Bottle Bioassays Test Resistance to Sumithrin in Central Massachusetts

by Frank H Cornine III

The Central Massachusetts Mosquito Control Project (CMMCP) has conducted bottle bioassays for eight years, beginning in 2005 (excluding 2006), to determine if pesticide resistance has been developing in local mosquito populations. Using procedures recommended by the United States Centers for Disease Control and Prevention (CDC), the results with unexposed mosquitoes were compared to those collected from areas serviced by the CMMCP adulticide program.

With environmental changes, mosquito species have the potential to change their distribution, bringing disease to new areas (Brogdon and McAllister 1998; Simsek 2003). These diseases include malaria, dengue, yellow fever and Rift Valley fever (McAbee *et al* 2003; Simsek 2003). Faced with these new threats, vector control personnel must be aware of the dynamics of local mosquito species in order to reduce the risk of human infection.

Insecticide resistance may have a major impact on the ability of public health agencies to effectively control vector-borne disease (Brogdon and McAllister 1998). Studies have correlated agricultural and pest control insecticide application to resistance development in select populations of mosquitoes (Rodriguez *et al* 2005). Despite research that has shown resistance in specific mosquito populations, the actual impact of this on vector control is not well 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 is that resistance seems to be localized. In a study in Guatemala, certain mosquito populations only a few kilometers apart varied greatly in the presence and levels of resistance, including the actual mechanism for the resistance (Brogdon and McAllister 1998).

These unknowns about the level of resistance in vector populations have reinforced the need to study pesticide resistance at 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. If resistance is observed, then a change in application rate or rotation to a different class of insecticide may need to be considered. CMMCP used resmethrin (Scourge® 18% + 54%, EPA Reg. No. 432-667) for ULV applications since 1988, before switching to sumithrin (Anvil® 10+10 ULV, EPA Reg. No. 1021-1688-8329) in 2007. Both products are synthetic pyrethroids that use piperonyl butoxide (PBO) as a synergist. CMMCP had previously used malathion, an organophosphate, a different chemical class of active ingredient (Nauen 2006).

The bottle assay procedure used by CMMCP is modeled after the CDC method, where a baseline for resistance is established using specimens collected from an area without known pesticide exposure (CDC 2002). This data is then plotted against data from mosquito populations in areas where CMMCP records show past adulticide usage occurred. This determines if any degree of resistance has developed to the current CMMCP adulticide product.

To start, clean 250 ml Wheaton bottles were lined with 1 ml of various concentrations of Anvil 10+10 ULV – 8.868µg/ml, 22.17µg/ml, 44.34µg/ml and 88.68µg/ml (micrograms of active ingredient per milliliter) – which were diluted with pesticide grade acetone obtained from Thermo Fisher Scientific, Inc; see Figure 1. Approximately 10 to 15 field collected mosquitoes were introduced into each bottle by mechanical aspiration. These captured

Figure 1: Equipment used for bottle bioassay tests.

mosquitoes were primarily *Coquillettidia perturbans* (>90%), with *Anopheles quadrimaculatus* and *An punctipennis* also observed at lower frequency; see Figure 2. The percentage knockdown was recorded at 5-minute intervals, up to 100% knockdown. Control bottles were lined with acetone only and percentage knockdown was observed at 5-minute intervals, up to an hour. Several trials were conducted at each insecticide concentration until a concentration was found that created a timely mortality curve that reached total knockdown in around 30 minutes. Once the sumithrin baseline concentration was determined, it could be used against the exposed mosquito populations with control bottles running simultaneously.

Figure 2: Bottle bioassay specimens are counted and identified to species.

Mosquitoes collected for the bottle assays were facilitated by the use of several CDC light traps baited with CO_2 at a flow rate of 500 ml per minute. Standard collection nets were used to collect the mosquitoes, which were provided a simple food source until resistance testing took place, usually within a couple of hours. Flashlight aspirators were used to transfer mosquitoes from the collection cages to the assay bottles.

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 year, CMMCP has no record of using adulticides in this area. Once the baseline concentration had been determined using these historically unexposed mosquitoes, collections were made at several other sites that had received 2 to 15 adulticide events over the previous couple of years. These potentially resistant mosquitoes were then tested against the baseline concentration for the unexposed population, as well as using control bottles coated with only acetone. Over the past seven seasons of resistance surveillance, several collection sites have been used, with slight modifications from year to year depending on habitat and seasonal population changes. The knockdown percentage was plotted against time interval to determine the presence of resistance in these populations, compared to those historically unexposed. If any specimens survived longer than those of the baseline group, this could represent some degree of resistance has developed.

