Bottle Assays of Field Collected Mosquitoes for Level of Resistance to ANVIL® 10+10 in Central Massachusetts (Update 2015)

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ABSTRACT

In 2015 the Central Mass. Mosquito Control Project continued conducting bottle bioassays, which test the potency of a substance on live specimens, to determine if pesticide resistance has been developing in local mosquito populations. Using procedures recommended by the Center for Disease Control and Prevention, the results of unexposed mosquitoes were compared to those collected from areas serviced by the CMMCP adulticide program. This was the ninth season of resistance surveillance by CMMCP in this manner. It was determined that the level of resistance in local mosquito populations does not warrant any procedural or insecticide changes at this time. 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 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 vector-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 if possible.

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)(CDC 2010; Petersen 2004), which is used as a synergist[1]. In this ongoing study to monitor resistance levels in its service area, CMMCP continued conducting bottle assays in the summer of 2015 for ANVIL® 10+10.

METHODS

The bottle assay procedure used by CMMCP was modeled after the CDC method (CDC 2010), where a baseline for resistance is 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 CMMCP records show past insecticide usage has occurred. This will determine if any degree of resistance has developed to the current CMMCP 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

[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).

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 unexposed mosquitoes, collections were made at several other sites that had varying number of adulticide events (~2-15) over the previous couple of years. These potentially resistant mosquitoes were then run against the baseline concentration from the unexposed population, as well as control bottles coated with only acetone. Over the past eight seasons of resistance surveillance, several collection sites have been used, with slight modifications year to year depending on habitat and seasonal population changes.

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 assays that resulted in the optimal concentration of the ANVIL® 10+10 was 22.17µg/ml, which corresponded with data from previous studies (Petersen 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 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).

Figure 1: 2007 Time-% Knockdown Curves of Bottle Assays for ANVIL® 10+10 (22.17µg/ml)

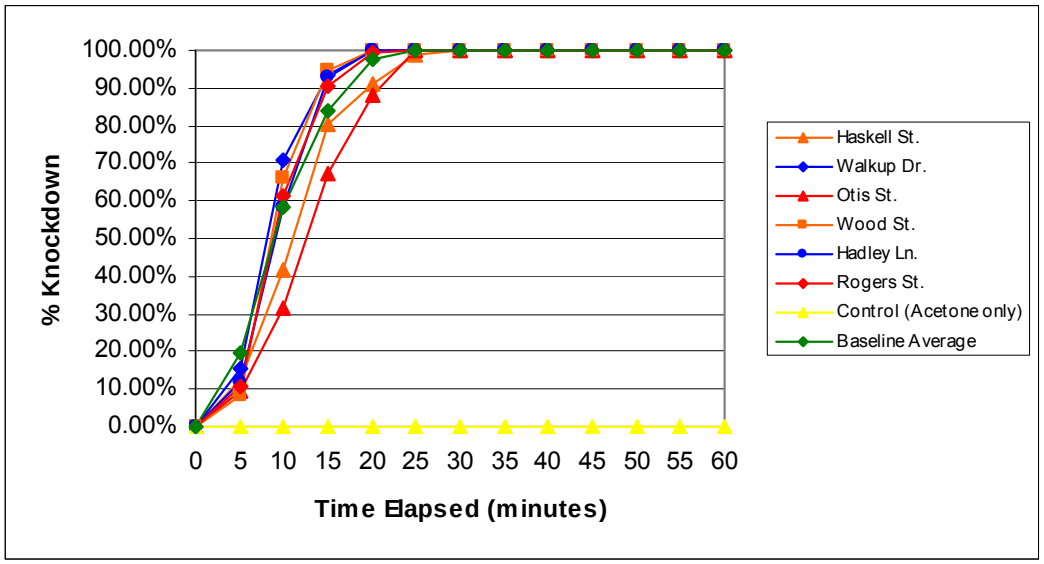
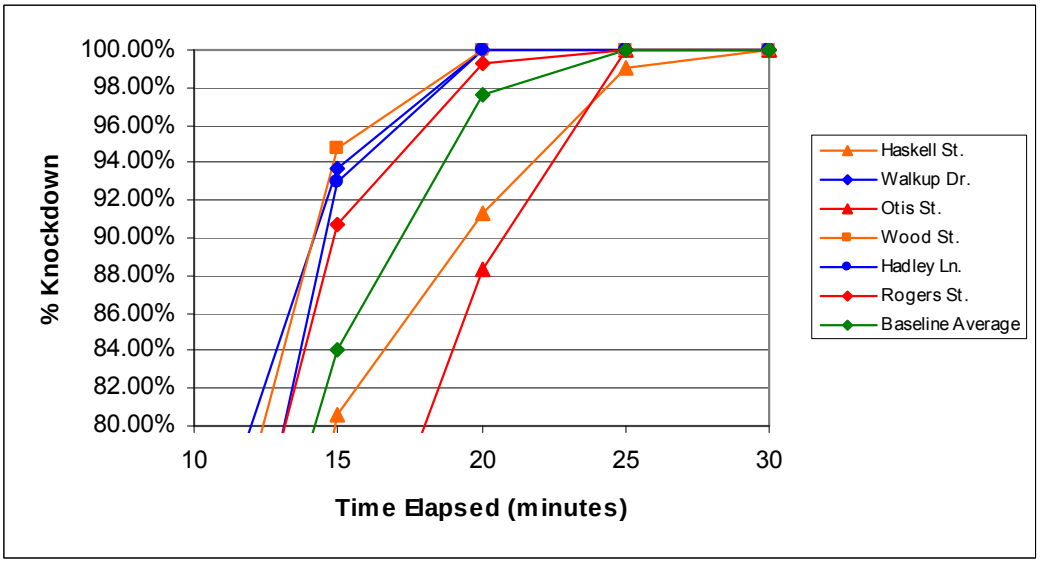


Figure 2: 2007 Time-% Knockdown Curves of Bottle Assays (2) for ANVIL® 10+10 (22.17µg/ml)



The bottle assays preformed in 2008 resulted in similar findings to 2007. Of the 13 trial sets, 6 had specimens that did not reach 100% knockdown by the 25 minute mark. However, these findings were not significant and all had

knockdown rates at the 25 minute mark of over 97.22%. Again, the acetone only coated bottles had zero knockdown effect (Figure 3).

