BOTTLE BIOASSAYS OF FIELD COLLECTED MOSQUITOES FOR LEVEL OF ETOFENPROX RESISTANCE (2023 UPDATE)

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ABSTRACT

The Central Massachusetts Mosquito Control Project has continued performing bottle bioassays to help monitor local mosquito populations for pesticide resistance. CMMCP staff used technical grade etofenprox and protocols from the Centers for Disease Control and Prevention during the 2023 season. *Coquillettidia perturbans* was the primary species tested in these bottle bioassays, and is also the predominant mosquito species in the area. Results of this season were consistent with those of the past several years. Although this data was developed using a mixture of field collected species, the information will be shared with other mosquito control and resistance surveillance organizations to help benefit the pesticide resistance surveillance community.

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 2023 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).

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.

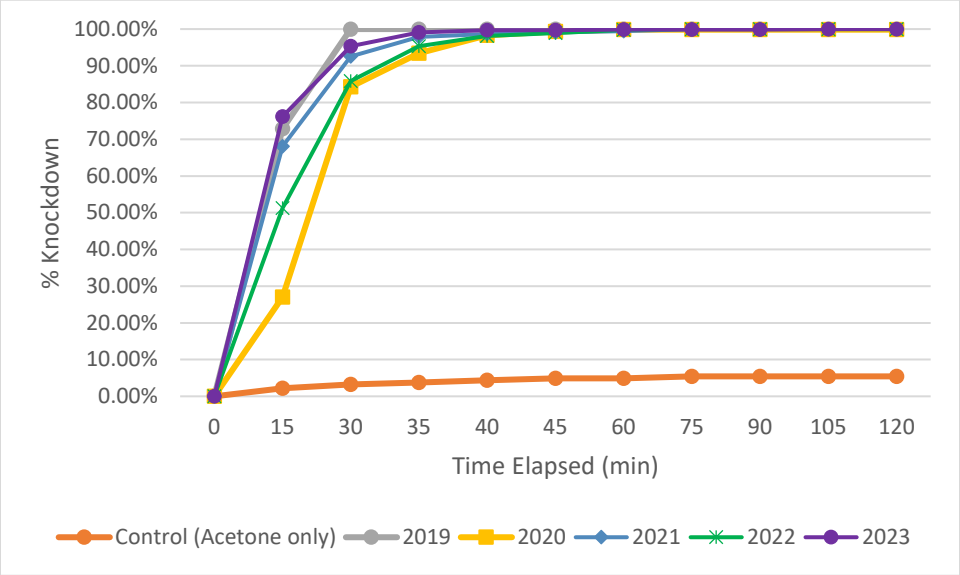
DISCUSSION

Once again, *Coquillettidia perturbans* was the predominant field collected species used in these bottle bioassays. *Cq. perturbans* is the most abundant species in the CMMCP service area, so this trend is not surprising. Unfortunately, the CDC has not established a standardized time estimate for 100% knockdown of *Cq. perturbans*, however there is a general etofenprox concentration per bottle of 12.5µg regardless of species. The CDC has published mortality times for *Ae. aegypti, Ae. albopictus*, *Cx. molestus, Cx. pipiens, Cx. tarsalis*, and *Cx. quinquefasciatus*, ranging at points from 15-105 minutes for etofenprox. In establishing these diagnostic times, the CDC utilizes standardized mosquito specimens, raised and developed in laboratory settings. Conversely, the specimens used by CMMCP are collected from surveillance traps and are not all of the same species, age or metabolic

stage. These variations will contribute to the differences in the knockdown curves observed over the past four seasons (Figure 1).

The goal for CMMCP in 2024 will be to locally obtain *Culex pipiens* egg rafts, and in the laboratory setting, rear the mosquitoes for use in the bottle bioassays. This will enable CMMCP to use the species-specific CDC diagnostic mortality time to better gauge local resistance. This process will also allow for specimens to of uniform age and metabolic status, in additional to being of a single target species. Etofenprox will likely be the active ingredient tested again in these bottle bioassays as Zenivex® E4 is currently slated to be used as the primary adulticide by CMMCP in 2024. If the adulticide product is changed for the 2024 season, these bottle bioassays can be modified to use that particular active ingredient. The results of these bottle bioassays will be shared with other mosquito control and resistance surveillance organizations to help benefit the pesticide resistance surveillance community.

Figure 1: Yearly Etofenprox Concentration Knockdown Curves



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