Final Report

FDACS Project: "*Impact of Aerially Applied Naled on Monarch Butterflies*"

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ABSTRACT

This study was limited to one spray trial due to severe damages inflicted on the Florida State University Panama City (FSU PC) butterfly and milkweed rearing facilities by Hurricane Michael. No acute mortality linked to a single swath of naled (Dibrom®) applied at 0.66 oz. per acre was observed in caged adult monarch butterflies (*Danaus plexippus plexippus*) set out at 0, 100, 500, 1000, and 1500 ft. in three replicated transects at Panama City Beach, FL. There was no mortality in the upwind controls. Laboratory bioassays of second instar larvae fed naled-treated milkweed leaves (*Asclepias incarnata*) for four days experienced little acute mortality that could be definitively linked to the insecticide. The mortality of caged 5-6 day-old female *Aedes taeniorhynchus* adults exposed at the same time and locations varied considerably and was attributed to differences in vegetation and application coverage. Mortality at 48 hrs. posttreatment in one transect ranged from 71-77% out to 1000 ft. and dropped to 55% at 1500 ft. Mortality in the other two transects ranged widely with 100% mortality at 100 ft. and 48% at 1000 ft. Mortality at all the other distances did not exceed 20%. Control mortality was <5% in one transect and zero in the other two.

Introduction

The monarch, *Danaus plexippus plexippus*, is one of the most widely recognized butterflies in the Northern America. It is the state insect/butterfly in Alabama, Georgia, Idaho, Illinois, Minnesota, Texas, West Virginia, and Vermont. Its annual migratory flights bring it in close contact and regular visibility with people. In Florida, monarchs are most abundant in the fall when migrating populations traverse the northeastern U.S. to south Florida and west across the Florida Panhandle and Gulf Coast to overwinter in Mexico (Harvey et al. 2012). In the spring, a smaller migratory flight is made to return north. Along these migratory flight ways, monarchs deposit eggs that hatch into larvae (i.e., caterpillars) that feed on milkweed, their primary host plant. Milkweed is an herbaceous perennial containing over 130 known species (Fishbein 2001). It is typically found along road margins, wetlands, and in other ubiquitous locations routinely treated for mosquito control.

Since 1995, North American populations of monarch butterflies have plummeted over 90% compared to the 20-year average (The Center for Biological Diversity, Center for Food Safety, The Xerces Society, and Lincoln Brower 2014). This decline has been attributed to several factors including: habitat destruction, pesticides, disease, and, climate change.

In 2014, the U.S. Fish & Wildlife Service (USFWS) was petitioned under the Endangered Species Act to protect the monarch as an endangered or threatened species (same reference above). In the intervening years, the USFWS has undertaken an assessment to determine if sufficient scientific evidence justifies this classification. On December 15, 2020, the USFWS will formally announce their findings (USFWS 2018). If found warranted, the agency could propose regulations "deemed necessary and advisable to provide for the conservation of this threaten species (i.e., 4(d) rule)." These regulations could severely limit mosquito pesticide applications in and near monarch habitats.

Naled (trade name: Dibrom®) is one of the most important mosquito adulticides applied aerially over vast acreages of Florida and other states for controlling pestiferous and disease-bearing mosquitoes. There is very little known about its effects on monarch butterflies. The purpose of this project was to assess the acute impact of aerially-applied naled on larval and adult monarch butterflies in an operational mosquito control setting.

Extensive butterfly adult dermal and larval ingestion assays have been performed in the laboratory to determine lethal dosages of the mosquito control insecticides, permethrin, naled, and its metabolite dichlorvos, to five native Florida species: common buckeye (Junonia coenia), painted lady (Vanessa cardui), zebra longwing (Heliconius charitonius), atala hairstreak (Eumaeus atala), and white peacock (Anartia jatrophae) (Hoang et al. 2011) and (Hoang and Rand 2015a, 2015b). By correlating these data with pesticide plant leaf deposition data following mosquito spraying, the authors were able to perform an environmental risk assessment for the insecticides. They found the greatest mortality risk was to the caterpillar growth stages and that exposure dosages from mosquito control spraying of these insecticides were likely to result in significant mortalities and other adverse effects.