Figure 3: 2008 Time-% Knockdown Curves of Bottle Assays for ANVIL® 10+10 (22.17µg/ml)

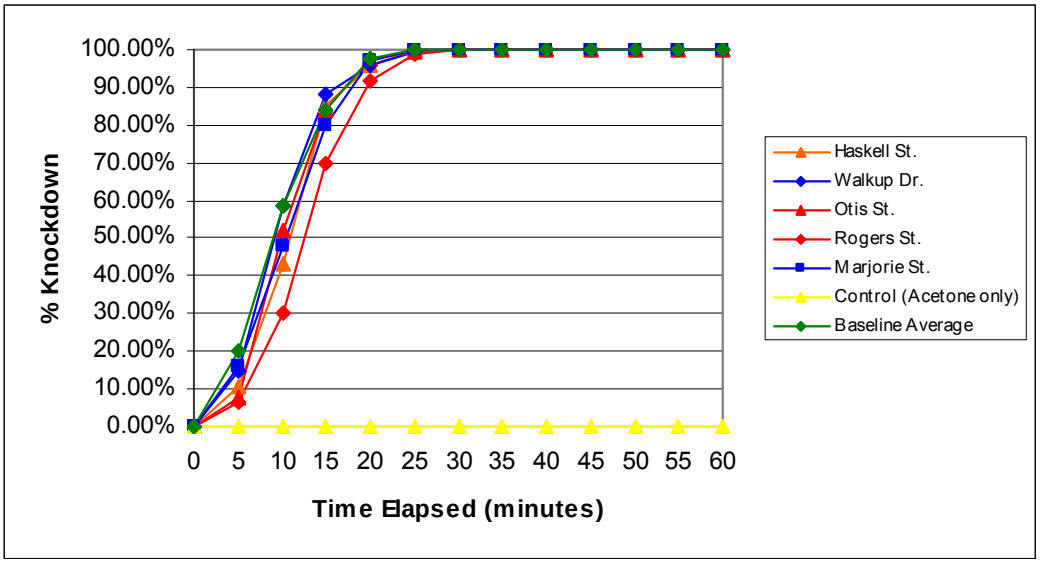
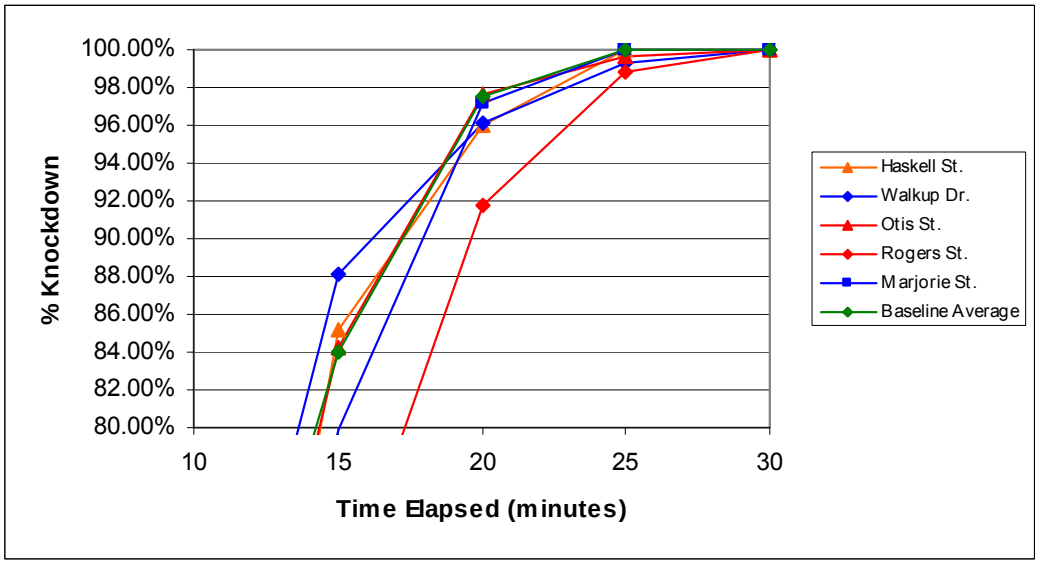


Figure 4: 2008 Time-% Knockdown Curves of Bottle Assays (2) for ANVIL® 10+10 (22.17µg/ml)



Bottle assays preformed in 2009 had trials where the specimens did not reach complete knockdown until the 35 minute mark (Figures 5, 6). Of all specimens tested in the 2009 trials, 99.72% of specimens were knocked down at the 30 minute mark or earlier. As with previous seasons, the acetone only coated bottles had zero knockdown effect (Figure 5).

