RESISTANCE TO SCOURGE® INSECTICIDE IN THE MOSQUITO POPULATIONS OF FOUR TOWNS IN THE CENTRAL MASS. MOSQUITO CONTROL PROJECT SERVICE AREA: WESTBOROUGH, BILLERICA, TEWKSBURY, AND WILMINGTON

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ABSTRACT

The Central Massachusetts Mosquito Control Project (CMMCP) has been using a synthetic pyrethroid called resmethrin, trade name Scourge®, since the early 1990's to control adult mosquito populations in its service area. The current CMMCP policy is to accept service requests from residents and town officials for adult mosquito control and to perform limited, targeted applications, and not perform random, area-wide spraying as was the standard procedure for adult mosquito control in Massachusetts decades ago. As part of our Standard Operating Procedures manual and as a function of an Integrated Mosquito Management (IMM) plan, surveillance is analyzed before any product is applied to justify the application. This can be in the form of landing rate counts or data collected from mosquito traps. Resistance to a class of chemicals has been noted in other areas of the world, and we will attempt to determine if any mosquito species or any collections of mosquitoes from a given area will show resistance to the synthetic pyrethroid class of chemicals. If resistance is noted, this may affect the product choice for vector-borne disease control, as well as the reduction of nuisance levels of mosquitoes. Initial results from 2005 show a minor potential for resistance, but no change in product usage is recommended at this time. There is no indication that resistance levels will increase due to the limited, sporadic nature of the CMMCP adulticide program, but further study would be prudent.

INTRODUCTION

The purpose of this study was to determine whether or not resistance to resmethrin was developing in the mosquito populations at the most frequently sprayed properties. If significant resistance were present, it could necessitate changing from resmethrin to a different product registered in Massachusetts and accepted by the Centers for Disease Control (CDC) for mosquito control. This would be particularly important in the case of an outbreak of mosquito-borne diseases such as West Nile Virus (WNV) or Eastern Equine Encephalitis (EEE). If a particular species of mosquito or a mosquito collection from a given area has been shown to be resistant to the pyrethroid class of chemicals, then vector suppression may need to be done using different products and procedures. The towns of Westborough, Billerica, Tewksbury and Wilmington were chosen for the study because these are the only towns in the CMMCP service area where WNV or EEE have been found either in mosquitoes, horses or humans. WNV was found in a collection of mosquitoes in Westborough in 2003, and that same year a woman in the area of the virus positive mosquito also contracted WNV. Horses in Billerica and Wilmington were identified to have been infected with EEE in 2004, and mosquitoes positive for EEE were found in Tewksbury in 2002.

METHODS AND MATERIALS

Five study sites were chosen in each town, from among the most frequently sprayed properties in the town according to the CMMCP database of service requests. The frequency of spraying was determined from a database of spray requests from 1998 to the present. Because the towns vary in their overall mosquito population and the number of requests is determined by the property owners, no two sites had exactly the same number of requests in each season. The number of spray requests varied considerably. The most frequently sprayed property had been sprayed 38 times over the eight year period, while the least frequently sprayed property had only been sprayed four times, all in 2004. Most of the properties had been sprayed between 12 and 25 times since 1998. All but two of the properties were at private homes; one was a public recreational area, and another was a wetland area at the end of a cul-de-sac.

The cul-de-sac site was chosen because at that site mosquitoes had been found to be positive for West Nile in 2003 and this was in the neighborhood of the human WNV case in 2003.

A control site was chosen in the town of Westborough in a swamp bordering an organic farm. This site has never been sprayed for mosquitoes by CMMCP, and to the best of our knowledge the town and the property owners did not apply any insecticides in that area. It was presumed that mosquitoes from this site would have no resistance to resmethrin having never been exposed to any insecticides.

At each site, live adult mosquitoes were collected using two CDC-style traps (John W. Hock Company) baited with carbon dioxide at 20 psi. The traps were set early in the morning and collected the following morning. Traps were set for one or two nights, depending on how many mosquitoes were collected. The number of mosquitoes tested for each site varied from 30 to 152; for most sites it was approximately 50.

The resistance testing was conducted according to the bottle bioassay procedure described by Brogdon and McAllister[1]. Scourge® insecticide (18% Resmethrin + 54% piperonyl butoxide synergist, lot no. 465-0815) manufactured by Bayer Environmental Science Company was diluted in acetone to make a 0.005% solution, and was evenly applied to coat the insides of 250ml Wheaton bottles (Fisher Scientific Company). Each bottle was coated with 1ml of acetone and 1ml of Scourge solution, containing 9.05 μg of resmethrin and 27.02 μg of piperonyl butoxide. This dosage was determined by testing seven batches of mosquitoes from the control site with Scourge/acetone solutions of different strengths. Controls consisted of bottles coated only with acetone.

After the mosquitoes were aspirated from the trap cage and introduced into the coated bottles, each bottle was checked at five-minute intervals, and the number of mosquitoes knocked down was recorded. A mosquito was considered knocked down if it could not regain a standing position when knocked off its feet by gently tapping or shaking the bottle. Knock-down was chosen as the standard rather than overall mortality because resmethrin may cause the mosquitoes to twitch even after they are dead, making the time of death difficult to determine exactly.

RESULTS

No mosquitoes were knocked down in the acetone-only control bottles. The mosquitoes from the unsprayed control site were all knocked down within ten minutes, with 96% knocked down after only five minutes.

The mosquitoes from the recreational area site survived the longest, reaching 100% knocked down only at 35 minutes. At one other site, 100% were knocked down at 25 minutes. At twelve sites, 100% were knocked down at 20 minutes, and at six sites, 100% were knocked down at 15 minutes. Sample graphs are included in this presentation, and all data is available on the CMMCP website at http://www.cmmcp.org/2005resistance.htm.

DISCUSSION AND CONCLUSIONS

At all of the study sites, the mosquitoes survived longer than those from the control site. The majority of the samples contained some individual mosquitoes that survived at least twice as long as the control mosquitoes. This would seem to indicate that some resistance to resmethrin may be developing in the populations surveyed.

However, another bottle bioassay study of resistance to various insecticides, including resmethrin, found resistant mosquitoes surviving for up to three hours.[2] In comparison, mosquitoes that survive for 20 to 35 minutes do not seem to be very resistant to resmethrin. If resistance is developing in the CMMCP service area, it appears to be at an early stage. A change of insecticide is not recommended at this time, although continued monitoring of resistance would be a wise course of action. Greater resistance could develop at a later date.

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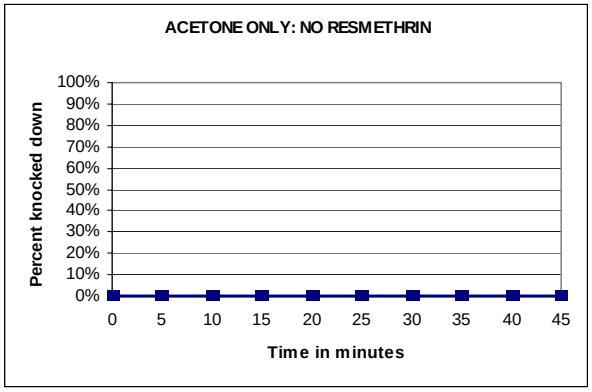
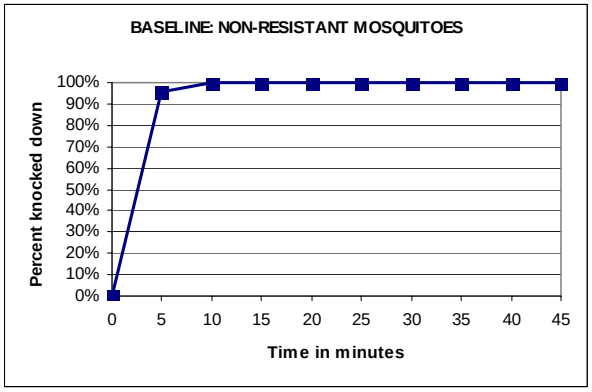
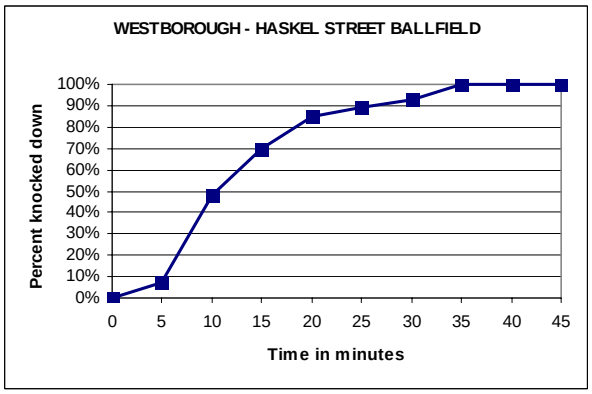
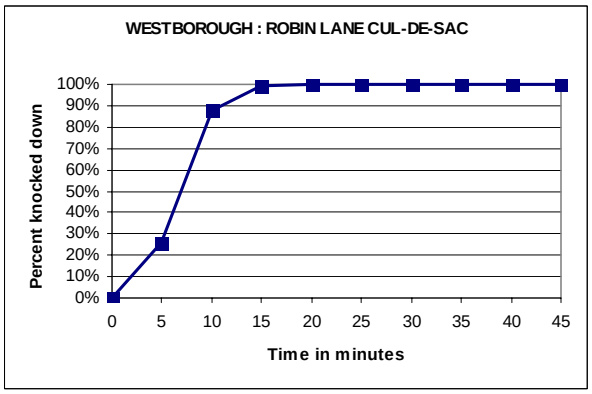
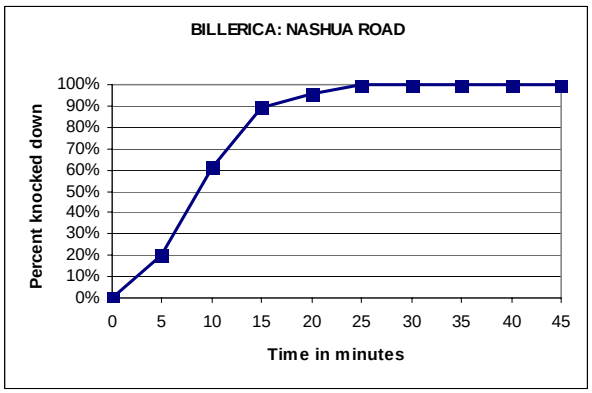
2. McAbee RD, Kang KD, Stanich MA, et al. Pyrethroid tolerance in *Culex pipiens pipiens* var *molestus* from Marin County, California. Pest Manag Sci 60:359-368 (2003).

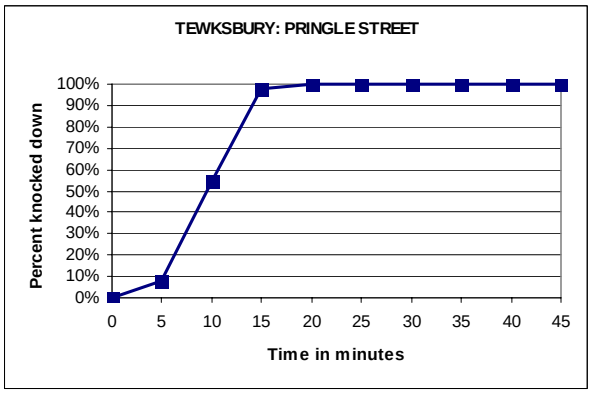












2005 CMMCP LARVAL CONTROL PROGRAM
PRODUCT EFFICACY EVALUATION

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ABSTRACT

The Central Massachusetts Mosquito Control Project (CMMCP) has used the bio-pesticide *Bacillus thuringiensis* var. *israelensis* (Bti) since the late 1980's as the product of choice for mosquito larval control in area wetlands. The product efficacy evaluations performed each year are designed not only to check for Bti efficacy, but to monitor our application methods and amounts. Most areas showed nearly 100% control; some areas done by helicopter required follow up applications by ground equipment around the perimeter of the targeted wetlands. Our website at www.cmmcp.org has detailed information on this program. Bti will continue to be the foundation of our larval control program.

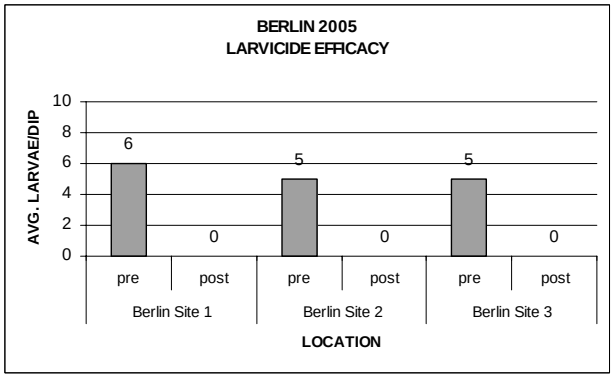
INTRODUCTION

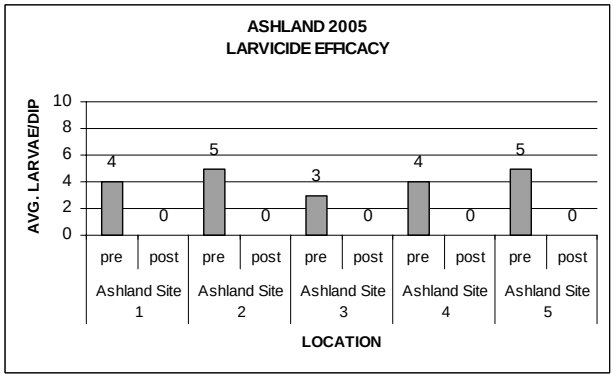
Larval control is the basis of an Integrated Mosquito Management (IMM) plan. Control of mosquitoes in the larval stage can show higher efficacy and lower non-target effects than other control methods, especially adulticiding. Broad-spectrum, chemical products available for larval control such as organophosphates have been used in the past by CMMCP, but we recognize the benefits of biocontrols such as Bti and have chosen this product over other products available for use, such as Abate®. Significant research has been done on Bti by researchers, university staff and mosquito control programs for many years, and the benefit-to-risk ratio is well documented.

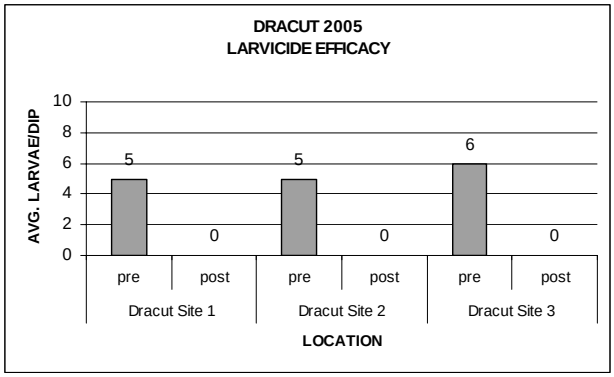
MATERIALS AND METHODS

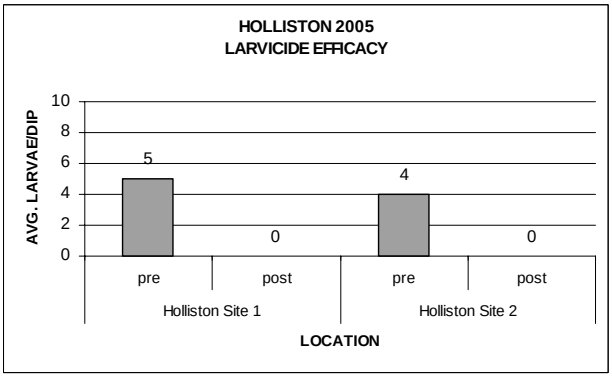
Granular Bti can be applied in a variety ways. It can be broadcast by hand, where the applicator can access areas not available to truck mounted equipment or aerial applications due to a heavy tree cover (canopy). Aircraft, either fixed wing or helicopter, can be used to deliver it to large, widespread areas inaccessible to any truck or hand applications because of size or location.
Label rates for applications are followed closely, and can range from 5 to 20 lbs per acre. Higher rates are recommended when late 3rd and early 4th instar larvae predominate, mosquito populations are high, water is heavily polluted, and/or algae are abundant.

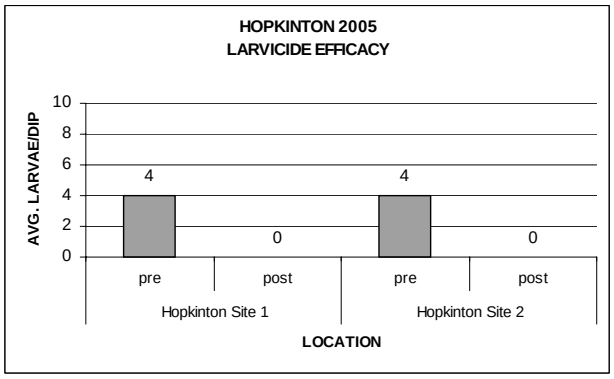
The following graphs show pre- and post treatment data for 29 sites in 8 member towns from 2005. 2 sites are from the aerial program, and the other 6 are from ground applications. Post monitoring data is done from 24 to 48 hours after the original treatment. The website has detailed information on the collection data, with types of area, product amounts, larval instars, etc.

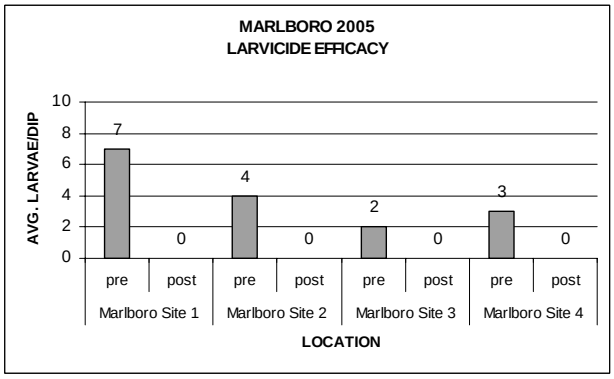


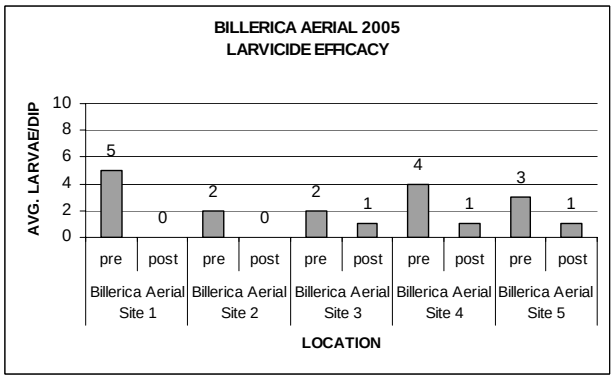


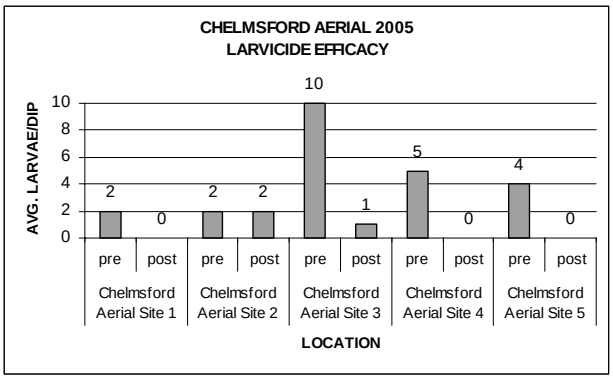












RESULTS & DISCUSSION

All sites done by hand showed 100% control. A wider study of hand applications would certainly show lower efficacy, but 90% on average is not unexpected based on previous results. The aerial application showed a range of 0% to 100% control. The wide range of results is not surprising; our aerial efficacy program is more intensive, with 20 dips per every 250 acres set up as recoverable dip stations. Dip stations near the perimeter of the wetlands, roads or residential areas will not show good control due to the buffer zone employed by the helicopter pilots. Hand applications around the perimeters are often used to increase total efficacy from an area. Surveys conducted by the ground crews during post-monitoring noted that where Bti granular product was observed, very effective larval control was achieved.

NOTE: REFERENCES FOR CLAIMS STATED HEREIN ARE AVAILABLE UPON REQUEST

A PRELIMINARY STUDY OF THE ATTRACTIVENESS OF OVITRAP CUPS IN COLLECTING CONTAINER SPECIES IN MASSACHUSETTS

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ABSTRACT

Using ovitraps to collect species data and to test for efficacy is a technique that has been well documented in many areas of mosquito control and research. A quick search on the Google website (www.google.com) using "Mosquito+Ovitrap" as the keywords turns up a wealth of information. In 2005 we employed ovitrap cups following the US Public Health Service & the Air Force Institute for Operational Health guidelines. Results were not entirely surprising, with *Oc. j. japonicus* as the predominant species, although *Oc. triseriatus* did not appear as anticipated, nor were any *Culex* identified. Ovitraps may be employed in the future by CMMCP to monitor population trends among the container species and for adulticide efficacy studies.

INTRODUCTION

The goal at CMMCP this year was simple and straightforward – will mosquitoes use ovitraps if presented, and what species will we expect to sample? Can ovitraps be used as a device to monitor container species and check for adulticide efficacy?

MATERIALS & METHODS

Ovitraps have been used at CMMCP in the past using coffee cans and seed germination paper. Dark colors are preferred by many container species of mosquitoes for oviposition (AFIOH website, Surveillance Methods/Ovitrap Collections). The coffee cans were painted black, but a source of seed germination paper can be hard to find once available stock is used up. To save on labor and to use materials readily available, the ovitraps will be designed according to the standards written by the US Public Health Service (figure 1). Black plastic cups with the CMMCP logo and "Mosquito Ovitrap" printed on the front were secured. Standard 6" hardwood tongue depressors and 8" natural (unbleached) paper towels were used to make the ovipaddle. A section of paper towel 8" square was cut and folded in half, then wrapped around the tongue depressor and secured at the top and bottom with office staples. A quarter-inch hole should be drilled in the cup 3.5 inches up from the bottom to prevent rainwater from overfilling the ovitrap. The cups may need to be secured in the area using a variety of methods such as a stone on the bottom, wired to a tree, etc.

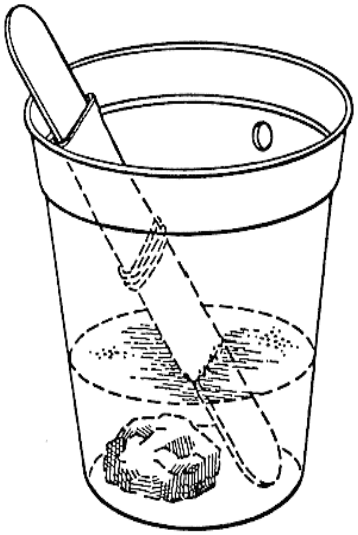

Figure 1

10 ovitraps were placed in a wooded area with other container-breeding sources such as tarps, cans, etc. Each cup was filled with approximately 10 ounces of water from a nearby pond, and the cups were allowed to season for 1 week without the ovipaddle. After 1 week, the ovipaddles were placed in the cups and allowed to remain for 2 additional weeks. After 2 weeks the ovipaddles were collected, and the larvae present in the cups was placed in a single breeding chamber and reared to adult to determine species (table 1). The ovipaddles were allowed to desiccate and then the egg clusters on each ovipaddle were counted (table 2).

Several ovipaddles were submerged in water at a later date to encourage the eggs to hatch with the intent of identifying these to species. However most of the eggs did not successfully hatch, possibly due to extreme desiccation or exposure to high temperatures. The Air Force Institute for Operational Health recommendations are to place each ovipaddle in a sealed plastic bag, which should slow desiccation if the intent is to hatch at a later date (AFIOH website, Sorting, Packaging and Shipping Specimens).

Table 1:

ADULT IDENTIFICATION OF ALL HATCHED LARVAE IN OVITRAPS
Oc. j. japonicus (11 male, 13 female)

Table 2:

OVIPADDLE EGG COLLECTION COUNT			
PADDLE 1:	350+	PADDLE 6:	350+
PADDLE 2:	275+	PADDLE 7:	175+
PADDLE 3:	200+	PADDLE 8:	350+
PADDLE 4:	250+	PADDLE 9:	250+
PADDLE 5:	200+	PADDLE 10:	350+
AVERAGE PER CONTAINER – 275			

CONCLUSION

As expected, *Oc. j. japonicus* dominated the collections. This species prefers cleaner water with less tannins than their counterparts *Oc. triseriatus* and *Culex* (Rutgers University, New Jersey Mosquito/Biology & Control website), and the pond water used would favor this species. If collections of *Oc. triseriatus* and *Culex* are the intended targets as well as *Oc. j. japonicus*, then water containing bacteria and tannins such as a hay infusion used in gravid traps should be used in the ovitraps.
Gravid females seemed to prefer to oviposit the eggs on the folded margins and on the dimples present on the oviposition substrate. The ovitraps collected an average of 275 eggs each over a 2 week period, and may be a useful device to monitor efficacy and to check for population trends among the container species especially if hay infusion water is used. The ovipaddles could also be collected and stored to be hatched at a later time for species composition, educational demonstrations, to check for larvicidal product efficacy, etc.

REFERENCES

Air Force Institute for Operational Health (AFIOH) website:
http://www.brooks.af.mil/afioh/Health%20Programs/rsrh_ent_methods.htm

Rutgers University, New Jersey Mosquito/Biology & Control website:
http://www-rci.rutgers.edu/~insects/njspp.htm

AERIAL MOSQUITO LARVAL CONTROL PROGRAM – SPRING 2007

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ABSTRACT

Aerial application of *Bacillus thuringiensis israelensis (Bti)* has become a standard procedure in many Massachusetts mosquito control programs. CMMCP used rotary aircraft over selected wetlands in Chelmsford, Billerica and Boxborough. Emergence of spring species of mosquitoes was reduced by 86.91% in targeted wetlands.

OBJECTIVE

To reduce the emergence of spring brood mosquito species *Ochlerotatus excrucians* and *Ochlerotatus abserratus* in the towns of Billerica, Chelmsford & Boxborough, with a secondary goal of reducing the early emergence of *Oc. canadensis* from the treated wetlands. *Oc. excrucians* and *Oc. abserratus* emerge in vast numbers from the targeted wetlands and result in significant adulticide requests in the early summer months. *Oc. canadensis* emerge throughout the late spring into summer, and can transmit important diseases like EEE to horses and humans[1].

MATERIALS AND METHODS

Using helicopter applications of a granular form of *Bacillus thuringiensis israelensis* (Bti), a non-reproducing bacteria used for the control of mosquito larvae, the total numbers of spring brood mosquitoes can be reduced. This is the same product used as part of our regular ground larval control program. First registered as an insecticide by the EPA in 1983, this form of the soil bacteria is active for approximately 48 hours and causes toxins in the mosquito larvae gut when ingested[2]. The product chosen for the aerial larvicide was Vectobac G®. The suggested rate range provided by the manufacturer is 2.5-10lbs/acre, with CMMCP applying at 5lbs/acre. This is the standard application rate used by other mosquito control districts in Massachusetts. Maps of the treatment sites using GIS software will be prepared for the contractor. Our staff will collect pre- and post larval surveillance data. Wetlands that were chosen for the aerial application were based on several aspects including historical larval collection data, wetland acreage, resident density, and other factors. A contract has been established with North Fork Helicopters out of Cutchogue, New York. The contract price per acre has been established at $14.15. This contractor has been involved in aerial applications of Bti for several years.