Field studies have been conducted to evaluate the acute mortality effects of ground-applied ULV resmethrin applied as an adulticide and permethrin applied as a barrier treatment against larval and adult monarch butterflies (Oberhauser et al. 2006 and 2009). The authors concluded both insecticidal treatments caused butterfly mortality the extent to which was dependent on the proportion of host plants (milkweed) treated in a given area.

Only one field study has been conducted on aerial ULV application of naled against butterflies (Zhong et al). The aim of this project conducted in the Florida Keys was to evaluate the impact of naled applied at 100 and 150 ft. altitudes to the endangered Miami blue butterfly, *Cyclargus thomasi bethunebakeri*. The authors found that butterfly larval survival was significantly reduced in the spray zone compared to drift and control zones. Although spray trials were repeated several times over the years, there was no replication of the treatment during each trial. The Miami blue butterfly is considerably smaller than the monarch. According to the literature, the adult monarch has a wing length at least 2X greater than the Miami blue. Monarch larvae are also much larger than the Miami blue. As such, one would anticipate differences in dose response.

There has not been any field studies to assess the impact of aerially-applied naled on monarch butterflies. Considering the popularity of the monarch and the growing concern about diminishing populations, this study addresses this issue in a proactive manner.

Methodology

Hurricane Michael impact

Numerous spray trials were planned; however, only one could be completed before the contract end date as agreed upon per FDACS. Hurricane Michael struck Panama City on October 10, 2018 destroying all of the outdoor plant and butterfly rearing facilities and shut down the FSU

PC campus. It took several months to clear the area of fallen trees and debris and to rebuild the greenhouses to reinitiate milkweed and butterfly rearing. Strong spring winds unimpeded by the fallen trees, made it difficult to keep the new greenhouses intact. To combat this, we constructed one plant greenhouse in a courtyard surrounded by buildings to protect it from the winds. We also installed a wooden fence to protect two additional greenhouses used to grow plants and as a butterfly flight house at the original site (Fig. 1).

Fig. 1. Monarch butterfly flight house.

Study area, plants, mosquitoes, and butterflies

Three spray transects configured west to east were located just east of Breakfast Point Residential Development, at the north end of Gulf Blvd on Gayle's Trail, and on St. Joe Company land west of the Panama City Beach Industrial Park near Beach Mosquito Control District's (BMCD) new facility in Panama City Beach, Florida (Figs. 2 & 3). The FSU PC campus, located 6 miles upwind from the nearest spray transect, served as the control site. Swamp milkweed, *Asclepias incarnata*, monarch butterflies, *Da. plexippus plexippus,* and black salt marsh mosquitoes, *Aedes taeniorhynchus,* were reared in incubators and green houses at FSU PC. The milkweed was sourced from seeds and cuttings provided by the US Fish & Wildlife Service, St. Marks National Wildlife Refuge and plants from native nurseries in North Walton and Leon Counties. *Aedes taeniorhynchus* eggs were obtained from the USDA Center for Medical, Agricultural, and Veterinary Entomology in Gainesville, Florida and reared in the P.I.'s lab at 12:12 L/D, 27°C, and 40-60% R.H. Feral monarch eggs and caterpillars were collected from nearby residential flower gardens to produce a supply of adults and caterpillars for the tests.

