

Final Research Report to FDACS

Project Title: Improving Mosquito Control Efficacy of Aerial ULV Permethrin Applications while Monitoring for Non-target Impacts (FDACS Contract # 012015)

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ABSTRACT

Public Health Entomology Research and Education Center at Florida A & M University, in collaboration with Beach Mosquito Control District, Florida Department of Agriculture and Consumer Services and Bayer Environmental Science, organized a multi-agency team to perform field work for this study located in Panama City Beach, Florida. The aim of the study was to achieve satisfactory mosquito control (>90% mortality) by delivering adequate aerial ULV spray concentration in a targeted spray zone, while limiting impacts to non-target aquatic organisms (i.e, fish). Lab-reared adult salt marsh mosquitoes, *Ochlerotatus taeniorhynchus*, were used for the field mosquito assay which consisted of >50 adult female mosquitoes in holding cages. In addition, lab-reared juvenile and adult of mosquitofish *Gambusia holbrooki* were used for the field non-target bioassay which consisted of at least 20 fish in plastic lined pans with 10 cm of water. Samples (water, yarns and filter papers) from the 12 experimental stations (9 treatment and 3 control stations) were collected and analyzed for naled residue using Gas Chromatography.

The mosquito control efficacy was excellent with four out of the five trials exceeding 90% average mortality. The second trial averaged 78% mosquito mortality. Average mortality in adult fish were similar (i.e., no significant difference) between the control and treatment stations and ranged from 5.3 to 16.8% during the 5-day observation period (post application) Less than 1% of the juvenile fish died in permethrin exposed bioassays compared to no mortality in the controls. No detectable permethrin residues were found in water samples from any of the field bioassays. Permethrin residues were detected and quantified from filter paper and yarn samples in both the spray zone and control zone. The amount of permethrin residue deposited on filter paper ranged from 80 to 600 $\mu\text{g}/\text{m}^2$. The amount of permethrin residue collected on yarn ranged from 9 – 249 $\mu\text{g}/\text{yarn}$. Overall, mosquito mortality appeared to correlate with permethrin residue on yarn, with a level of 80 – 100 $\mu\text{g}/\text{yarn}$ resulting in >90% mortality for caged mosquitoes. This research demonstrated that the aerial ULV application of permethrin is effective in controlling adult mosquitoes without causing unacceptable risk to non-target fish.

Introduction

Background Information

In 2001, West Nile (WN) virus began emerging in many Florida counties, presenting a highly publicized health threat to people and animals. One of the most widely used tools within the State to control adult mosquitoes and reduce mosquito-transmitted diseases was, and continues to be, ultra-low-volume (ULV) application of insecticides. Permethrin products are among the registered mosquito adulticides that are routinely used today for ground ULV application. Currently, the labels for adulticides containing permethrin prohibit aerial application in the State of Florida due to concerns over potential impacts to aquatic organisms. However, some mosquito control programs have expressed interest in applying permethrin aerially for enhanced management of disease vector populations. As a consequence, the Florida Coordinating Council on Mosquito Control (FCCMC) has been considering submitting a recommendation to the Florida Department of Agriculture and Consumer Services (FDACS) to allow for aerial ULV applications of permethrin in the State of Florida.

Permethrin is known to be extremely toxic to many aquatic species. For example, the LC₅₀ values for rainbow trout and blue gill were determined to be 3 and 5 micrograms per liter (µg/L) of permethrin, respectively, in 96h laboratory fish bioassays (Coats and O'Donnell-Jeffery, 1979). Tietze et al. (1995) reported 48h LC₅₀ values for grass shrimp, inland silverside, sheepshead minnow and mosquitofish were 0.049, 2.86, 3.02 and 4.29 ppb of permethrin, respectively. With such low LC₅₀ levels for permethrin, there are concerns that large-scale aerial ULV application of permethrin may result in impacts to non-target aquatic species. Our previous aerial research indicated that smaller droplets (<30µm) produced by a high-pressure nozzle system can decrease environmental deposition of insecticides and, therefore, significantly reduce mortality to non-target organisms such as fiddler crabs and honeybees (Zhong et al. 2003, 2004). We postulate that non-target impacts could be minimized if permethrin ground deposition is reduced to non-toxic levels during aerial application.

In 2005, FDACS funded a permethrin research project (FDACS Contract # 00955), directed by the Public Health Environmental Research and Education Center (PHEREC), Florida A&M

University (FAMU), to provide ‘real-world’ data on whether adequate mosquito control can be achieved through aerial application of permethrin without causing unacceptable non-target impacts. The Pesticide Environmental Impact Section (PEIS) at FAMU/HEREC brought together and oversaw a multi-organizational team that included 20+ members to conduct this permethrin research.

For the past two years, the Beach Mosquito Control District (BMCD) has conducted specially permitted aerial ULV spray missions using permethrin formulations to support this field research. Our research team assessed mosquito control efficacy and non-target impacts (i.e., fish mortality) through the use of field bioassays. We also collected and quantitatively analyzed field samples to determine concentrations of permethrin residue in the air influx (yarn), deposition (filter paper) and water (bioassays pans).

In 2005-2006, our initial aerial research trials with permethrin ULV spray demonstrated mosquito control efficacy ranging from approximately 60 – 100% at multiple open field stations using either AquaReslin or Permanone 30-30 (formulations provided by Bayer Environmental Sciences), even though permethrin was aerially applied at the maximum label dose of 8.2 milliliter per hectare (ml/ha) or 0.007 pounds per acre (lb/acre). The high probability of lower efficacy could have been anticipated considering that several of the sample stations were located in wooded areas; however, the low efficacy for several stations in open areas was a concern. A review of the data set showed reduced residues in the air influx, indicating that the aerial application did not adequately reach the target stations. Although no adverse impacts were demonstrated during these field trials, in order to make the non-target assessment valid, adequate and consistent mosquito control efficacy is essential.

Our preliminary conclusion was that inadequate permethrin coverage may have been the result of: 1) droplets not effectively penetrating the canopy at the woodland sites; 2) the aircraft equipped with an integrated Wingman™ GX / AIMMS-20 system [Aircraft Integrated Meteorological Measurement System], (Adapco, Inc., Sanford, FL) could not adequately adapt to changes in wind direction given the pre-set operating conditions, that is, the spray line was not always perpendicular to the wind direction; 3) wind speeds aloft were too low, resulting in poor

dispersion of the insecticide in the air column and increased insecticide ground deposition; 4) the spray altitude was too high, which facilitated insecticide drift away from the target zone; and 5) the location of the spray boom in relation to the helicopter rotor may have reduced the influence of the aircraft vortices in dispersing the spray droplets.

In 2007, this project was funded by FDACS (Contract No: 012015) to target the mosquito control efficacy issues while evaluating the potential for non-target impacts on fish. During our research planning meeting on March 2007, we determined that delivering adequate permethrin concentration to the target zone was an essential requirement for the new aerial ULV research conducted later that same year. We revised our previous research methodology to address the inadequate permethrin coverage and improve mosquito control efficacy by:

- 1) Eliminating the previous 'woodland area' test stations. The current aerial research is limited to open area locations only (Fig. 1).
- 2) Configuring the integrated Wingman™ GX / AIMMS-20 system using an octagonal box shape instead of the pre-set square box to increase flexibility for offset baseline adjustments (Figs. 2 thru 6).
- 3) Spraying during more stable atmospheric conditions at wind speeds of 2.24 – 5.36 meter per second (m/s) or 5 – 12 miles per hour (mph) aloft.
- 4) Lowering the spray altitude from 76 to 46 meters (m) or from 250 to 150 feet (ft) to increase the aerosol concentration at the target zone.

In addition, we expanded the scope of our earlier non-target testing as suggested by FCCMC members by:

- Adding juvenile fish in plastic pans to assess the possible impacts of permethrin exposure to juveniles during the aerial spray trials. The revised research plan incorporated non-target bioassays using both adult and juvenile mosquitofish.

Research Objectives

The aim of our 2007 research was to improve insecticide coverage in the target zone to achieve over 90% mosquito control efficacy and while assessing non-target impacts. Our specific objectives are 1) evaluate control efficacy through bioassay of caged adult mosquitoes

(*Ochlerotatus taeniorhynchus*); 2) assess non-target impacts through field bioassays using both adult and juvenile mosquitofish (*Gambusia holbrooki*); 3) measure the amount of permethrin residue in the air influx, deposited on the ground and in water; 4) evaluate possible correlations between permethrin concentrations in the air and mosquito mortality; and 5) evaluate possible correlations between permethrin deposition and fish mortality.

