BOTTLE ASSAYS OF FIELD COLLECTED MOSQUITOES FOR LEVEL OF RESISTANCE TO ANVIL® 10+10 IN CENTRAL MASSACHUSETTS (UPDATE 2017)

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ABSTRACT

In 2017 the Central Massachusetts Mosquito Control Project conducted bottles assays to monitor synthetic pyrethroid resistance in 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 ANVIL® 10+10 was specifically examined as it is the primary adulticide product of CMMCP. Results of this surveillance indicated that the level of resistance in local mosquito populations does not warrant any procedural or insecticide changes at this time. CMMCP will continue bottle assays of local mosquito populations to monitor the levels of synthetic pyrethroid resistance, helping ensure the efficacy of current control practices.

INTRODUCTION

Faced with new and emerging vector-borne diseases, public health officials must be aware of pesticide resistance and how that may impact their ability to address these diseases. In addition to encouraging novel diseases, resistance could also elevate historic vector-borne diseases that were once considered contained (Brogdon 1998). Studies indicate that past agricultural and pest control activities may have contributed to the development of present day resistance (Rodriguez 2005). Increasing regulatory restrictions on pesticide products and fewer chemical class options to utilize may also be contributing to the level of resistance in vector populations (Brogdon 1998).

Despite examples of resistance being well documented, resistance surveillance programs vary greatly across regions and because of this the negative impacts resistance may have on control efforts is in reality unknown. Mosquito control agencies in Massachusetts are an example of this situation. Organizations in the Commonwealth run the spectrum from zero resistance surveillance, to long running programs on pesticide resistance in mosquitoes. Another issue that clouds the accuracy of resistance surveillance is that the degree of resistance and actual mechanism can be localized. A documented case exists of two mosquito populations, separated by only few miles that actually differ in resistance mechanism (Brogdon 1998). The unknowns involving insecticide resistance reinforce the efforts of CMMCP to create baseline data and monitor for early resistance. In the event CMMCP observes resistance, interventions can take place timely enough to ensure continued success in supporting public health.

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

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. 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 ANVIL® 10+10. The final sumithrin solution concentrations were 2.217µg/ml, 4.434µg/ml, 8.868µg/ml, and 22.17µg/ml. In addition to the bottles coated with sumithrin, 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.

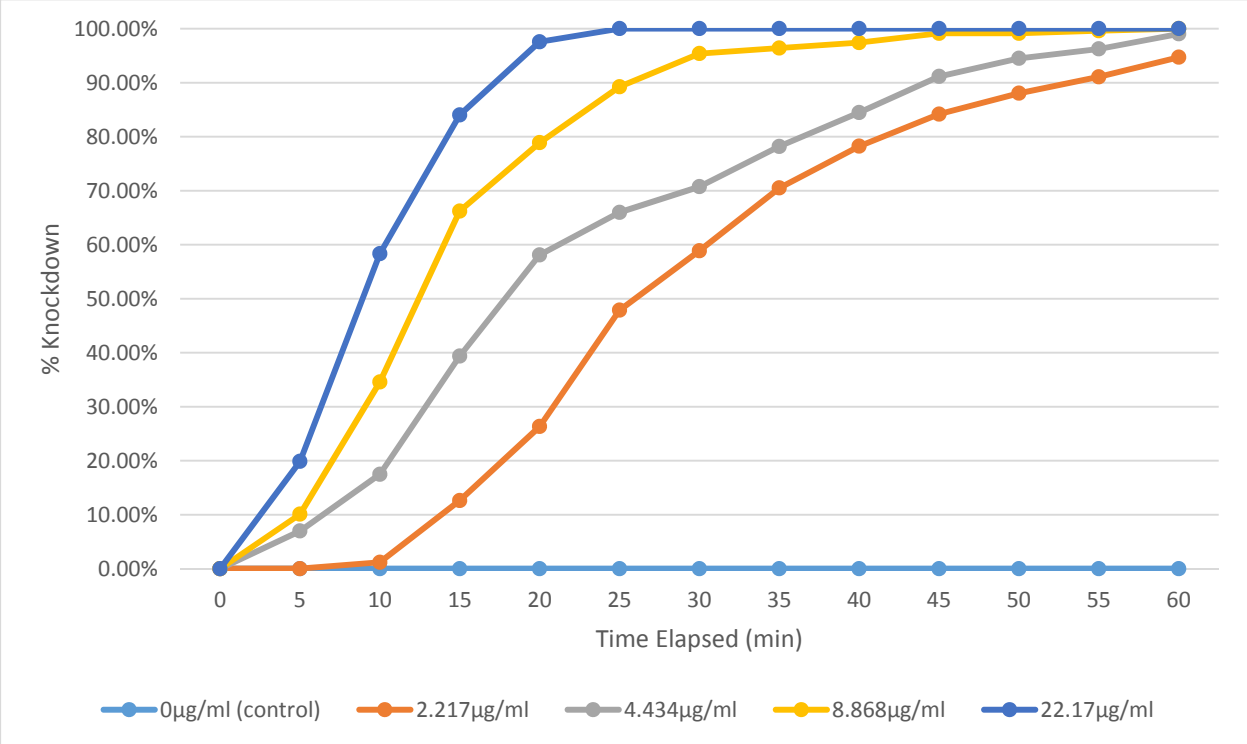
With these local unexposed mosquitoes collected, coated bottles created, and mosquitoes aspirated into the bottles, specimen knockdown percentage was recorded several intervals, up to 100% knockdown. For the untreated control bottles lined with only acetone (zero ANVIL® 10+10), knockdown percentage was observed up to two hours. Each one of the diluted sumithrin 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

The baseline component of the bottle assays that resulted in the optimal concentration of sumithrin was 22.17µg/ml, which corresponded with data from previous studies (Petersen 2004). The other concentrations of sumithrin produced either too fast or too slow mortality curves (Figure 1).

