BOTTLE BIOASSAYS OF FIELD COLLECTED MOSQUITOES FOR LEVEL OF ETOFENPROX RESISTANCE IN CENTRAL MASSACHUSETTS (2020 UPDATE)

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ABSTRACT

To continue surveillance for pesticide resistance in local mosquito populations, the Central Massachusetts Mosquito Control Project conducted bottle bioassays during the 2020 season. The Centers for Disease Control and Prevention once again supplied CMMCP with technical grade etofenprox and direction for performing the bioassays on locally trapped adult mosquitoes. As in previous seasons, *Coquillettidia perturbans* was the primary species used in the bioassays. The resistance surveillance data created from these etofenprox bottle bioassays was shared with the CDC, which along with results from other mosquito control organizations across the United States, form a better understanding of the current pesticide resistance level in mosquitoes nationally.

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 2020 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.

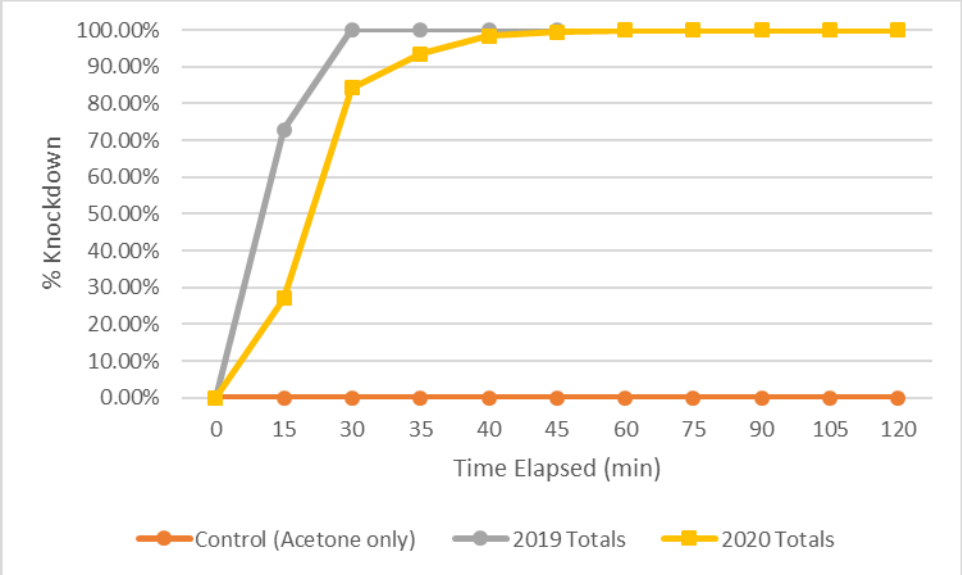
DISCUSSION

The species composition of the field collected mosquitoes used during the

2020 bottle bioassays were overwhelmingly *Coquillettidia perturbans*. Although the CDC has not published a standardized time estimate for 100% knockdown of *Cq. perturbans*, they have set a standard etofenprox concentration per bottle of 12.5µg regardless of species. The published mortality times for six species (*Ae. aegypti, Ae. albopictus, Cx. molestus, Cx. pipiens, Cx. tarsalis, and Cx. quinquefasciatus*) range from 15-105 minutes for etofenprox. All of these knockdown estimates from the CDC are determined using lab reared mosquitoes. This is somewhat problematic as CMMCP utilizes mosquitoes collected from surveillance traps and these specimens from the field are typically not all of the same species, nor are they of the same age or metabolic characteristics. Unfortunately, all of these variables can create different resistance outcomes during the bottle bioassays. This issue may have contributed to the different in knockdown curves of the past two seasons (Figure 1). Future data will show whether this is a trend towards resistance or simple variation from field collected specimens.

To help address the issues present with using field collected mosquitoes, CMMCP staff is planning on collecting egg rafts from local larval habitats, rearing the larvae, and using the adults in bottle bioassays. If this method is successful it will eliminate the potential age discrepancies of the specimens, feeding stage, and species composition. Even if bottle bioassays continue with field collected mosquitoes, CMMCP staff will continue to examine the local etofenprox resistance levels, as Zenivex® E4 will be used by CMMCP in 2021. However, if the primary adulticide product changes, CMMCP will alter the subsequent bottle bioassays to examine that particular active ingredient according to the CDC practices. Future data will once again be submitted to the CDC to help assist in the greater understanding of resistance in mosquitoes nationally.

Figure 1: Yearly Etofenprox Concentration Knockdown Curves



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