Study Hypothesis

Adequate mosquito control efficacy (>90%) can be achieved through permethrin aerial ULV spraying without resulting in mosquitofish mortality.

Materials and Methods

Study Area and Sampling Stations

A multi-organizational research team was organized by FAMU/PEIS to evaluate permethrin aerial ULV applications in 2007. We conducted five mosquito and non-target field bioassays. For each aerial trial, the research team was divided into three groups to ensure rapid collection of residue samples and field bioassays. The field trials were carried out at Frank Brown Park, Panama City Beach, Florida (Fig. 1). The study area, located less than 2 miles from the Gulf Coast, is typical of the complex coastal environments treated by the BMCD. The target zone occupied an area of approximately 1-square-mile within the park and was covered by a larger 2-square mile spray zone. A total of twelve experimental stations were established including nine open field stations within the target zone and three control stations about 7 miles east of the park and away from the target zone. Global positioning system (GPS) coordinates for the treatment and control stations are given in Table 1.

Permethrin Application

The five aerial ULV trials occurred from mid-July through mid-November 2007. BMCD applied permethrin 30-30 (Bayer Environmental Sciences, Research Triangle Park, NC) mixed with ULV oil (1:1) using a Bell OH-58C helicopter according to the insecticide label instructions. The district's helicopter is configured with an integrated spray management system – Wingman™ GX / AIMMS-20 system (Aircraft Integrated Meteorological Measurement System)

developed by Adapco, Inc., Sanford, FL. This system combines real-time, onboard meteorological input at release height with flow control using an embedded computer. Flow and meteorological conditions are recorded and fed into the AGDISP spray-drift model (USDA Forestry Service) to calculate real-time navigation offsets for spray application optimization.

A high-pressure system was used for the application. This system operated at 1,350 pounds per square inch (psi) and was configured with 7 Bete-15 impinger nozzles (Bete Nozzles, Thomas Agency, Winter Park, FL). The nozzle system was characterized by Jonathan Hornby (Lee County Mosquito Control District) using a Malvern laser-diffraction technique. BMCD personnel calibrated the spray equipment, with assistance from Bayer and Adapco representatives, following industry protocols. During the first aerial trial (7/17/2007), spray droplets were collected using spinning slides positioned at station 1 in the target zone. The droplets were later counted by diameter measurement under the microscope using a procedure developed by the Mosquito Adulticide Section (MAS) at PHEREC. The volume mean diameter size 50 (DV_{50}) was calculated following MAS protocols. Approximately 250 drops were measured for each of the two spinning slides.

Permanone 30-30 was diluted 1:1 with ULV oil and applied at 3.95 liters per minute (1.04 gallons per minute) from a release height of 46 m (150 feet) above ground level with an aircraft speed of 140 kilometers per hour (87 miles per hour). This resulted in an application rate of 8.2 ml/ha (0.007 lb/acre) – the maximum rate allowed on the label. Aerial sprays were applied after dark during 'favorable weather conditions' (in terms of wind speed and direction) based on real-time AGDISP modeling.

Weather Data

BMCD collected all weather data to support the permethrin aerial ULV trials. Weather data were monitored and recorded as part of routine operations by the spray helicopter's real-time onboard meteorological system (AIMMS-20). Weather conditions recorded included wind direction and speed, and temperature at flight altitude.

Field Efficacy Bioassay

Laboratory-reared salt marsh mosquitoes *Oc. taeniorhynchus* were used for our field efficacy bioassay for this permethrin aerial research. Pupae or 4th larval instars were obtained from the Mosquito Larvicide Section (MLS) at PHEREC about one week prior to the scheduled field trial. We reared the pupae/larvae to emergence in a temperature- (22 - 25 °C) and humidity- (> 80 %) controlled insectary provided by the Disease Ecology and Control Section (DECS) at PHEREC. Once emergence began, the remaining pupae and/or larvae were sequentially moved into a new holding cage each day to document the age of the adult *Oc. taeniorhynchus* mosquitoes. Newly emerged adults were fed well water and a 10% sugar water solution, both of which were refreshed each day.

The morning of the bioassay field test, about 50+ female adult mosquitoes, 3 – 5 days old, were transferred by gentle aspiration from holding cages into a cylindrical screened exposure cage, which was modified from plastic world health organization (WHO) bioassay cage. Test caged mosquitoes were maintained with a sugar water moistened cotton ball in the insectary until time for the field trial. In the early evening, caged mosquitoes were transported to the field where one exposure cage was deployed at each of the 12 test stations. The caged mosquitoes were collected from the field ~50 minutes after the aerial application to allow sufficient time for the permethrin spray droplets to settle. The mosquitoes were transferred to a clean plastic WHO bioassay cage, maintained with sugar water on a cotton ball and brought back to the insectary at PHEREC for observation. Mosquito mortality was evaluated 12 hours post treatment following PEIS bioassay protocols.

Non-target Bioassay

Mosquitofish, *Gambusia holbrooki* (Girard 1859), were used for our field non-target bioassay. We reared and maintained the fish stock outdoors in concrete plots at the PHEREC facility. On the day before a trial, small plastic pans (41 × 31 × 23 centimeters [cm], L × W × H, Sterilite Co, Townsend, MA) covered with 13 gallons plastic liners (Hefty Easy Flaps, 71cm × 60cm, L × W, Pactiv Corporation, Lake Forest, IL) were filled with approximately 8.35 liters (10.2 cm depth) of well water and stocked with mosquitofish. Two plastic pans were prepared for each test station. One pan held 20 adult female fish and the second pan held 30 – 35 juvenile fish. These fish were maintained with food and aeration for the upcoming field bioassay.

During field deployment for the aerial trials, two fish bioassay pans (adults and juveniles) were placed on the ground at each test and control station. All exposed and control fish pans were collected approximately 50 minutes after treatment and promptly transported back to the PEIS laboratory to continue the 132-hour observation period. Adult and juvenile fish mortality counts were made at 12 hours post treatment and then at 24-hour intervals following PEIS bioassay protocols. During the observation in laboratory, fish were held with food and aeration in the same exposed pans without water changes, and dead fish were removed when first observed.

Collection of Water Samples from Exposed Fish Bioassay Pans

In conjunction with the fish bioassay, an 800-ml aliquot (a grab sample) water sample was collected from each of the juvenile fish pans. The water samples were preserved and extracted by adding 30 ml of hexane to prevent further residue degradation. After sample processing, an aliquot of the hexane extract from each water sample was analyzed for permethrin by gas chromatography (GC).

Collection of Field Residue Samples

At each sampling station, a fuzzy yarn string collector (Jiffy, Lion Brand Yarn Co., New York, NY) was placed vertically 1.5 meter [m] (~5 feet) above ground level to monitor for permethrin residue drift through the air. This residue collector consists of 6.7m (~22 feet) of fuzzy yarn strung in a zigzag pattern onto a rectangular plastic frame (45 × 45cm). A filter paper collector was positioned at each treatment and control station as well. One filter paper (24 cm, Whatman, Maidstone, United Kingdom) was pinned to a small aluminum foil-covered styrofoam board and this assemblage was placed horizontally on the ground to collect permethrin residue deposition. All the residue samples were collected ~50 minutes after the aerial treatment was completed. Each residue sample was removed from either the yarn frame or the styrofoam board using clean forceps and fresh gloves and placed into a clean 40-ml Pyrex screw-top culture tube (Corning Glassworks, Corning, NY).

Quality control of residue sampling in the field was monitored by spiking 100 microliters [μ l] of a known permethrin concentration (206 μ g/ml in hexane) onto clean yarn and filter paper at the

start of the aerial application. Two yarns and two filter papers were spiked at the control station located ~7 miles away from the treatment area. The matrix spike and control blank samples were subjected to similar environmental conditions and exposure times in the field as those treatment samples. After the field collection, all samples were immediately transported to PHEREC laboratory for permethrin residue analysis. To prevent permethrin residue degradation, 30 ml of hexane was added to each of the samples upon arrival at the laboratory.