Figure 1: ANVIL® 10+10 Concentration Knockdown Curves for Baseline Determination



The bottle assays from the 2017 season produced a knockdown curve simlar to the original baseline average for many locations, and slightly slower for a few areas (Figures 2, 3). Despite the slower mortality curve, the average reduction at the 30 minute threshold was 98.28%. The reduction shown in the control bottles was not significant and consistent with other projects.

Figure 2: 2017 Time-% Knockdown Curves of Bottle Assays for ANVIL® 10+10 (22.17µg/ml)

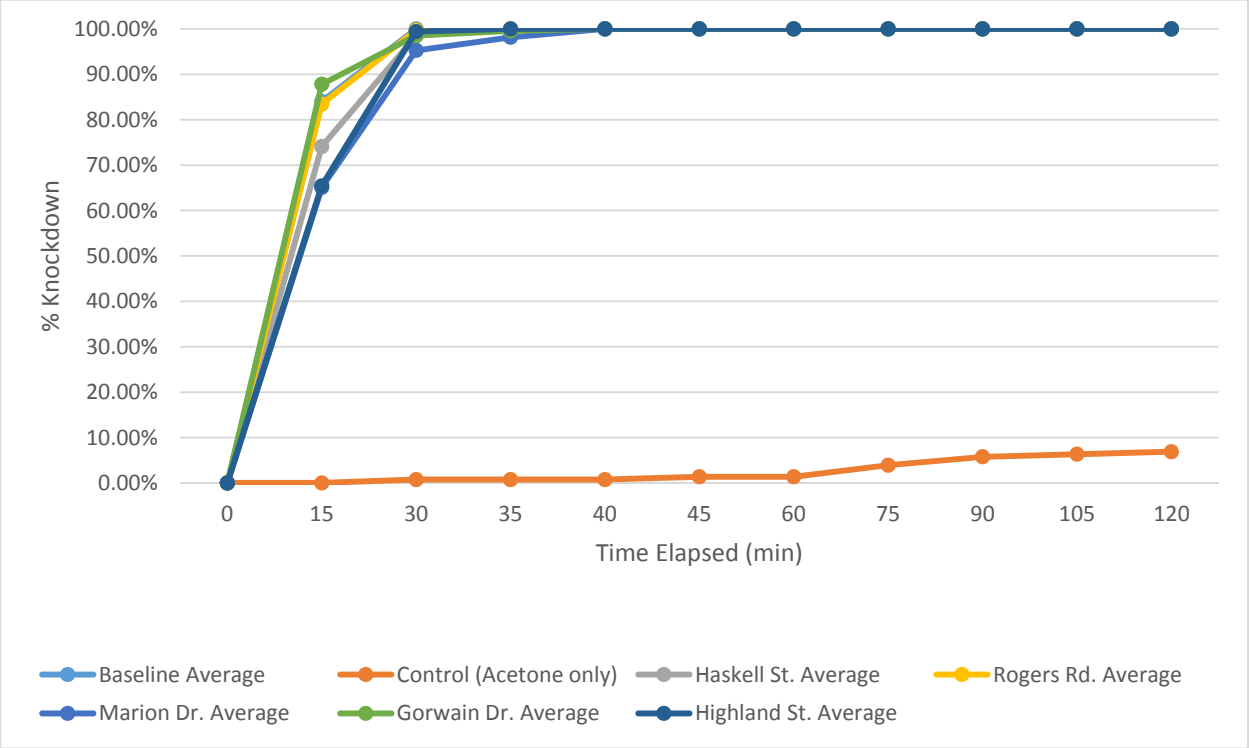
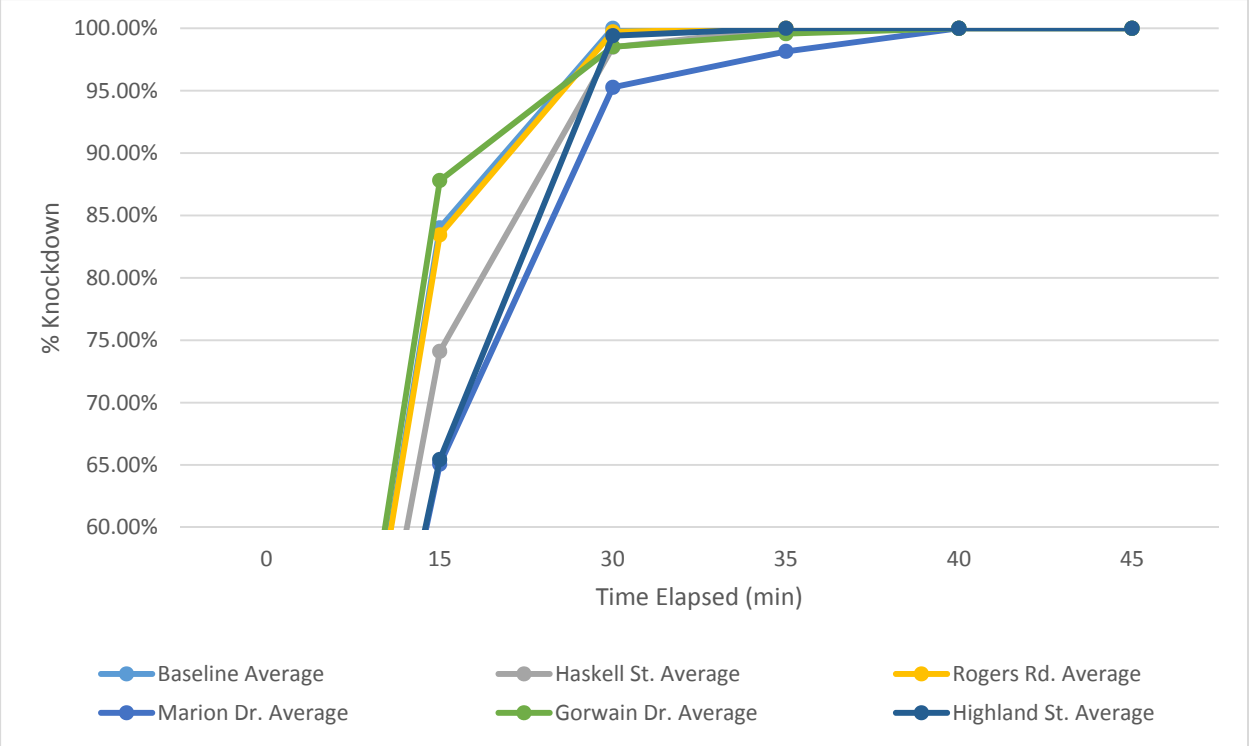