DISCUSSION

Snowmelt and spring weather conditions typically contribute to standing pools of water that lend themselves to become ideal mosquito breeding habitat. Control of mosquito larvae in their aquatic habitat is a major focus of the integrated pest management program employed by the Central Mass. Mosquito Control Project (CMMCP). In response, CMMCP has organized an aerial larval control program using rotary aircraft (helicopter) in addition to the standard ground larval control program performed in all member cities and towns. The towns that participate in this program pay for this service under a separate appropriation than the program of mosquito control that is currently offered. This program is targeted at reducing the numbers of two “spring brood” species, *Ochlerotatus abserratus* and *Ochlerotatus excrucians*, in addition to reducing secondary target species *Ochlerotatus canadensis*. The spring brood species greatly contribute to significant mosquito populations experienced by residents in May through June, and *Oc. canadensis* is a concern for the transmission of EEE and WNV.

Although we have identified potential areas of mosquito breeding, we will only target these areas after confirmation is received from field observations (pre-surveillance). All areas will be sampled using established procedures and protocols established in the mosquito control Generic Environmental Impact Report. An average of 1 mosquito per dip over 10 dips will be used as a threshold to determine if an

[1] http://www.mass.gov/dph/wnv/mosquito_descriptions_arbo_activity.pdf
[2] http://www.epa.gov/pesticides/health/mosquitoes/larvicides4mosquitoes.htm

application in that area is warranted, and we will have at least 1 dip station per 250 treated acres, with a control site as a monitoring device. Once confirmation in a wetland has been recorded, the area will be designated for treatment.

LARVAL MONITORING

Before any applications are made, recoverable dip stations (RDS) were established so that larval monitoring could be conducted at the same location both pre- and post application. In each town, RDS were placed outside the chosen treatment areas to act as a control site along with several RDS inside the treatment areas for mortality comparison. At each RDS there were 10 dip sites, where field technicians would record the number of mosquito larvae as well as their life stage (instar); then the larvae were returned to the RDS. All dip sites were clearly marked so that they could be accurately located and sampled again after the application. Species identified in the larval stage were *Oc. provocons* (potential vector of WNV), *Oc. abserratus* and *Oc. canadensis* (potential vector of EEE). All larvae were in the targeted instar range, 2-3rd instar.

SITE SELECTION

Specific wetland types over 1 acre have been outlined on the maps. These wetland types (wooded swamp deciduous, conifer & mixed; shallow marsh; shrub swamp) have been identified as the preferred habitat for the target species. Any sites under 5 acres that we identify as not suitable for this application will be removed for consideration and monitored and treated if necessary as part of our regular ground larval control program.

THE APPLICATION

Weather and mosquito larval instar are the determining factors in choosing the dates of application. Historically we have performed this program during the third week of April. This timeframe has shown to be a consistent time of year when larvae are in the target life stage, the second and third instar. According to the USGS, the water table in March was at normal levels, but rain in April caused them to rise to above normal[3]. A comment from the USGS website states:

> *"Ground-water levels were generally above normal (highest 25 percent of levels for April) throughout Massachusetts and Rhode Island. New record-high ground-water levels for the month of April were measured in 10 wells in Massachusetts and 10 wells in Rhode Island; each of these wells has 10 or more years of monthly measurements."*

A majority of this rain in April fell during our typical application week (4.1 inches that week compared to 7.02 inches for the entire month) and caused us to postpone the application until the following week. The aerial larvicide took place over the course of two days, April 23rd for the town of Chelmsford, and April 24th for both Billerica and Boxborough. A landing zone in Chelmsford serves as the base of operations for Chelmsford & Billerica, and we secured permission from Stow Airport to use their facility as a base of operations for Boxborough. Approximately 713 acres were treated in Chelmsford, 557 acres in Billerica, and 562 acres in Boxborough. The Vectobac® material was transported to site prior to each application, and CMMCP staff members were on hand to load the helicopter.

Notifications were placed in the Lowell Sun for Chelmsford & Billerica, and The Beacon for the town of Boxborough for the week of April 19, 2007, as required under 333CMR 13.04:

> *"no applications of pesticides by aircraft shall be made unless the following conditions have been met: (a) Notification of the proposed application has been given by the Contracting Entity to the public residing on adjacent lands by publication of a notice in a newspaper of general circulation normally used by the municipality for legal notices not later than two days before the application and no sooner than ten days before application"[4].*

CONCLUSIONS

In general, the application was a success. Overall numbers show an 86.91% reduction in areas where Bti granules were present post-treatment. Control sites showed a 22.52% increase in larval activity. One deficiency noted in the program this year is the lack of Bti granules present in some of the RDS; this can be a result of irregular wetland shapes as well as the buffer zones pilots will employ around roads, yards and other areas of exclusion. For the 2008 application we may change the location or setup of some of the RDS, and will be sure to notify the pilot of the RDS locations.

[3] http://ma.water.usgs.gov/current_cond/cwrc_statements.htm

[4] http://www.mass.gov/agr/legal/regs/pesticides_regulations_list.htm

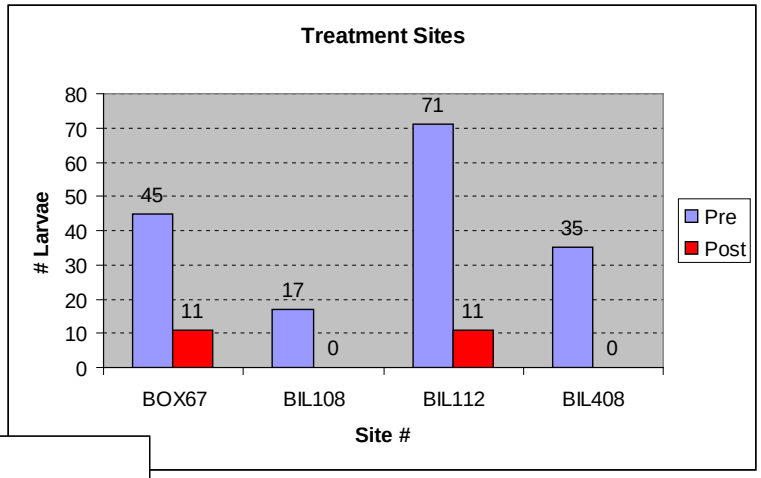
ACKNOWLEDGEMENTS

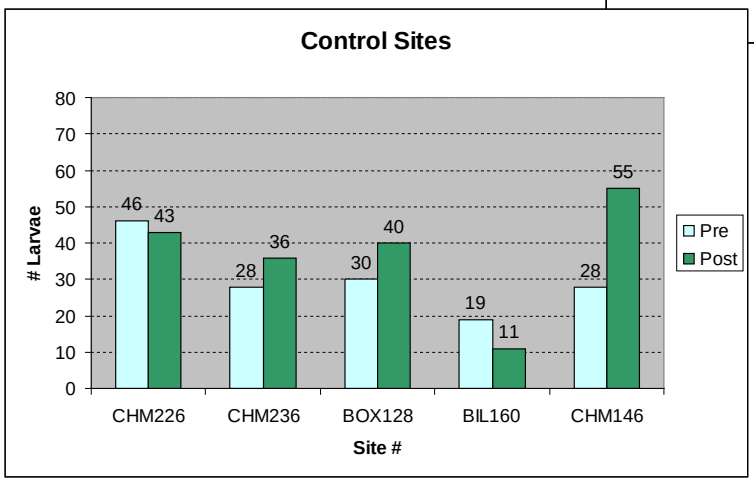
The authors would like to thank the towns of Chelmsford, Billerica and Boxborough; North Fork Helicopters for doing the application; Clarke Mosquito Control Products for supplying the Bti; the CMMCP Commission, and most importantly the CMMCP staff for pre and post monitoring, wetland delineations, map generation and loading the aircraft.

OVERALL RESULTS:

Treatment Sites	24Hr Pre	48Hr Post	Observed Change
BOX67	45	11	- 75.55%
BIL108	17	0	- 100.00%
BIL112	71	11	- 84.51%
BIL408	35	0	- 100.00%
Overall:	168	22	**- 86.91%**
Control Sites	**24Hr Pre**	**48Hr Post**	**Observed Change**
CHM226	46	43	- 6.52%
CHM236	28	36	+ 28.57%
BOX128	30	40	+ 33.33%
BIL160	19	11	- 42.11%
CHM146	28	55	+ 96.43%
Overall:	151	185	**+ 22.52%**

Data shown above is from RDS sites where Bti granules were present post-treatment. Control Sites include data from RDS sites where there was no evidence of Bti granules in area during surveys conducted post-treatment.





CENTRAL MASS. MOSQUITO CONTROL PROJECT

AERIAL MOSQUITO LARVAL CONTROL PROGRAM
~
SPRING 2007

Frank H. Cornine III, *Field Biologist*
Timothy D. Deschamps, *Executive Director*

OBJECTIVE:
To reduce the emergence of spring brood mosquito species *Ochlerotatus excrucians* and *Ochlerotatus abserratus* in the towns of Billerica, Chelmsford & Boxborough, with a secondary goal of reducing the early emergence of *Oc. canadensis* from the treated wetlands. *Oc. excrucians* and *Oc. abserratus* emerge in vast numbers from the targeted wetlands and result in significant adulticide requests in the early summer months. *Oc. canadensis* emerge throughout the late spring into summer, and can transmit important diseases like EEE to horses and humans[1].

MATERIALS AND METHODS:
Using helicopter applications of a granular form of *Bacillus thuringiensis israelensis* (Bti), a non-reproducing bacteria used for the control of mosquito larvae, the total numbers of spring brood mosquitoes can be reduced. This is the same product used as part of our regular ground larval control program. First registered as an insecticide by the EPA in 1983, this form of the soil bacteria is active for approximately 48 hours and causes toxins in the mosquito larvae gut when ingested[2]. The product chosen for the aerial larvicide was Vectobac G®. The suggested rate range provided by the manufacturer is 2.5-10lbs/acre, with CMMCP applying at 5lbs/acre. This is the standard application rate used by other mosquito control districts in Massachusetts. Maps of the treatment sites using GIS software will be prepared for the contractor. Our staff will collect pre- and post larval surveillance data. Wetlands that were chosen for the aerial application were based on several aspects including historical larval collection data, wetland acreage, resident density, and other factors. A contract has been established with North Fork Helicopters out of Cutchogue, New York. The contract price per acre has been established at $14.15. This contractor has been involved in aerial applications of Bti for several years.

DISCUSSION:
Snowmelt and spring weather conditions typically contribute to standing pools of water that lend themselves to become ideal mosquito breeding habitat. Control of mosquito larvae in their aquatic habitat is a major focus of the integrated mosquito management program employed by the Central Mass. Mosquito Control Project (CMMCP). In response, CMMCP has organized an aerial larval control program using rotary aircraft (helicopter) in addition to the standard ground larval control program performed in all member cities and towns. The towns that participate in this program pay for this service under a separate appropriation than the program of mosquito control that is currently offered. This program is targeted at reducing the numbers of two “spring brood” species, *Ochlerotatus abserratus* and *Ochlerotatus excrucians*, in addition to reducing

[1] http://www.mass.gov/dph/wnv/mosquito_descriptions_arbo_activity.pdf
[2] http://www.epa.gov/pesticides/health/mosquitoes/larvicides4mosquitoes.htm

secondary target species *Ochlerotatus canadensis*. The spring brood species greatly contribute to significant mosquito populations experienced by residents in May through June, and *Oc. canadensis* is a concern for the transmission of EEE and WNV.

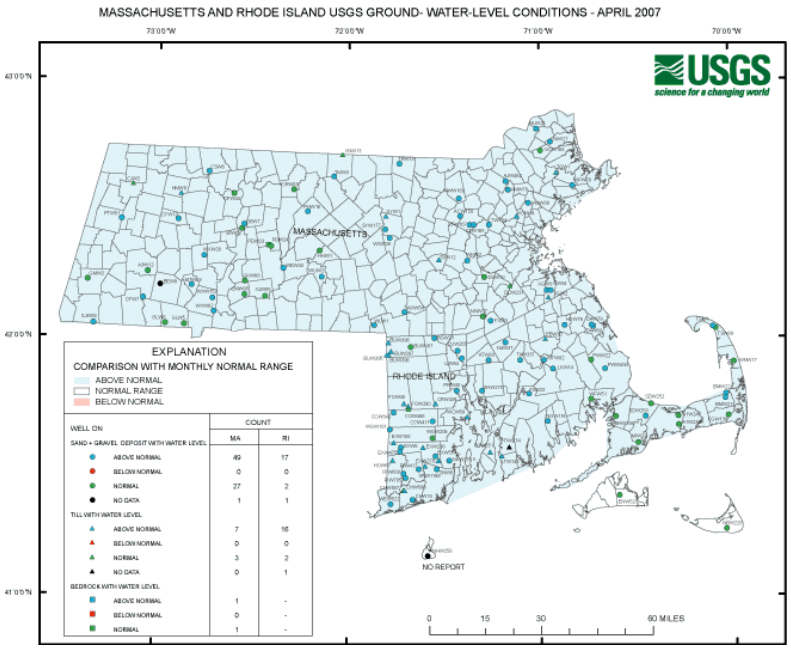
Although we have identified potential areas of mosquito breeding, we will only target these areas after confirmation is received from field observations (pre-surveillance). All areas will be sampled using established procedures and protocols established in the mosquito control Generic Environmental Impact Report. An average of 1 mosquito per dip over 10 dips will be used as a threshold to determine if an application in that area is warranted, and we will have at least 1 dip station per 250 treated acres, with a control site as a monitoring device. Once confirmation in a wetland has been recorded, the area will be designated for treatment.

LARVAL MONITORING:

Before any applications are made, recoverable dip stations (RDS) were established so that larval monitoring could be conducted at the same location both pre- and post application. In each town, RDS were placed outside the chosen treatment areas to act as a control site along with several RDS inside the treatment areas for mortality comparison. At each RDS there were 10 dip sites, where field technicians would record the number of mosquito larvae as well as their life stage (instar); then the larvae were returned to the RDS. All dip sites were clearly marked so that they could be accurately located and sampled again after the application. Species identified in the larval stage were *Oc. provocons* (potential vector of WNV), *Oc. abserratus* and *Oc. canadensis* (potential vector of EEE). All larvae were in the targeted instar range, 2-3rd instar.

SITE SELECTION:

Specific wetland types over 1 acre have been outlined on the maps. These wetland types (wooded swamp deciduous, conifer & mixed; shallow marsh; shrub swamp) have been identified as the preferred habitat for the target species. Any sites under 5 acres that we identify as not suitable for this application will be removed for consideration and monitored and treated if necessary as part of our regular ground larval control program.



THE APPLICATION:

Weather and mosquito larval instar are the determining factors in choosing the dates of application. Historically we have performed this program during the third week of April. This timeframe has shown to be a consistent time of year when larvae are in the

target life stage, the second and third instar. According to the USGS, the water table in March was at normal levels, but rain in April caused them to rise to above normal[3]. A comment from the USGS website states:

> *"Ground-water levels were generally above normal (highest 25 percent of levels for April) throughout Massachusetts and Rhode Island. New record-high ground-water levels for the month of April were measured in 10 wells in Massachusetts and 10 wells in Rhode Island; each of these wells has 10 or more years of monthly measurements."*

A majority of this rain in April fell during our typical application week (4.1 inches that week compared to 7.02 inches for the entire month) and caused us to postpone the application until the following week. The aerial larvicide took place over the course of two days, April 23rd for the town of Chelmsford, and April 24th for both Billerica and Boxborough. A landing zone in Chelmsford serves as the base of operations for Chelmsford & Billerica, and we secured permission from Stow Airport to us their facility as a base of operations for Boxborough. Approximately 713 acres were treated in Chelmsford, 557 acres in Billerica, and 562 acres in Boxborough. The Vectobac® material was transported to site prior to each application, and no problems in loading were noted.

The authors would like to thank the CMMCP Commission, residents and town officials in the towns of Billerica, Boxborough and Chelmsford.

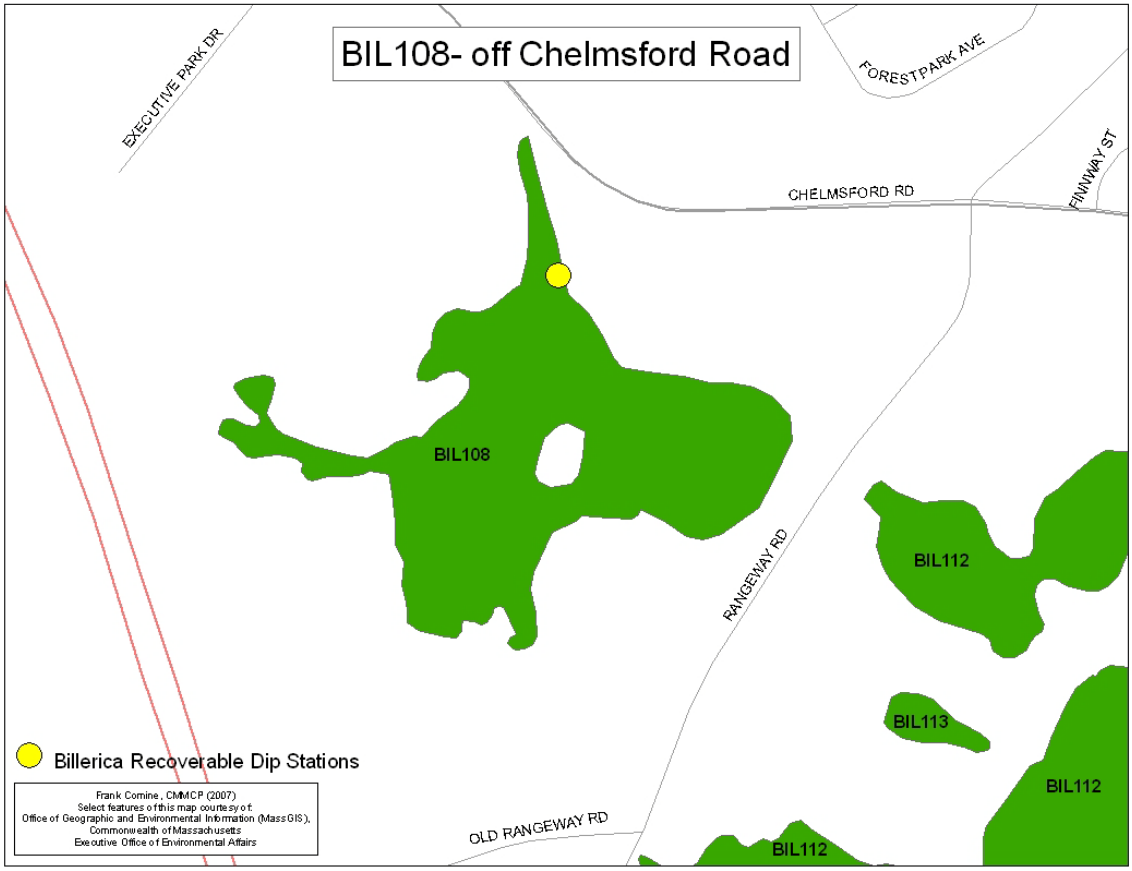
[3] http://ma.water.usgs.gov/current_cond/cwrc_statements.htm

OVERALL RESULTS:

Treatment Sites	24Hr Pre	48Hr Post	Observed Change
T1	45	11	-75.55%
T3	17	0	-100.00%
T4	71	11	-84.51%
T5	35	0	-100.00%
Overall:	168	22	-86.91%
Control Sites	**24Hr Pre**	**48Hr Post**	**Observed Change**
C1	46	43	-6.52%
C2	28	36	28.57%
C3	30	40	33.33%
C4	19	11	-42.11%
C5	28	55	96.43%
Overall:	151	185	+22.52%

Data shown above is from RDS sites where Bti granules were present post-treatment. Control Sites include data from RDS sites where there was no evidence of Bti granules in area during surveys conducted post-treatment.

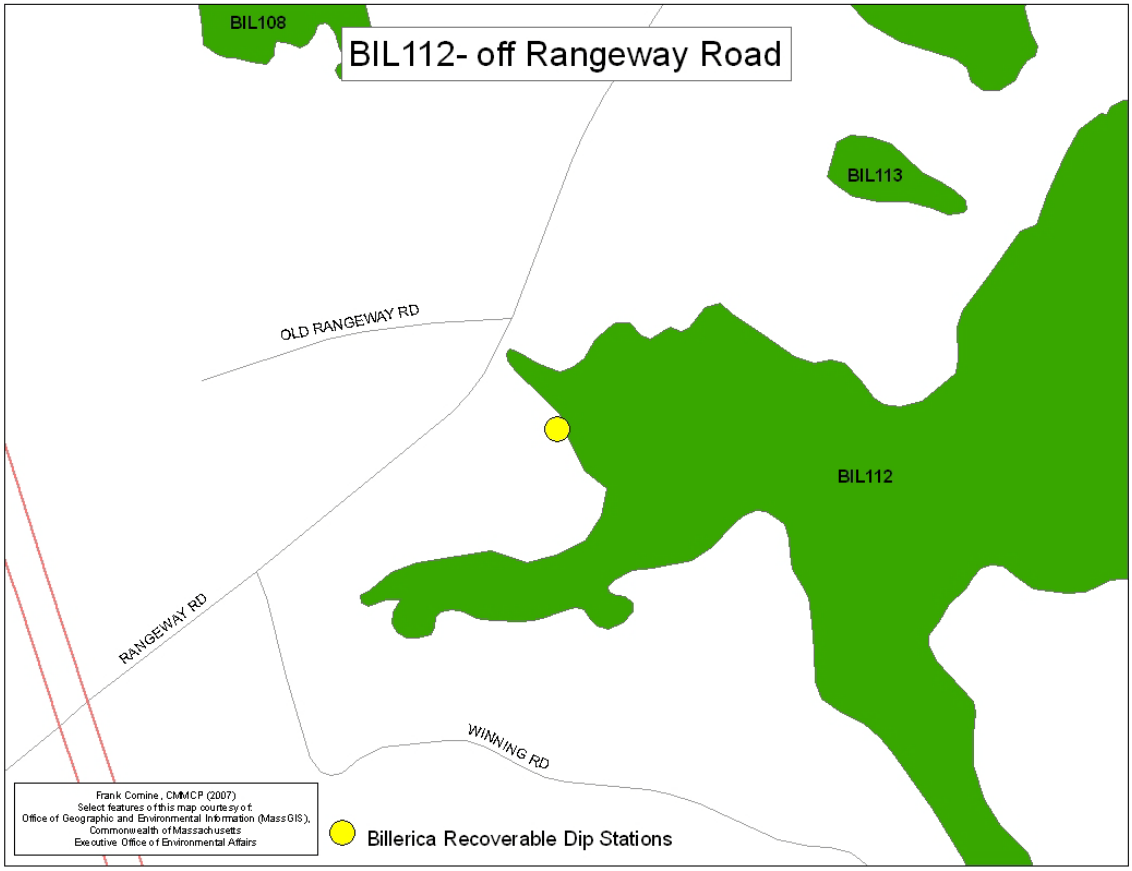
Town of Billerica
Recoverable Dip Stations Data



This dip station showed excellent efficacy, exhibiting 100% reduction in mosquito larvae form the pre-application survey to the 48 hours post-application. There were Bti granules present at the RDS during the post survey

Dip Site #	18-Apr	23-Apr	24-Apr	25-Apr	26-Apr
1	1	3	Application	0	0
2	1	1		0	0
3	1	4		0	0
4	2	1		0	0
5	0	1		0	0
6	0	2		0	0
7	0	1		1	0
8	0	1		0	0
9	0	1		1	0
10	0	2		0	0
Total:	**5**	**17**		**2**	**0**

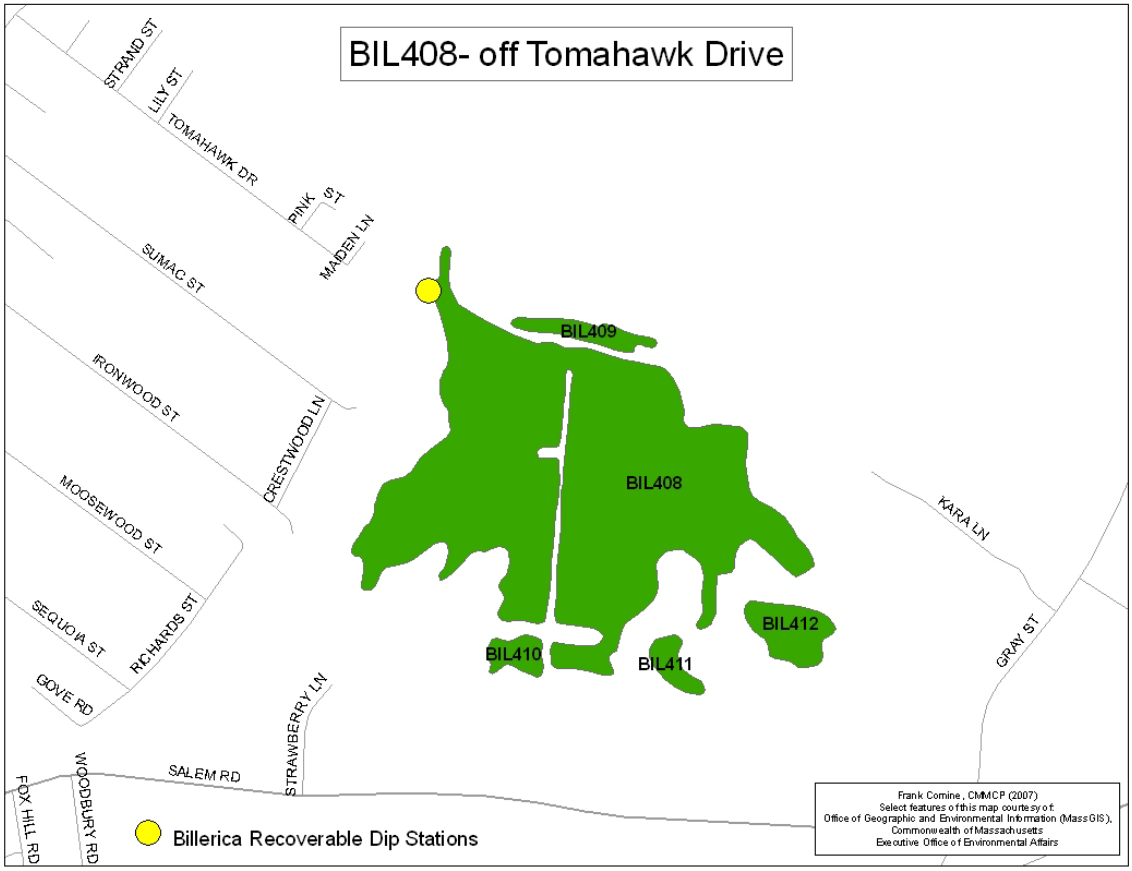
Town of Billerica
Recoverable Dip Stations Data



At this RDS there was an 84.51% reduction in mosquito larvae, although no 48 hour post survey was completed. If allowed another 24 hour period, the Bti granules may have increased this larvae reduction even further. Field technicians reported the presence of Bti granules at BIL112.