Permethrin Residue Analysis

Residue samples were processed and extracted the following day after collection. After sample extracting, an aliquot of the hexane extract from each sample was analyzed for permethrin according to our laboratory GC method. An injection volume of 1 μ l was used for all standards and samples. A Varian 3400 gas chromatograph configured with an electron capture detector (ECD) and an 8200 autosampler (Varian Analytical Instruments, Walnut Creek, CA) was used. The compounds were separated on a Zebron ZB-5 capillary column (15 m \times 0.25-mm inner diameter, 0.25- μ m film thickness) (Phenomenex $\text{\textcircled{R}}$, Torrance, CA). A Dell Computer (Dell Computer Corporation, One Dell Way, Round Rock, TX) was used for data acquisition. The computer was equipped with data-handling software Star Chromatography Workstation, Version 4.51 (Varian Analytical Instruments). The GC injector was operated isothermally at 220 $^{\circ}$ C in splitless mode, and the detector block was maintained at 300 $^{\circ}$ C. The GC column oven temperature was initially held at 215 $^{\circ}$ C for 0.5 minute [min], then ramped at 20 $^{\circ}$ C/min to 240 $^{\circ}$ C and held for 3.75 min, and ramped again at 25 $^{\circ}$ C/min to 300 $^{\circ}$ C and held for 2 min, for a total analysis time of 9.90 min per sample. Retention time of the permethrin group (including cis- and trans-permethrin) was 5.025 min. The GC method was streamlined to calculate permethrin 'group' concentrations so that reported values are reflective of both the cis- and trans-isomers.

An eight-point calibration curve (five data point minimum was acceptable), covering a permethrin concentration range from ~0.25 – ~1.20 μ g/ml, was generated for quantitative analysis of the samples. The criteria for the curve was at least 0.995 ($R^2 \geq 0.995$) correlation coefficients to maintain the data accuracy. The analytical standard used for the calibration was obtained from Bayer Crop Sciences US and was certified as 49.5% pure permethrin dissolved in cyclohexane. The analytical-grade permethrin was dissolved in a small volume of hexane to

make a primary stock standard solution at 2.68 mg/ml. This stock solution was subsequently diluted to prepare calibration solutions. A new calibration curve was generated if analysis of a mid-range continuous calibration (CC) failed to meet the $100 \pm 10\%$ target concentration. During analysis, CC at a mid-point calibration concentration was added at 10-sample intervals to verify the instrument detector signal stability. The recovery criterion for CC was $100 \pm 10\%$. Laboratory control and field spikes were used to check for extraction efficiency and field degradation of permethrin. The recovery criterion for these spike samples was $100 \pm 20\%$. The blank samples (field, laboratory and instrument) were used to check for possible contamination. Samples were diluted as needed so that permethrin concentrations fell within the range of the calibration curve.

Data Analysis: A randomized complete block statistical design was used for this permethrin field study. Data were blocked by spray date (each aerial ULV spray mission) so that meteorological variations were kept to a minimum. Adult mosquitofish mortality data were characterized by analysis of variance (ANOVA) using STATISTICA software (StatSoft, Inc. 2001). A significance level of $P < 0.05$ was selected for all statistical tests. For fish mortality data, comparisons between treatment stations (stations exposed to permethrin spray) and control stations were used to evaluate the impact of the aerially applied permethrin on adult mosquitofish. Mosquito mortality and residue data were analyzed and graphed by Microsoft® Office Excel (Microsoft Corporation, Seattle, WA).

Results and Discussion

Aerial Application and Droplet Size

This aerial ULV application research was conducted in open field with Permanone 30-30, a permethrin formulation provided by Bayer Environmental Sciences. Delivering adequate permethrin concentration to target zone was an essential requirement for 2007 field research. To accomplish that, we configured the integrated Wingman™ GX / AIMMS-20 system using an octagonal box shape instead of the pre-set square box to increase direction flexibility for offset baseline adjustments. The new configuration positioned the aircraft spray line perpendicular to eight wind directions instead of 4 directions for all 5 spray trials (Fig. 2 – 6). Also, the spray

altitude for all trials was lowered by BMCD from 76 to 46 m and the all treatment stations were in open areas. The specific parameters of each field aerial application trial were listed on Table 2. With the exception of the second trial, wind speeds were generally favorable for aerially spraying (2.1 – 5.8 m/s). For the second trial (7/24/07), however, wind speeds became calm after the application was initiated. Wind direction was steady for all field trials.

Before the 2007 field trials began, the droplet spectrum for BMCD's nozzle system was measured using Malvern laser-diffraction by Jonathan Hornby at Lee County mosquito control district, Florida. Statistics for the droplet size spectrum were: $DV_{50} \leq 22\mu\text{m}$; $DV_{10} \leq 10\mu\text{m}$ and $DV_{90} \leq 48\mu\text{m}$. The droplet sizes were smaller, however, when measured in field during the spray mission. During the first aerial trial (7/17/2007), spray droplets were collected using two spinning slides positioned in the target zone at station 1. The droplet size spectrum was DV_{50} value was $8.75\ \mu\text{m}$, ($DV_{10} = 2.97\ \mu\text{m}$ and $DV_{90} = 39.94\ \mu\text{m}$) following MAS protocols.

Mosquito Efficacy Bioassay

Overall, the use of caged adult mosquitoes proved to be an effective measure of adulticide efficacy with less than 10% mortality found in all control stations and in all five trials (Fig 7). Figure 7 summarizes overall control efficacy for caged mosquitoes (*Oc. Taeniorhynchus*) at the nine treatment and three control stations. The average mortality 12 hours after the spray applications were 93 ± 2.4 (trial 1), 78 ± 7.6 (trial 2), 95 ± 2.5 (trial 3), 95 ± 1.8 (trial 4), and 95 ± 3.7 (trial 5), demonstrating excellent control efficacy. The specific results of mosquito mortality at each testing stations were summarized in Table 3. Although the average mortality for the second trial (7/24/2007) was less than desirable ($78 \pm 7.6\%$), the efficacy for four out of the nine stations (4, 6, 8 and 9) still exceeded 90% (Table 3). The reduced efficacy in the second trial was likely the result of the reduction in wind speed (0.51 m/s, Table 2) after the spray mission was initiated. The reduction of wind speed created poor dispersal of the permethrin droplets in the air column which then resulted in higher ground deposition. This result was confirmed by the lower permethrin residues found on yarn (air-influx and droplet dispersal; Table 4) and higher residue on filter paper (ground deposition; Table 5). In addition, one of the sampling stations (station 7) in trial 2 had a vegetation barrier (trees) upwind that likely block the path of the spray droplets (Fig 1.). This station had lower levels of permethrin residues on both the yarn (Table 3) and the

filter paper (Table 4). It should be noted that these efficacy results were obtained for a specific location (Panama City Beach, Florida) and under specific local weather conditions (Table 2). To achieve the similar efficacy results for other locations, application methods used by other mosquito control districts may require adjustments to account for local geographic and meteorological conditions.

Permethrin residue results

The permethrin residues in water samples were below our instrument detection limits ($< 1\mu\text{g/L}$). No permethrin residues were detected in water samples from the fish bioassay pans (60 samples tested) that were exposed to the spray drift. The permethrin concentrations in air influx were represented by the concentrations on yarn sample collectors (Table 4). The overall concentration ranged from 8.96 – 248.82 $\mu\text{g/yarn}$. The mean and standard errors of permethrin concentrations recovered from yarn collectors were 156 ± 17 , 61 ± 10 , 107 ± 13 , 87 ± 15 and 113 ± 18 $\mu\text{g/yarn}$ for trial 1, 2, 3, 4 and 5 respectively. The lower residue concentration in air influx (61 ± 10 $\mu\text{g/yarn}$) may account for the lack of mosquito control efficacy in second trial (Figs. 7 and 9). Table 5 showed the ground deposition of permethrin residues as it was monitored by filter paper collector. The overall ground deposition ranged from 80.37 to 602.52 $\mu\text{g/m}^2$. The mean and standard errors of permethrin ground deposition were 196 ± 13 , 491 ± 35 , 223 ± 17 , 167 ± 22 and 224 ± 18 $\mu\text{g/m}^2$ for trials 1, 2, 3, 4 and 5 respectively. The higher ground deposition (491 ± 35 $\mu\text{g/m}^2$) in second trial appeared to be associated with lower residue on the yarn collectors (61 ± 10 $\mu\text{g/yarn}$) (Fig. 9). The highest ground deposition (603 $\mu\text{g/m}^2$) (Table 5) in station 9 of the second trial did not cause either significant adult fish mortality (Table 6) or juvenile fish mortality (Table 8). No permethrin residues were found in any samples from the control stations for any of the trails.