Figure 5: 2009 Time-% Knockdown Curves of Bottle Assays for ANVIL® 10+10 (22.17µg/ml)

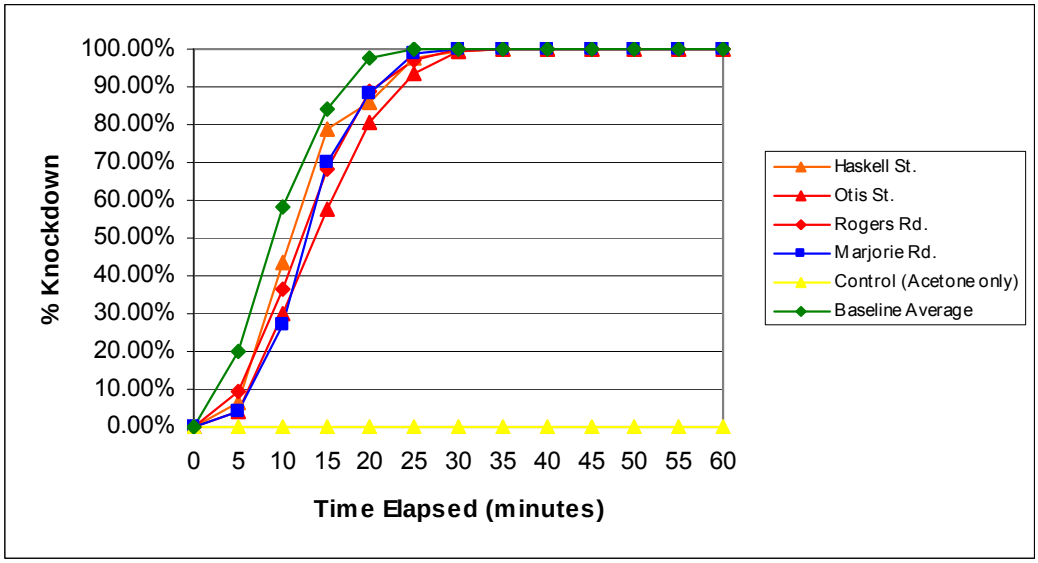
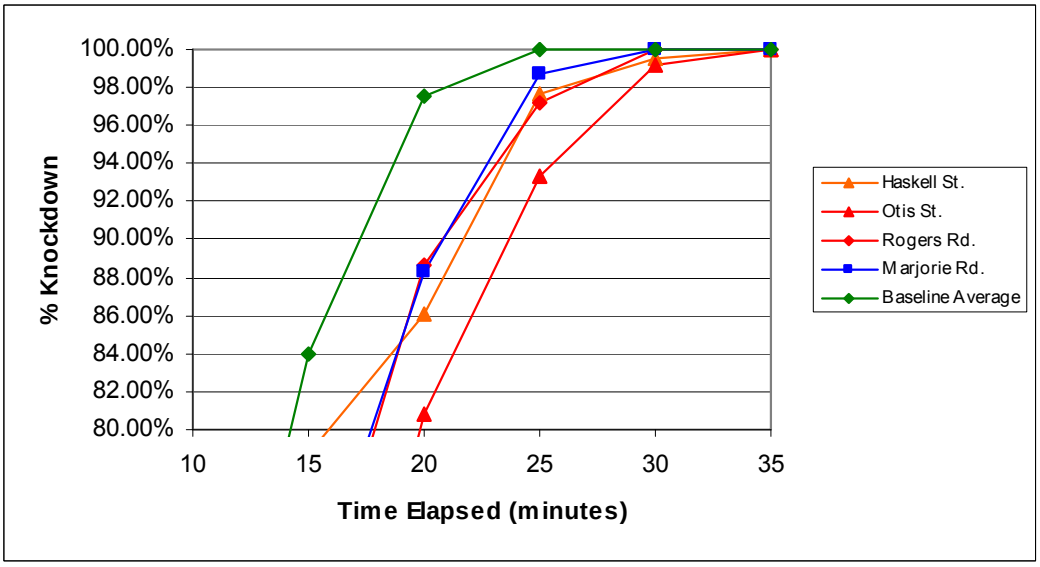


Figure 6: 2009 Time-% Knockdown Curves of Bottle Assays (2) for ANVIL® 10+10 (22.17µg/ml)



The bottle assays performed in 2010 showed an increase in the knockdown rate compared to the previous year (Figures 7, 8). At the 20, 25, and 30 minute mark, the knockdown percentages were 98.52%, 99.86%, and 100% of the specimens respectively. This rate is more consistent with the baseline average and also with the trials conducted in 2007 and 2008. The acetone only control exhibited zero knockdown effect on the specimens (Figure 7).

Figure 7: 2010 Time-% Knockdown Curves of Bottle Assays for ANVIL® 10+10 (22.17µg/ml)

