BOTTLE ASSAYS OF FIELD COLLECTED MOSQUITOES FOR LEVEL OF RESISTANCE TO ZENIVEX® E20 IN CENTRAL MASSACHUSETTS 2016

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ABSTRACT

In 2016 the Central Massachusetts Mosquito Control Project conducted bottles assays to establish a diagnostic baseline concentration for etofenprox resistance on the local mosquito population. The process is outlined by the Center for Disease Control and Prevention, with CMMCP initially using naïve field collected adult mosquitoes to create the standard concentration for which all susceptible mosquitoes would be tested against. Resistance to etofenprox was examined because the primary adulticide product of CMMCP is Zenivex® E20, of which etofenprox is the active ingredient. Through these bottle assays, the baseline concentration for etofenprox resistance was determined to be 18µg/ml for mosquitoes of the CMMCP service area and can now be utilized in future seasons to monitor the local resistance.

INTRODUCTION

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 has been well documented, the scope of the issue and its real world 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 create baseline data

and monitor for early resistance detection. In the case of observed resistance, adulticide protocols could be modified to ensure continued efficacy.

The primary adulticide product used by CMMCP during the 2017 season was Zenivex® E20 (Wellmark International, Schaumburg, IL) (EPA Reg No. 2724-791), a synthetic pyrethroid that utilizes the active ingredient etofenprox. Prior to this season CMMCP had used another synthetic pyrethroid, ANVIL® 10+10 (Clarke Mosquito Control Products, Inc., Roselle, IL) (EPA Reg. No. 1021-1688-8329). Unlike Zenivex® E20, ANVIL® 10+10 uses the active ingredient sumithrin along with the synergist piperonyl butoxide (PBO). The absence of PBO in Zenivex® E20 is one of its perceived benefits over ANVIL® 10+10. As this was the first season CMMCP primarily used Zenivex® E20, a diagnostic baseline concentration for etofenprox needed to be established, which was the goal of the 2017 bottle assays.

MATERIALS & METHODS

The procedure used for these bottle assays comes from the Centers for Disease Control and Prevention (CDC 2010). A reference, or baseline, concentration needs to be determined by using adult mosquitoes that originate from an area that has been excluded from pesticide exposure, CMMCP or otherwise. Using the diagnostic information established from these naïve specimens against mosquito populations from the CMMCP service area, one can gauge if resistance has developed and to what degree. Determining the baseline concentration for bottle assays begins by lining clean 250ml Wheaton bottles (Wheaton Science Products, Millville, NJ) with various 1ml dilutions of the product being analyzed. The solutions used in this project were created using pesticide grade acetone (Thermo Fisher Scientific, Inc., Fair Lawn, NJ) and undiluted Zenivex® E20, finishing at etofenprox concentrations of 3µg/ml, 6µg/ml, 12µg/ml, 18µg/ml, and 24µg/ml. In addition to the bottles coated with etofenprox, untreated bottles were created using only the pesticide grade acetone to establish a control for the assays.

For the baseline concentration to be determined, it is important that the mosquito specimens used have not been unexposed to synthetic pyrethroids, whether through the CMMCP program or other activities. To meet this criteria, CDC light traps (John W. Hock Co., Gainesville, FL) were deployed in wetlands surrounding a local organic farm property. This location has been identified and treated as a pesticide exclusion since 2006. Once the labeled bottles were coated and sufficiently dried, approximately 10-15 adult mosquitoes were aspirated into each bottle mechanically. The CDC light traps used compressed carbon dioxide gas as an attractant at a release rate of 500cc/min. 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 assay bottles.