Non-target mosquitofish bioassays

Mosquitofish bioassays were observed for 132 hrs (5.5 days) post application. Adult fish mortality is summarized in Table 6. For all five trials, the average adult fish mortality was less than 15% in the treated zone (stations 1 – 9) and less than 17% in control zone (stations 10 – 12). Individual sample station mortality ranged from 0 – 40% in treated zone and 0 – 35% in control zone. The highest fish mortality at treated zone was 40% at station 3 during the 4th trial

(9/12/2007) and 35% at control station 10 during the 3rd trial (8/9/2007). Table 7 shows the statistical data for adult fish mortality. There were no significant differences in adult fish mortality between permethrin exposure (treatment) and non-exposure (control) group ($F = 0.171$; $df = 1$; $P = 0.681$). Also, there are no significant differences in adult fish mortality among the five trials ($F = 1.74$; $df = 4$; $P = 0.156$). The pooled data for all trails also indicates that adult fish mortality was not significantly different among treatments and controls ($F = 1.42$; $df = 5$; $P = 0.231$; $R^2 = 0.116$).

We found an average mortality of less than 1% for juvenile fish in permethrin treated sample stations compared to no mortality in control station throughout all 5 trials (Table 8). This limited mortality may have resulted in permethrin exposure, but the lacks of deaths in the treated stations are inadequate for statistical comparison. Overall, the data suggests that aerial application of permethrin has little or no affect on juvenile fish survival even under worst case conditions (shallow water in open habitat).

The variable adult fish mortality in both the treatments and the control stations may have resulted from stress and/or aggressive behavior of the fish. Some of the dead fish found in the pans contained wounds that were likely caused by the other fish. Stress resulting from being in a confined environment may have also contributed to the deaths. Stress can contribute to increased aggression, and increased use of dissolved oxygen. Given that almost all of the juvenile fish survived during the trials and observation period, indicates that the loading of the much larger adult fish in the containers may have been too high resulting in either loss in dissolved oxygen or excessive ammonia (highly toxic to fish) produced from fish waste. Overall, we consider the survival in the adult fish to adequate (>85% average) for all the field trails. Based on the results from this study, aerial ULV applications of permethrin did not result in significant treatment related mortality in either adult or juvenile mosquito fish.

Conclusion

The control efficacy of caged adult mosquitoes was excellent (>90%) against laboratory reared-salt marsh mosquitoes, *Oc. taeniorhynchus*, for four out of the five permethrin aerial ULV trials.

The second trial was performed under suboptimal conditions (i.e., lack of wind) resulting in reduced efficacy. The impact of mosquito ULV aerial spray to mosquitofish, *G. holbrooki*, both adults and juveniles, was minimal as determined by our bioassays. This research supported our initial hypothesis that we are able to apply permethrin insecticide using current aerial ULV technology to effectively control adult mosquitoes with minimal impact on non-target fish. We believe that using high-pressure nozzle systems, making applications at an application altitude of 46 m (150 feet) and with a wind speed between 2.1 - 5.1 m/s (4 – 10 mph), will not only provide excellent efficacy in controlling adult mosquitoes, but also should not result in an unacceptable risk to non-target aquatic organisms including fish in shallow water bodies (worst-case exposure scenario). Although potential effects to aquatic invertebrates were not addressed in this particular study, results from the spray deposition residue data can be used to assess impacts to these more sensitive aquatic organisms. Overall, potential impacts to aquatic organisms from the aerial application of permethrin will likely be greatly mitigated by the limited deposition of pesticide when using high pressured nozzle systems that produce droplets less than 50 µm and the extremely high affinity of permethrin to bind to sediments. Therefore, we recommend that FDACS approve aerial application of permethrin to control adult mosquitoes in the State of Florida. In addition, we also recommend that individual mosquito control districts, who wish to conduct aerial ULV applications of permethrin, monitor efficacy and collect residue samples using similar methods described in this report in order to provide a better understanding of the effect of various spray scenarios (variations in weather, geographic location and application technology) in achieving adequate efficacy against adult mosquitoes. Increasing efficacy not only enhances overall control of mosquitoes, but also minimizes impacts to non-target aquatic organisms by reducing deposition and subsequent exposure.

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Table 1. GPS Coordinates for the treatment and control stations during permethrin aerial ULV field spray trials, Panama City Beach, FL., 2007.

<i>Research Testing Stations</i>	<i>Global Positioning System (GPS) Coordinates</i>	
	<i>Latitude</i>	<i>Longitude</i>
1	30°13'39.04"	85°52'29.52"
2	30°13'38.53"	85°52'22.21"
3	30°13'38.95"	85°52'16.47"
4	30°13'43.46"	85°52'23.70"
5	30°13'43.88"	85°52'20.36"
6	30°13'46.41"	85°52'22.21"
7	30°13'53.45"	85°52'26.36"
8	30°13'59.75"	85°52'29.46"
9	30°13'59.81"	85°52'33.39"
10 (control) ¹	30°12'4.07"	85°44'51.97"
11 (control) ¹	30°12'4.02"	85°44'52.20"
12 (control) ¹	30°12'3.99"	85°44'52.42"

¹ Control stations (10 – 12) are located approximately 7 miles east of the spray zone and away from the treatment area.

Table 2. Summary of weather and application parameters during permethrin aerial ULV field spray trials, Panama City Beach, FL, 2007.

<p>BMCD¹ Helicopter: Bell OH-58C</p> <p>Spray Management System: Wingman™ GX/AIMMS-20</p> <p>Permanone 30-30 dosage: 7.94 g/hectare (0.007 lb/acre)</p> <p>Spray Area Dimensions: 3.2 × 3.2 kilometers (2 × 2 miles)</p> <p>Target Zone: 1.6 × 1.6 kilometers (1 × 1 miles)</p>	<p>High-pressure Equipment: 7 impingement nozzles @ 1350psi</p> <p>Nozzle Droplet Spectra by Malvern laser-diffraction: DV₁₀ ≤10µm; DV₅₀ ≤22µm; DV₉₀ ≤48µm</p> <p>Swath Width: 321.87 meter (1,056 feet)</p> <p>Altitude of Application: 45.72 meters (150 feet)</p> <p>BMCD Pilot: Brad Gunn</p>
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<i>Spray Parameters</i>	<i>Spray Dates</i>				
	<u>7/17/2007</u>	<u>7/24/2007</u>	<u>8/7/2007</u>	<u>9/12/2007</u>	<u>9/19/2007</u>
Wind Speed (miles/hour)	6.0-9.0	1.0-6.0	4.0-10.0	6.0-13.0	9.0-13.0
Wind Speed (meters/second)	3.1-4.6	0.51-3.1	2.1-5.1	2.7 – 5.8	4.0 – 5.8
Wind Direction ²	245 (WSW)	210-230 (SW)	240-265 (W)	215-230 (SW)	360-010 (N)
Spray Path	N – S	NW – SE	N – S	NW – SE	N – S
Air Temperature (°F)	82	80	80	81	81
Humidity	71%	70%	85%	77%	52%

¹ BMCD = Beach Mosquito Control District

² Direction from which the wind is blowing, in degrees, referenced to north (0 degrees).

Table 3. Summary of percent mortality¹ of cage female adult mosquitoes, *Ochlerotatus taeniorhynchus*, on 12h after initial field exposure following permethrin aerial ULV spray applications, Panama City Beach, FL, 2007.