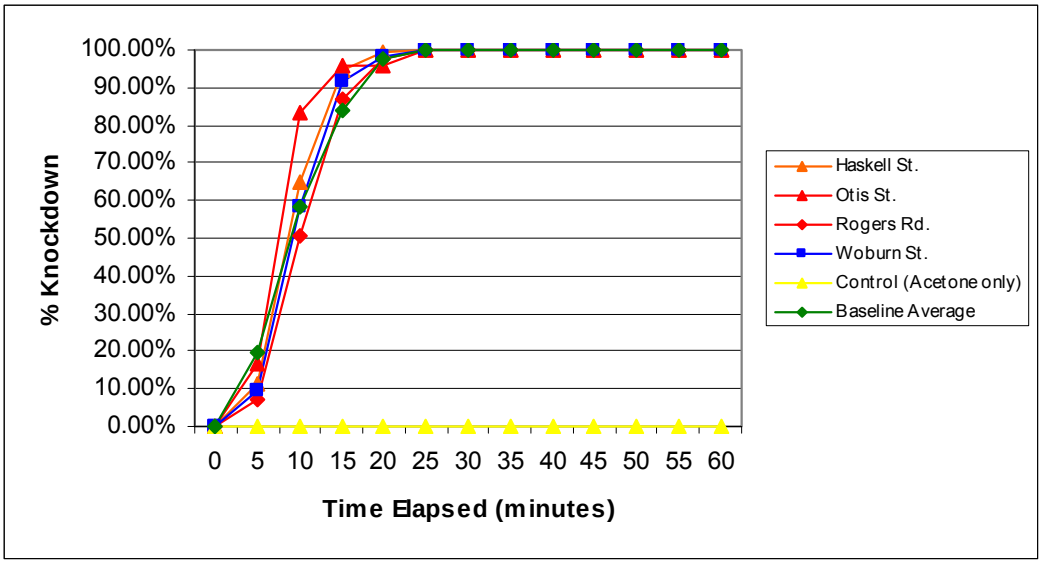
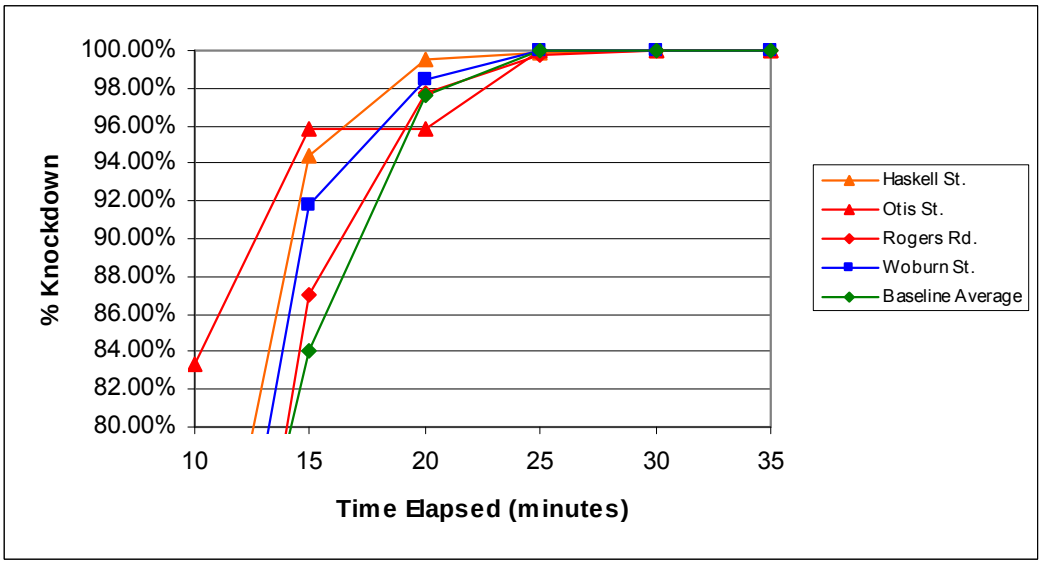


Figure 8: 2010 Time-% Knockdown Curves of Bottle Assays (2) for ANVIL® 10+10 (22.17µg/ml)



The 2011 bottle assays were very similar to the previous year, with all sites within the spectrum of the baseline average (Figures 9, 10). Overall, all of the specimens were knocked down by the 30 minute mark, with 97.60% and 99.69% down at the 20 and 25 minute marks respectively. The control bottles coated with acetone alone had zero knockdown effect as one would expect (Figure 9).

Figure 9: 2011 Time-% Knockdown Curves of Bottle Assays for ANVIL® 10+10 (22.17µg/ml)

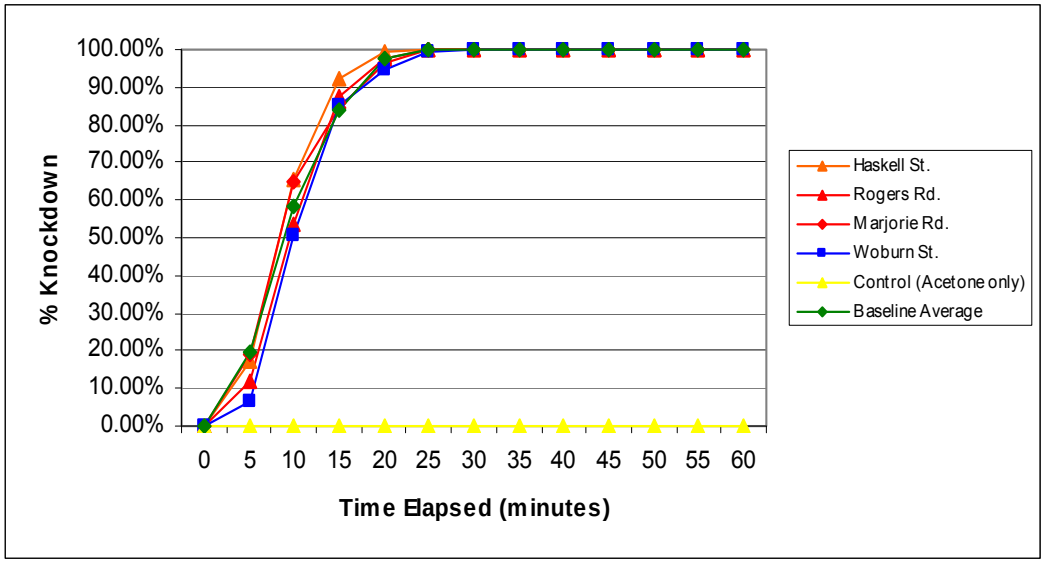
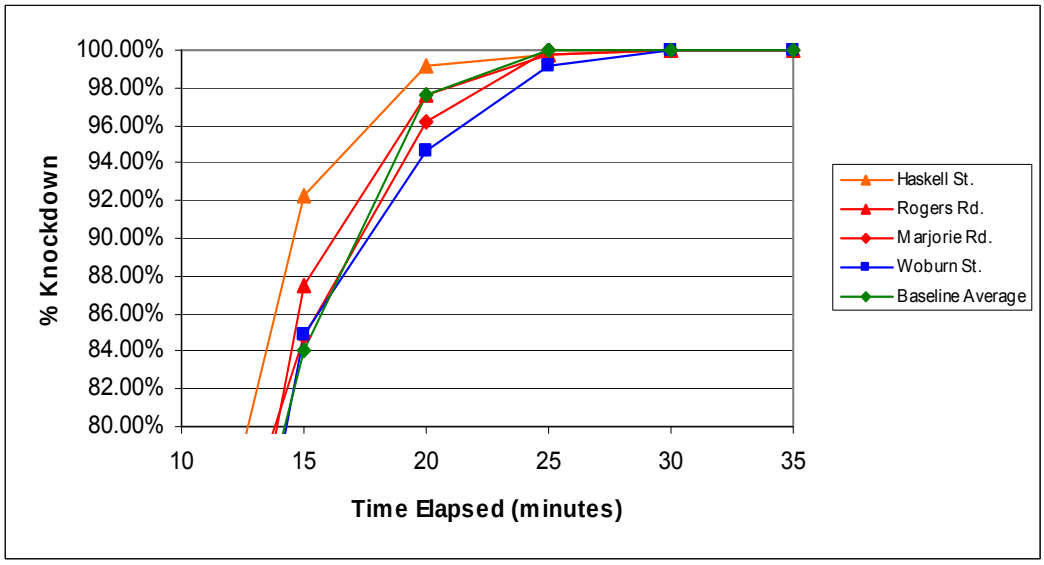