Dip Site #	23-Apr	24-Apr	25-Apr
1	5	Application	10
2	8		0
3	7		1
4	12		0
5	7		0
6	7		0
7	1		0
8	5		0
9	12		0
10	7		0
Total:	**71**		**11**

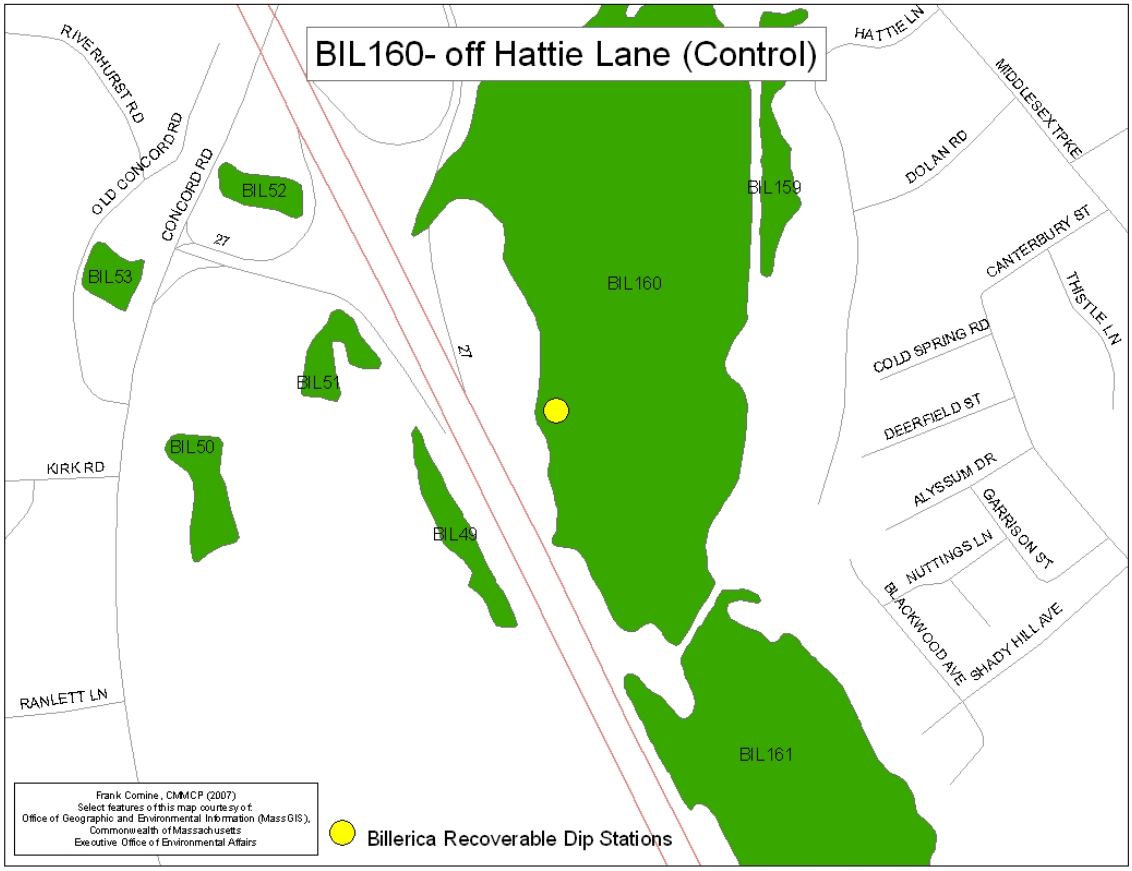
Town of Billerica
Recoverable Dip Stations Data



Similar to BIL108, this site had a 100% reduction in mosquito larvae although this site reached it only 24 hours after application. As with the other two Billerica test sites, Bti was visibly present.

Dip Site #	18-Apr	23-Apr	24-Apr	25-Apr
1	0	2	Application	0
2	0	5		0
3	0	1		0
4	0	2		0
5	0	5		0
6	0	3		0
7	0	5		0
8	0	8		0
9	0	2		0
10	0	2		0
Total:	**0**	**35**		**0**

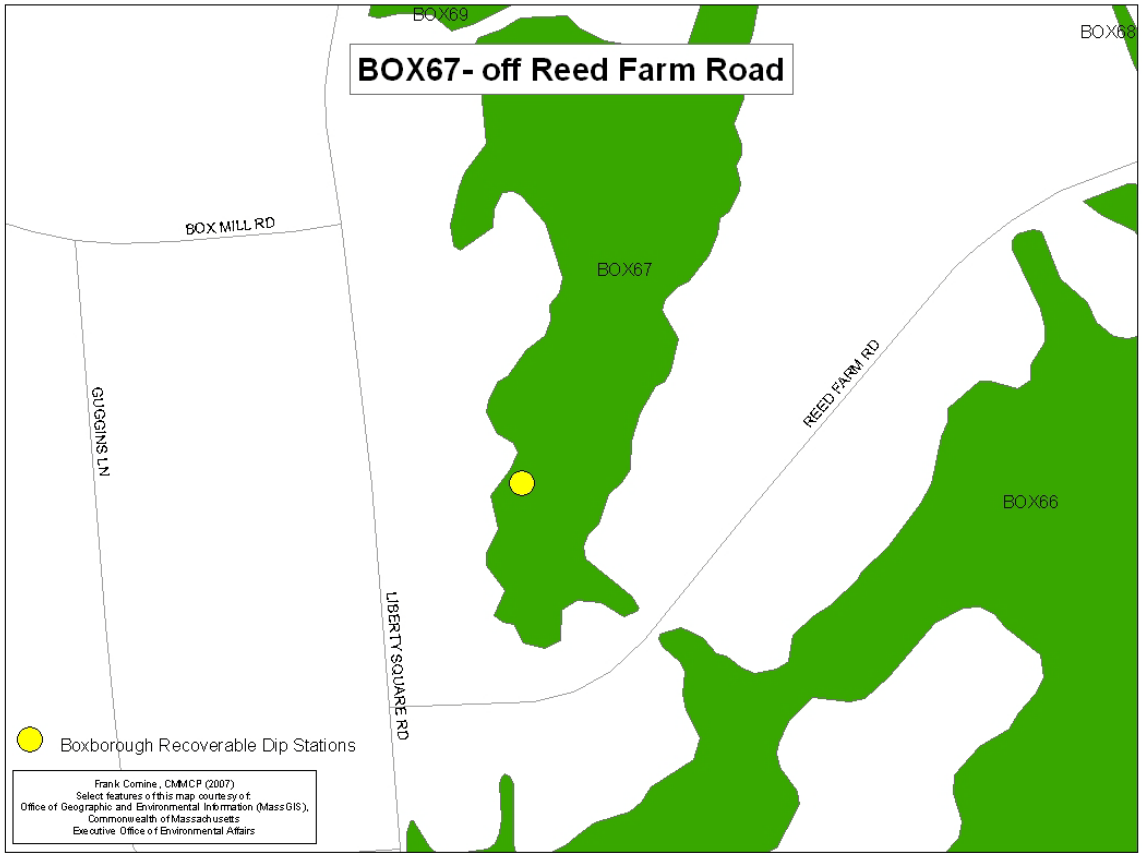
Town of Billerica
Recoverable Dip Stations Data



This RDS was setup as the designated control site for the town. This wetland was not treated in the aerial application, but had 42.11% fewer mosquito larvae post application. There were no observed dead larvae in the samples, as is the case in many of the treated sites. Because of this absence, one could speculate that the reduction may be due in part to larval hatching.

Dip Site #	18-Apr	23-Apr	24-Apr	25-Apr
1	0	1	Application	0
2	0	3		1
3	0	4		0
4	0	1		3
5	0	2		0
6	0	1		4
7	0	1		2
8	0	2		0
9	0	1		1
10	0	3		0
Total:	**0**	**19**		**11**

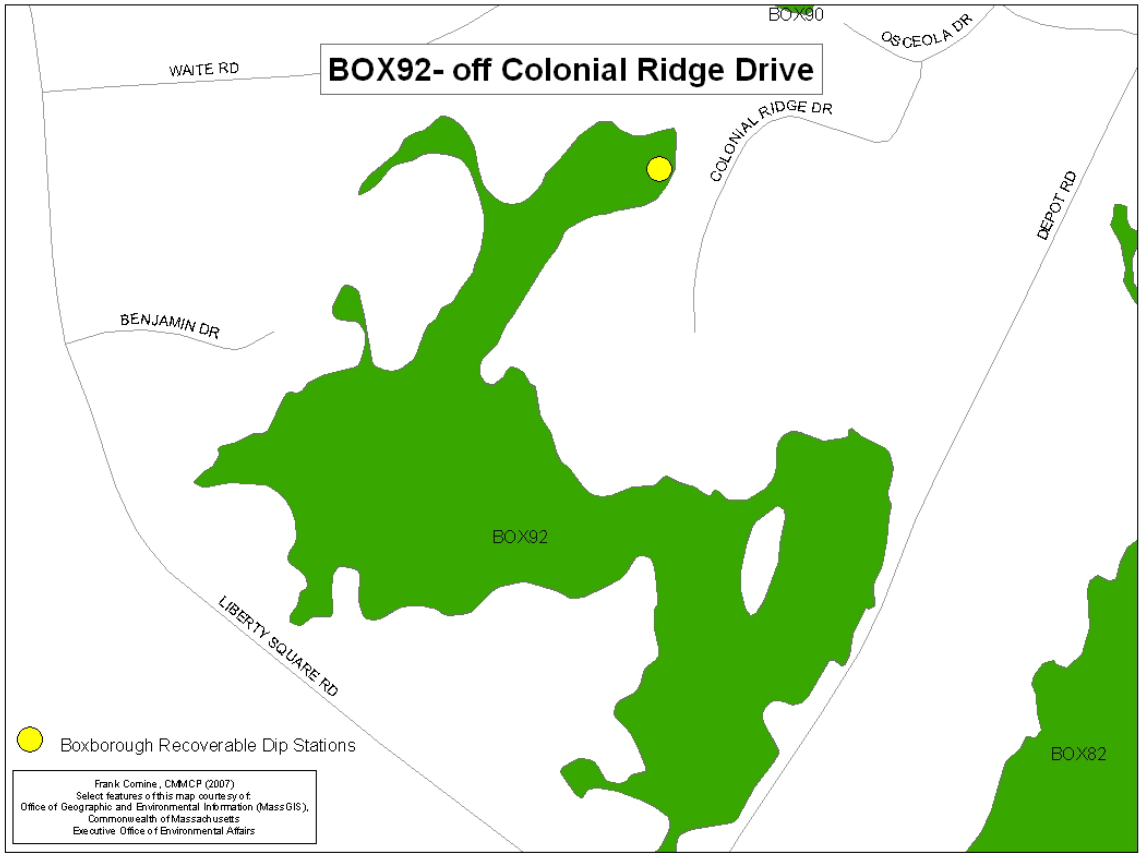
Town of Boxborough
Recoverable Dip Stations Data



This RDS in Boxborough displayed a 75.56% reduction in the number of mosquito larvae post-application. Bti was not found directly in all the dip sites, possibly due to the applicators buffer zone around the proximity of residential areas. Despite the lack of Bti granules at every dip site, the overall reduction was very good at this site.

Dip Site #	18-Apr	23-Apr	24-Apr	25-Apr	26-Apr
1	1	6	Application	1	0
2	1	3		2	1
3	1	7		0	0
4	1	3		3	4
5	1	4		2	3
6	1	4		0	0
7	1	10		1	1
8	1	3		5	0
9	1	2		0	0
10	3	3		1	2
Total:	**12**	**45**		**15**	**11**

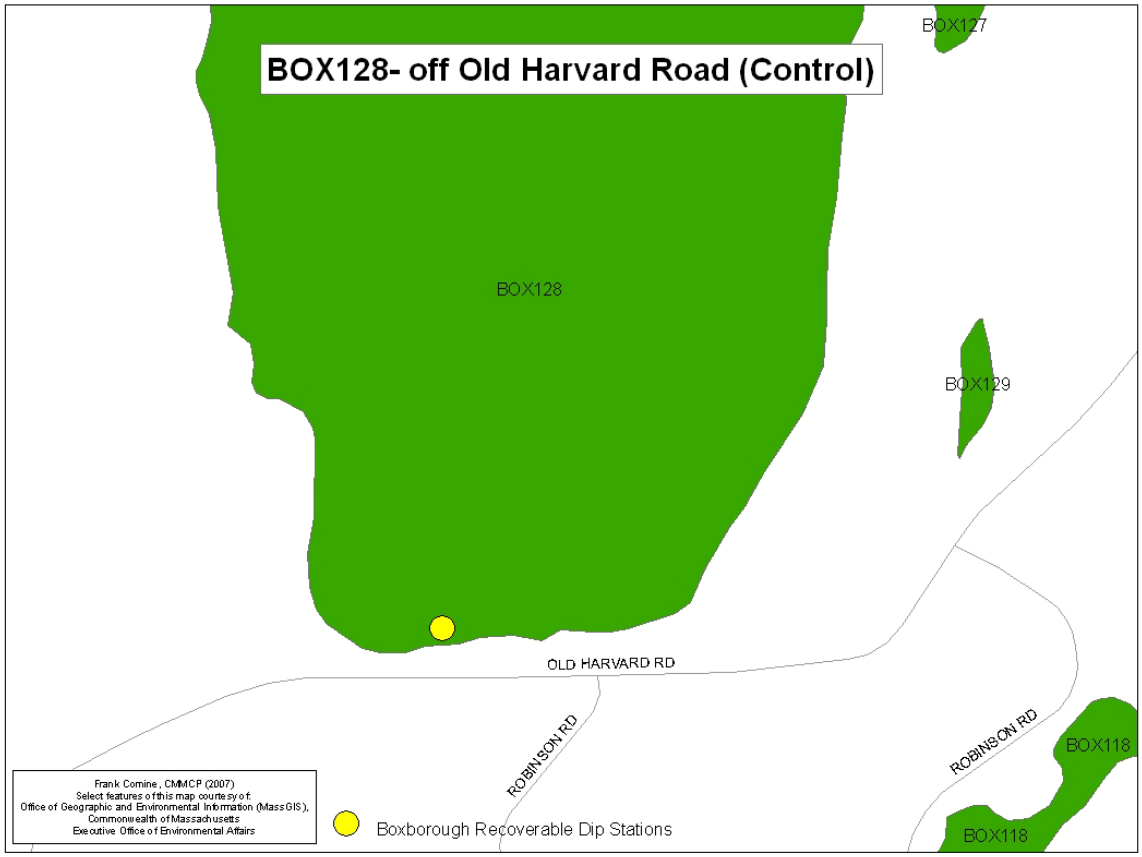
Town of Boxborough
Recoverable Dip Stations Data



This RDS had an overall reduction in mosquito larvae of 30.77%, although there were no Bti granules found at the dip sites. The shape of this wetland body may have contributed to the apparent absence of product. When technicians sampled other areas of BOX92 in response to the lack of granules at the RDS, Bti was found and no live larvae were observed.

Dip Site #	20-Apr	23-Apr	24-Apr	25-Apr	26-Apr
1	5	4	Application	5	2
2	5	12		4	3
3	2	7		3	4
4	2	11		5	4
5	3	8		4	4
6	4	10		8	2
7	4	7		4	8
8	4	14		10	30
9	4	10		10	3
10	3	8		8	3
Total:	**36**	**91**		**61**	**63**

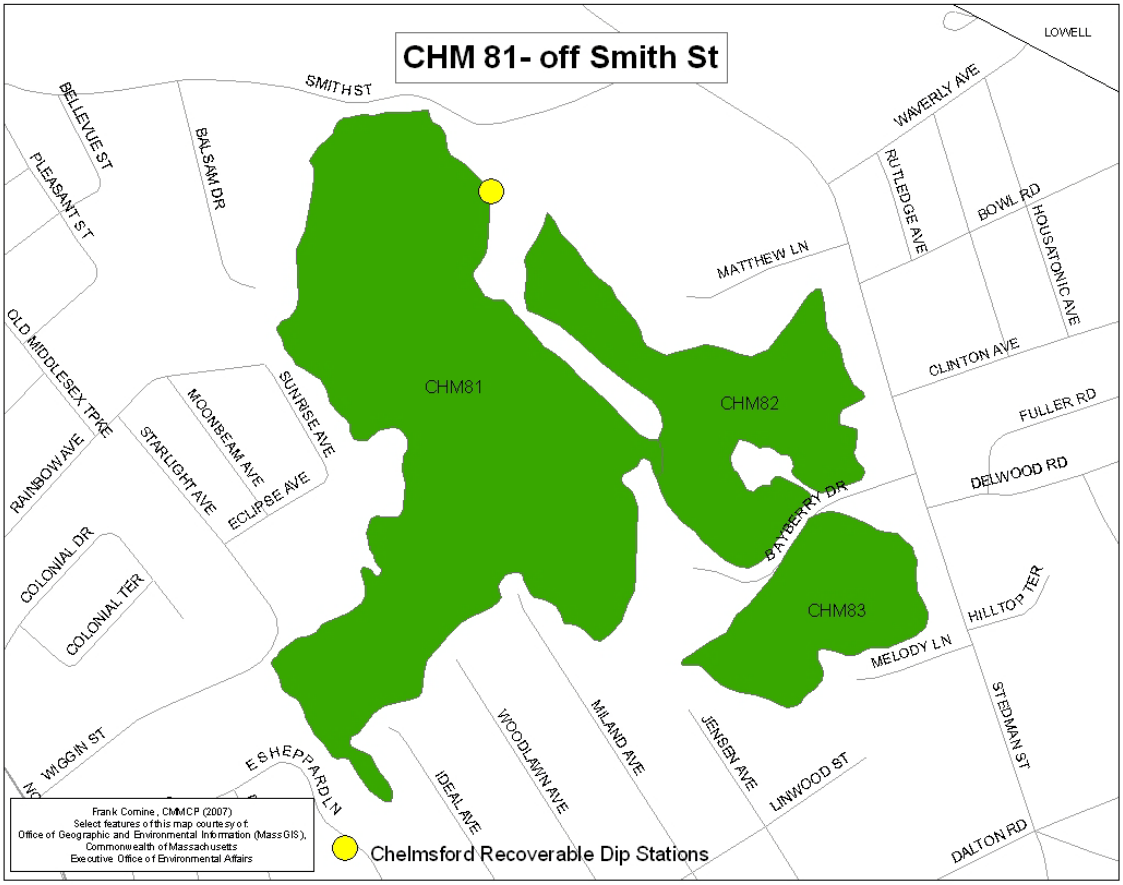
Town of Boxborough
Recoverable Dip Stations Data



This control site for Boxborough showed an increase of 33.33% from before the application to 48 hours after. These results are what may be expected from an untreated area.

Dip Site #	23-Apr	24-Apr	25-Apr	26-Apr
1	2	Application	2	2
2	3		2	2
3	2		4	2
4	5		3	4
5	4		5	6
6	2		3	3
7	4		5	3
8	3		3	4
9	2		5	6
10	3		5	8
Total:	**30**		**37**	**40**

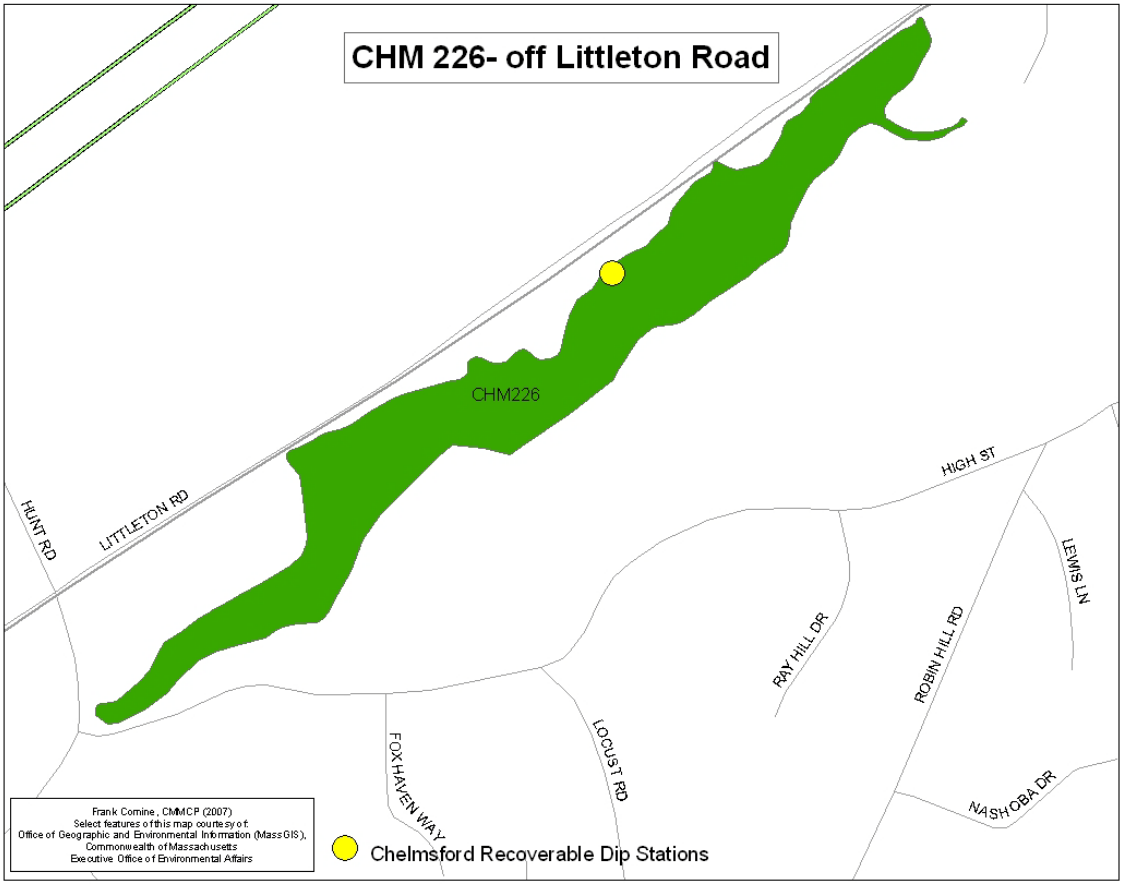
Town of Chelmsford
Recoverable Dip Stations Data



This RDS in Chelmsford had a 30.00% reduction in mosquito larvae, despite not having Bti present at the actual dip stations. Field technicians noticed granules in the area but not directly in the RDS. In the rest of the area where Bti was present, the reduction can be presumed to have been even greater.

Dip Site #	18-Apr	23-Apr	24-Apr	25-Apr
1	1	Application	0	2
2	3		0	1
3	1		1	1
4	2		2	1
5	2		1	1
6	2		3	5
7	1		0	1
8	3		2	0
9	1		2	1
10	4		3	1
Total:	**20**		**14**	**14**

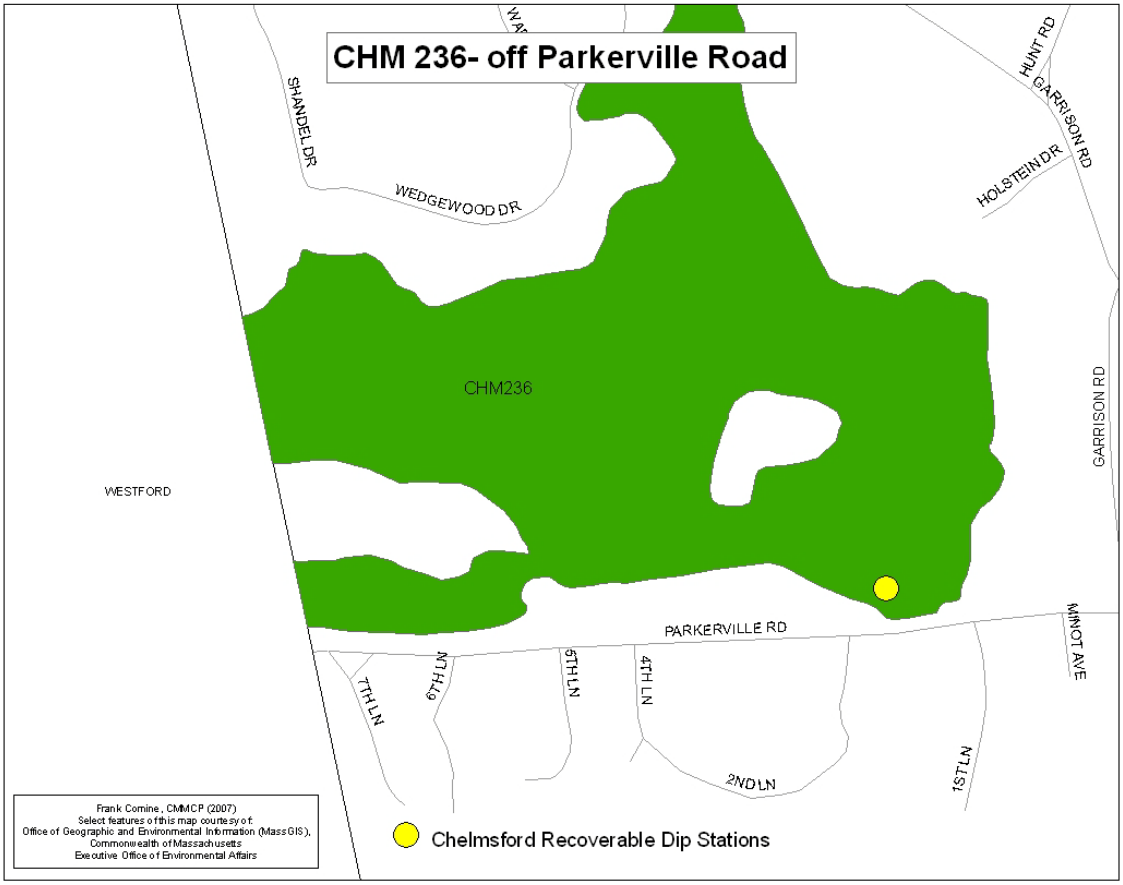
Town of Chelmsford
Recoverable Dip Stations Data



The observed reduction in mosquito larvae at this RDS was much lower than some of the others. This is not an indication of the effectiveness of the Bti, because technicians did not notice Bti at the RDS. The area right at the RDS could have been excluded due to the judgment of the pilot doing the application.