<i>Research Testing Station</i>	<i>Cumulative Percent Mortality (%) after 12-h Post Treatment</i>				
	<i>Spray Dates</i>				
	<u>7/17/2007</u>	<u>7/24/2007</u>	<u>8/7/2007</u>	<u>9/12/2007</u>	<u>9/19/2007</u>
1	79.49	86.67	100.00	83.64	100.00
2	100.00	57.81	77.27	95.65	100.00
3	97.73	77.78	89.80	100.00	100.00
4	98.28	96.88	98.11	100.00	100.00
5	87.76	71.43	98.04	100.00	98.99
6	96.00	100.00	100.00	92.59	65.88
7	84.38	28.81	98.08	93.55	95.73
8	96.67	89.83	91.53	98.78	95.50
9	96.67	94.00	98.15	93.65	100.00
Mean ± St Error	93 ± 2.4	78 ± 7.6	95 ± 2.5	95 ± 1.8	95 ± 3.7
10 (control) ²	2.13	9.30	0.00	3.45	0.00
11 (control) ²	0.00	3.33	1.69	0.00	0.00
12 (control) ²	3.33	10.00	1.82	1.59	2.25
Mean ± St Error	1.8 ± 1.0	7.5 ± 2.1	1.2 ± 0.6	1.7 ± 1.0	0.7 ± 0.8

¹Data was not adjusted by Abbott's formula

²Control stations (10 – 12) are located approximately 7 miles east of the spray zone and away from the treatment area

Table 4. Permethrin residue concentrations ($\mu\text{g}/\text{yarn}$) measured on fuzzy yarn collectors following aerial ULV mosquito spray applications, Panama City Beach, FL, 2007.

<i>Research Testing Station</i>	<i>Permethrin Residue ($\mu\text{g}/\text{yarn}$) Detected by GC Analysis</i>				
	<i>Spray Dates</i>				
	<u><i>7/17/2007</i></u>	<u><i>7/24/2007</i></u>	<u><i>8/7/2007</i></u>	<u><i>9/12/2007</i></u>	<u><i>9/19/2007</i></u>
1	129.06	97.38	93.66	30.58	219.90
2	203.10	59.84	69.79	47.14	131.28
3	248.82	33.98	97.05	114.02	109.51
4	115.62	38.39	180.39	97.42	114.11
5	148.08	84.65	157.74	80.88	109.68
6	179.94	47.77	112.83	101.58	8.96
7	143.64	15.44	75.33	46.524	138.69
8	165.06	91.74	93.30	87.588	98.18
9	69.21	78.66	83.87	179.385	89.70
Mean \pm St Error	156 \pm 17	61 \pm 10	107 \pm 13	87 \pm 15	113 \pm 18
10 (control) ¹	ND	ND	ND	ND	ND
11 (control) ¹	ND	ND	ND	ND	ND
12 (control) ¹	ND	ND	ND	ND	ND
Mean \pm St Error	ND	ND	ND	ND	ND

$\mu\text{g}/\text{yarn}$ = micrograms per yarn collector (22 feet of fuzzy yarn)

GC = gas chromatography

ND = Not detected – the concentration is below method detection limit

¹ Control stations (10 – 12) are located approximately 7 miles east of the spray zone and away from the treatment area.

Table 5. Permethrin residue concentrations ($\mu\text{g}/\text{m}^2$) measured on filter paper collectors following aerial ULV spray applications, Panama City Beach, FL., 2007.

<i>Research Testing Stations</i>	<i>Permethrin Residue ($\mu\text{g}/\text{m}^2$) Detected by GC Analysis</i>				
	<i>Spray Dates</i>				
	<u><i>7/17/2007</i></u>	<u><i>7/24/2007</i></u>	<u><i>8/7/2007</i></u>	<u><i>9/12/2007</i></u>	<u><i>9/19/2007</i></u>
1	230.31	427.92	310.48	83.69	344.30
2	184.81	496.62	194.96	181.10	215.92
3	N/A	511.41	169.69	271.35	230.97
4	148.94	524.40	279.58	151.72	210.34
5	176.33	561.21	264.79	160.08	212.27
6	195.62	536.94	216.78	147.28	211.60
7	247.68	242.44	199.01	80.37	241.25
8	159.68	514.26	175.20	170.03	212.93
9	205.31	602.52	200.07	259.02	139.66
Mean \pm St Error	196 \pm 13	491 \pm 35	223 \pm 17	167 \pm 22	224 \pm 18
10 (control) ¹	ND	ND	ND	ND	ND
11 (control) ¹	ND	ND	ND	ND	ND
12 (control) ¹	ND	ND	ND	ND	ND
Mean \pm St Error	ND	ND	ND	ND	ND

$\mu\text{g}/\text{m}^2$ = micrograms per square meter

GC = gas chromatography

N/A = Not applicable because sample was lost during field collection

ND = Not detected – the concentration is below method detection limit

¹ Control stations (10 – 12) are located approximately 7 miles east of the spray zone and away from the treatment area.

Table 6. Summary of percent mortality¹ of adult mosquitofish, *Gambusia holbrooki*, after 132 hours following permethrin aerial ULV mosquito spray applications, Panama City Beach, FL., 2007.

<i>Research Testing Station</i>	<i>Cumulative Percent Mortality (%) 132-h Post Treatment</i>				
	<i>Spray Dates</i>				
	<u><i>7/17/2007</i></u>	<u><i>7/24/2007</i></u>	<u><i>8/7/2007</i></u>	<u><i>9/12/2007</i></u>	<u><i>9/19/2007</i></u>
1	10.0	26.3	21.1	0.0	10.0
2	20.0	5.0	20.0	5.0	5.0
3	5.0	0.0	20.0	40.0	5.0
4	0.0	10.0	15.8	10.5	0.0
5	5.0	10.0	0.0	5.0	0.0
6	0.0	10.0	10.0	16.7	10.0
7	20.0	10.5	4.8	27.3	15.0
8	10.0	15.0	10.0	15.0	0.0
9	5.3	15.0	15.0	15.0	20.0
Mean ± St Error	8.4 ± 2.5	11.3 ± 2.4	13.0 ± 2.4	14.9 ± 4.1	7.2 ± 1.4
10 (control) ²	0.0	10.5	35.0	10.0	10.0
11 (control) ²	4.8	20.0	14.3	14.3	10.0
12 (control) ²	11.1	20.0	0.0	10.0	10.0
Mean ± St Error	5.3 ± 3.2	16.8 ± 3.2	16.4 ± 10.2	11.4 ± 1.4	10 ± 0.0

¹ Data was not adjusted by Abbott's formula

² Control stations (10 – 12) are located approximately 7 miles east of the spray zone and away from the treatment area

Table 7. Statistical comparison of percent adult fish mortality after 132 hours following permethrin aerial ULV mosquito spray, Panama City Beach, FL, 2007. The data were analyzed using ANOVA (STATISTICA software, StatSoft. Inc. Tulsa, OK. 2001).

<i>Level of Factor</i>	N	Mean	Std. Dev.	Std. Err.	-95%	+95%
	60	11.22	8.58	1.11	9.01	13.44
Treatment	45	10.96	8.66	1.29	8.36	13.56
Control	15	12.00	8.58	2.22	7.25	16.75
Spray trial 1	12	7.60	6.95	2.01	3.18	12.02
Spray trial 2	12	12.69	7.10	2.05	8.18	17.20
Spray trial 3	12	13.83	9.89	2.86	7.55	20.12
Spray trial 4	12	14.07	10.72	3.10	7.25	20.88
Spray trial 5	12	7.92	6.20	1.79	3.98	11.86

Table 8. Summary of percent mortality¹ of juvenile mosquitofish, *Gambusia holbrooki*, after 132 hours following permethrin aerial ULV mosquito spray applications, Panama City Beach, FL., 2007.

<i>Research Testing Station</i>	<u><i>Cumulative Percent Mortality (%) 132-h Post Treatment (OR?) Exposure</i></u>				
	<i>Spray Dates</i>				
	<u><i>7/17/2007</i></u>	<u><i>7/24/2007</i></u>	<u><i>8/7/2007</i></u>	<u><i>9/12/2007</i></u>	<u><i>9/19/2007</i></u>
1	0.0	10.0	0.0	0.0	0.0
2	0.0	0.0	2.9	0.0	0.0
3	0.0	0.0	0.0	0.0	0.0
4	0.0	0.0	0.0	2.9	0.0
5	0.0	0.0	6.5	0.0	2.8
6	0.0	0.0	0.0	0.0	0.0
7	0.0	0.0	0.0	0.0	0.0
8	0.0	0.0	0.0	0.0	0.0
9	0.0	0.0	0.0	0.0	3.0
Mean ± St Error	0	1.1 ± 1.1	1.0 ± 0.8	0.3 ± 0.3	0.6 ± 0.4
10 (control) ²	0.0	0.0	0.0	0.0	0.0
11 (control) ²	0.0	0.0	0.0	0.0	0.0
12 (control) ²	0.0	0.0	0.0	0.0	0.0
Mean ± St Error	0.0	0.0	0.0	0.0	0.0

¹ Data was not adjusted by Abbott’s formula)

² Control stations (10 – 12) are located approximately 7 miles east of the spray zone and away from the treatment area.