Figure 10: 2011 Time-% Knockdown Curves of Bottle Assays (2) for ANVIL® 10+10 (22.17µg/ml)



The bottle assay results from the 2012 season continued to reflect the baseline averages (Figures 11, 12). Overall, 99.94% of the specimens were knocked down by the 30 minute mark, with 96.23% and 99.74% down at the 20 and 25 minute marks respectively. The acetone only coated bottles had zero knockdown effect (Figure 11).

Figure 11: 2012 Time-% Knockdown Curves of Bottle Assays for ANVIL® 10+10 (22.17µg/ml)

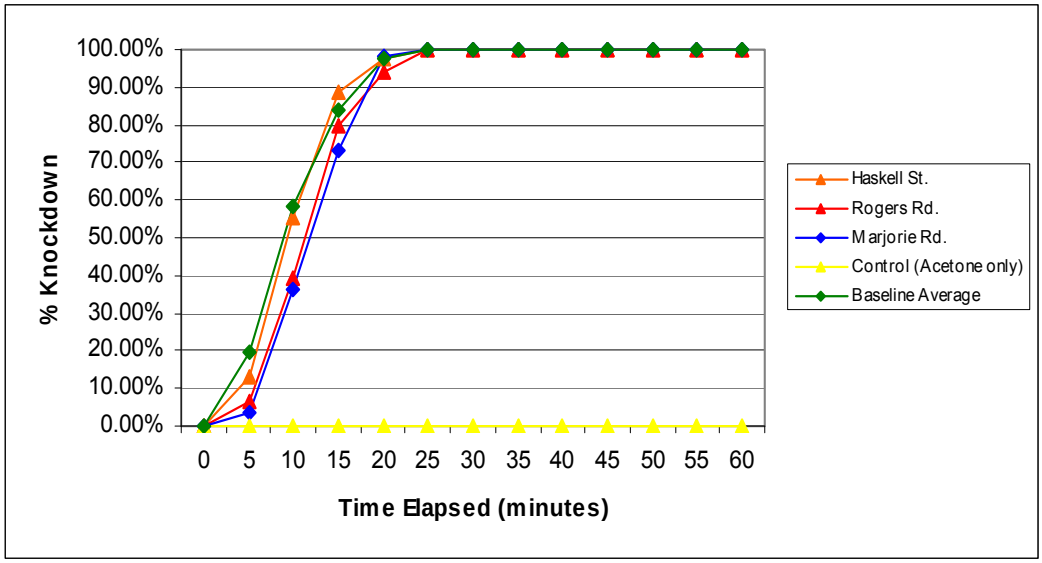
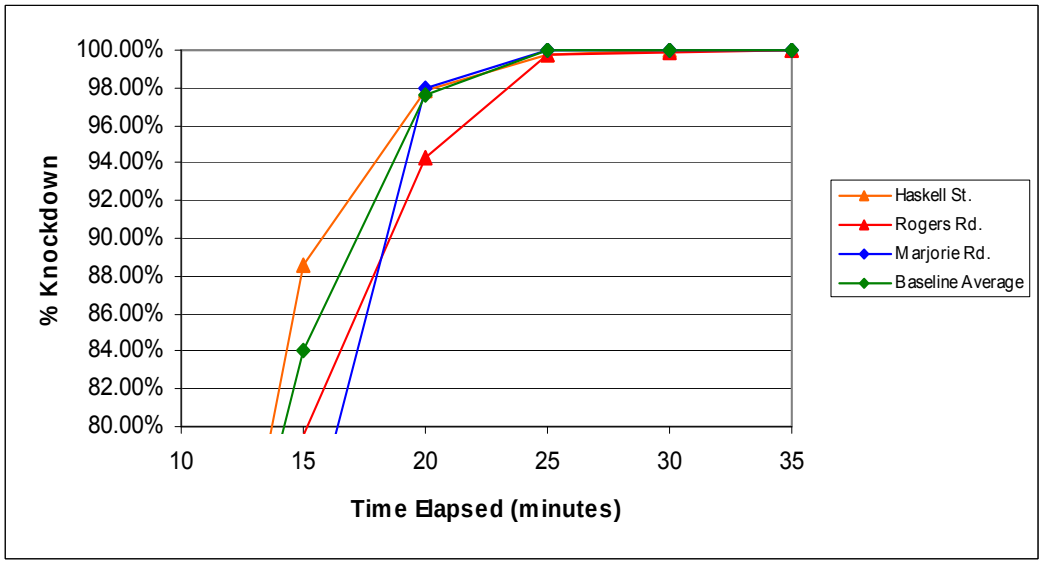


Figure 12: 2012 Time-% Knockdown Curves of Bottle Assays (2) for ANVIL® 10+10 (22.17µg/ml)



The bottle assay results from the 2013 season were slightly off the baseline averages (Figures 13, 14). Overall, 97.78% of the specimens were knocked down by the 30 minute mark, with 93.13% down at the 25 minute mark. The few remaining individual specimens became knocked down shortly after. The acetone only coated bottles had zero knockdown effect (Figure 13).