Figure 3: 2017 Time-% Knockdown Curves of Bottle Assays for ANVIL® 10+10 (22.17µg/ml)



DISCUSSION

Resistance surveillance using bottle assays continue to indicate that the level of resistance in the mosquito populations from the CMMCP service area does not warrant a change of pesticide or application protocol at this time. The CMMCP adulticide program is primarily request-only applications in localized, targeted areas, which may contribute to these findings. Additionally, the CMMCP service area includes 41 cities and towns that are not completely contiguous. Within this region are non-member municipalities that have no organized mosquito control program. All of these characteristics lower the potential for local mosquitoes to develop resistance by limiting exposure to synthetic pyrethroids. The quick degradation and low residual nature of the insecticide product may also could contribute to low resistance development.

CMMCP had used Scourge® (resmethrin) (Bayer Environmental Science, Montvale, NJ) (EPA Reg. No. 432-667), for their ULV applications since 1988 before switching to ANVIL® 10+10 in 2007. Zenivex® E20 was utilized as the CMMCP primary adulticide product in the 2016 season, before returning to using ANVIL® 10+10 this past season. All three of these products are synthetic pyrethroids, with only ANVIL® 10+10 and Scourge® containing piperonyl butoxide (PBO) as a synergist (CDC 2010; Petersen 2004). Before using either of those synthetic pyrethroids, CMMCP had been using Malathion, an organophosphate (Nauen 2007). As a diagnostic baseline concentration for the ANVIL® 10+10 product had previously been established by CMMCP, field collected mosquitoes from the CMMCP service area were used this season against this concentration. At the point CMMCP changes products, the resistance surveillance program will make the appropriate alterations and develop the proper investigative dosage for the bottle assays.

Since mosquitoes used for this resistance surveillance are field collected as opposed to lab reared, there is some inherent variability. These captured adult mosquitoes are at various metabolic stages and therefore process synthetic pyrethroid exposure at different rates. Controlled food sources create more regulated and uniform digestion stages when using lab reared mosquitoes for bottle assay testing. Another significant factor in using colonized mosquito populations is that only one specific species of mosquito is tested, opposed to the field collected mosquitoes that may contain numerous species. Bottle assays using field collected mosquitoes may simulated real world results more accurately but the lab reared mosquitoes would have more consistent, specific findings. With the CMMCP laboratory undergoing renovations, it is anticipated that future bottle assays will take place in more consistent, climate controlled conditions, which would benefit this resistance surveillance program.

The continuation of bottle assays in future seasons will provide additional data for resistance management in the CMMCP service area. In conclusion, the cumulative results of the bottle assay research show that the level of resistance in local mosquito populations does not currently warrant a change in protocol or product. The slight decrease in knockdown rate observed in select sites this past season only reinforces the importance of this program moving forward. Resistance surveillance is

essential to ensure current control practices remain effective in protecting the public health.

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