Figure 1. Aerial ULV research site at Panama City Beach, Florida – Treatment Stations Locations (T1 – T9).

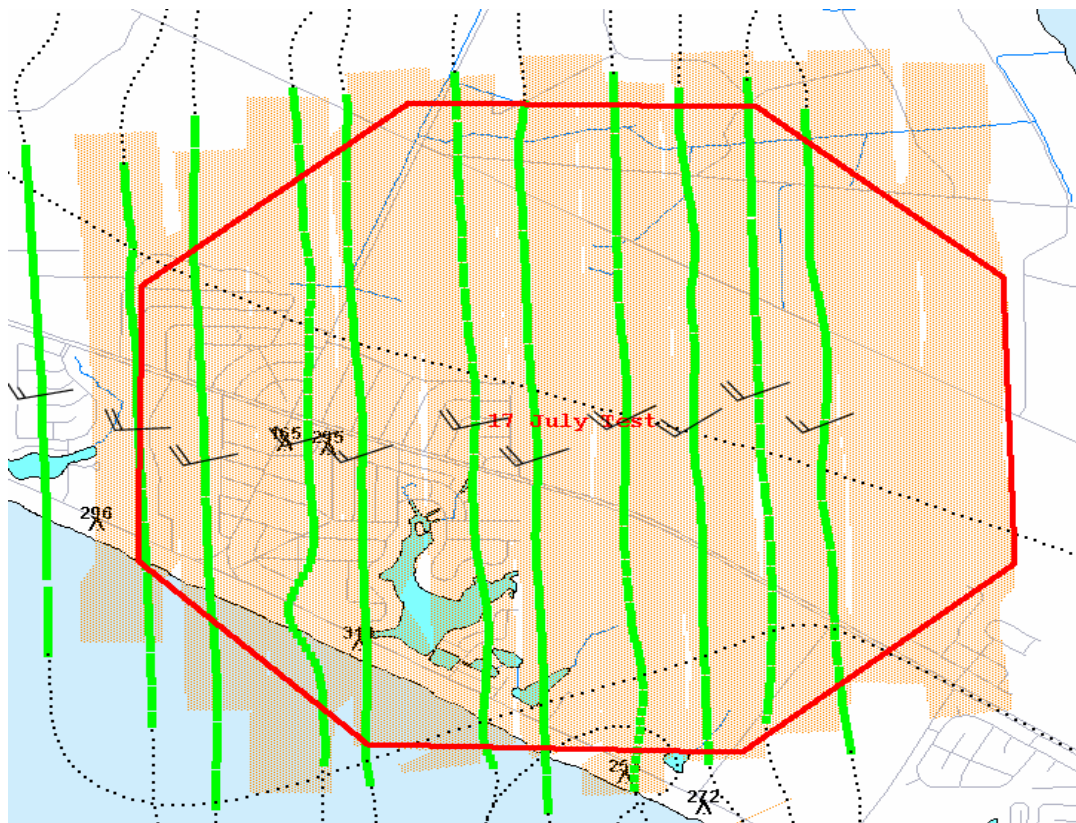


Figure 2. BMCD aerial ULV treatment record for the 1st field aerial ULV trial conducted on July 17, 2007. The map shows the spray off-set, swath placement, wind direction and speed.

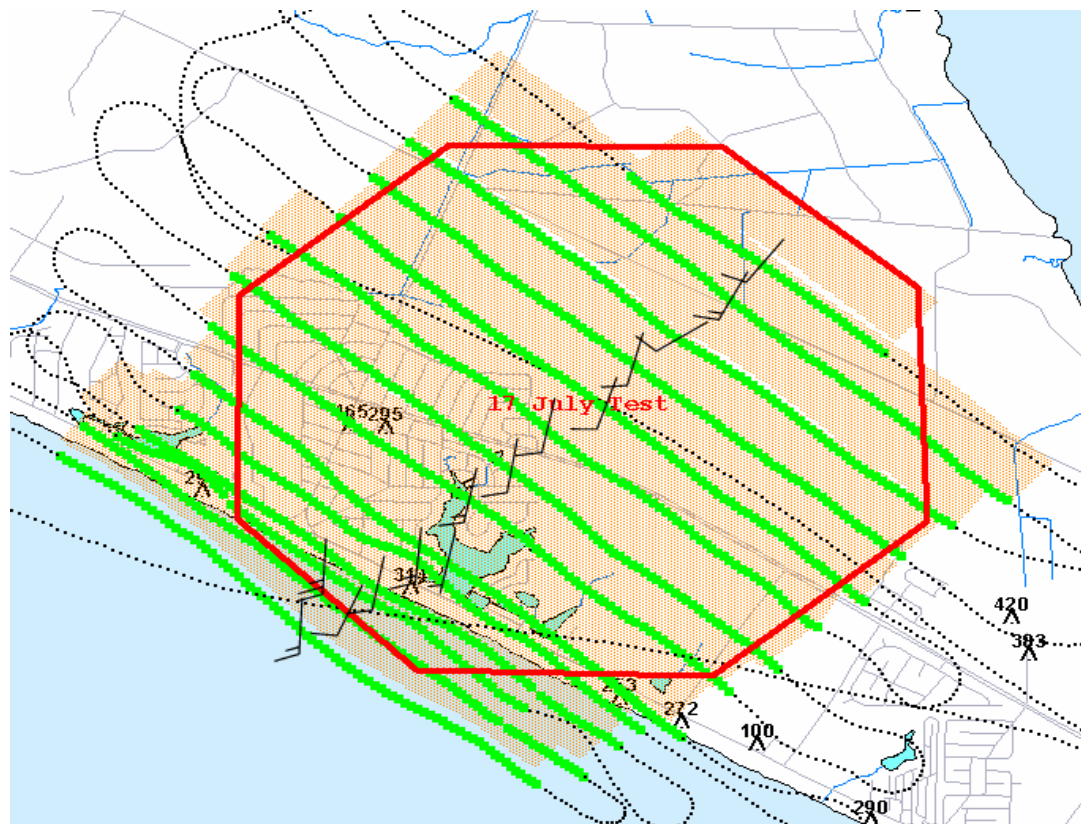


Figure 3. BMCD aerial ULV treatment record for the 2nd field aerial ULV trial conducted on July 24, 2007. The map shows the spray off-set, swath placement, wind direction and speed.

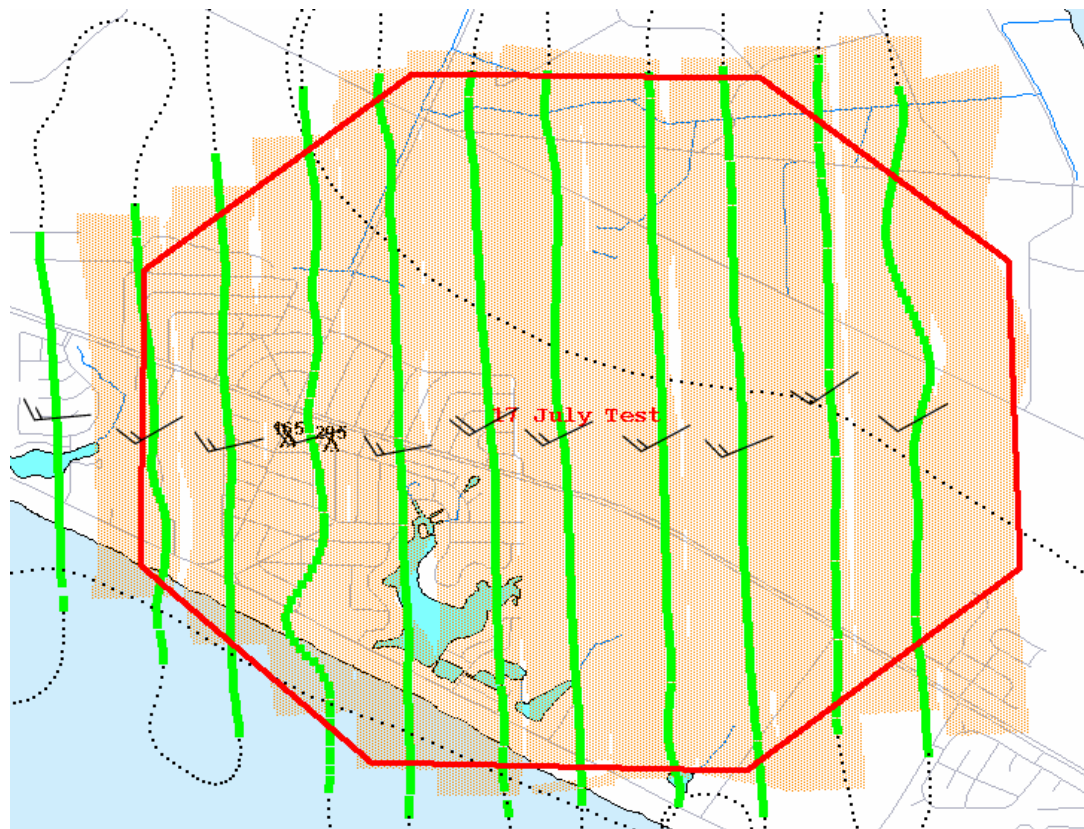


Figure 4. BMCD aerial ULV treatment record for the 3rd field aerial ULV trial conducted on August 7, 2007. The map shows the spray off-set, swath placement, wind direction and speed.