Figure 13: 2013 Time-% Knockdown Curves of Bottle Assays for ANVIL® 10+10 (22.17µg/ml)

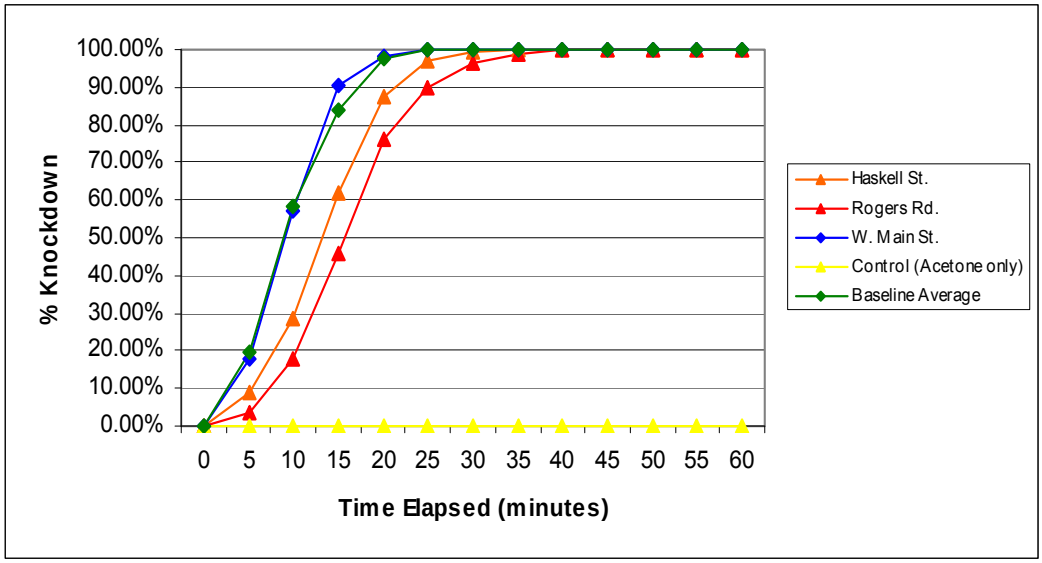
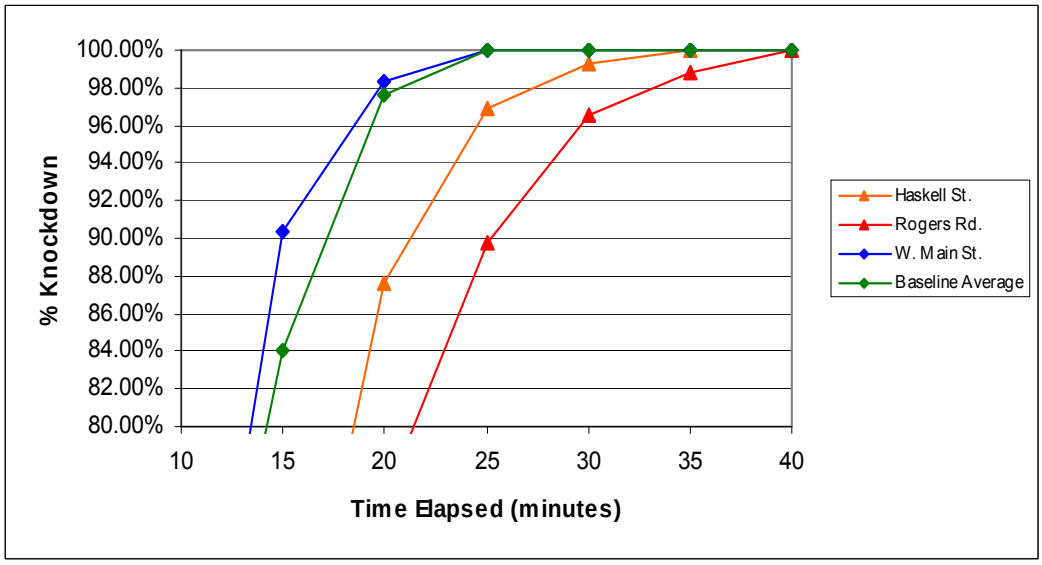


Figure 14: 2013 Time-% Knockdown Curves of Bottle Assays (2) for ANVIL® 10+10 (22.17µg/ml)



The bottle assay results from the 2014 season indicated a slower knockdown curve compared to the original basement average (Figures 15, 16). Despite this reduction, overall there remained a 96.26% knockdown at the 30 minute mark. Few individual mosquito specimens remained after this point for varying amounts of time. The acetone only coated bottles had negligible knockdown effect as the bottle assay control (Figure 15).