Dip Site #	18-Apr	23-Apr	24-Apr	25-Apr
1	7	Application	6	6
2	3		6	5
3	1		2	4
4	7		5	5
5	3		0	4
6	3		2	4
7	10		4	6
8	5		4	5
9	5		3	2
10	2		3	2
Total:	**46**		**35**	**43**

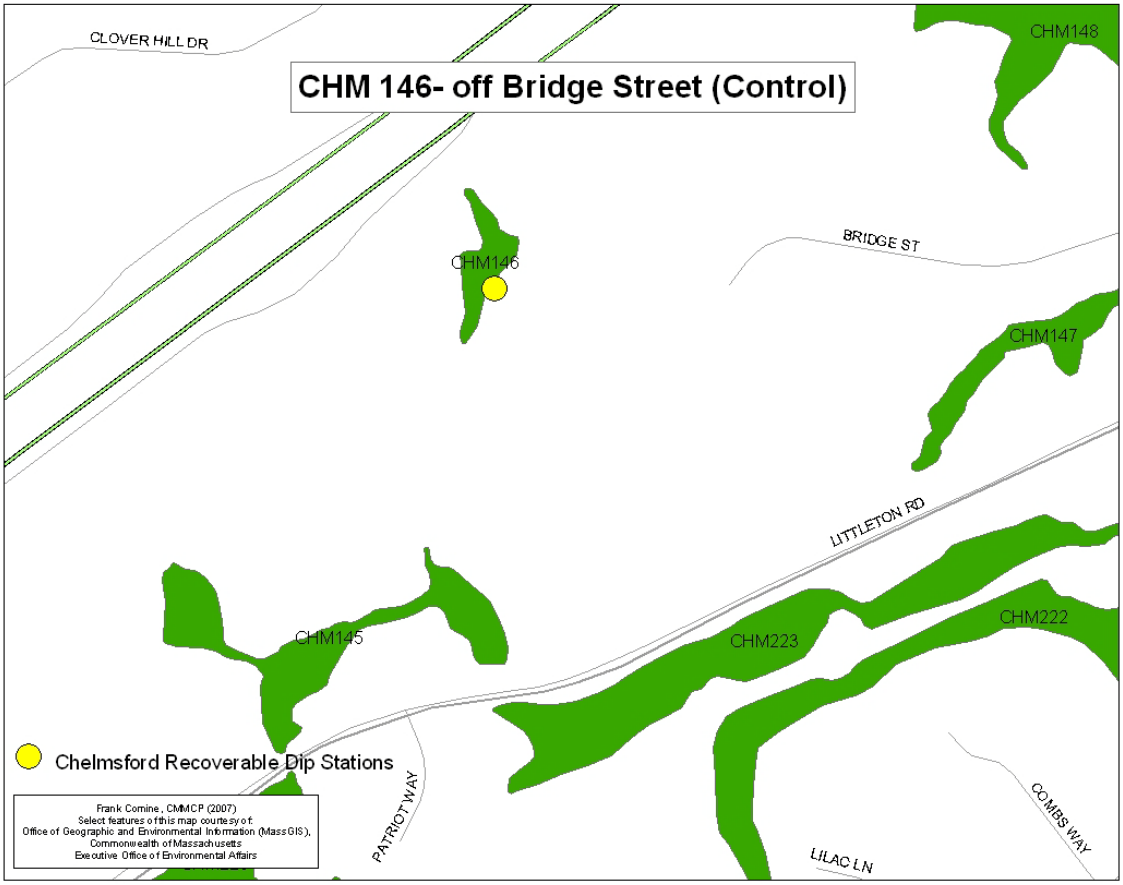
Town of Chelmsford
Recoverable Dip Stations Data



This RDS was very similar to CHM226, where there was no Bti present in the dip station area. The increase in mosquito larvae reflects the lack of Bti granules.

Dip Site #	18-Apr	23-Apr	24-Apr	25-Apr
1	3	Application	2	4
2	1		2	3
3	3		4	3
4	1		5	2
5	4		3	4
6	1		4	3
7	5		5	5
8	2		4	3
9	4		3	4
10	4		3	5
Total:	**28**		**35**	**36**

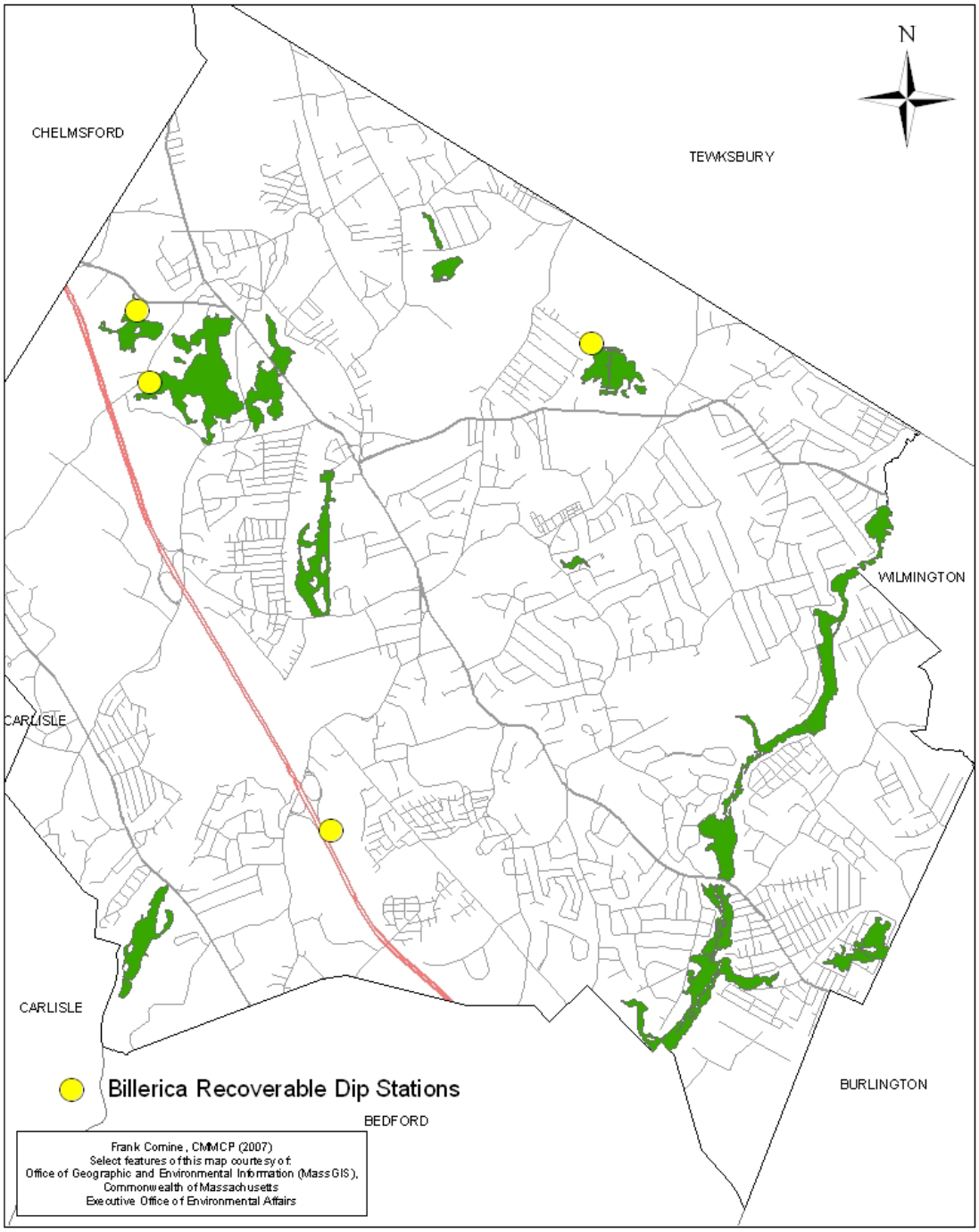
Town of Chelmsford
Recoverable Dip Stations Data



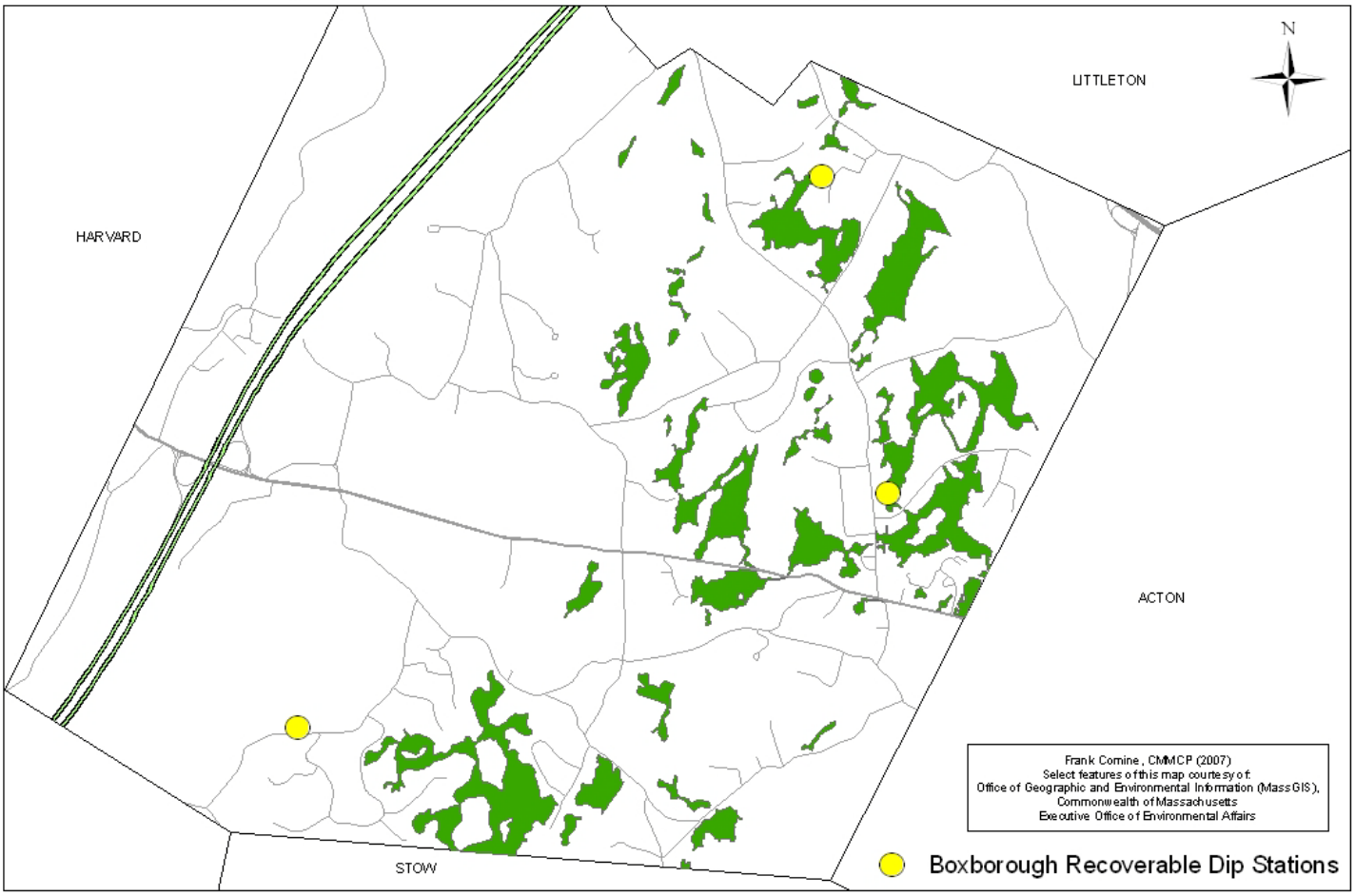
This was the designated control RDS for the town of Chelmsford. This site was not treated with Bti and the results greatly reflected that. The mosquito larvae sampled almost doubled from pre application with a 96.43% increase.

Dip Site #	18-Apr	23-Apr	24-Apr	25-Apr
1	2	Application	4	2
2	1		3	4
3	6		4	8
4	3		4	5
5	1		7	3
6	2		6	5
7	5		7	6
8	4		11	7
9	1		3	7
10	3		4	8
Total:	**28**		**53**	**55**

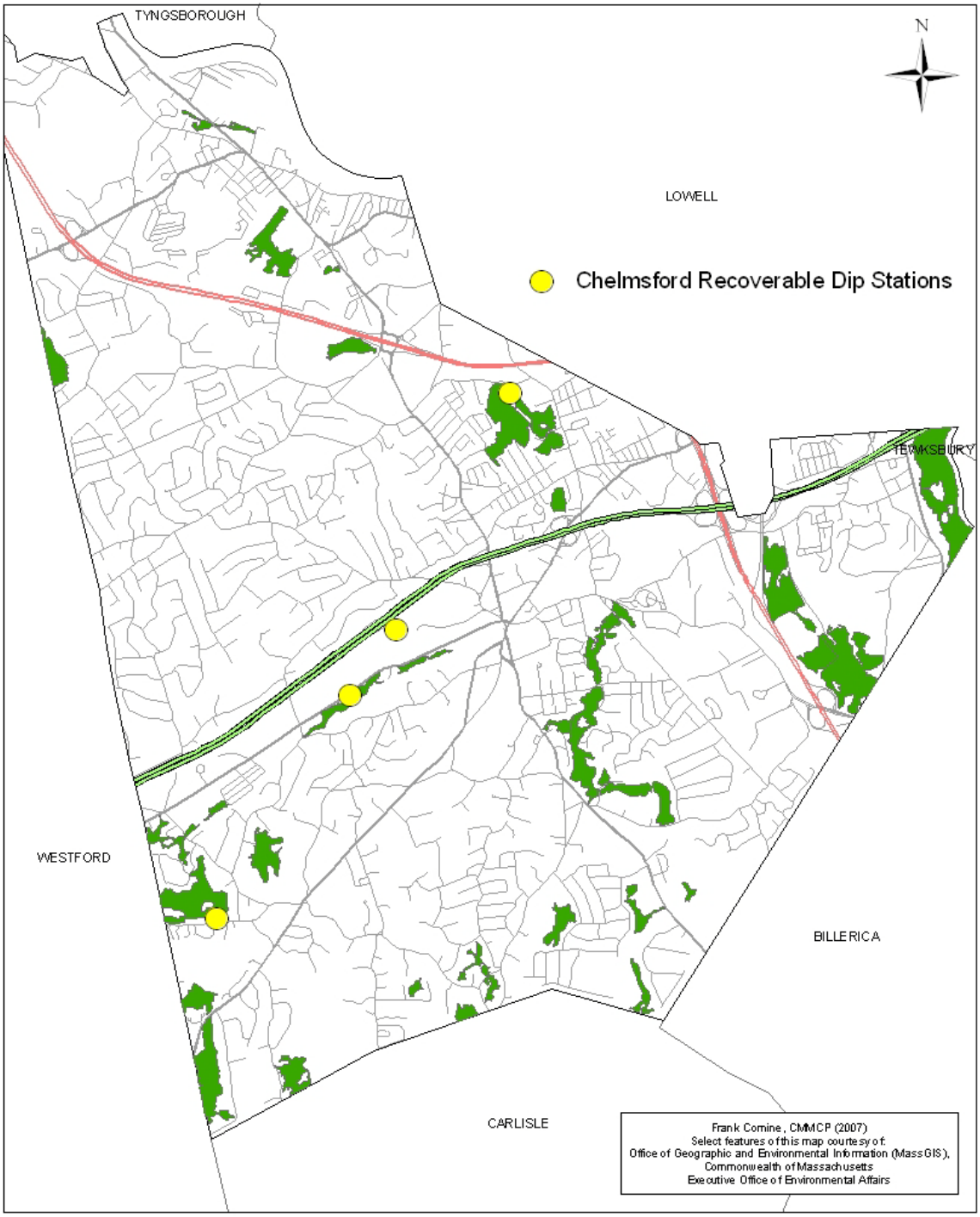
Billerica Wetlands Treated in 2007 Spring Aerial Larvicide



Boxborough Wetlands Treated in 2007 Spring Aerial Larvicide



Chelmsford Wetlands Treated in 2007 Spring Aerial Larvicide

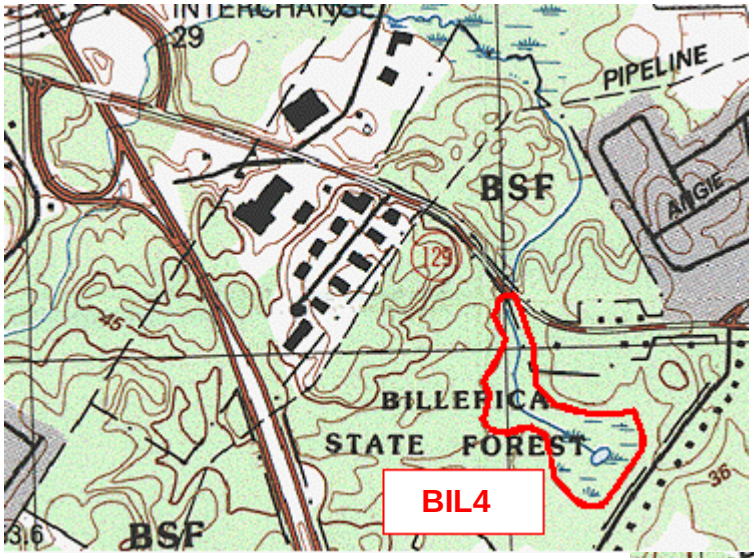


BILLERICA AERIAL APPLICATION FOR THE CONTROL OF SPRING BROOD MOSQUITO LARVAE

APRIL 2002

In 2001 the Town of Billerica contacted the Central Massachusetts Mosquito Control Project and began a program of aerial applications of *Bacillus thuringiensis israelensis* (Bti) in large-scale area wetlands to reduce the emergence of spring brood mosquitoes, most notably *Ochlerotatus abserratus, Oc. excrucians* and *Oc. canadensis*. This program continued in 2002, and the contractor selected this year was North Fork Helicopters based in Cutchogue, NY after a competitive bid process.

The following summary will show the areas treated by helicopter in Billerica, and the surveillance gathered pre- and post application. Data was collected at the same site pre- and post, and larval counts are noted in the narration below.



BIL4 - *off Chelmsford Road*

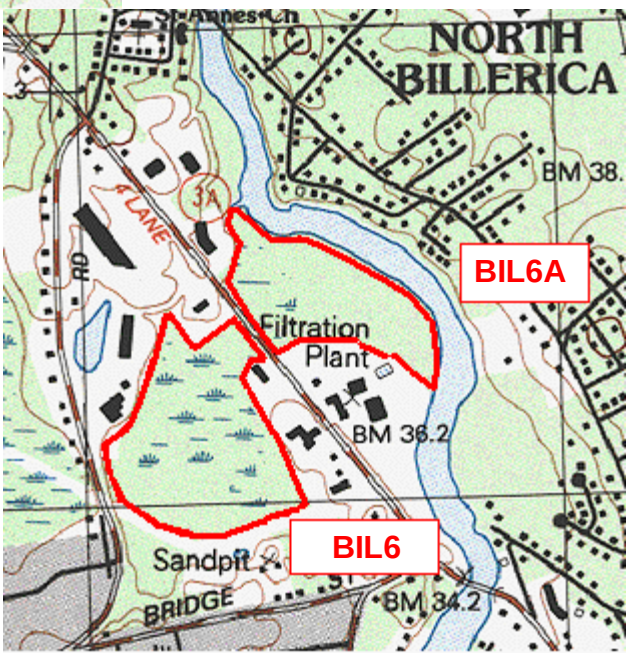
Pre-application data in 2 dip stations showed 2nd and 3rd instar larvae averaging 3 per dip at both stations. Post application surveillance did not show any active larvae in this area.

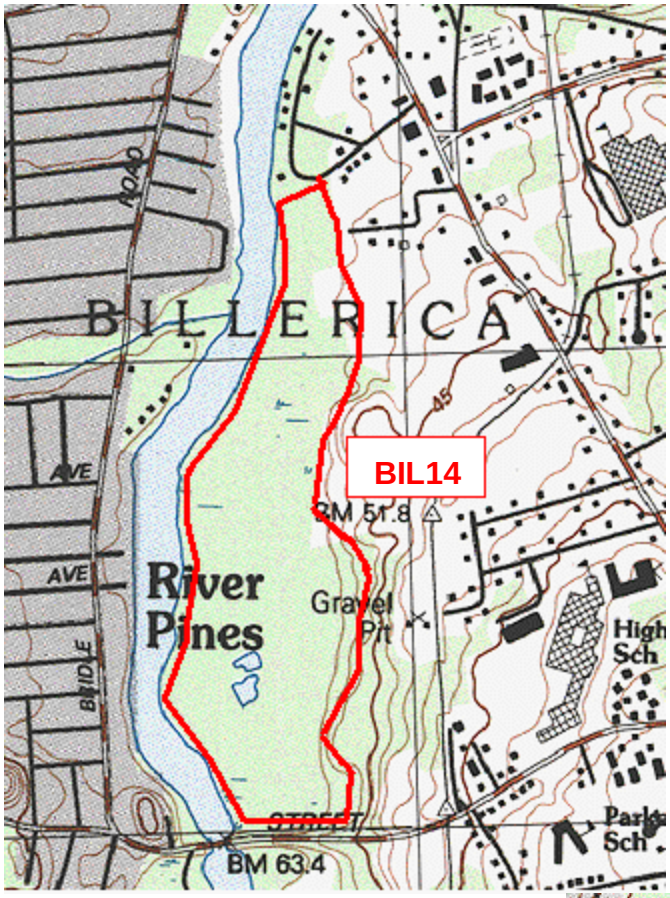
BIL4 - 5 acres

BIL6 & 6A - *off Boston Road*

Pre-application data in 2 dip stations showed 1st, 2nd and 3rd instar larvae averaging 2 per dip at both stations. Post application surveillance did not show any active larvae in these areas, but a few mosquito pupae were noted, indication the larvae were advancing through their life cycle at a rapid pace.

BIL6 - 30 acres
BIL6a - 11 acres





BIL14 - *off Simmons Lane & Todd Lane*

Pre-application data in 2 dip stations off Simmons Lane showed 3rd and 4th instar larvae, with 20 per dip at one site, and 4 per dip (average) at the other. Post application dips showed only scattered pupae and a few 4th instar larvae remaining in these areas.

Dip stations off Todd Lane showed 2nd and 3rd instar larvae, pre-application, averaging 3 and 8 per site. Post application showed no breeding at 1 station, and only one 4th instar larvae at the other.

BIL14 - 78 acres

BIL32 - *off Shawsheen Road, Seminole Road & Sheridan Street*

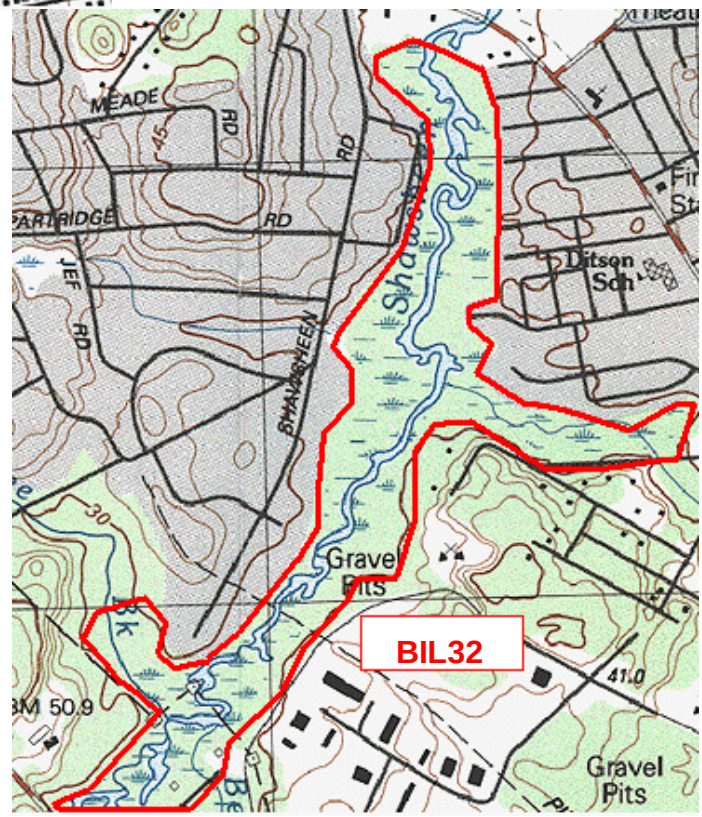
This area is a large floodplain of the Shawsheen River in the Pinehurst section of town. 6 dip stations were monitored for this application.

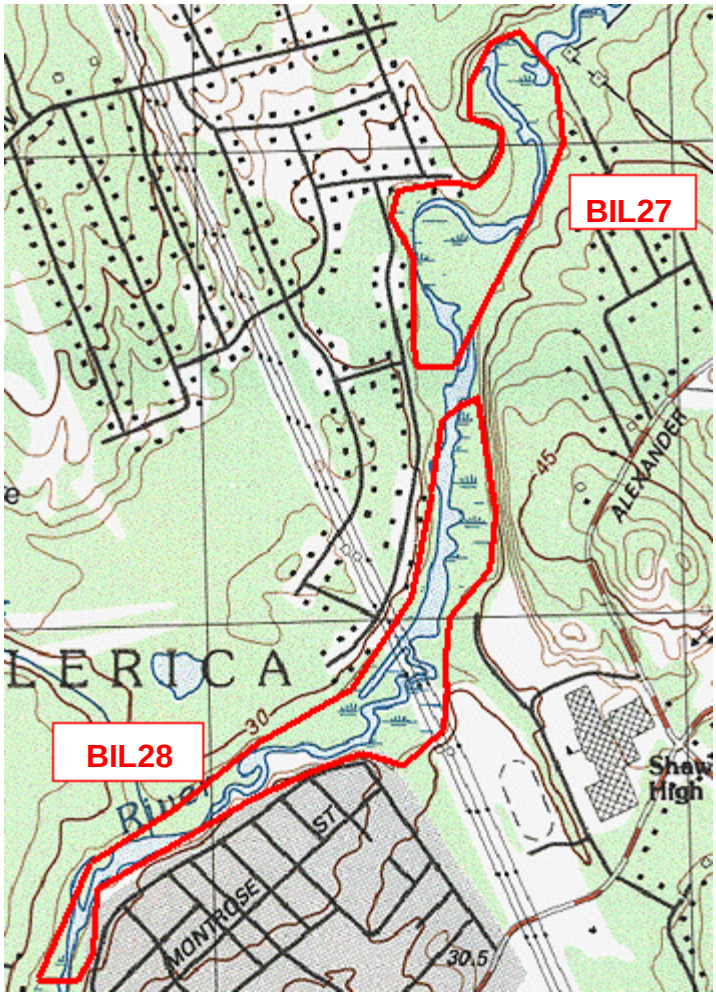
Seminole Rd. #1:
pre- 4/dip 2nd & 3rd
post - no larvae found in area

Shawsheen Rd. #1,2&3:
pre - 20/dip, 2nd & 3rd (sites 1&2) 15/dip 2nd & 3rd (site 3)
post - no larvae, 6/dip 2nd & 3rd, no larvae

Sheridan Rd. #1 & 2:
pre - 4/dip 2nd & 3rd, 16/dip 2nd $ 3rd
post - 3/dip 2nd, no larvae

BIL32 - 85 acres





BIL27 & 28 - *off Sachem Street & Islington Road*

Pre-application data off Sachem Street shows 3/dip average, 1st instar. Post treatment data shows no mosquito breeding found in this area.

Data for the Islington Road dip station shows 18/dip, 2nd instar, pre-application. Post application follow up data reveals 3rd instar larvae in the area, at and average of 2 per dip.

BIL27 - 105 acres
BIL28 - 70 acres

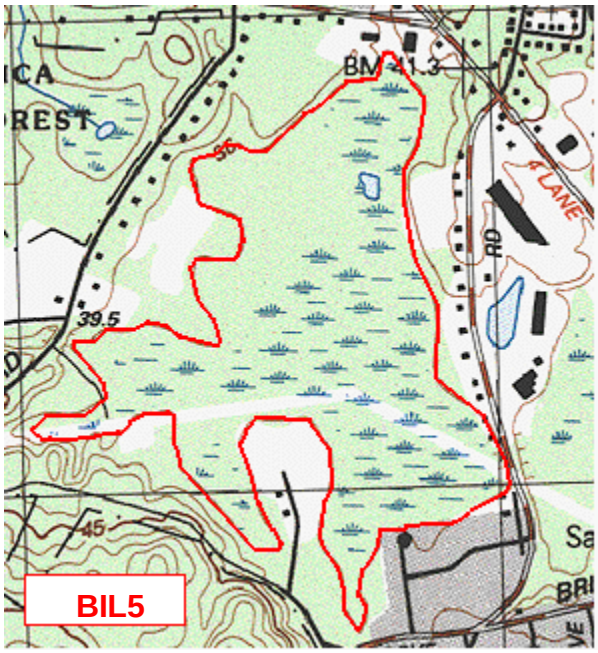
CONCLUSION

Data collection at the dip stations shows this application to be a success. Most areas did not show active larvae after the application. Reduced dip counts in some areas may be due to the fact that the pilot will have buffer zones around roads, houses, etc, and also due to the fact that Bti is not as effective on later (4th) instar larvae. Other areas in Billerica were also treated, and dipping was done only to confirm the presence of larvae - success in these areas would be expected to equal the areas listed above. Maps of the areas are on the following pages in Appendix A.

