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

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ABSTRACT

During the summer of 2007, the Central Mass. Mosquito Control Project (CMMCP) conducted bottle assays, which test the potency of a substance on live specimens, to determine if pesticide resistance had been developing in local mosquito populations. After using procedures developed by the Center for Disease Control and Prevention (Center for Disease Control and Prevention 2002), the results of naive mosquitoes were compared to those collected from areas serviced by our adulticide program. It was determined that the level of resistance in local mosquito populations does not warrant any procedural or insecticide changes at this time. Of the numerous sites sampled, only one showed a very low degree of resistance. Despite these findings, CMMCP will continue bottle assays of local mosquito populations to monitor the levels of resistance so that if indications of resistance are observed, proper actions could be implemented to ensure control effectiveness.

INTRODUCTION

With environmental changes, mosquito species, as well as other arthropods, have the potential to change their current distribution and bring disease with them to new areas (Brogdon 1998; Simsek 2003). These possible diseases include malaria, dengue, yellow fever and Rift Valley Fever among others (McAbee 2003; Simsek 2003). Faced with these new threats, vector control personnel must be aware of the dynamics of local mosquito species in order to lessen the threat of human infections.

Resistance to pesticides can have a major impact on the abilities of public health officials against arthropod-borne disease (Brogdon 1998). It has been shown that some past agricultural and pest control use of insecticides has led to the development of resistance of these chemicals in select populations of mosquitoes (Rodriguez 2005). This resistance is predicted to be the basis for future reemergence of vector-borne diseases, and also impair the control efforts in these situations (Brogdon 1998).

There are several factors that may have contributed to this development, including the narrowing scope of insecticides available for public health use, along with increasing restrictions from regulatory agencies (Brogdon 1998). Resistance to pyrethroids in particular could be due in part to past use of DDT in some areas, with the resistance mechanism being similar for both (Brogdon 1998; McAbee 2003). This cross-resistance, as observed between pyrethroids and DDT, is becoming more prevalent as the existing resistance mechanisms are being enhanced in the target insects (Brogdon 1998).

Despite research that has shown resistance in specific mosquito species, the actual impact of this on vector control is not known due to several issues. One is the lack of information about the current resistance levels, due in part to the wide variety of surveillance programs and data collection efforts. Another factor, and potentially more important, is that resistance seems to be localized. In one study, certain mosquito populations that were only a few kilometers apart varied greatly on the presence and levels of resistance, including the actual mechanism for the resistance (Brogdon 1998).

These unknowns about the level of resistance in vector species have reinforced the need to study pesticide resistance by CMMCP. The goals of this research will be to create baseline data for control efforts, detect early resistance, and to observe the current effects of control strategies (Brogdon 1998). If resistance is observed, then a change in application rates or a change to a different class of insecticides may need to be considered.

To control adult mosquitoes, CMMCP uses ANVIL® 10+10 (Clarke Mosquito Control Products, Inc., Roselle, IL) (EPA Reg. No. 1021-1688-8329), a synthetic pyrethroid composed of 10% SUMITHRIN® (Sumitomo Chemical Company, Ltd., Osaka, Japan)(d-phenothrin) and 10% piperonyl butoxide (PBO)(Center for Disease Control and Prevention 2002; PHEREC 2001), which is used as a synergist[1]. In this ongoing study to monitor resistance levels in its service area, CMMCP conducted bottle assays in the summer of 2007 for ANVIL® 10+10.

METHODS

The bottle assay procedure used by CMMCP was modeled after the CDC method (Center for Disease Control and Prevention 2002), where a baseline for resistance was established using specimens collected from an area without any historical adulticide exposure. This data could then be plotted against data from mosquito populations in areas where our records show past insecticide usage has occurred. This will determine if any degree of resistance has developed to our current adulticide product.

To start, clean 250ml Wheaton bottles (Wheaton Science Products, Millville, NJ) were lined with 1ml of various concentrations of ANVIL® 10+10 (8.868µg/ml, 22.17µg/ml, 44.34µg/ml, and 88.68µg/ml), which were diluted with pesticide grade acetone (Thermo Fisher Scientific, Inc., Fair Lawn, NJ). Approximately 10-15 field collected mosquitoes were introduced into each bottle by mechanical aspiration and % knockdown was recorded at 5 minute intervals, up to 100% knockdown. For control bottles lined with only acetone, (zero ANVIL® 10+10) % knockdown was observed at 5 minute intervals up to an hour. Each pesticide concentration assay had several trials until a concentration was found that created a timely morality curve that reached total knockdown around 30 minutes. Once the ANVIL® 10+10 baseline concentration was determined, it could be used against the exposed mosquito populations, with control bottles running simultaneously.