Figure 15: 2014 Time-% Knockdown Curves of Bottle Assays for ANVIL® 10+10 (22.17µg/ml)

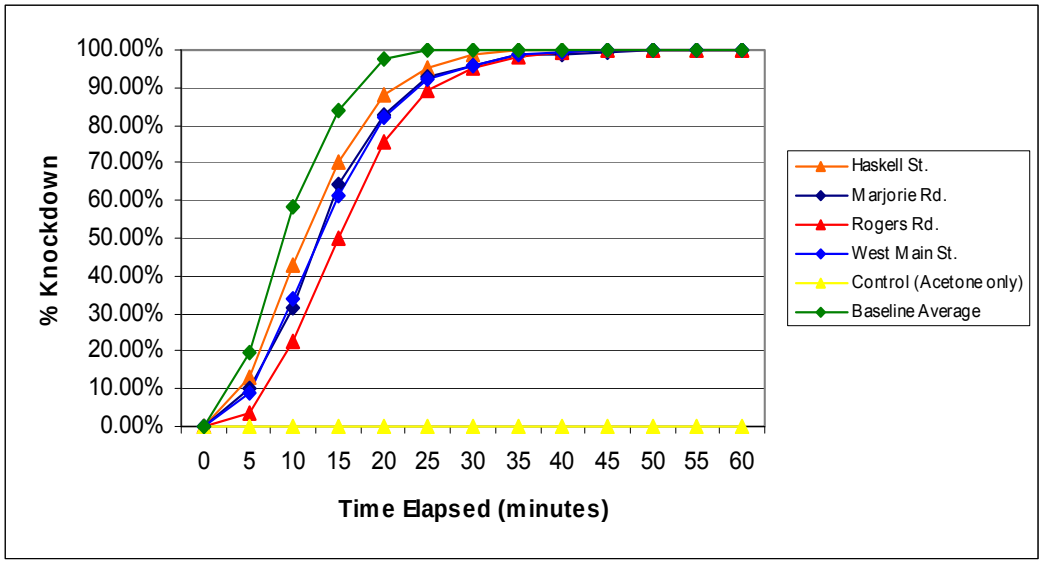
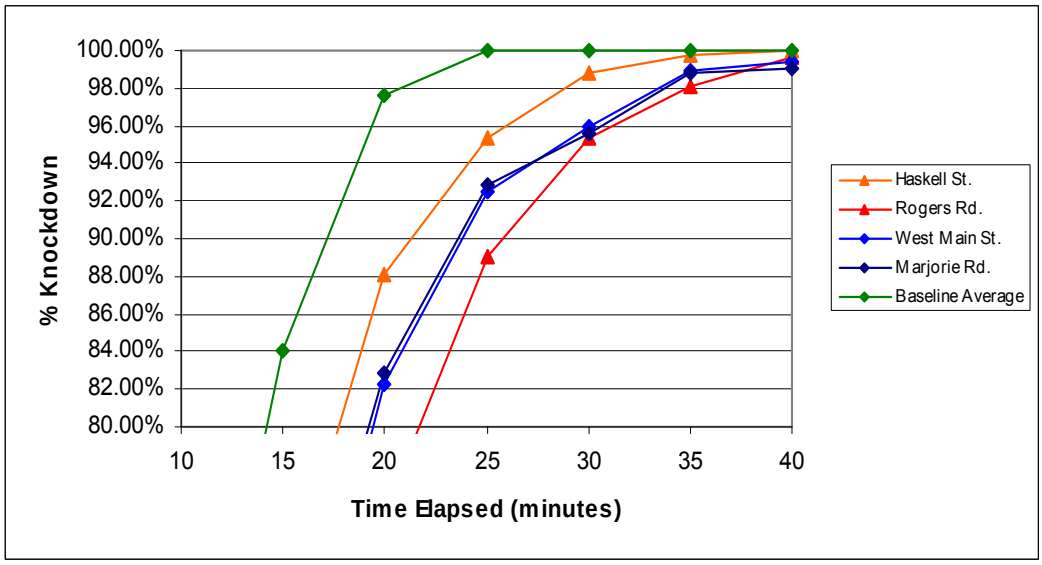


Figure 16: 2014 Time-% Knockdown Curves of Bottle Assays (2) for ANVIL® 10+10 (22.17µg/ml)



The 2015 bottle assay results indicated a slower knockdown curve compared to the original basement average (Figures 17, 18). Despite this reduction, complete knockdown was experienced by the 30 minute mark, with 96.84% down at the 25 minute mark. There was not significant knockdown of specimens within the acetone only control bottles (Figure 17).

Figure 17: 2015 Time-% Knockdown Curves of Bottle Assays for ANVIL® 10+10 (22.17µg/ml)