These sites were chosen due to the proximity to residential areas, adult collections over the past few years, and service calls to this office. Total acreage for this application was on or about 600 acres. Acreage listed is approximate, and does not reflect the drought we have experienced in 2002. Field inspections have shown sites to be smaller this year than in past years.

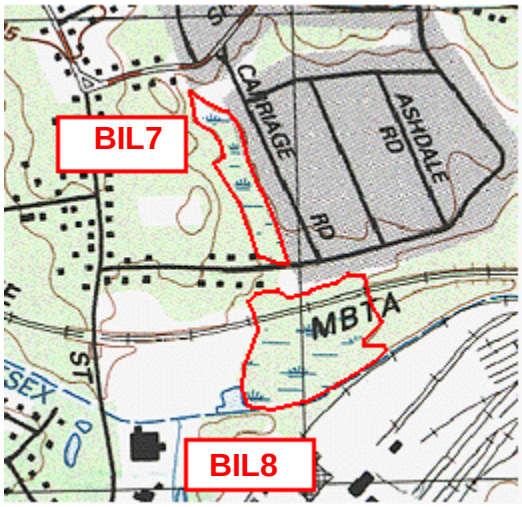
We look forward to continuing this application in the future, and expanding this program in neighboring communities to the benefit of all residents.

Appendix A



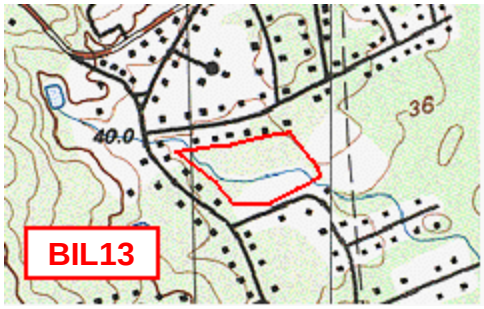
BIL5 - between Rangeway Road & Treble Cove Road

BIL5 - 103 acres



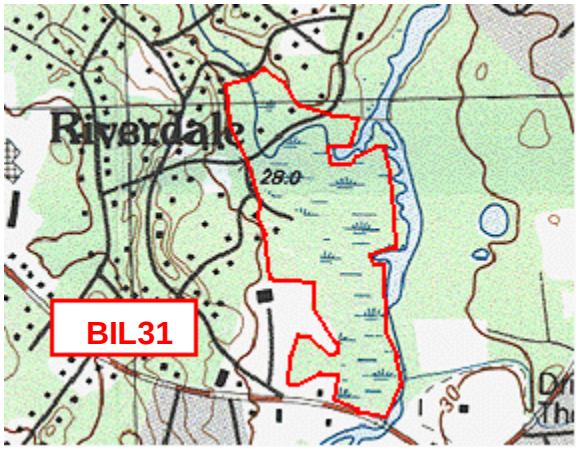
BIL7 & BIL8 - Carriage Road area

BIL7 - 10 acres
BIL8 - 10 acres



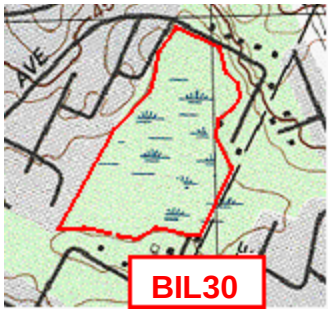
BIL13 - off Allen Road

BIL13 - 23 acres



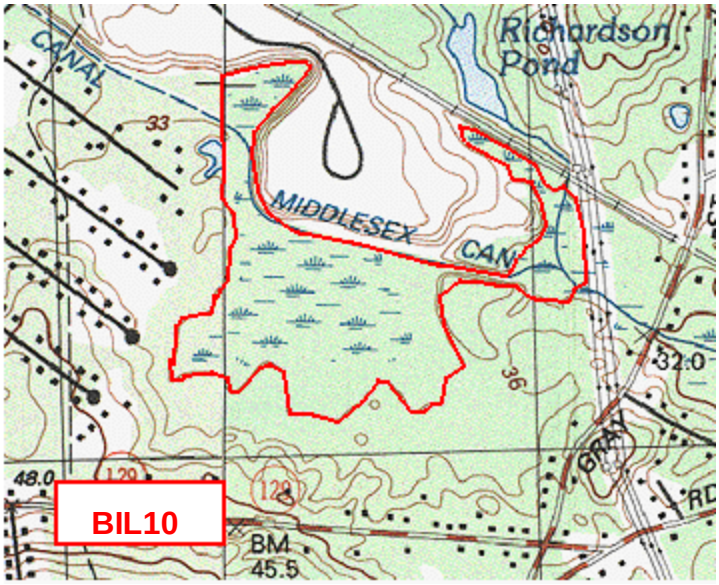
BIL31 - Shawsheen River floodplain, Riverdale section

BIL31 - 42 acres



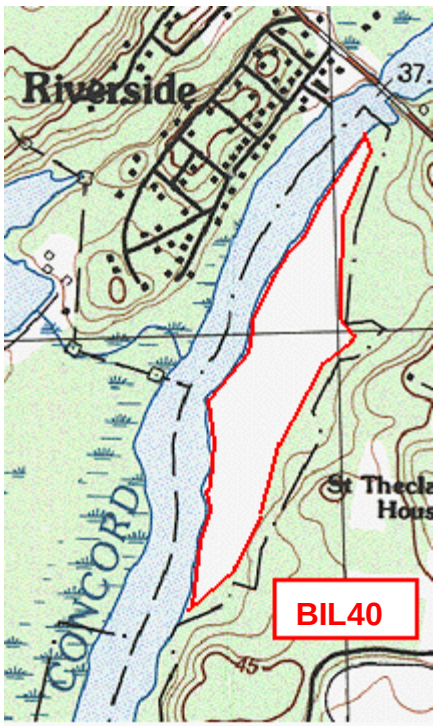
BIL30 - off Pinedale Avenue

BIL30 - 35 acres



BIL10 - off Pond Street

BIL10 - 67 acres



BIL40 - Concord River floodplain, Riverside section

BIL40 - 45 acres

Environmental Influences for the Tree Canopy Preference of *Culex pipiens* and *Culiseta melanura*

Senior Thesis
December 2005

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ABSTRACT

In our study we found that when mosquito collections were made on the same night and location, with one trap placed in the tree canopy and the other trap at ground level, there were a significantly higher number of *Culex pipiens* and *Culiseta melanura* in the tree canopy traps. These two trap levels also exhibited no significant difference in temperature, although it was determined that there was a significantly higher relative humidity at the ground level than in the canopy. This difference in relative humidity was also found to not be significantly correlated with the collections. By learning more about the biology of *Culex pipiens*, as well as other mosquitoes, we will be able to devise more effective methods to hamper their negative effects on humans without impacting other parts of the ecosystem.

INTRODUCTION

Since the discovery of West Nile virus (WNV) in the United States in 1999, much emphasis has been placed on learning more about its transmission and characteristics of the specific mosquito species involved (Kulasekera, 2001; Nasci, 2001; Kilpatrick, 2005). The first known human case of WNV was reported in New York City, in August of 1999. After this first case there were an additional 61 humans positive for WNV in New York, from August to October of 1999, consisting mostly of elderly people (Enserink, 2000; Rappole, 2000). As of March 2005, WNV has infected over 17,000 and killed over 670 people in North America (Kilpatrick, 2005). From its initial discovery, WNV quickly spread across the U.S. and has made its way down into Mexico and Central America (Knight, 2003).

An important vector of WNV in the United States is the mosquito species *Culex pipiens* (Goddard, 2002; Anderson, 2004; Kilpatrick, 2005). It has been suggested that these mosquitoes act as hosts for overwintering flaviviruses such as WNV, until they reemerge in the spring (Goddard, 2002). Some studies suggest that *Culex pipiens*, along with *Culex restuans*, may in fact be responsible for up to 80% of human WNV infections in the northeast United States (Kilpatrick, 2005). Previously believed to feed mainly on birds, and therefore reducing their likelihood of infecting humans, *Culex pipiens* are now thought to more commonly feed on humans than previously thought (Kilpatrick, 2005). By learning more about the biology of *Culex pipiens*, as well as other mosquitoes, we will be able to devise more effective methods to hamper their negative effects on humans without impacting other parts of the ecosystem.

The vast majority of female adult mosquitoes require a blood meal to begin development of each clutch of eggs, and obtain this from a variety of sources (Bates, 1949; Knight, 2003). Most mosquito species will feed on warm-blooded animals after receiving cues to induce biting. These signals include carbon dioxide and ammonia, especially when coupled with a temperature and moisture level similar to breath (Bates, 1949). Respiration of animals, along with color, motion, and smell to a lesser degree attract the mosquitoes to feed upon various hosts (Bates, 1949). Some mosquitoes exhibit host preference while others do not. For example, past studies have the *Culex pipiens* species preferentially feeding on birds, but also feeding on assorted mammals (Nasci, 2001).

Once they have acquired their blood meal necessary for egg development, mosquitoes may use many different types of areas for breeding, including irrigated agricultural lands, shallow isolated pools, dumping areas, and wetlands (Knight, 2003). After obtaining a blood meal, the female mosquitoes will usually have a resting period before oviposition. It has been shown that mosquitoes don't lay eggs randomly but instead may lay eggs where there are fewer predators present (Kiflawi, 2003).

In many aspects of mosquito life history, temperature seems to play a very influential role. Low air temperatures in the winter lead many mosquito adults to enter a hibernation state and high temperatures in the summer can also lead to decreased adult mosquito activity (Knight, 2003). As noted before, temperature also plays an important role in the feeding habits of mosquitoes. *Culex pipiens* have been shown to prefer host temperatures between 32° C and 43°C, with temperatures above 49°C and below 30°C

showing less attraction. Temperature also seems to have an effect on oviposition, with mosquitoes avoiding water temperatures outside the range of 20°C to 30°C (Bates, 1949).

There are several common trapping methods for adult mosquitoes. These include gravid traps that simulate oviposition habitat, light traps, and carbon dioxide traps with the latter two possibly being combined. With carbon dioxide being a major attractant for mosquitoes, yields from these traps are especially clean, containing almost no unwanted insects. The traps with light alone can produce many kinds of non-targeted insect species, which can slow research.

Culex pipiens, as well as other mosquito species, has been discovered to prefer inhabiting tree canopies, or at least seem to frequent tree canopy height. The specific reasons for this behavior are not clear although it has been speculated that they may be influenced by temperature, humidity, light, as well as the potential feeding of nesting birds (Anderson, 2004). This project was geared toward gathering data on two of these possibilities, temperature and humidity. My hypothesis is that *Culex pipiens* and *Culiseta melanura* will both show a significant preference for the canopy level, but that this will not have a significant relationship with either temperature or humidity.

METHODS

Data collection for the project was started in late May 2005 and ended September 2, 2005. There were three different sites, two in Westborough, MA, and the other in neighboring Hopkinton. The two sites in Westborough were located off of Rogers St. (42°16.427'N, 071°36.033'W) and Hopkinton Rd. (42°15.709'N, 071°35.812'W), while the Hopkinton site was located off of Woods St. (42°15.354'N, 071°35.149'W).

Trapping involved using two CDC light/CO_2 mosquito traps (John W. Hock Co., model 512) with net collection bags, one placed approximately 6.5 meters into the air and the other about 1.5 meters high at the same site. Carbon dioxide was used as the only means of attractant, with the light feature of the traps being disabled to avoid non-target insects. The CO_2 tanks were adjusted with regulators to 15psi. On each trap there was a temperature/relative humidity data logger (Onset 64K HOBO Pro RH/Temp Logger) that logged each every 40 seconds while the trap was collecting.

The traps were set and collected overnight and retrieved approximately 24 hours later and set again usually at one of the other sites, with new collection bags, new batteries and new CO_2 tanks. The data logger information was downloaded and reset at each retrieval. The specimen collections were knocked down and stored in a refrigerator until identification. The specimens were identified as *Culex pipiens*, *Culiseta melanura*, or "other," by using the Darise mosquito index (1981) and a dissecting microscope.

The data collected from the data loggers and mosquito identification was then used in several ANOVAs to determine whether there were significantly different findings for the two trap levels, three trap sites, any interaction between those factors, and also for the temperature and relative humidity of the two trap levels. Significantly different

mosquito numbers were then put through a test for normality and then a Spearman correlation test to determine if they were associated with any of the two possible environmental influences that were tested.

RESULTS

There were 42 viable collections made, which included both canopy and ground traps along with complete temperature and relative humidity data sets (Tables 1-4). An ANOVA for the number of *Culex pipiens* caught was performed against the two trap levels and the three sites. It was then determined that there was a significantly higher number of these mosquitoes caught in the canopy traps than in the ground traps (Figure 1), but no significant difference between any of the sites and any interactions within the trap levels and sites (Table 5).

Table 1: Descriptive Statistics for *Culex pipiens* Collections

Variable	N	Mean	Median	TrMean	StDev	SE Mean
CUL (Canopy)	42	5.98	3.00	5.03	7.65	1.18
CUL (Ground)	42	2.43	1.00	1.37	6.70	1.03
	Min.	Max.				
CUL (Canopy)	0.00	33.00				
CUL (Ground)	0.00	43.00				

Table 2: Descriptive Statistics for *Culiseta melanura* Collections

Variable	N	Mean	Median	TrMean	StDev	SE Mean
MEL (Canopy)	42	11.31	5.00	8.030	20.00	3.09
MEL (Ground)	42	2.00	1.00	1.579	2.98	0.46
	Min.	Max.				
MEL (Canopy)	0.00	123.00				
MEL (Ground)	0.00	15.00				

Table 3: Descriptive Statistics for Average Temperature (°C) Readings

Variable	N	Mean	Median	TrMean	StDev	SE Mean
Ave. Temp. (Canopy)	42	19.278	20.325	19.596	4.622	0.713
Ave. Temp. (Ground)	42	19.070	19.825	19.380	4.462	0.689
	Min.	Max.				
Ave. Temp. (Canopy)	6.700	25.980				
Ave. Temp. (Ground)	6.780	25.400				

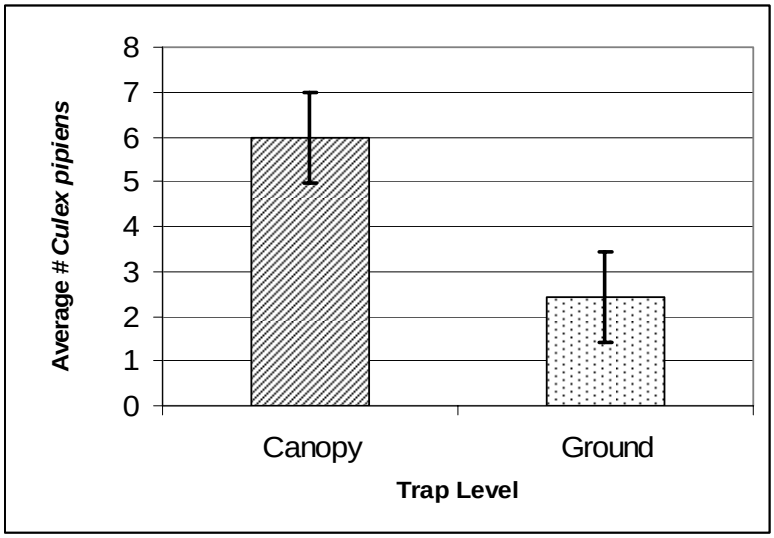
Table 4: Descriptive Statistics for Average Relative Humidity (%H) Readings

Variable	N	Mean	Median	TrMean	StDev	SE Mean
Ave. RH (Canopy)	42	84.57	84.94	84.98	8.04	1.24
Ave. RH (Ground)	42	88.21	87.84	88.56	6.82	1.05
	Min.	Max.				
Ave. RH (Canopy)	58.20	98.41				
Ave. RH (Ground)	67.14	99.45				

Table 5: Analysis of Variance for *Culex pipiens*

Source	DF	SS	MS	F	P
Canopy/Ground	1	264.3	264.3	5.30	0.024
Site	2	145.2	72.6	1.46	0.240
Interaction	2	200.2	100.1	2.01	0.141
Error	78	3889.9	49.9		
Total	83	4499.6			

Figure 1: Average # *Culex pipiens* at Canopy and Ground Trap Levels



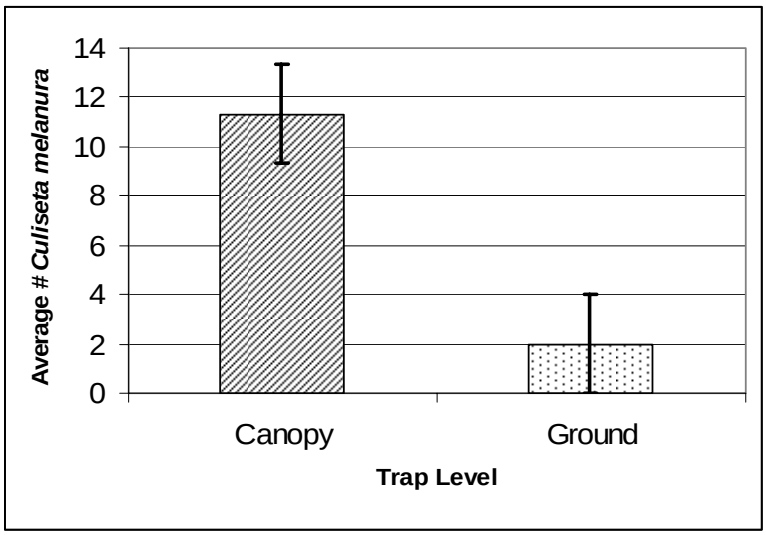
Similar results were found when an ANOVA was performed for the number of *Culiseta melanura* caught against the two trap levels and the three sites. There was a significantly higher amount of *Culiseta melanura* mosquitoes found in the canopy traps as opposed to the ground traps (Figure 2). Again, there was no significant difference

between the number caught from the three sites or any interactions between the levels and sites (Table 6).

Table 6: Analysis of Variance for *Culiseta melanura*

Source	DF	SS	MS	F	P
Canopy/Ground	1	1820	1820	8.72	0.004
Site	2	329	164	0.79	0.458
Interaction	2	150	75	0.36	0.700
Error	78	16278	209		
Total	83	18577			

Figure 2: Average # *Culiseta melanura* at Canopy and Ground Trap Levels



When two-way ANOVAs were used with average temperature and average relative humidity against the trap levels and different sites, it was determined that there was a significant difference in the relative humidity readings of the canopy and ground level traps, but not in those of the average temperature of the two levels. There was no significant difference in the environmental factors between each site and also no significant difference in any interaction between trap level and site (Tables 7, 8).

Table 7: Analysis of Variance for Average Temperature

Source	DF	SS	MS	F	P
Canopy/Ground	1	0.9	0.9	0.04	0.835
Site	2	49.4	24.7	1.17	0.315
Interaction	2	0.2	0.1	0.00	0.995
Error	78	1642.8	21.1		
Total	83	1693.3			

Table 8: Analysis of Variance for Relative Humidity

Source	DF	SS	MS	F	P
Canopy/Ground	1	277.6	277.6	5.09	0.027
Site	2	270.7	135.3	2.48	0.090
Interaction	2	27.5	13.7	0.25	0.778
Error	78	4253.9	54.5		
Total	83	4829.6			

A test for normality showed that the mosquito collection data was not normal, and so the resulting Spearman correlation test showed that there was not a significant positive correlation between the *Culex pipiens* and *Culiseta melanura* canopy preference and the significantly different relative humidity of the two levels (Table 9; Figures 3, 4).

Table 9: Spearman Correlation Test for Relative Humidity and Mosquito Collections (Canopy:Ground)

RH Difference: CUL Difference	P-value = .280
RH Difference: MEL Difference	P-value = .943

Figure 3: Scatter Plot of Relative Humidity vs. # *Culex* Differences (Canopy-Ground)

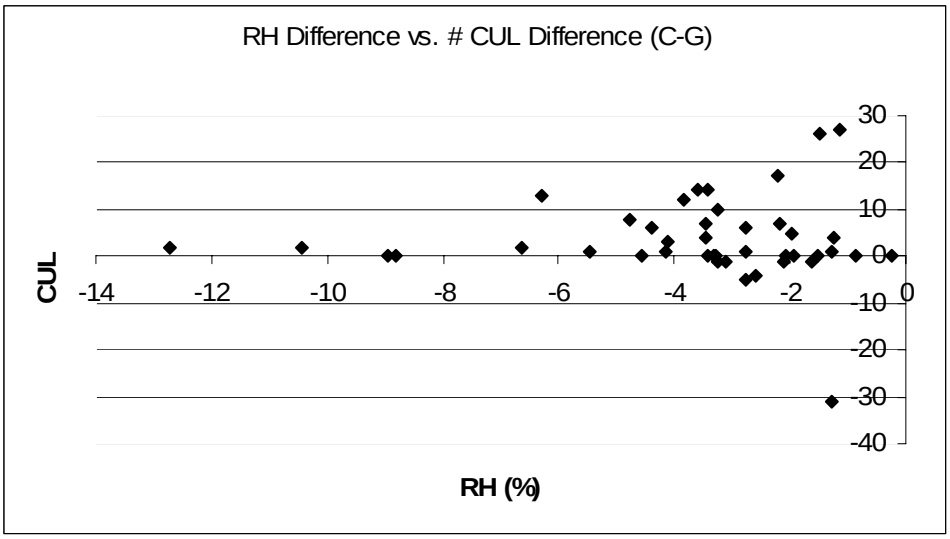
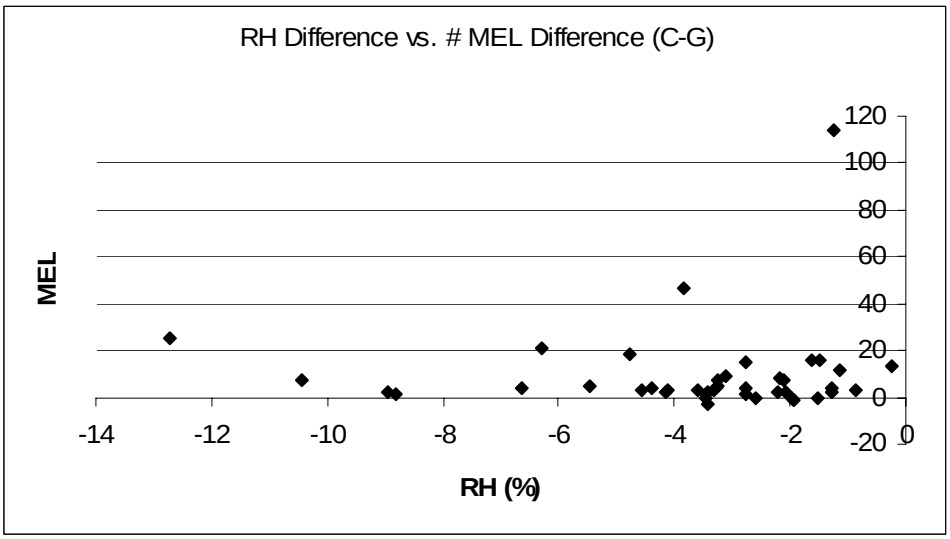


Figure 4: Scatter Plot of Relative Humidity vs. # *Culiseta melanura* Differences (Canopy-Ground)



DISCUSSION

Our collections exhibited the canopy preference shown by *Culex pipiens* and other mosquitoes in previous studies (Table 5, 6; Figure 1, 2; Anderson, 2004). Our study also showed that there was not a significant difference in the average temperatures of the two traps level (Table 3). However, the relative humidity of the two levels did prove to be significantly difference (Table 4), leading us to perform a correlation, which showed that there was not a significant relationship between relative humidity and collections (Table 9).

Through the lack of a correlation, I believe our results seem to support the idea that the canopy preference is due more to the feeding habitat of these mosquitoes on roosting birds than abiotic environmental influences. The preference for obtaining blood meals through birds by *Culex pipiens* seems to be more behavioral than being influenced by certain environmental factors, temperature and relative humidity in this case. Our results support the possibility that these target mosquitoes are present in the canopy not because of the proposed abiotic factors but more likely because the dominant feeding patterns and the location of these organisms.

Because of the susceptibility of *Culex pipiens* and *Culiseta melanura* to acquire and transmit West Nile virus and also other diseases including Eastern Equine Encephalitis, it is important to know where they are predominantly located and also the reasons why. Previous studies along with this one seem to indicate that these mosquito species do prefer canopy level, which could be very influential in the control aspect of mosquito (Anderson, 2004). With the right thermal currents, a mosquito control application could be administered so that it would rise through the canopy, eliminating

those targets before any virus is allowed to transfer and build in bird hosts. Lessening the amount of virus that bird host populations are exposed to could significantly decrease the chances of a mosquito with bird and mammal feeding preference to obtain virus and transmit it to humans.

Similar research of canopy preference of *Culex pipiens*, *Culiseta melanura* and other mosquito species, may lead mosquito surveillance projects to change their trapping protocol. By shifting the focus of surveillance techniques to the canopies as opposed to the standard ground level, there could be an increased chance of finding infected mosquitoes before they have a chance to infect birds, which would begin to build up the virus in themselves. Finding these infected mosquitoes before they have a chance to infect birds would give mosquito control projects a head start on signaling potentially high risk areas, and taking any proper actions.

These ideas were relevant during this project as one of the collections from a canopy trap was found to have West Nile virus. Signs were posted and a press release was announced, allowing local residents to take their own precautions to avoid contracting WNV. In response to these findings more traps were established in the local area, which later in the season resulted in a positive Eastern Equine Encephalitis pool of mosquitoes. These traps were located in an area that was frequented by children and senior citizens, emphasizing the importance of identifying it for infectious mosquitoes early.

In conclusion this study reinforces the canopy preference for *Culex pipiens* and *Culiseta melanura*. It was also found that there was no correlation between the canopy preference and canopy temperature and relative humidity. This finding leads one to

believe that the canopy preference exhibited by these mosquitoes is influenced by something else, host availability being among the possibilities.