The collection of mosquitoes for the bottle assays were facilitated by the use of several CDC light traps (John W. Hock Co., Gainesville, FL), baited with CO_2 at a flow rate of 500ml/min. ABC standard collection nets (Clarke Mosquito Control Products, Inc., Roselle, IL) were used to contain the mosquitoes, along with a simple food source, until resistance testing took place, which was usually within a couple of hours. The mechanical aspiration from the collection cages to the assay bottles was enabled by the use of a flashlight aspirator (BioQuip Products, Inc., Rancho Dominguez, CA).

The baseline mosquitoes were collected from an area located near an organic farm. This site has been an official exclusion property since 2006, but even prior to that CMMCP has no record of using adulticide products there. Once the baseline concentration had been determined using these naive mosquitoes, collections were made at several other sites that had varying number of adulticide events (~2-15) over the previous couple of years. We used six different locations, with two sites having multiple collections and trial sets. These potentially resistant mosquitoes were then run against the baseline concentration from the unexposed population, as well as control bottles coated with only acetone.

After conducting bottle assays on the collected mosquitoes against the baseline concentration, the knockdown percentage was plotted against the time interval to determine if any degree of resistance was forming in these populations compared to those unexposed. If any specimens survived longer than those of the baseline group, this could represent some degree of resistance has developed.

RESULTS

The baseline component of the bottle assay that resulted in the optimal concentration of the ANVIL® 10+10 was 22.17µg/ml, which corresponded with data from previous studies (PHEREC 2001). Using this concentration, it was found that only one assay of eight trial sets had specimens that did not reach 100% knockdown before the 25 minute mark. This particular site, Haskell Street, had an average of 98.9% knockdown at the 25 minute mark, and by the next time interval did reach 100% knockdown. Both Otis Street locations had a slower curve than the rest of the sites, although they still reached 100% knockdown at 25 minutes like the baseline population. As one would expect, the control bottles coated with only acetone had zero knockdown effect (Figures 1, 2).

[1]Synergist- Additional substance that will assist in the elimination of certain resistance mechanisms; PBO synergist eliminates oxidase activity (Center for Disease Control and Prevention 2002).

Figure 1: Time-% Knockdown Curve of Bottle Assay for ANVIL® 10+10 (22.17µg/ml)