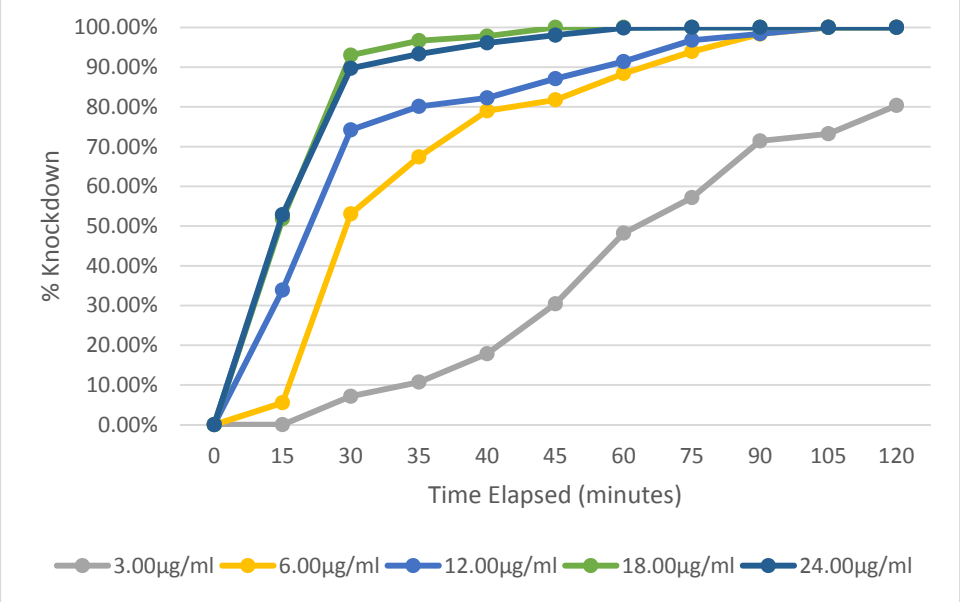
With these local unexposed mosquitoes collected, coated bottles created, and mosquitoes aspirated into the bottles, specimen knockdown percentage was recorded at 5 minute intervals, up to 100% knockdown. For the untreated control bottles lined with only acetone

(zero Zenivex® E20), knockdown percentage was observed at 5 minute intervals for up to two hours. Each one of the diluted etofenprox solutions underwent several trials until a concentration was found that produced morality curve around 30 minutes for total knockdown. This baseline concentration, once determined, could eventually be used against the exposed mosquito populations, along with untreated control bottles running concurrently. Potential differences between the plotted knockdown curves of the treatment mosquito populations and the original 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.

RESULTS

To determine the baseline concentration for etofenprox resistance in local mosquito populations, concentrations of 3µg/ml, 6µg/ml, 12µg/ml, 18µg/ml, and 24µg/ml were tested. The diagnostic baseline concentration of Zenivex® E20 was determined to be 18µg/ml. The other concentrations of etofenprox produced either too fast or too slow mortality curves (Figure 1). Future bottle assays will be conducted using only the 18µg/ml etofenprox diagnostic concentration.

Figure 1: Zenivex® E20 Concentration Knockdown Curves for Baseline Determination



DISCUSSION

It was important to determine the baseline concentration of etofenprox against local mosquito species, as this was the first season where Zenivex® E20 was the primary adulticide used by CMMCP. Prior to this year ANVIL® 10+10, with the active ingredient sumithrin and synergist piperonyl

butoxide, had been the principal product used. Now that a diagnostic baseline concentration has been established CMMCP can use it against field collected mosquitoes from the CMMCP service area. As long as Zenivex® E20 is used as the primary adulticide product, resistance surveillance will continue using this baseline concentration against local mosquito populations. When CMMCP changes products, the resistance surveillance program will make the appropriate shift.

The diagnostic baseline concentration for Zenivex® E20/etofenprox against local mosquito populations was determined to be 18µg/ml, but results were somewhat variable. This may have been a byproduct of the mosquitoes being field collected opposed to lab reared. Adult mosquitoes collected from the field may be at various metabolic stages and process exposure to synthetic pyrethroids at different rates. If lab reared mosquitoes are used instead, their food source intake can be more readily regulated and uniform for bottle assay testing. Another potential factor in variability could have been in the solutions used for determining the baseline concentration. Zenivex® E20 product was used, but alternatively technical etofenprox could have been diluted into solution, which may have been more stable and accurate over the course of bottle assays. CMMCP will investigate obtaining etofenprox and comparing the two sources. The diagnostic concentration for etofenprox determined this season will be utilized in resistance surveillance throughout the CMMCP service area in the 2017 season.

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