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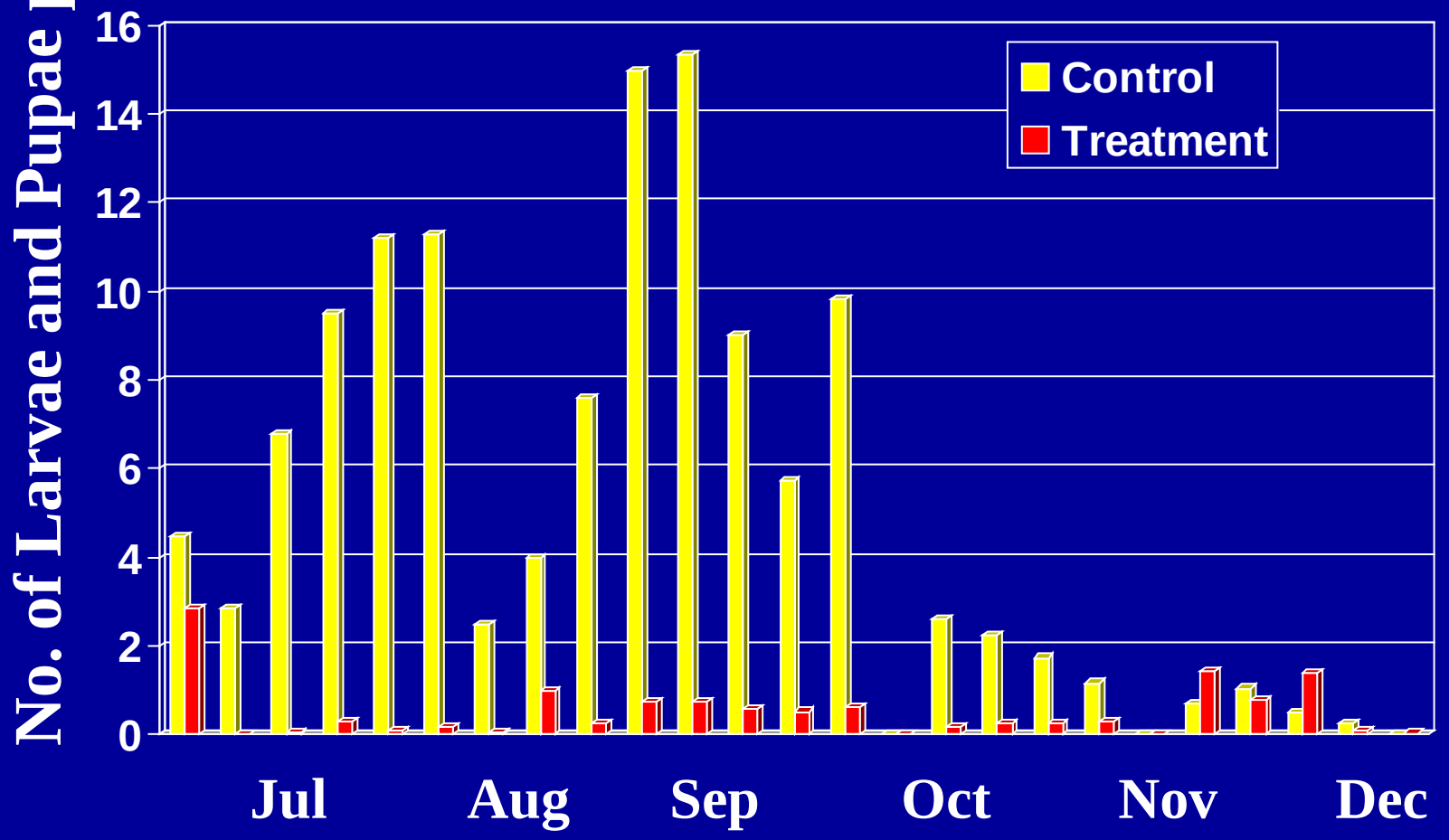
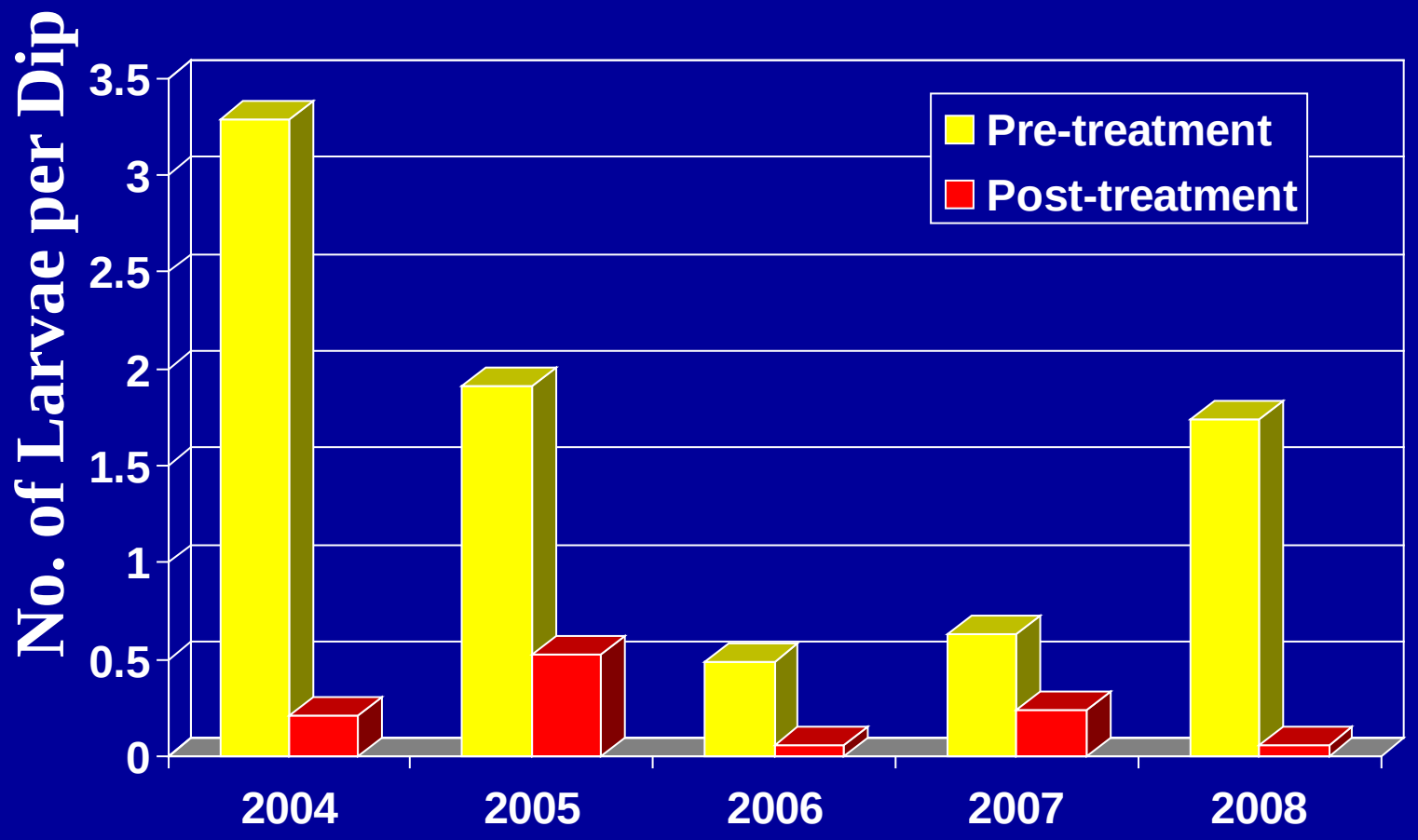
Timothy McGlinchy
Central Massachusetts Mosquito Control Program

Effect of Vectolex WSP against *Culex pipiens/restuans* larvae in Newton catchbasins



Efficacy of Aerial *Bti* Applications



EFFICACY OF THE CENTRAL MASS. MOSQUITO CONTROL ADULTICIDE PROTOCOL – 2007

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ABSTRACT

During the summer of 2007, the Central Mass. Mosquito Control Project (CMMCP) conducted field trials for the efficacy of their adulticide product and procedure. By observing natural mosquito populations during seven week-long trial sets at application areas and control sites, it was determined that the current protocol results in a 2-3days of control before returning to pre-application levels. These results are believed to be due to the low residual nature of the product used for control and a rapid reinfestation by neighboring mosquito populations. Recent emergence of new mosquitoes is also a possibility although more unlikely than the previous scenarios. Despite these findings, the application rates are considered sufficient for non-vector control situations at this time, but it is proposed that changes in the protocol involving insecticide rates and target areas could increase the efficacy of this program during vector control situations.

INTRODUCTION

At the forefront of any vector control operation should be the efficacy of their practices. As one of several tools in any mosquito control project's integrated pest management (IPM) plan, adulticide applications are no different (Crockett 2002). Like many mosquito control projects, CMMCP uses ULV machines, the basis of which is to use the smallest effective amount of insecticide product (Mount 1998). Currently 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).

During the 2007 season, CMMCP applied ANVIL® 10+10 at a flow rate of 1.9oz at 15mph, which results in the application of .0012lbs of active ingredient per acre. This is the lowest active ingredient rate suggested on the product label (CMMCP 2007). In order to maintain proper application equipment standards, CMMCP conducts a ULV Sprayer Maintenance and Calibration Program as part of the Standard Operating Procedures Manual[MSOffice1]. This ongoing program involves monthly droplet size tests and flow rate calibration, and well as other general maintenance actions for the ULV machines such as spray head flushing and ultrasonic cleaning. In striving to monitor the strength of their protocols, CMMCP conducted an efficacy review of the 2007 adulticide program.

Efficacy trials of the past tend to use caged mosquitoes over natural populations because of their rapid, economical, and more standardized results. Despite these differences, studies have shown that the percent reduction of caged mosquitoes is the same as the reduction of the natural populations (Mount 1998). Any poor results of a ULV application could be caused by an ineffective insecticide dosage, mosquito resistance to that insecticide, unfavorable weather conditions, reduced target coverage due to dense vegetation, or quick repopulation of the area (Curtis 1996; Efird 1991; Mount 1998).

Mosquito insecticide resistance has become an issue in recent years. Routine resistance surveillance is needed to ensure that resistance is not impacting the efficacy of ULV applications (Brogdon 1998). CMMCP has started routine resistance surveillance and the results indicate that resistance to the current insecticide does not seem to be an issue with the mosquito populations in the CMMCP service area (Cornine 2007).

Weather conditions can also have a great impact on the effectiveness of an ULV application. Important factors include wind direction and velocity, temperature and temperature gradients (Mount 1998). Wind direction and velocity are important in that they are needed to create the

drift for the adulticide across the target area. Velocities of 1-7mph are ideal with gusts of no more than 11mph. Ambient temperatures are important to the efficacy of ULV applications in that they influence mosquito activity as well as possibly compromising the effectiveness of the insecticide itself.

Another temperature factor is the temperature gradients in the atmosphere which can impact the inversion of the application product into the elevated levels of tree canopies (Mount 1998). This can be important for vector control efforts due to the fact that certain potential vector species of Eastern Equine Encephalitis (EEE) and West Nile virus (WNV) tend to congregate in the canopies, namely *Culiseta melanura* and *Culex pipiens* (Anderson 2004). These meteorological factors all play a part in the mosquito control efficacy of ULV applications. With these factors in mind, it is generally more advantageous to perform applications in the evenings due to mosquito activity and weather conditions (Mount 1998).

Vegetated areas can also be a factor in the efficacy of a ULV application (Mount 1998). It has been noted that a higher dosage rate may have to be used to obtain the same control level for areas where there is heavy vegetation compared to open spaces (Curtis 1996). This is due in part to the idea that the size and amount of droplets at the regular dosage rates may be unable to physically reach the adult mosquitoes in the vegetative cover. In fact, it has been reported that for a ULV application in vegetative areas compared to open spaces, the effectiveness of the normal dosage rates can be reduced by over 4 times (Curtis 1996; Mount 1998). With cost and environmental impact in mind, mosquito control personnel tend to use insecticide at the lowest suggested rates, but in situations where there is dense cover for adult mosquitoes these rates may be less effective, resulting in the need for additional applications, increasing costs and impact overall (Curtis 1996). Dense housing, fencing and other wall structures can also have similar impacts on the effectiveness of ULV ground applications as vegetation (Mount 1998).

A major problem with efficacy studies using natural populations is that mosquitoes in neighboring areas may repopulate the area after the application (Efird 1991; Mount 1998). In areas where the street layout allows a large coverage area, ULV applications can provide increased control over smaller targeted areas because of the possibility of reinfestation. In these small target areas situations, additional and more frequent applications may have to be made for adequate control (Mount 1998).

Keeping all of these factors in mind, CMMCP personnel conducted efficacy trials of the 2007 adulticide program to help determine what limiting factors may be present and if any procedural changes are consequently needed.

METHODS

To test the efficacy of the CMMCP standard adulticide procedure, two sites were chosen per week for seven weeks with mosquito collections made for both sites every weekday evening. One of these sites was selected to be sprayed in the standard manner while the other is not sprayed and is used as the control site. Collections were made for each site Monday through Friday with the experimental site being adulticided on Wednesday evenings. Test sites were chosen from service requests received, while the control sites were selected from nearby areas that the residents were informed that their property would be treated as an exclusion area for that week. Of the seven weeks of trials, four were at residential sites, two at recreational locations, and one was at a transfer station.

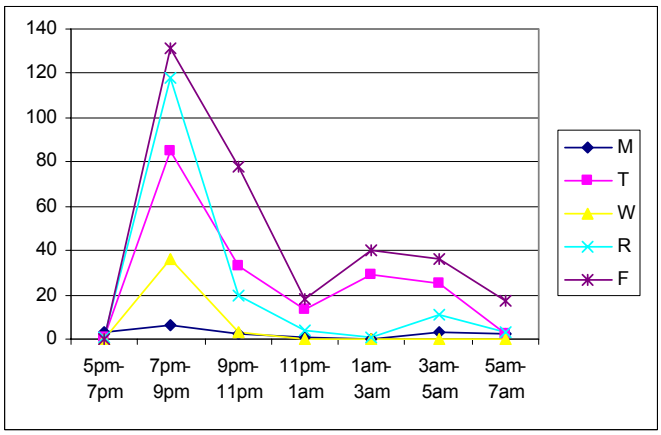
Using model 512 CDC miniature light traps baited with CO_2 (500ml/min), along with model 1512 collection bottle rotators (John W. Hock Co., Gainesville, FL), we were able to make the nightly collections that could be identified as to what time period the specimens were captured. There were seven collection time periods used for this project, programmed for 2 hour intervals from 5pm to 7am in order to observe the peak mosquito activity times as well as to have greater detail on the impact of the application.

Specimens were counted by site and collection period, with the weekly data for each site plotted. After plotting the data for both sites during the week, the graphs could be compared to help determine to what affect the adulticide application had on the local mosquito population. Then we compare for both sites, the two days before the application, the day of the application, and also the two days after the application. On the evening of applications, field technicians noted the time, temperature and wind direction prior to beginning.

EFFICACY TRIAL #1

Looking at each week's corresponding site collections, we can observe a couple trends. For MMWR 23 collections, the pre-application evening for the application site was more than double of the night of the application. This observation was different from the control site, which showed an increase during the application evening. Despite this decrease, levels for the two post-application evenings were much higher for both sites. A rain event during the first evening looks to have negatively impacted the collections from both sites, but especially from the application site (Figures 1-4).

Figure 1: MMWR 23 Application Site Collections (6/4-6/8)



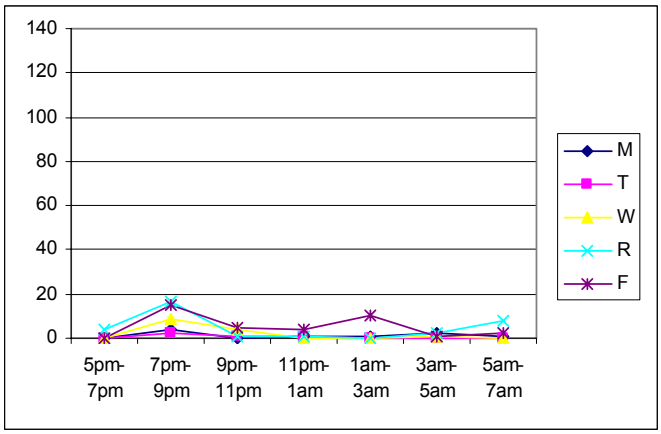
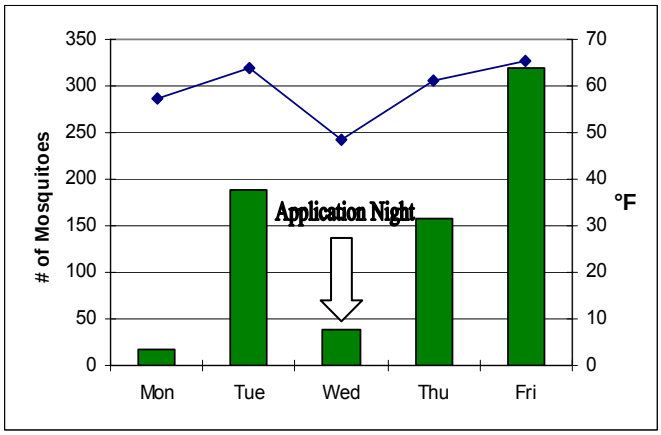


Figure 2: MMWR 23 Control Site Collections (6/4-6/8)

Figure 3: MMWR 23 Application Site Nightly Collection Totals With Midnight Temperatures (°F) (6/4-6/8)



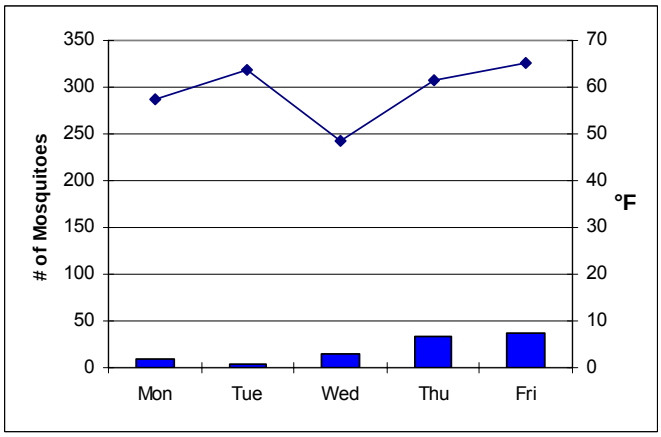
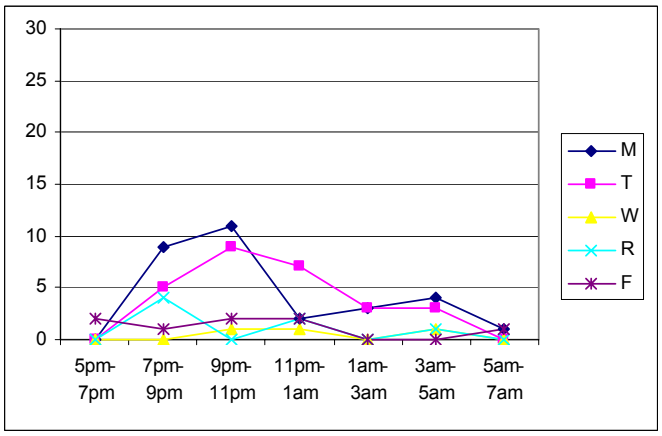


Figure 4: MMWR 23 Control Site Nightly Collection Totals With Midnight Temperatures (°F) (6/4-6/8)

EFFICACY TRIAL #2

The results from MMWR 24 were affected by evening temperatures on the application night that were much cooler than those for the rest of the week. This midweek dip in temperatures may have altered the results of the application. The post-application collections were relatively lower than pre-application, although the results were shown for both the control and application sites (Figures 5-8).

Figure 5: MMWR 24 Application Site Collections (6/11-6/15)



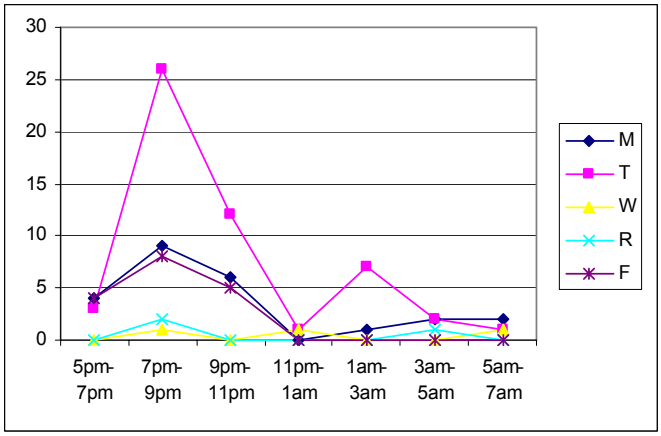
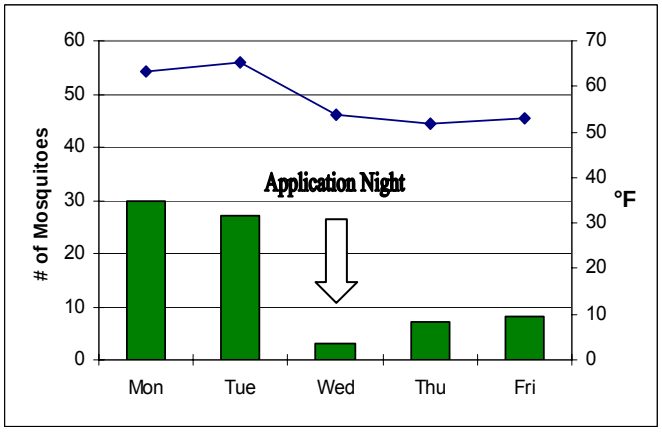


Figure 6: MMWR 24 Control Site Collections (6/11-6/15)

Figure 7: MMWR 24 Application Site Nightly Collection Totals With Midnight Temperatures (°F) (6/11-6/15)



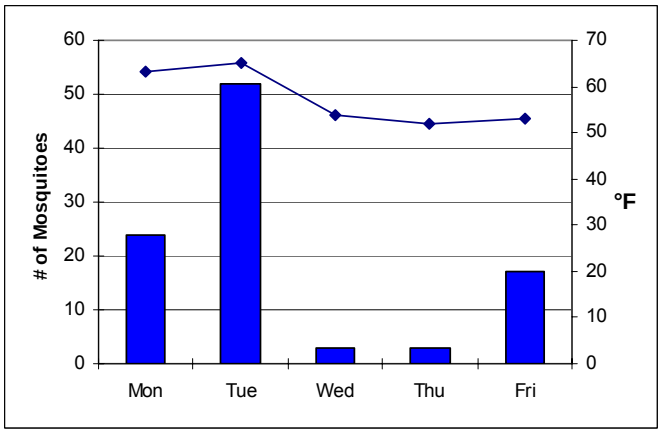
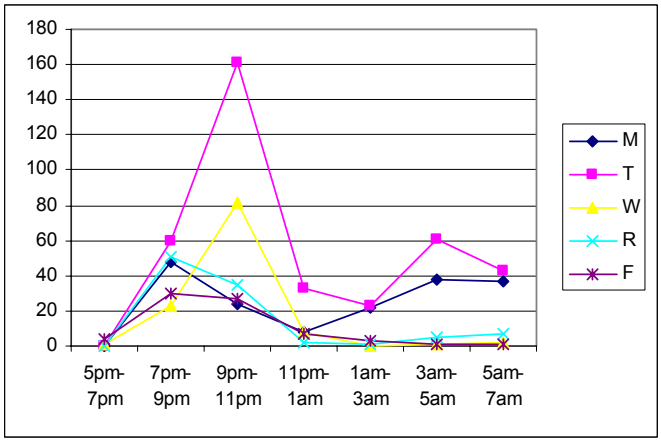


Figure 8: MMWR 24 Control Site Nightly Collection Totals With Midnight Temperatures (°F) (6/11-6/15)

EFFICACY TRIAL #3

The control obtained for MMWR 25 was relatively good for the application night as well as the two evenings after, although the second post application night had a dip that was similar to that of the control site. A rain event was recorded for the first post-application evening, possibly impacting the collection numbers of both sites. Field technicians noted the wind traveling from the spray origin toward the application site trap location (Figures 9-12). Despite these factors, the MMWR 25 trial showed good control.

Figure 9: MMWR 25 Application Site Collections (6/18-6/22)



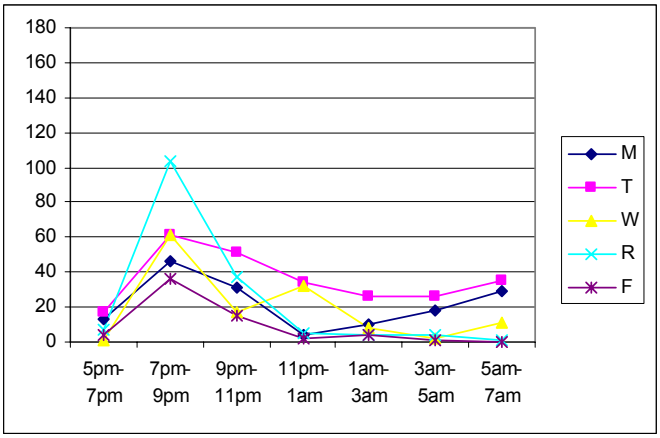
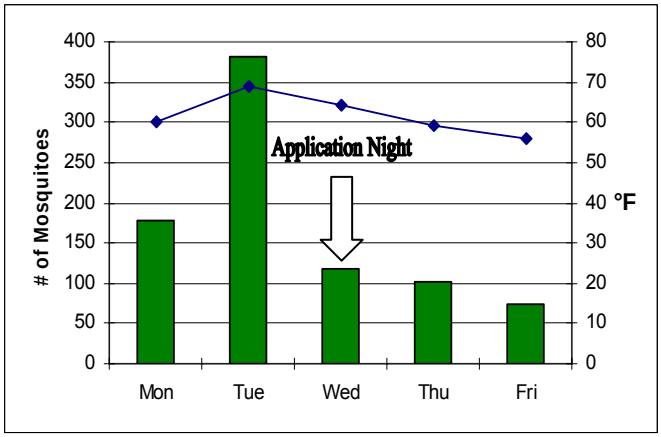


Figure 10: MMWR 25 Control Site Collections (6/18-6/22)

Figure 11: MMWR 25 Application Site Nightly Collection Totals With Midnight Temperatures (°F) (6/18-6/22)



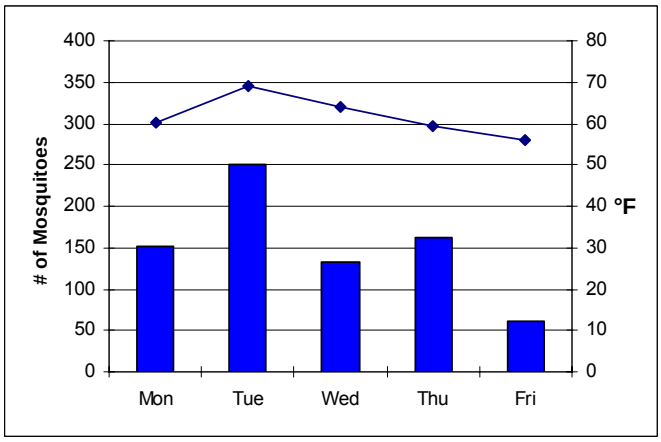
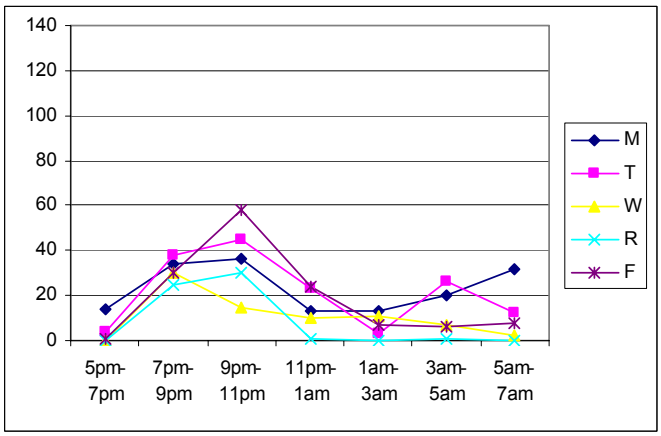


Figure 12: MMWR 25 Control Site Nightly Collection Totals With Midnight Temperatures (°F) (6/18-6/22)

EFFICACY TRAIL #4

For the MMWR 28 trial set, the application night control was good, but eventually it returned to pre-application levels, which mirrored the control site population changes as well. Field technicians noted that there was very little wind present during the application, and it was followed by a brief rain event as well. These observations may have influenced the results of the application (Figures 13-16).

