible prior to submission, mosquito identification can be done at the laboratory with prior arrangement, but it will increase the turnaround time of the results.
  - Mixed species submissions should be submitted in rigid containers.
  - Do not pool mosquitoes from multiple collection dates or collection locations in the same container.
  - In mixed species submissions, non-*Aedes* mosquitoes will not be tested.

- **Shipping/Transport**
  - Samples should be shipped to the laboratory immediately after collection, and shipped overnight on ice packs.
  - U.S. Postal shipping address: BADDL – P.O. Box 458006, Kissimmee, FL 34745-8006
  - Physical delivery address: BADDL – 2700 N. John Young Parkway, Kissimmee, FL 34741-1266

- **Turnaround Time (TAT)**
  - Arbovirus real-time PCR test results can be expected within 5 business days following receipt of the sample. Please contact the Molecular Diagnostics Section at the Bronson lab (321-697-1400) with any questions.