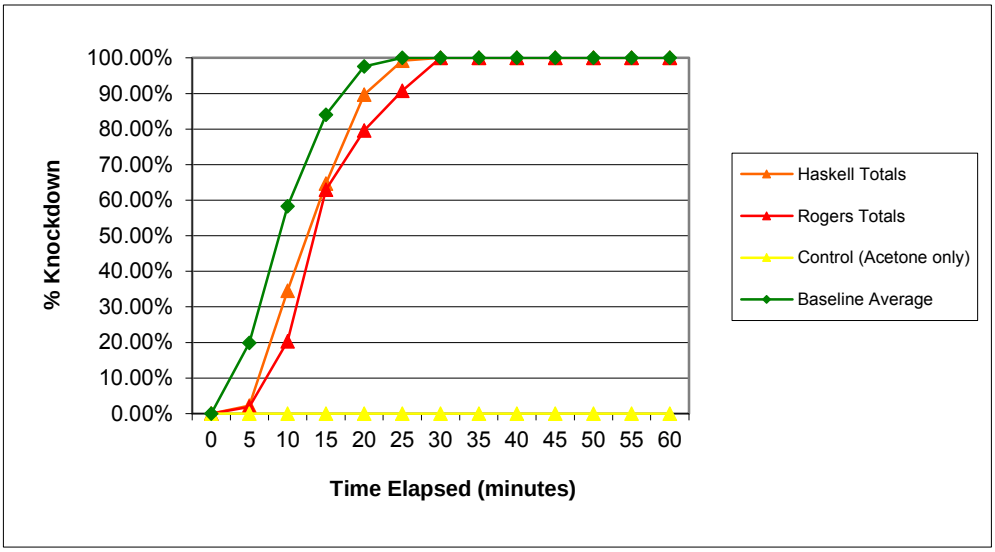
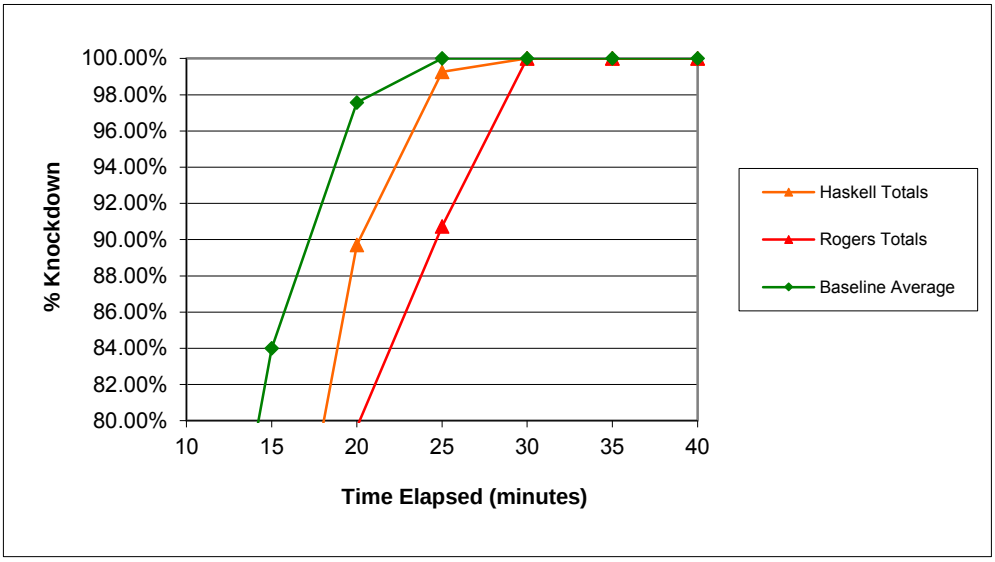
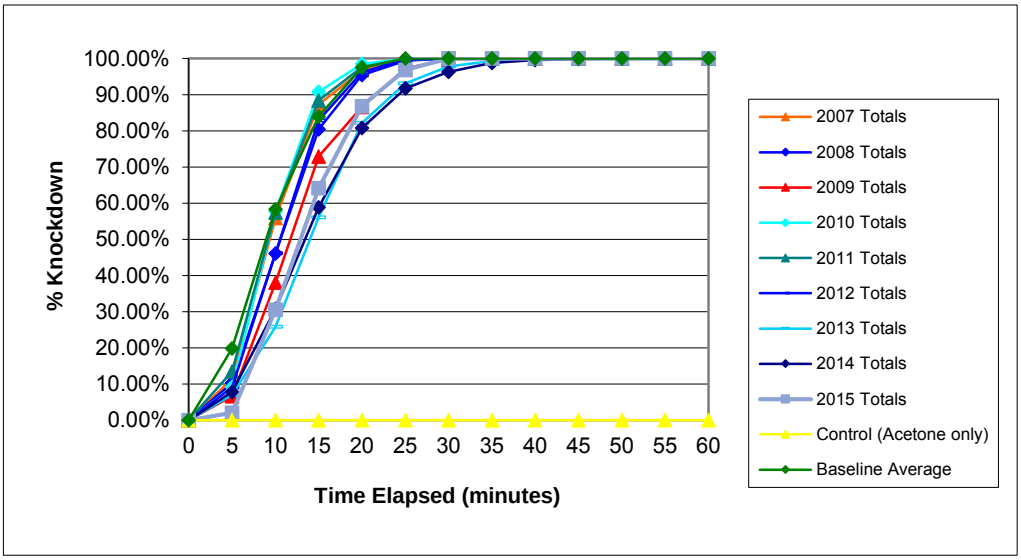


Figure 18: 2015 Time-% Knockdown Curves of Bottle Assays (2) for ANVIL® 10+10 (22.17µg/ml)



Looking at the yearly totals from the nine seasons of bottle assays, one can observe that the knockdown rate has been relatively consistent around the baseline average. Four years, 2009 and the past three seasons 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 observed (Figure 19).

Figure 19: Yearly Comparison of Time-% Knockdown Curves of Bottle Assays for ANVIL® 10+10 (22.17µg/ml)



DISCUSSION

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 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 41 cities and towns, with non-member municipalities having 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® (CDC 2010; Petersen 2004). Before using either of those synthetic pyrethroids, CMMCP had been using Malathion, an organophosphate, which is of a different chemical class (Nauen 2007).

Bottle assays in subsequent seasons will provide additional data for resistance management in the CMMCP service area. In conclusion, the results of the bottle assay

research conducted since 2007 show that the level of resistance in the local mosquito populations tested does not warrant a change in protocol or product. The slight decrease in knockdown rate observed the past three seasons is noted, and only reinforces the importance of this program moving forward. As shown this past season, resistance surveillance is a vital tool to ensure control practices remain effective in protecting the public health.

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Simsek FM. 2003. Seasonal Population Dynamics and Breeding Habitat Diversity of *Culex pipiens* Linnaeus, 1758 (Diptera: Culicidae) in Gölbasi District, Ankara, Turkey. *J Ent Res Soc* 5(1):51-62.