BOTTLE BIOASSAYS OF FIELD COLLECTED MOSQUITOES FOR LEVEL OF ETOFENPROX RESISTANCE IN CENTRAL MASSACHUSETTS (2022 UPDATE)

FRANK H. CORNINE III
Central Mass. Mosquito Control Project
111 Otis Street Northborough, MA 01532
(508) 393-3055 • www.cmmcp.org • cornine@cmmcp.org

ABSTRACT

During the summer of 2022, the Central Massachusetts Mosquito Control Project once again conducted bottle bioassays to help monitor for local pesticide resistance. Using technical grade etofenprox obtained from the Centers for Disease Control and Prevention, CMMCP staff examined local adult mosquito populations for potential pesticide resistance. The majority of specimens tested in these bottle bioassays were *Coquillettidia perturbans*, as this is the predominant area species. Results did not indicate a change in product or protocol at this time. This information was added to data from other mosquito control and resistance surveillance organizations, contributing to a nationwide pesticide resistance network. This CDC database can be used to help inform national directives on resistance management.

BACKGROUND

Pesticide resistance in this age of vector-borne disease can hamper the ability of public health officials to successfully control threats. Potential resistance may also lead to the reemergence of several diseases that would have been otherwise contained through control measures (Brogdon 1998). Current resistance in select mosquito populations may be the result of historical insecticide use in the agricultural and pest control industries (Rodriguez 2005). The bygone use of DDT for example, could have contributed to current resistance to synthetic pyrethroids, due to the mechanism for resistance being similar for both (Brogdon 1998; McAbee 2003). Another associated and contributing factor may be the contracting classes and options for public health insecticides as well as growing regulatory restrictions (Brogdon 1998).

Although examples of pesticide resistance have been well documented, the scope of the issue and its genuine impact on public health control activities is not known. This is partially due to varying levels of resistance surveillance programs that currently exist. This factor is shown in Massachusetts where some organized mosquito control agencies conduct zero resistance surveillance, while others have limited to well-developed programs collecting data on pesticide resistance in mosquitoes. In addition to the variety of resistance surveillance programs, resistance also appears to be quite localized which further clouds the impact. One noted example involved two separate mosquito populations that not only differed in resistance levels, but also resistance mechanism, all despite being only a few miles apart (Brogdon 1998). These continuing uncertainties surrounding

insecticide resistance have supported CMMCP efforts to monitor for detection of early resistance. In the case of observed resistance, adulticide protocols could be modified to ensure continued efficacy.

The primary adulticide product used by CMMCP during the 2022 season was Zenivex® E4 (Wellmark International, Schaumburg, IL) (EPA Reg No. 2724-807), a synthetic pyrethroid that utilizes the active ingredient etofenprox. Prior to using this product CMMCP had used another synthetic pyrethroid, ANVIL® 10+10 (Clarke Mosquito Control Products, Inc., Roselle, IL) (EPA Reg. No. 1021-1688-8329). Unlike Zenivex® E4, ANVIL® 10+10 uses the active ingredient sumithrin along with the synergist piperonyl butoxide (PBO). The absence of PBO in Zenivex® E4 is one of its advantages over ANVIL® 10+10.

MATERIALS & METHODS

The procedure used for these bottle bioassays comes from the Centers for Disease Control and Prevention (CDC 2010). Using the CDC diagnostic concentration established from naïve specimens against mosquito populations from the CMMCP service area, potential resistance can be observed. In these bottle bioassays, clean 250ml Wheaton bottles (Wheaton Science Products, Millville, NJ) were lined with the baseline etofenprox concentration of 12.5µg/ml. The solutions used in this project were created using pesticide grade acetone (Thermo Fisher Scientific, Inc., Fair Lawn, NJ) and technical grade etofenprox supplied by the CDC. In addition to the bottles coated with etofenprox, untreated bottles were created using only the pesticide grade acetone to establish a control for the bioassays.

Field collected mosquitoes were obtained by using CDC light traps (John W. Hock Co., Gainesville, FL) deployed in areas with a history of CMMCP adulticide applications. The CDC light traps used compressed carbon dioxide gas as an attractant at a release rate of 500cc/min. Once the labeled bottles were coated and sufficiently dried, approximately 10-15 adult mosquitoes were aspirated into each bottle mechanically. ABC standard collection nets (Clarke Mosquito Control Products, Inc., Roselle, IL) were used in conjunction with the CDC light traps and held the mosquitoes until introduction into the bioassay bottles.

With these local exposed mosquitoes aspirated into the bottles, specimen knockdown percentage was recorded at various intervals, up to 100% knockdown or ending at 120 minutes elapsed time. For the untreated control bottles lined with only acetone (zero etofenprox), knockdown percentage was observed at similar intervals. Potential differences between the plotted knockdown curves of the treatment mosquito populations and the established baseline group could be used to determine if resistance was forming in local mosquitoes. If test specimens survived longer than those of the baseline group, it could be an indication of resistance developing.