Figure 13: MMWR 28 Application Site Collections (7/9-7/13)



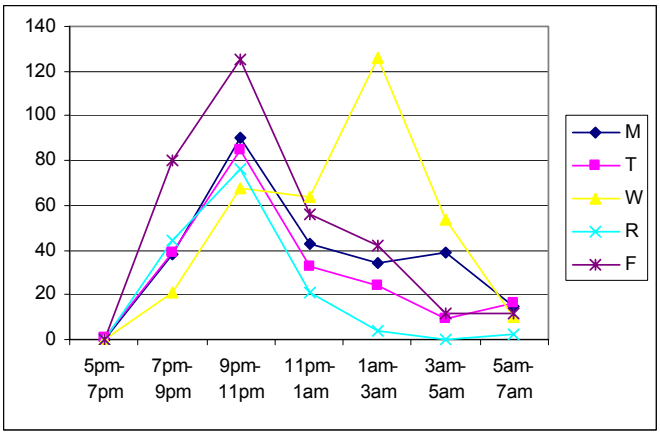
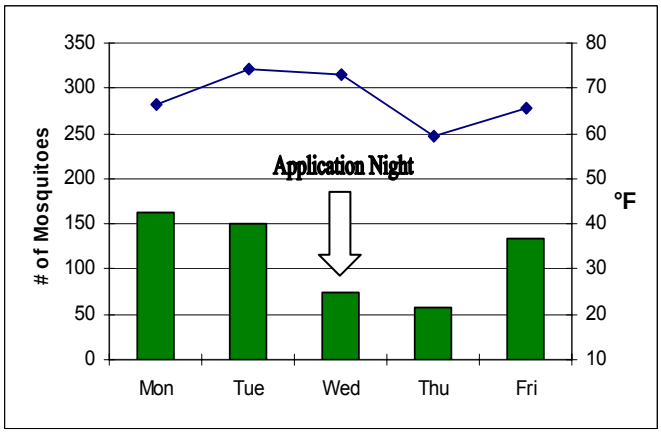


Figure 14: MMWR 28 Control Site Collections (7/9-7/13)

Figure 15: MMWR 28 Application Site Nightly Collections With Midnight Temperatures (°F) (7/9-7/13)



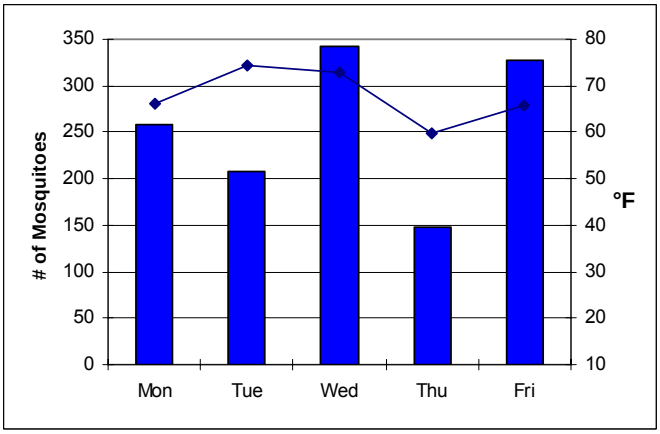
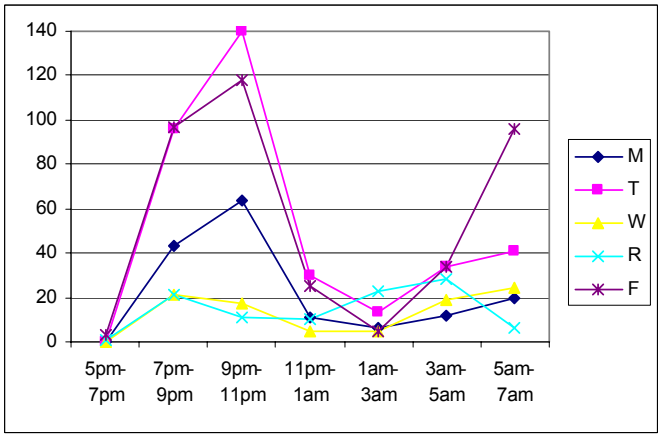


Figure 16: MMWR 28 Control Site Nightly Collections With Midnight Temperatures (°F) (7/9-7/13)

EFFICACY TRIAL #5

The MMWR 29 trial set showed good control for the application night and post-application evening before populations returned to pre-application levels. The post-application evening control may not be as significant because the corresponding night for the control site also had lowered levels from pre-application numbers. Field technicians also noted very little wind on the application evening, which could have hampered the results of the application (Figures 17-20).

Figure 17: MMWR 29 Application Site Collections (7/16-7/20)



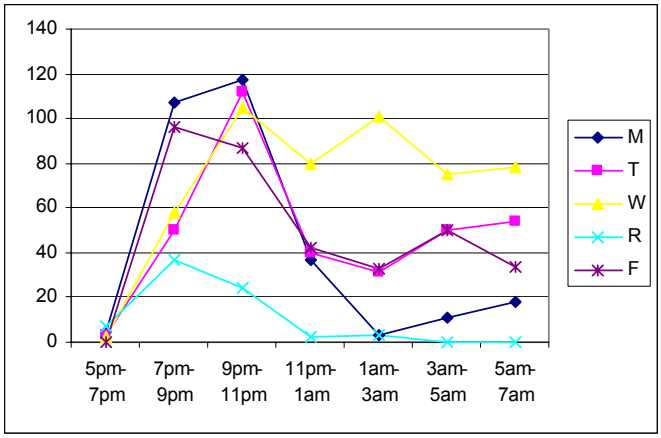
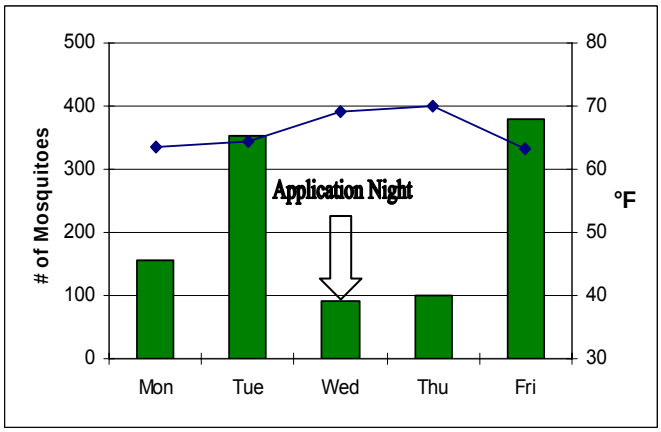


Figure 18: MMWR 29 Control Site Collections (7/16-7/20)

Figure 19: MMWR 29 Application Site Nightly Collections With Midnight Temperatures (°F) (7/16-7/20)



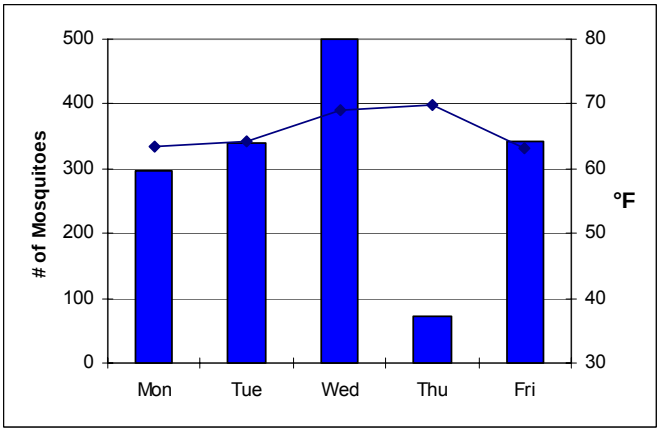
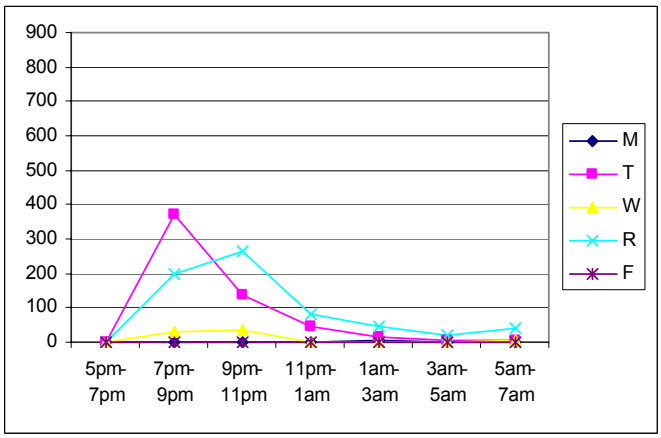


Figure 20: MMWR 29 Control Site Nightly Collections With Midnight Temperatures (°F) (7/16-7/20)

EFFICACY TRIAL #6

The MMWR 30 trial set had a similar trend to the previous weeks. The collections from the application night showed good control, but the post-application evening was not, eventually returning to pre-application levels. The wind direction was favorable during the application, although the wind speed was very low. A rain event on the last evening may have impacted the collections of that night (Figures 21-24).

Figure 21: MMWR 30 Application Site Collections (7/23-7/27)



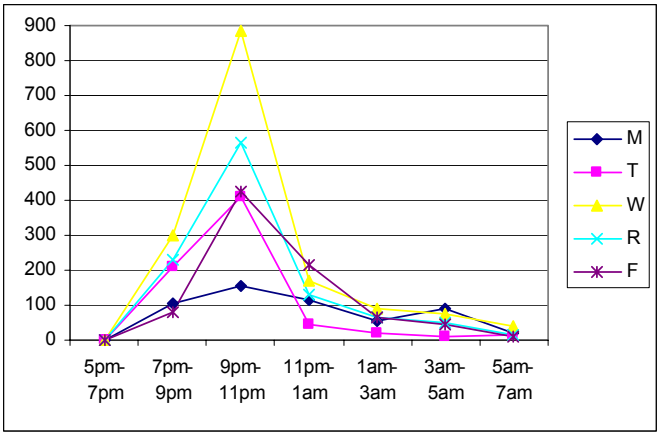
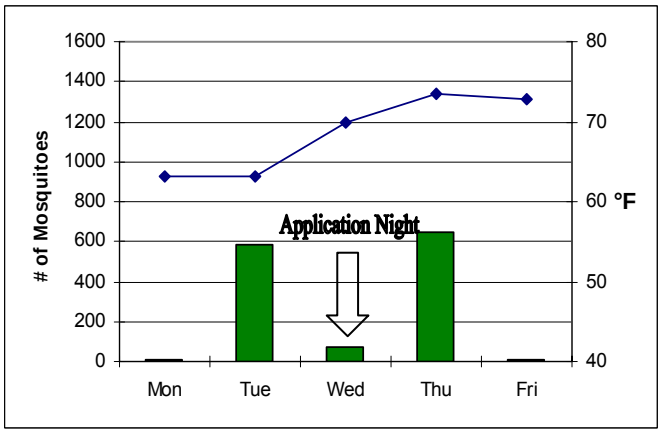


Figure 22: MMWR 30 Control Site Collections (7/23-7/27)

Figure 23: MMWR 30 Application Site Nightly Collections With Midnight Temperatures (°F) (7/23-7/27)



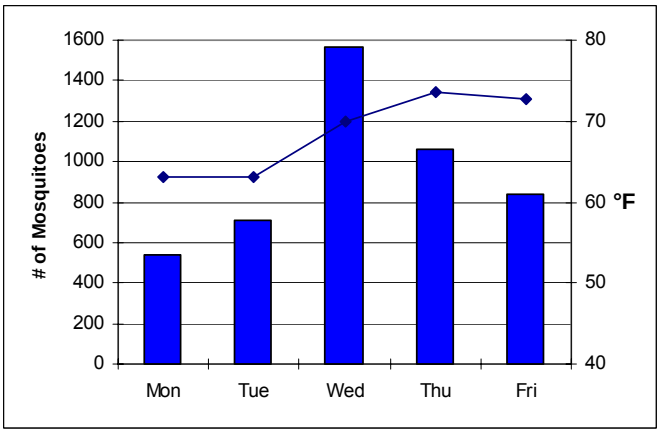
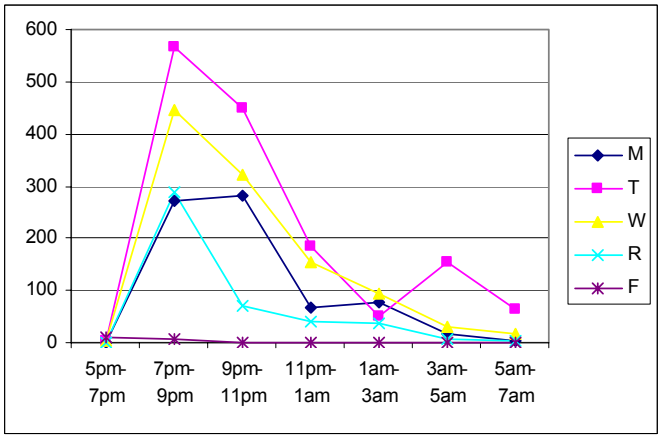


Figure 23: MMWR 30 Control Site Nightly Collections With Midnight Temperatures (°F) (7/23-7/27)

EFFICACY TRIAL #7

The MMWR 32 was the last trial set of the study and again showed similar results to the other weeks. The collections from the application night exhibited good control as did the two post-application collections. Observed wind direction during the application seemed favorable as well as wind speed. Despite the lower post-application collection numbers of the application site, the control site also exhibited similar changes during this time (Figures 25-28).

Figure 25: MMWR 32 Application Site Collections (8/6-8/10)



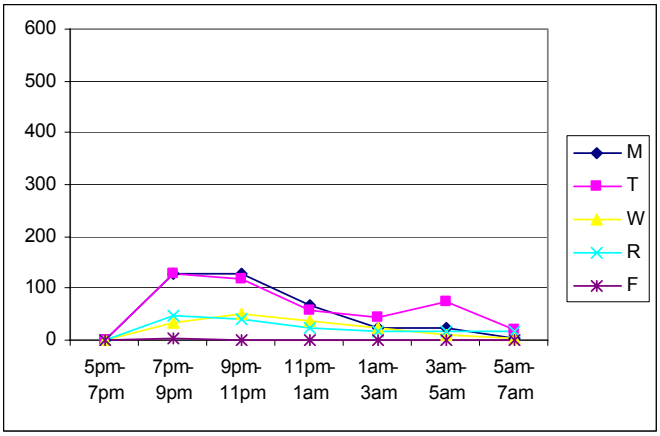
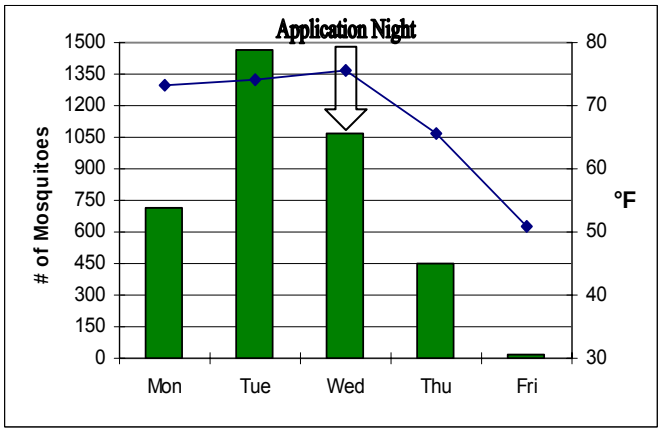


Figure 26: MMWR 32 Control Site Collections (8/6-8/10)

Figure 27: MMWR 32 Application Site Nightly Collections With Midnight Temperatures (°F) (8/6-8/10)



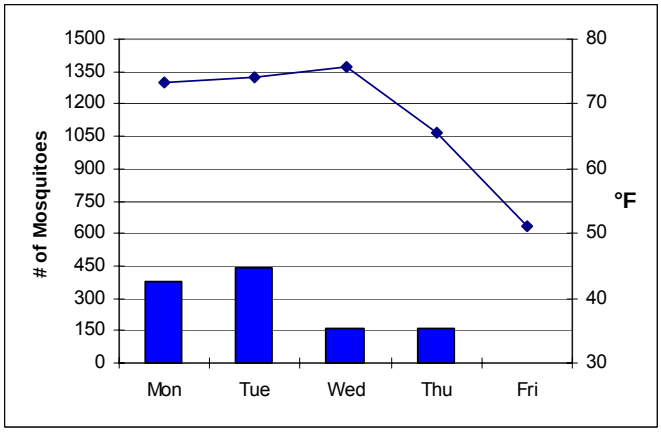


Figure 28: MMWR 32 Control Site Nightly Collections With Midnight Temperatures (°F) (8/6-8/10)

DISCUSSION

As previously discussed, many past efficacy studies of ULV applications involve using caged mosquitoes. Using caged mosquitoes has its advantages with many less variables, but doesn't necessarily give you an accurate picture of what's really happening. Caged mosquitoes can judge the efficacy of an adulticide product very well, but may not mimic the actual field results of an adulticide program. With our residential field trials one has to factor in the role that weather plays on natural mosquito populations, as well as irregular road design, vegetation and obstructions at the residence, and migration of neighboring mosquitoes to name a few. I think that many of these problems associated with natural mosquito population field trials were apparent in our study.

Overall, our study showed that control was achieved for approximately one to two nights before the mosquito populations returned to pre-application levels. This was similar to another study where one day post treatment control was good, but then after two days post-treatment populations began to return to pretreatment levels. Authors involved seemed to believe that this rebound was due to quick reinfestation of area and also some weather factors (Mount 1998).

I believe that the findings in this study were primarily a product of rapid reinfestation by neighboring mosquito populations. This migration of mosquitoes was made more easily due to the fact that the target areas were relatively small, and like other synthetic pyrethroids, leaves very little residual and has a rapid breakdown (Lesser 1998). This property of the chemical lends itself to a quicker reinfestation compared to that of a barrier treatment with higher residual characteristics. Even if the target areas were to be expanded, some of the site locations would not have provided a road network that would have allowed for a greater penetration of the insecticide into the forested areas. If applications were able to have been made on an adequate road design around the target site such as a street layout using a grid pattern with low to moderate foliage, control results would have been improved. Most of these locations also contained at least some vegetative cover, which could have impacted the results.

Despite these results it is not believed that a significant change in procedure is needed. Possibly a slight increase in dosage rate, which would still be under the allowable EPA and label rates, may improve control, especially in vegetative cover instances. This increase would also help in less than ideal weather conditions. Another possible change in procedure could be to try to increase coverage whenever allowable. Spray applicators always need to consider whether or not to apply when conditions are doubtful. Applications in unsuitable conditions may not be providing much control, wasting product and increasing the potential for negative environmental impact. If adjustments to the application procedure are not made, future spray events should not be expected to result in different control levels as compared to our study.

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and the Central Mass. Mosquito Control Project Commissioners

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WEST NILE VIRUS INTERVENTION – MARLBOROUGH, MA AUGUST 2007

On July 26, 2007 the Mass. Dept. of Public Health (MDPH) confirmed that a bird collected on July 24 tested positive for West Nile Virus (WNV). The Central Mass. Mosquito Control Project coordinated with MDPH and placed additional surveillance traps in the area the bird was found. CMMCP also expanded its treatment of catch basins in that area to reduce the larval population of *Culex* species and other mosquito species that prefer these areas for larval development. No spraying in the area was done at that time. A press release from MDPH was sent to all media outlets.

On August 1, 2007 MDPH confirmed that a collection of adult *Culex* mosquitoes collected July 30 tested positive for West Nile Virus. CMMCP issued a press release to local media outlets and the City of Marlborough.

Areas where the virus was identified were sprayed after sunset between 9pm and midnight on the evenings of August 1, 5 and 8, 2007 in coordination with the Marlborough Mayor's office, Department of Public Works and City Health Department. Concerns regarding the City's watershed area around Millham Reservoir were discussed, and the spray application was modified slightly to stay away from the area. The City began a study of the raw water from the intake pipe to monitor for any negative effects from the spray application. A map of the intended spray areas was posted on the CMMCP website, a street listing was recorded on the CMMCP phone system, and the City used a reverse 911 system to alert residents in the area.

The product used for spraying by CMMCP is called sumithrin (d-phenothrin), and is the same product used by MDPH in southeastern Mass. last year for the aerial spraying event and is the product we use as part of our standard adulticide program.

Data analysis have shown overall mosquito populations have been reduced, and additional mosquito testing has not identified new virus activity at this time. It is very important to note that while the risk from West Nile Virus may have been lowered to some extent; it is not at zero risk; residents need to be aware that WNV may still be in the area, and to use personal protection measures to further reduce your exposure and lower your risk from mosquitoes. WNV will continue to be a threat until freezing temperatures become predominant in the area.

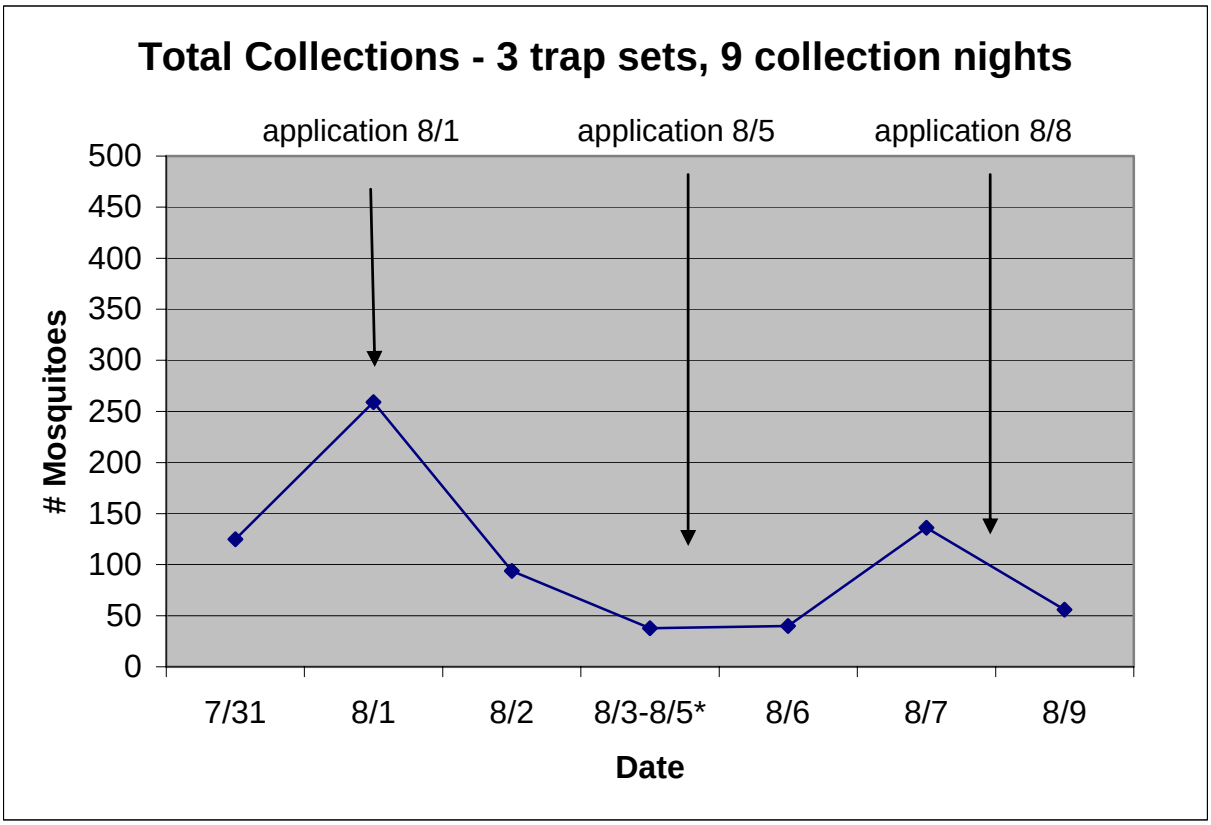
After the data was analyzed, CMMCP issued an additional press release outlining the success of this operation, noting that while risk has been reduced, it is not at zero.

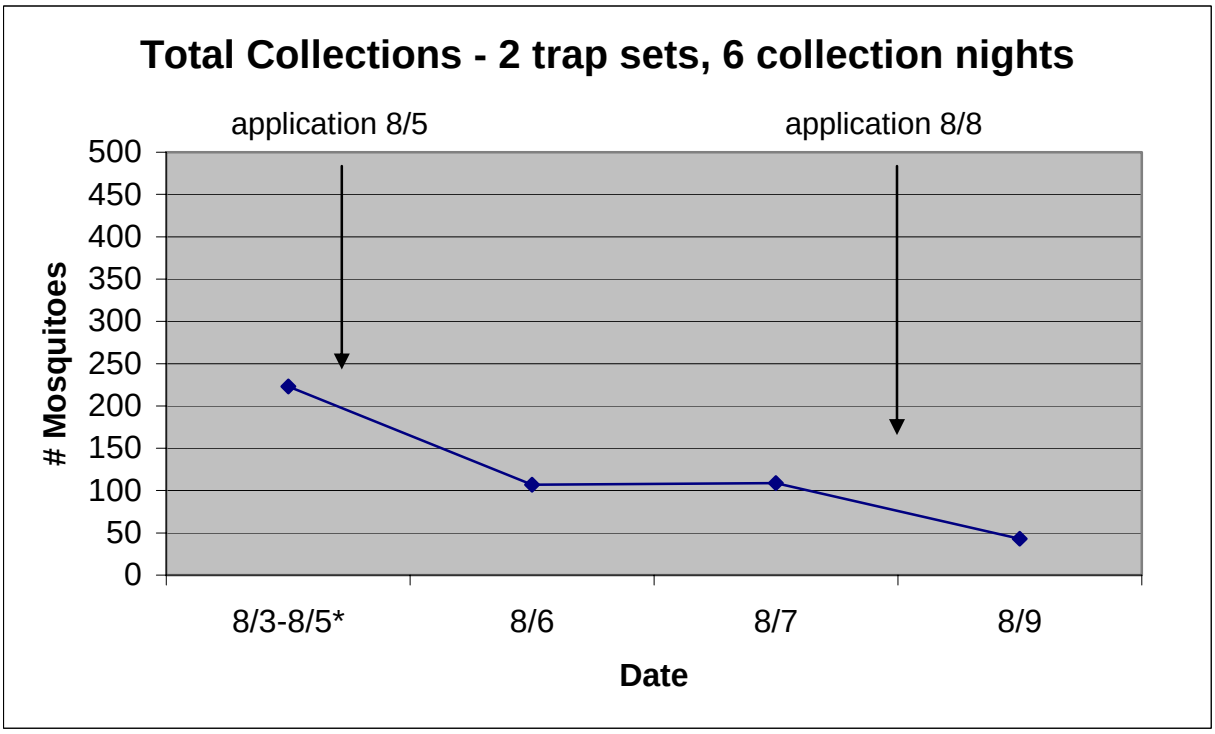
In 2006, there were three human cases of WNV, with no fatalities. While WNV can infect people of all ages, people over the age of 50 are at higher risk for severe disease. WNV is usually transmitted to humans through the bite of an infected mosquito.