The baseline component of the bottle assays resulted in an optimal Anvil 10+10 ULV concentration of 22.17µg/ml, which corresponded with data from previous studies (Petersen *et al* 2004). Using this concentration, it was found that in 2007 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 by 25 minutes, reaching 100% at 30 minutes. Another site, Otis Street, had a slower curve than the rest, although 100% knockdown occurred at 25 minutes like the baseline population. As one would expect, the control bottles coated with only acetone had zero knockdown effect.

The bottle assay results from the 2013 season were slightly off the baseline averages. Overall, 97% of the specimens were knocked down by 30 minutes, with 93% down by the 25-minute mark. The few remaining individual specimens were knocked down shortly thereafter.

Looking at the yearly totals from the seven seasons of bottle assays, the knockdown rate has been relatively consistent around the baseline average. Two seasons, 2009 and 2013, had knockdown rates that were slightly lower than the baseline average. The acetone-only coated bottles have consistently provided a proper control measure, with negligible knockdown effect, if any; see Figure 3.

The results of the bottle assays continue to indicate that the level of resistance in the populations of the local mosquitoes tested in the CMMCP service area is not significant enough to suggest that a change of pesticide or application protocol is needed. This is not necessarily surprising, considering the nature of the CMMCP adulticide program, which is primarily request-only in localized, targeted areas. The vast size of the CMMCP service area,

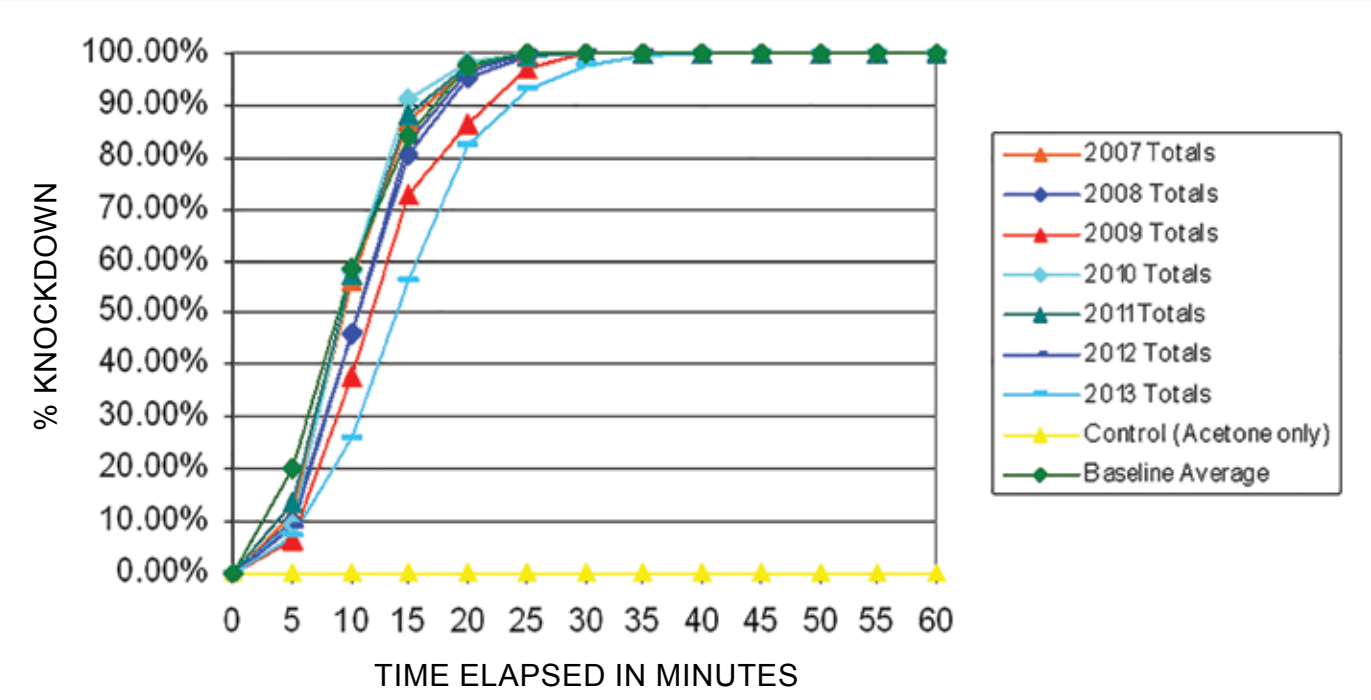

Figure 3: Graph comparing time vs knockdown curves of bottle bioassays using 22.17µg/ml sumithrin, 2007 - 2013.

encompassing 40 cities and towns, with non-member municipalities having no mosquito control programs scattered in and around them, could be another reason. 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 could also contribute to low resistance development.

Data from 2014 is being compiled and analyzed, and will be posted at http://www.cmmcp.org/research.htm when available. At this link you can also see other research and efficacy projects performed at our program since 2007. In addition, memoranda on bottle bioassay techniques using different formulations and active ingredients have been archived at http://floridamosquito.org/Archive/PHEREC/.

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