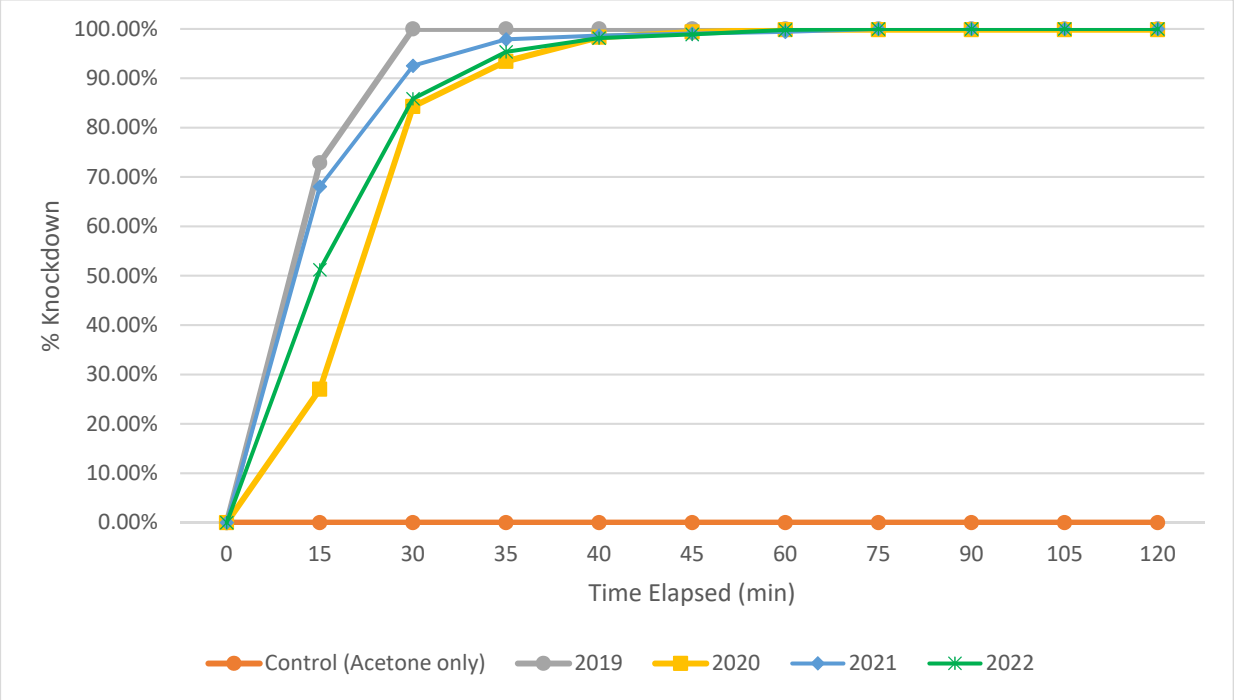
DISCUSSION

Coquillettidia perturbans accounted for the majority of field collected mosquitoes used in these 2022 bottle bioassays for resistance. Historically, this species has been the overwhelming target mosquito

of these trials. One issues with this *Cq. perturbans* tendency is that the CDC has not established a standardized time estimate for 100% knockdown of this species, although there is a general etofenprox concentration per bottle of 12.5µg regardless of species. The only species that the CDC has published mortality times for are *Ae. aegypti*, *Ae. albopictus*, *Cx. molestus, Cx. pipiens, Cx. tarsalis*, and *Cx. quinquefasciatus*. The knockdown times for these various species range widely from 15-105 minutes for etofenprox. Another important factor to note is that these CDC established mortality estimates are developed using mosquitoes reared in the lab. This is another potential issue for CMMCP as currently only mosquitoes collected from surveillance traps are currently used in bottle bioassays. Field collected specimens are typically not all of the same species, age or metabolic stage. These factors may have contributed to the variations in knockdown curves over the past four seasons (Figure 1).

In 2023 CMMCP will attempt to use adults for the bottle bioassays that were solely collected from locally egg rafts, and reared through the larval stages to adulthood. This process has been used in other CMMCP research projects and will negate any potential age, feeding stage, and species discrepancies of the specimens. Regardless of the source for adult mosquitoes used in the bottle bioassays, the project will continue next season, likely using etofenprox again as Zenivex® E4 will still be used by CMMCP in 2023. In the unexpected case that CMMCP changes primary adulticide product, the active ingredient used in the bottle bioassays will be adapted to that corresponding compound. Bottle bioassay results will continue to be shared with the resistance surveillance community.

Figure 1: Yearly Etofenprox Concentration Knockdown Curves



REFERENCES

Brogdon WG, McAllister JC. 1998. Insecticide Resistance and Vector Control. *Emerg Infect Dis* 4:605-613.

CDC. 2010. Guideline for evaluating insecticide resistance in arthropod vectors using the CDC bottle bioassays. Atlanta, GA: Center for Disease Control and Prevention [accessed January 10, 2019]. Available from: http://www.cdc.gov/malaria/resources/pdf/fsp/ir_manual/ir_cdc_bioassay_en.pdf

McAbee RD, Kang KD, Stanich MA, Christiansen JA, Wheelock CE, Inman AD, Hammock BD, Cornel AJ. 2003. Pyrethroid tolerance in *Culex pipiens pipiens* var *molestus* from Marin County, California. *Pest Manag Sci* 60:359-368.

Rodriguez MM, Bisset JA, DeArmas Y, Ramos F. 2005. Pyrethroid Insecticide-Resistant Strain of *Aedes Aegypti* From Cuba Induced by Deltamethrin Selection. *J Am Mosq Control Assoc* 21(4):437-445.