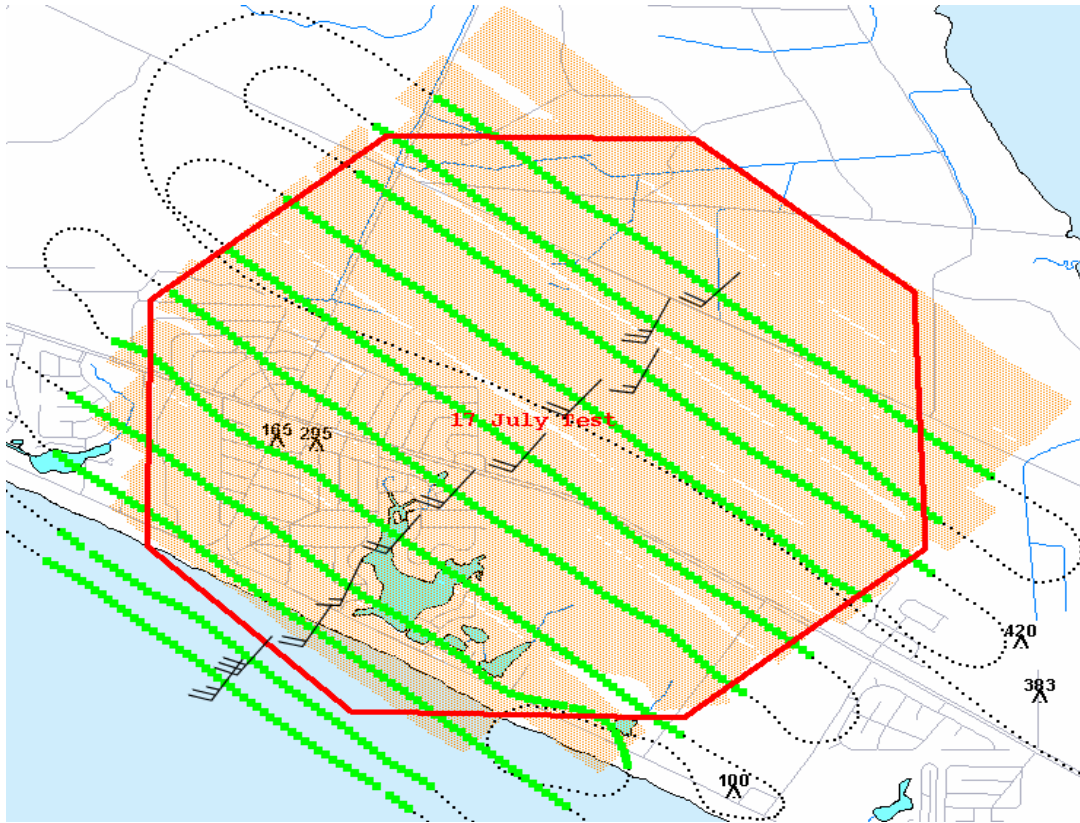


Figure 5. BMCD aerial ULV treatment record for the 4th field aerial ULV trial conducted on September 12, 2007. The map shows the spray off-set, swath placement, wind direction and speed.

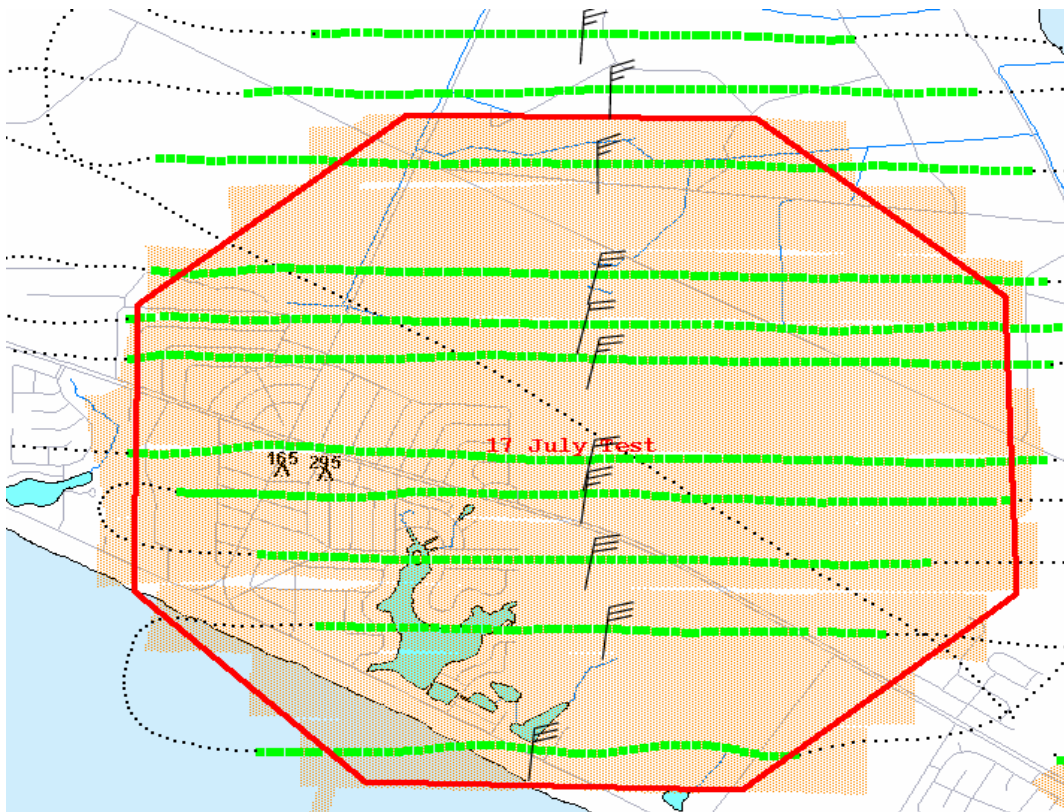


Figure 6. BMCD aerial ULV treatment record for the 5th field aerial ULV trial conducted on September 19, 2007. The map shows the spray off-set, swath placement, wind direction and speed.