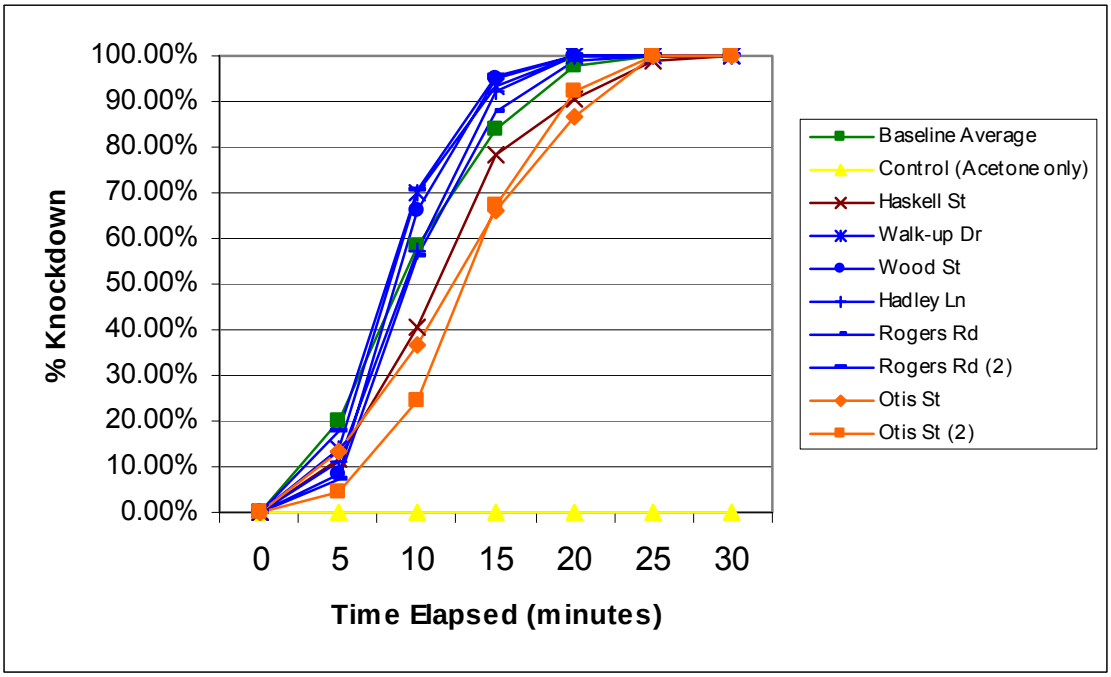
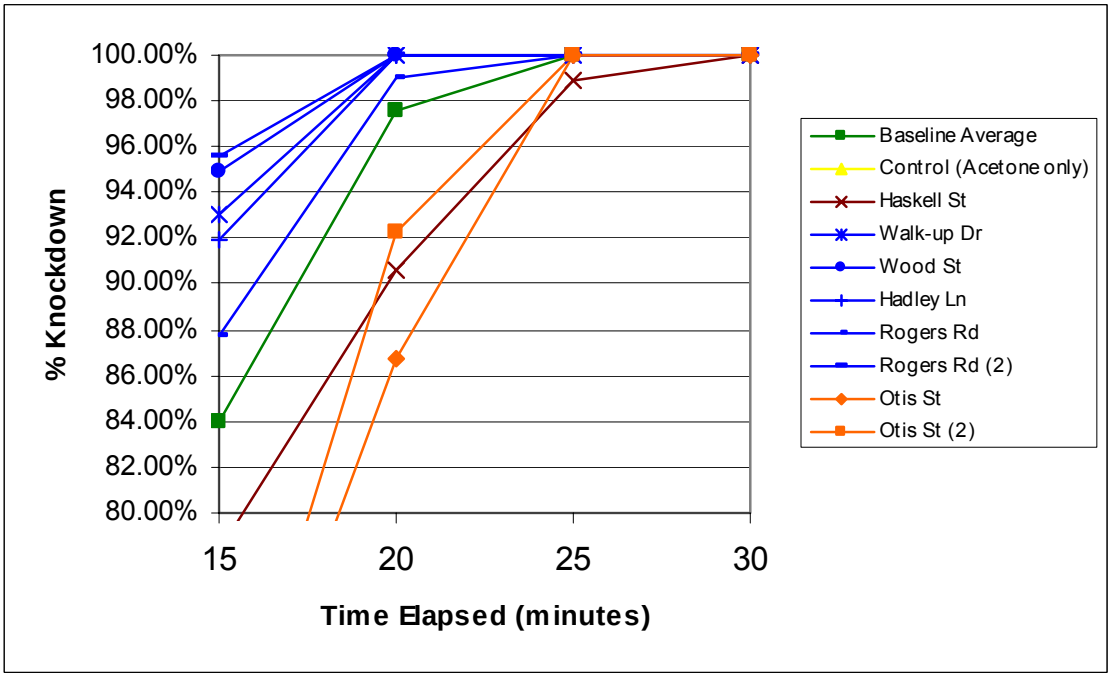


Figure 2: Time-% Knockdown Curve of Bottle Assay (2) for ANVIL® 10+10 (22.17µg/ml)



DISCUSSION

The results of the bottle assays indicate that the level of resistance in the populations of the local mosquitoes tested in the CMMCP service area is not significant enough where a change of pesticide or application protocol is needed at this time. This is not necessarily surprising considering the nature of the CMMCP adulticide program, which is primarily request-only in localized, targeted areas. Another reason would be the vast size of the CMMCP service area, encompassing 39 municipalities, with non-member cities and towns with no mosquito control program scattered in and around them. These factors contribute to local mosquito populations not being consistently exposed to a single class of insecticides, lessening the potential development of resistance. The rapid degradation and low residual nature of the insecticide also could contribute to low resistance development.

CMMCP had used resmethrin (Scourge® Bayer Environmental Science, Montvale, NJ) (EPA Reg. No. 432-667), for their ULV applications since 1988 before switching to ANVIL® 10+10 in 2007. Both products are synthetic pyrethroids. Both insecticides also use piperonyl butoxide (PBO) as a synergist, in different concentrations, with ANVIL® 10+10 using 10% PBO compared to 18% for Scourge® (Center for Disease Control and Prevention 2002; PHEREC 2001). Before using either of those synthetic pyrethroids, CMMCP had been using Malathion, an organophosphate, which is of a different chemical class (Nauen 2006).

Drought conditions in the latter part of 2007 impacted collection numbers, which hindered collections for additional bottle assay trials this season. Future bottle assays would provide more baseline data for resistance management in our service area.

In conclusion, the results of the bottle assay research conducted in the summer of 2007 showed that the level of resistance in the mosquito populations tested does not warrant a change in protocol or product, but monitoring for resistance should continue because it is considered a vital tool in resistance management.

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