Synopsis: **Timothy Deschamps**, *CMMCP Executive Director*
Mosquito identification: **Curtis Best**, *CMMCP Staff Entomologist*
Data compilation & analysis: **Frank Cornine**, *CMMCP Field Biologist*

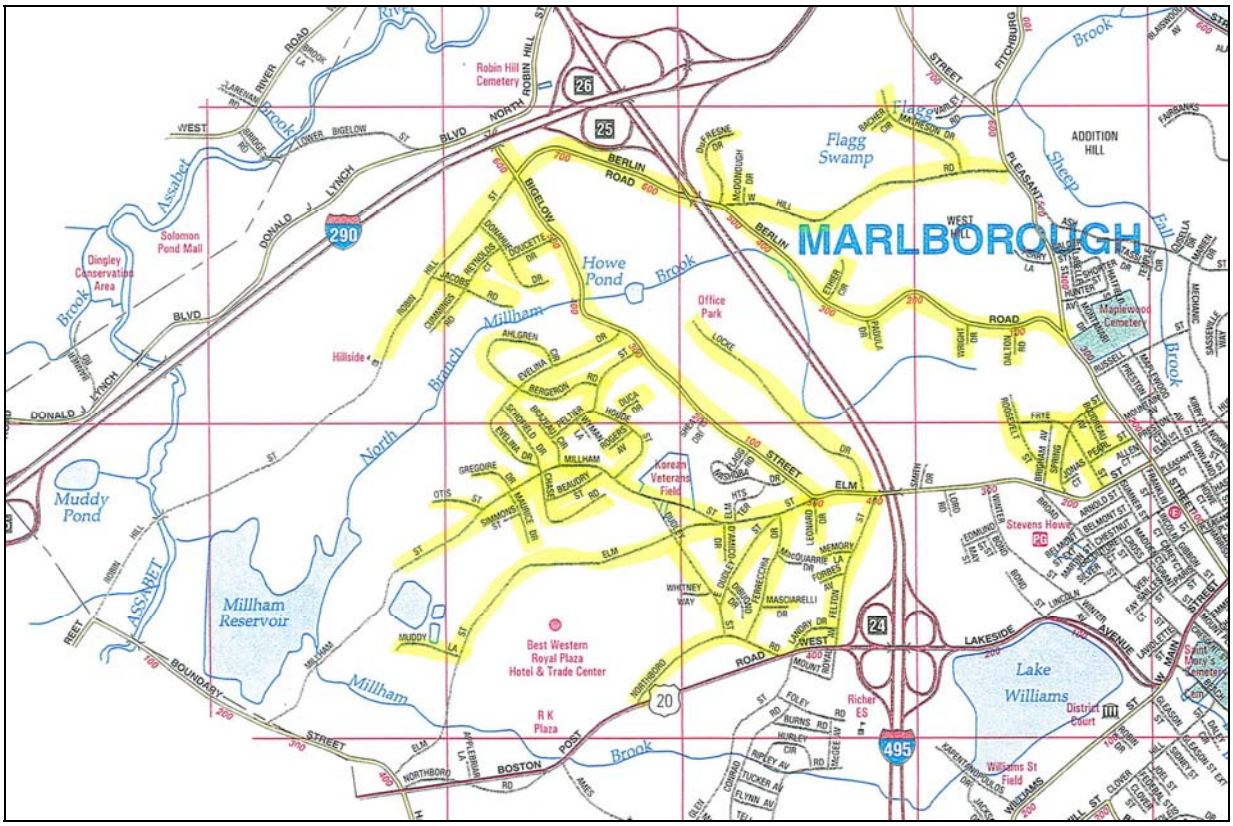
8/17/2007

Surveillance summaries, pre and post application





Application map



Street listing

Ahlgren Cir.
Bacher Cir.
Beaudry St.
Bergeron Rd.
Berlin Rd.
Bigelow St.
Boudreau Ave.
Brazeau Cir.
Brigham Ave.
Chase Rd.
Cleversy Dr.
Cummings Rd.
Dalton Rd.
D'Amico Dr.
Dibuono Dr.
Donahue Dr.
Doucette Dr.
Duca Dr.
Dudley St.
DuFresne Dr.
E. Dudley St.
Elm Hts.
Elm St.
Elm Ter.
Ethier Cir.
Evelina Dr.
Felton St.
Ferrecchia Dr.
Flagg Rd.
Forbes Ave.
Frye St.
Gregoire Dr.
Houde St.
Jacobs Rd.
Jonas Ct.
Joseph North Dr.
Landry Dr.
Leonard Dr.
Locke Dr.
MacQuarrie Dr.
Masciarelli Dr.
Matheson Dr.
Maurice Dr.
McDonough Dr.
Memory Ln.
Millham St.
Muddy Ln.
Nashoba Dr.
Northboro Rd.
Otis St.
Padula Dr.
Pearl St.
Peebles Way
Peltier St.
Reynolds Ct.
Robin Hill Rd.
Rogers Ave.
Roosevelt St.
Schofield Dr.
Simmons St.
Spring St.
West Hill Rd.
Whitney Way
Wright Dr.
Wyman Ln.

The Commonwealth of Massachusetts
State Reclamation & Mosquito Control Board
CENTRAL MASS. MOSQUITO CONTROL PROJECT
111 Otis Street Northborough, MA 01532
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Richard J. Day
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FOR IMMEDIATE RELEASE:
August 1, 2007

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Mosquito Collection in Marlborough Tests Positive for West Nile Virus

On July 26, 2007 the Mass. Dept. of Public Health (MDPH) confirmed that a bird collected on July 24 tested positive for West Nile Virus (WNV). The Central Mass. Mosquito Control Project coordinated with MDPH and placed additional surveillance traps in the area the bird was found. CMMCP also expanded its treatment of catch basins in that area to reduce the larval population of *Culex* species and other mosquito species that prefer these areas for larval development. No spraying in this area was done at that time. A press release from MDPH was sent to all media outlets.

On August 1, 2007 MDPH confirmed that a collection of adult *Culex* mosquitoes collected July 30 tested positive for West Nile Virus.

Areas around the surveillance trap are planned to be sprayed after sunset between 9pm and midnight on the evening of August 1, 2007 in coordination with the Marlborough Board of Health. A map of the intended spray areas is available on the CMMCP website for from its office. The product used for spraying by CMMCP is called sumithrin (d-phenothrin), and is the same product used by MDPH in southeastern Mass. last year for the aerial spraying event. Additional treatments in the same area may be planned for the evenings of August 3 and 5.

In 2006, there were three human cases of WNV, with no fatalities. While WNV can infect people of all ages, people over the age of 50 are at higher risk for severe disease. WNV is usually transmitted to humans through the bite of an infected mosquito.

“This species of mosquito is not likely to bite humans, but this confirmation reminds us that the virus is present in the environment, and we must do everything possible to avoid mosquito bites” said CMMCP Executive Director, Timothy Deschamps. “You can

help reduce mosquito populations around your home and neighborhood by emptying anything that collects water. Please use repellents according to label directions if you are outside in areas of high numbers of mosquitoes".

Personal protection measures have an important role to play in monitoring for WNV and protecting themselves and their loved ones.

Avoid Mosquito Bites – **Be Aware of Peak Mosquito Hours** – The hours from dusk to dawn are peak biting times for many mosquitoes. Consider rescheduling outdoor activities that occur during evening or early morning. Otherwise, take extra care to use repellent and protective clothing.

Clothing Can Help reduce mosquito bites. Although it may be difficult to do when it's hot, wearing long-sleeves, long pants and socks when outdoors will help keep mosquitoes away from your skin.

Apply Insect Repellent when you go outdoors. Use a repellent with DEET (N, N-diethyl-m-toluamide), permethrin, picaridin (KBR 3023), or oil of lemon eucalyptus [p-methane 3, 8-diol (PMD)] according to the instructions on the product label. DEET products should not be used on infants under two months of age and should be used in concentrations of 30% or less on older children. Oil of lemon eucalyptus should not be used on children under three years of age.

Mosquito-Proof Your Home – **Drain Standing Water** – Mosquitoes lay their eggs in standing water. Limit the number of places around your home for mosquitoes to breed by either draining or getting rid of items that hold water. Check rain gutters and drains. Empty any unused flowerpots and wading pools, and change water in birdbaths frequently.

Install or Repair Screens – Some mosquitoes like to come indoors. Keep them outside by having tightly-fitting screens on all of your windows and doors.

Report Dead Birds – Dead crows, blue jays or robins may be a sign that WNV is circulating among the birds and mosquitoes in an area. Call 1-866-MASS WNV to report a dead bird. By reporting dead birds, you can play an important role in monitoring WNV.

More information is available on the DPH website at http://www.mass.gov/dph/wnv/wnv1.htm. Information about WNV and EEE is also available by calling the DPH recorded information line at 1-866-MASS-WNV (1-866-627-7698).

For more information please call CMMCP at (508) 393-3055 or log on to our website at www.cmmcp.org.

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State Reclamation & Mosquito Control Board
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West Nile Virus Risk in Marlborough is Reduced but Not Eliminated

On July 26, 2007 a bird collected two days earlier tested positive for West Nile Virus (WNV), and on August 1 a collection of *Culex* mosquitoes was identified with this virus. In coordination with the Marlborough Mayor's office and the City Health Department, the Central Mass. Mosquito Control Project performed applications of sumithrin to the northwestern section of the city where the virus was found. Applications were done according to recommendations from the MDPH Vector Control plan for WNV, and after consultation with the State Reclamation & Mosquito Control Board. These applications were done in this area on the evenings of August 1, 5 and 8 from sunset to 11:00pm.

Surveillance from MDPH and CMMCP surveillance sites gathered before and after these applications show an overall reduction in mosquito numbers, and no new virus isolations have been identified thus far.

While this may indicate that the WNV risk has been reduced to some extent, the RISK LEVEL IS NOT AT ZERO and residents must listen for additional public health announcements, and perform personal protection measures to reduce their exposure to mosquitoes until freezing temperatures become widespread in the area.

"If additional virus is identified in the city, we will again coordinate with local and state officials to determine the appropriate response" said CMMCP Executive Director, Timothy Deschamps. "Weather, time of year, mosquito species and other factors influence the available interventions. You can reduce mosquito populations around your home by emptying any containers that collects water. Please use repellents according to label directions if you are outside in areas of high numbers of mosquitoes".

Personal protection measures have an important role to play in monitoring for WNV and protecting themselves and their loved ones.

Avoid Mosquito Bites – **Be Aware of Peak Mosquito Hours** – The hours from dusk to dawn are peak biting times for many mosquitoes. Consider rescheduling outdoor activities that occur during evening or early morning. Otherwise, take extra care to use repellent and protective clothing.

Clothing Can Help reduce mosquito bites. Although it may be difficult to do when it's hot, wearing long-sleeves, long pants and socks when outdoors will help keep mosquitoes away from your skin.

Apply Insect Repellent when you go outdoors. Use a repellent with DEET (N, N-diethyl-m-toluamide), permethrin, picaridin (KBR 3023), or oil of lemon eucalyptus [p-methane 3, 8-diol (PMD)] according to the instructions on the product label. DEET products should not be used on infants under two months of age and should be used in concentrations of 30% or less on older children. Oil of lemon eucalyptus should not be used on children under three years of age.

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For more information please call CMMCP at (508) 393-3055 or log on to our website at www.cmmcp.org.

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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)

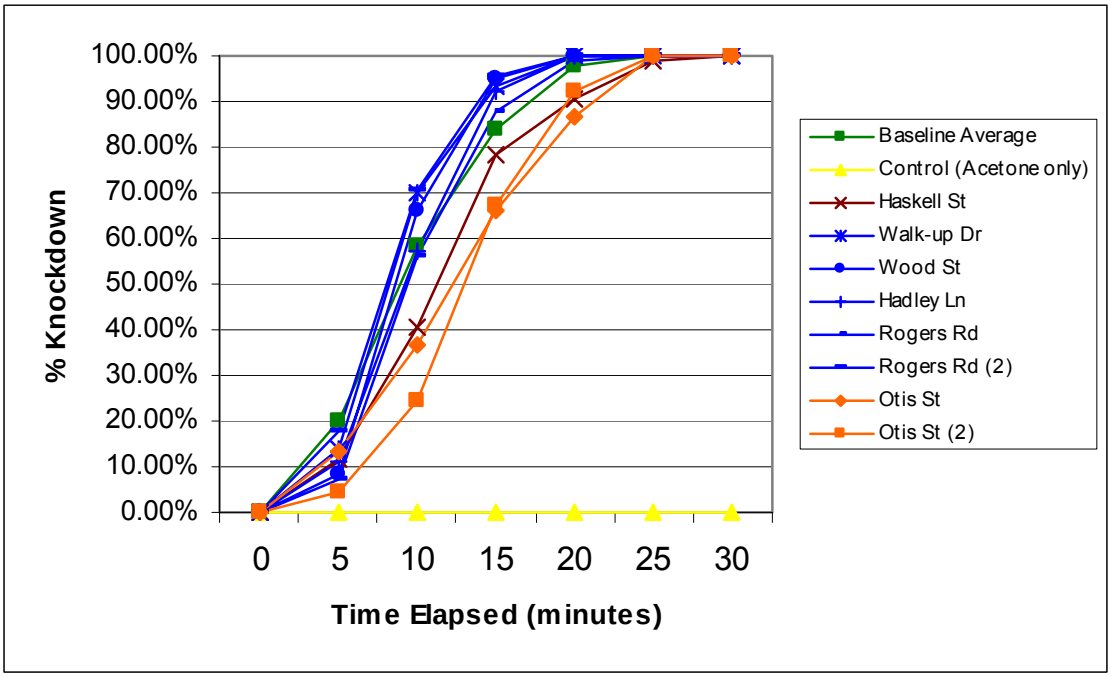
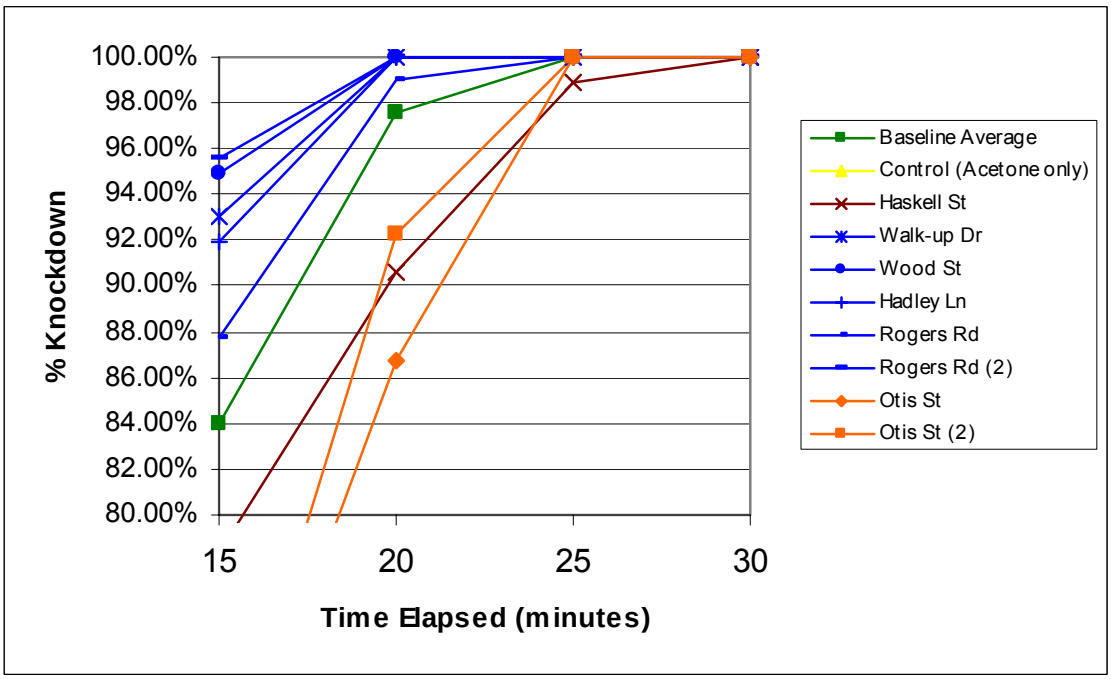


Figure 2: Time-% Knockdown Curve of Bottle Assay (2) for ANVIL® 10+10 (22.17µg/ml)



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.

ACKNOWLEDGEMENTS

I would like to thank the following people and groups for their help and guidance throughout this project:

Timothy Deschamps, Exec. Director
Curtis Best, Staff Entomologist
Peter Laptewicz, Intern
and the Central Mass. Mosquito Control Project Commissioners

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(Journal Name)

VectoBac G (Bti) Efficacy for Field Application of

***Ochlerotatus abserratus* Mosquito Larvae**

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DRAFT

Abstract

Bacillus thuringiensis israelensis (Bti) is a commonly used larvicide part of many mosquito control programs. We tested the potency of VectoBac G (Bti) after it had been stored away for a year. Third instar *Ochlerotatus abserratus* larvae were subjected to five treatments: two sub-lethal dosages of 2.5 and 5 lbs/acre, minimum and maximum label rate of 10 and 20 lbs/acre, and untreated control. Larval mortality was recorded at 1-hr, 2-hr, 24-hrs, and 48-hrs post exposure. After 24 hours, more than 92% mortality was observed in larvae exposed to all treatments of Bti. 100% mortality was recorded after 48 hours of exposure to all treatments of Bti. The untreated control group reported 2% mortality after 48 hours. In conclusion, VectoBac G was still very potent and effective after one year if stored properly.

DRAFT

Introduction

Bacillus thuringiensis is a naturally occurring rod-shaped soil bacterium. In the environment, it rests in a dormant stage as a spore until ingestion in an alkaline gut of an insect activates it. There it releases endotoxins that compromise the integrity of the insect gut lining, which eventually leads to death of the host. In 1976, a mosquito virulent strain, *Bacillus thuringiensis israelensis* (Bti) was discovered (Goldberg and Margalit, 1977).

Bacillus thuringiensis israelensis, a biological control agent, is very specific for mosquitoes with few nontarget effects limited to other dipterans within the suborder of Nematocera. In freshwater treatments Bti is noted to be moderately toxic to *Daphnia* crustaceans. There is no toxic or pathogenic association of Bti to estuarine or marine animals. In the more than 30 years of safe use of *Bacillus thurinigiensis*, the risk of toxicity of Bti to mammals is minimal to nonexistent (EPA.gov, 1998).

The Norfolk County Mosquito Control Project (NCMCP), located in Norwood, Massachusetts, conducts an extensive and calculated aerial larvicide program early in the mosquito season to suppress the imminent mosquito population as a part of its Integrated Pest Management (IPM) program that stresses preventative measures (source reduction, larviciding) above curative ones (adulticide spraying). The intent of this experiment was to test the shelf-life, potency, and efficacy of VectoBac G (Bti, Valent BioSciences), the primary larvicide for the Norfolk County Mosquito Control Project, after it had been stored away for a season or more.

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Materials and Methods

The recommended label rate for the application of VectoBac G is 2.5-10 lb/acre for 1st-2nd instar larvae and 10-20 lb/acre for 3rd-4th instars or when the water is heavily polluted. For our 3rd instar *Oc. abserratus* larvae we used the recommended label-rate treatments of 10 lb/acre and 20 lb/acre and sub-label rates of 25% and 50% of the minimum label rate (2.5 lb/acre and 5 lb/acre respectively). Since we had 17 in x 12 in tubs in the lab we converted the acreage application for square footage of these tubs (volume application rate is not described).

Application rate of 1 lb/acre

1 acre= 43,560 ft^2

1 lb/43,560 ft^2 = 2.29 x 10^{-5} lb/ ft^2

2.29 x 10^{-5} lb/ ft^2 x 453.6g/lb= 0.01 g/ft^2

Application rate of 1 lb/acre for 17 in x 12 in tubs

Area of tub= 17 in x 12 in= 204 in:

204 in^2 x 1 ft^2/ 12 in^2 = 1.42 ft^2

0.01 g/ft^2 x 1.42ft^2= 0.0148 g

Application rates for 1.42ft^2 tubs

2.5 lb/acre= 0.0148 g x 2.5= 0.037 g

5 lb/acre= 0.0148 g x 5= 0.074 g

10 lb/acre= 0.0148 g x 10= 0.148 g

20 lb/acre= 0.0148 g x 20= 0.296 g

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Three sets of 5 basins each (4 treatments and 1 control) were filled to a depth of ~1/2 in of field collected water. Before filling basins with water, we lined ours with white trash bags (due to the fact that our basins are black) to make the mosquito larvae more visible.

We collected and identified larvae from the field as *Ochlerotatus abserratus* (Figure 1). Fifty larvae were transferred into each basin for a total of 750 larvae in all 15 basins (Figure 2). Next, a few pinches of ground fish food (fish flakes) were added to each basin to encourage feeding activity. Immediately afterwards, the corresponding amounts of Bti were added to each treatment basin. This combination of feed and larvicide ensures that the larvae will ingest the Bti spores sooner. Larvae mortality was recorded in 1 hr, 2 hr, 24 hr, and 48 hr intervals. To control for cross-contamination among treatment and control basins, we used 5 different droppers labeled as control, or one of the four treatments to prod and check for live or dead larvae.

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Results

One hour after the treatments began the mortality rate for the following groups were: untreated control, 0.7%; 2.5 lb/acre, 0%; 5 lb/acre, 0%; 10 lb/acre, 4.7%; and 20 lb/acre, 8%. After two hours the mortality rates were: untreated control, 0.7%; 2.5 lb/acre, 2%; 5 lb/acre, 4%; 10 lb/acre, 8.7%; and 20 lb/acre, 18%. Twenty four hours post-treatment mortality rates were as follows: untreated control, 0.7%; 2.5 lb/acre, 92.7%; 5 lb/acre, 99.3%; 10 lb/acre, 99.3%; and 20 lb/acre, 100%. At forty eight hours, mortality rates were noted for the final time: untreated control, 2%; 2.5 lb/acre, 99.3%; 5 lb/acre, 100%; 10 lb/acre, 100%; and 20 lb/acre, 100% (Table 1, Figure 3).

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Discussion

After about a year in storage, VectoBac G was still very potent. Even at the sub-label rate for 3rd instar larvae, we achieved more than 99% larval mortality after 48 hours of exposure in ideal conditions. These results may vary in a more organic or polluted larval habitat.

The Norfolk County Mosquito Control Project protocol calls for 5 lb/acre of Bti via aerial application. In the laboratory setting, 5 lb/acre had a 100% mortality rate after 48 hours in ideal conditions. In our own field calculations and trials, we measured the true rate of application to be approximately 6.74 lb/acre.

Ochlerotatus abserratus larvae were used because they are one of the first mosquito species to emerge in the spring and were relatively abundant. Third instar larvae were used to replicate a real world simulation of NCMCP protocols for spring aerial larviciding. Mosquito larval habitats are monitored on a daily basis in the spring season, and upon discovery of 3rd instar larvae, aerial larviciding is initiated immediately. The NCMCP should be using 10 lb/acre according to label for 3rd instar larvae, but according to our data, 5 lb/acre resulted in 100% larval mortality 48 hours post-application. To maximize our resources, not only are we targeting 3rd instar *Oc. abserratus*, we aim to ensure that one aerial application can cover other emerging broods of mosquito larvae such as 1st-2nd instar *Oc. canadensis* and *Aedes cinereus*.

With the current condition of the economy and budget constraints of many mosquito control agencies, it is imperative that all surveillance and control programs maximize efficiency and cut costs as much as possible. An example would be to follow the minimum application rate or larvicide. For the NCMCP, this has cut down our costs for product and helicopter services. *Bacillus thuringiensis israelensis* is a naturally occurring biological control agent and is the most

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cost effective and environmentally friendly larvicide available. Bti is much more economical to manufacture compared to other larvicides (methoprene, *Bacillus sphaericus*), and is specific for mosquitoes with few nontarget effects (Hajek 2004).

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Table 1. Mortality rates per 150 *Ochlerotatus abserratus* mosquito larvae exposed to varying label rates of VectoBac G *Bacillus thuringiensis israelensis* granules.

	Control		2.5 lb/acre		5 lb/acre		10 lb/acre		20 lb/acre	
Time (H)	# larvae	% mortality	# larvae	% mortality	# larvae	% mortality	# larvae	% mortality	# larvae	% mortality
1	1	0.7%	0	0.0%	0	0.0%	7	4.7%	12	8.0%
2	0	0.7%	3	2.0%	6	4.0%	6	8.7%	16	18.7%
24	0	0.7%	136	92.7%	143	99.3%	136	99.3%	122	100%
48	2	2.0%	10	99.3%	1	100%	1	100%	0	100%
Total	3	2.0%	149	99.3%	150	100%	150	100%	150	100%

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Figure 1. Fifty *Ochlerotatus abserratus* in a cup ready to be transferred into a treatment basin.

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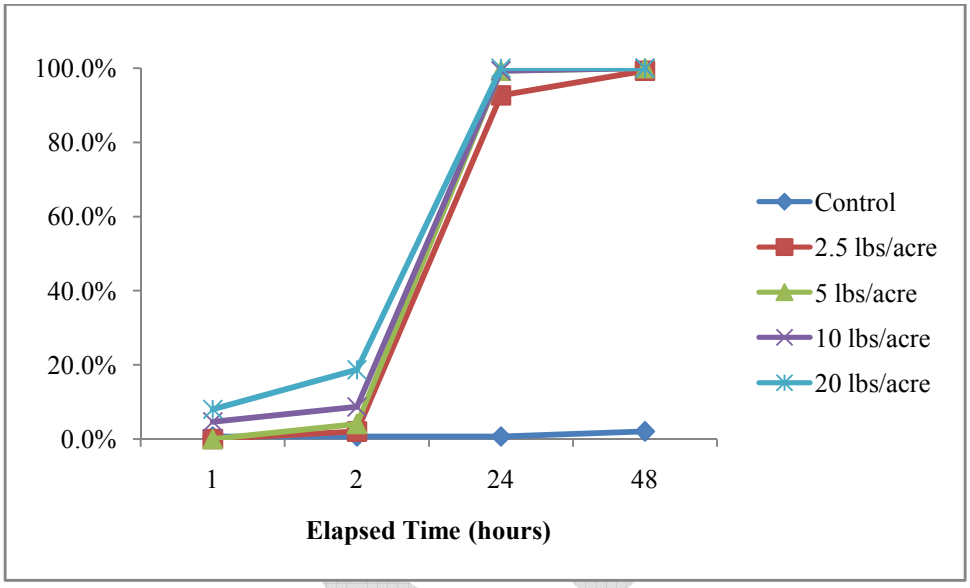
Figure 2. Two of the three sets of replicates in five treatment groups exposed to Bti: untreated control, 2.5 lb/acre (0.04 g), 5 lb/acre (0.07 g), 10 lb/acre (0.15 g), and 20 lb/acre (0.30 g). Basins are lined with white bags to facilitate identification of larvae condition.

Figure 3. Mortality rates per 150 *Ochlerotatus abserratus* mosquito larvae exposed to varying label rates of VectoBac G *Bacillus thuringiensis israelensis* granules.



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