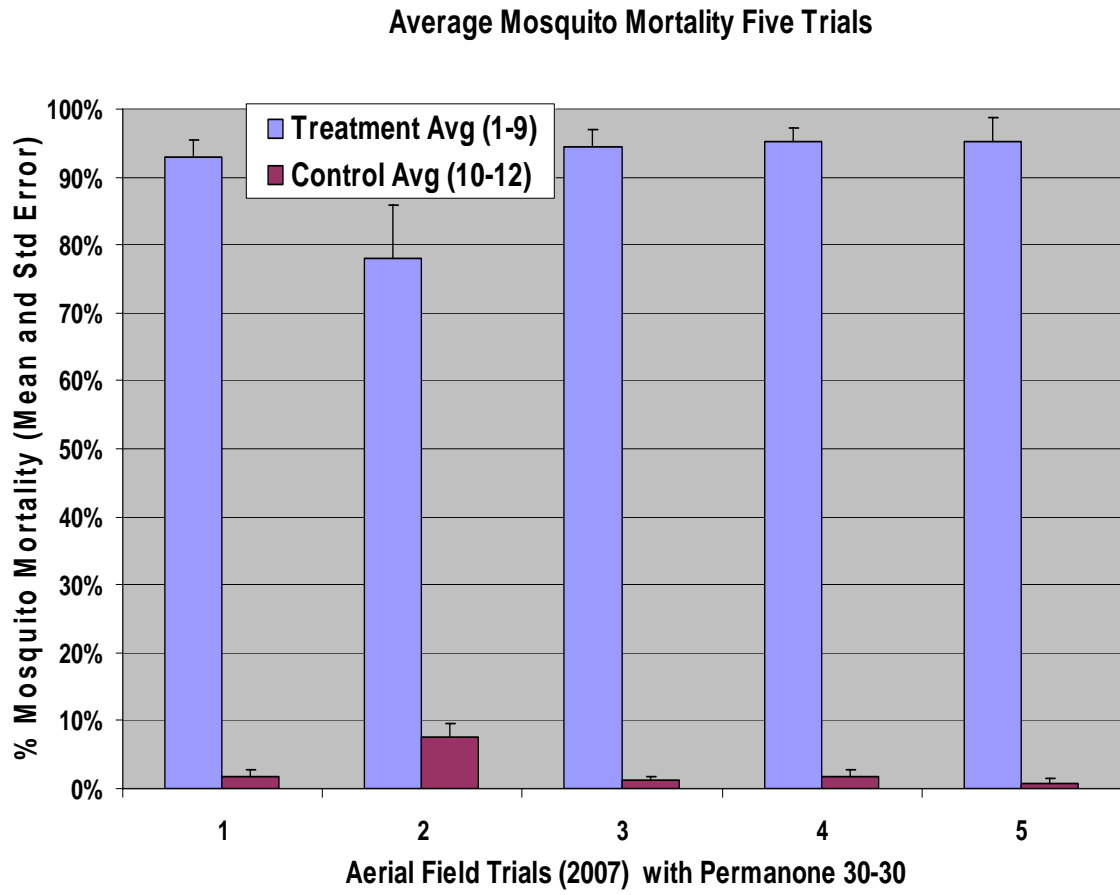


Figure 7. Average percent and standard error of adult mosquito (*Ochlerotatus taeniorhynchus*) mortality in five field aerial ULV trials.

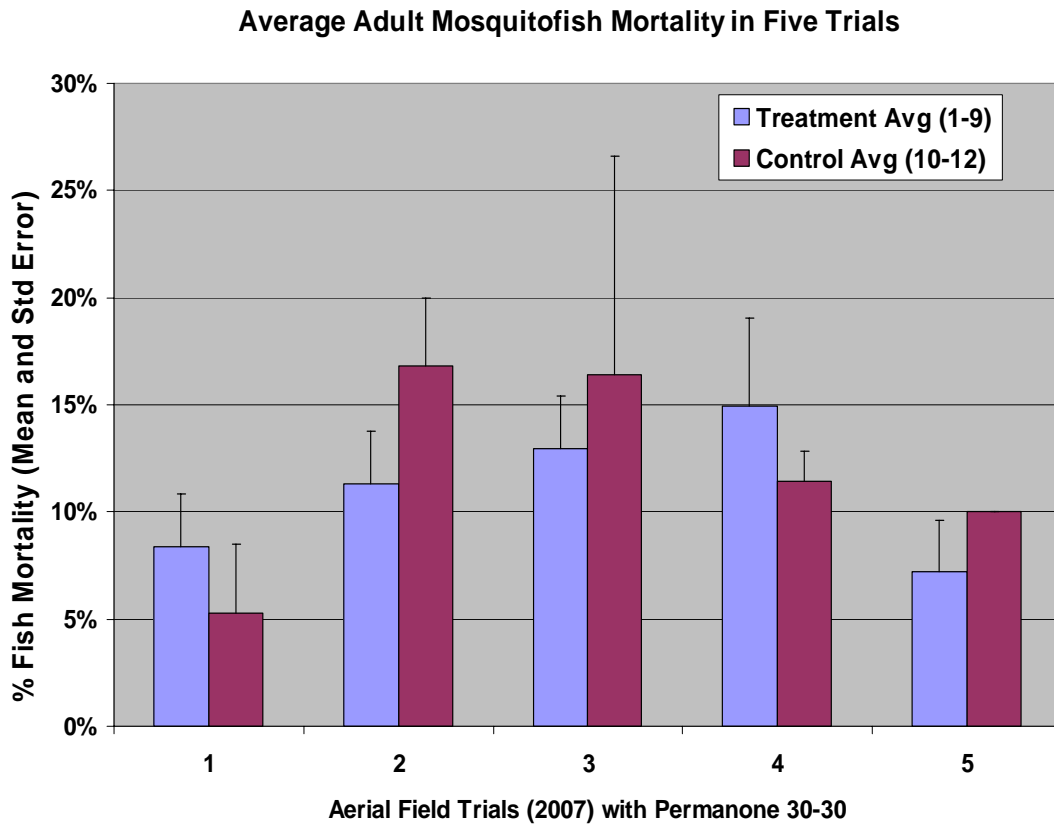


Figure 8. Average percent and standard error of adult mosquitofish (*Gambusia holbrooki*) mortality in five field aerial ULV trials.

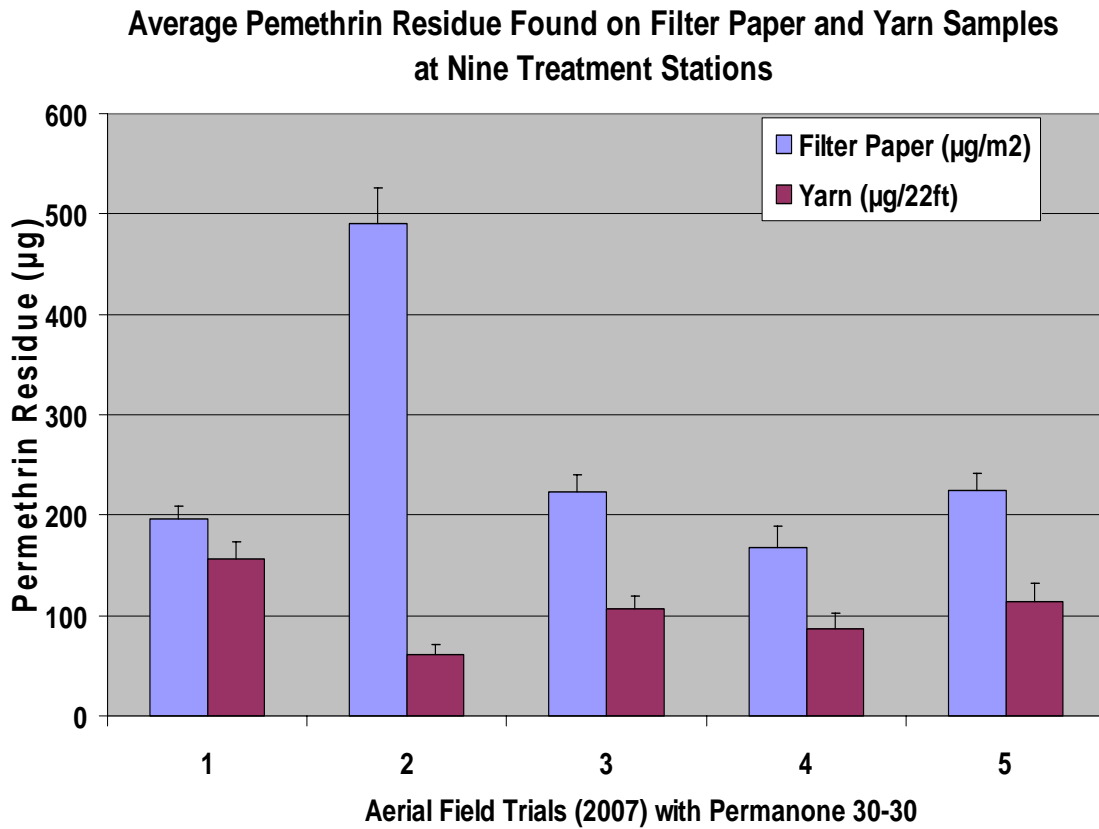


Figure 9. Average percent and standard error of permethrin residue found on samples of filter paper (ug/m²) and yarn (ug/22 ft) for each of five field aerial ULV trials at nine treatment stations.