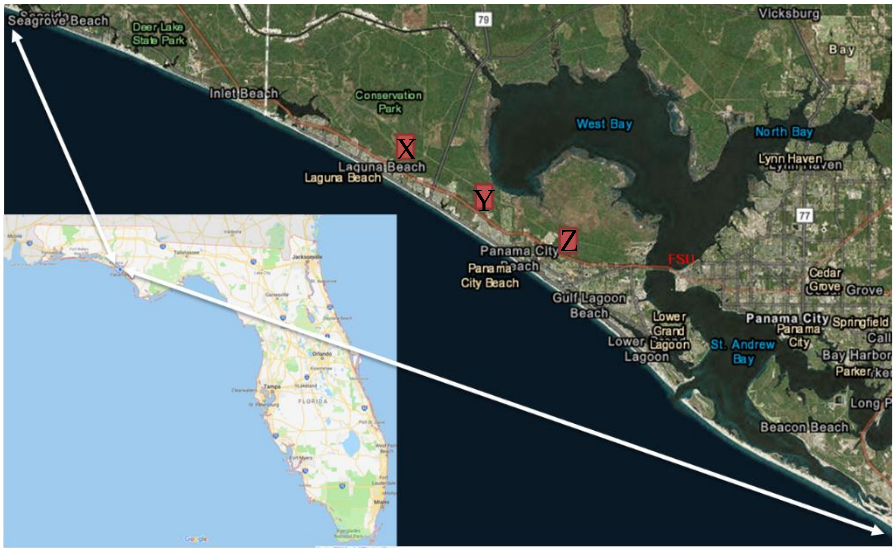

Fig. 2. Location of three 1500 ft. spray transects (X, Y, Z) in Panama City Beach, FL and control site at FSU in Panama City, FL.

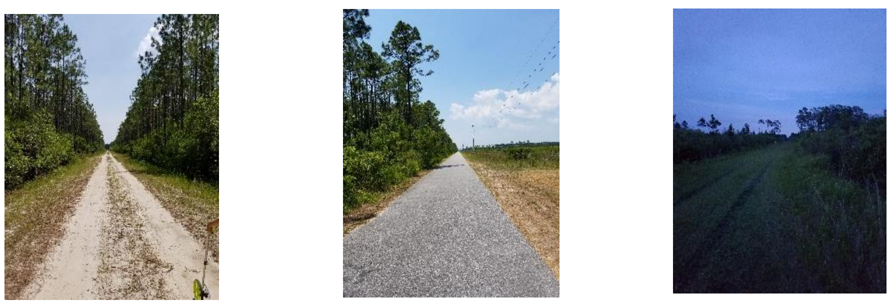

Fig. 3. Ground views of transects X, Y, and Z.

Research procedures

Aerial treatments were applied on June 12, 2019 to three replicate 1500 ft. transects located about three miles apart and positioned downwind from the spray swath (Fig.4). Potted milkweed plants, caged *Aedes taeniorhynchus* mosquitoes, and monarch butterfly adults, respectively, were positioned at the base and hung on five-foot PVC hangers at: 0, 100, 500, 1000, and 1500 ft. on each transect (Fig 5). Three similar hangers were located at FSU PC as negative controls.

The mosquito cages were as designed by Manatee County Mosquito Control District (Williams 2018). The butterfly cages were made by wrapping tulle cloth around three foam board rings, pinning the cloth to the rings, stapling a narrow vertical strip of cloth to bind the tulle around the rings, and wrapping rubber bands around the top and bottom ends.

Aerial treatments were made with two Micronair 5000 rotary nozzles mounted on the BMCD Bell 206 helicopter applying Dibrom (naled) at 0.66 oz. per acre at an altitude of 150 ft. and 86 mph (Fig. 6). Spray equipment was calibrated by BMCD personnel the morning of the test. Onboard GPS and weather data were recorded with Wingman and AIMMS 20 systems. Temperature, relative humidity, wind speed, and direction at 4 ft. and 35 ft. elevations were collected with Kestrel 5500 AG data loggers fitted with wind vanes and placed at the center of each transect on 35-ft. weather tower masts (Fig. 7). The Kestrels positioned on the top of the towers were connected by Bluetooth to cell phones with the Kestrel Link App to monitor real time temperature, wind speed, direction and other environmental data. Spray droplets were collected with Florida

Latham Bonds droplet samplers attached to the top of the PVC hangers at 0, 500, 1000, and 1500 ft. at each transect. A droplet sampler was also located on one of the three control PVC hangers at FSU PC. Droplets were analyzed by BMCD with the Leading Edge DropVision® program. Bioassay organisms were collected 50 minutes after the spray was applied, immediately transferred to clean holding containers, and provided 10% sugar water cotton balls for mosquitoes and 10% fructose cotton balls for butterflies. Pint size white cardboard containers fitted with 20X20 mesh screen tops were used for the mosquitoes. The butterflies were transferred into cages designed similar to the treatment cages except the bottoms were made from a solid plate of foam board with a small petri dish hot glued to it for holding and moistening the fructose cotton ball. The bioassays were placed in laboratory incubators at 12:12 L/D, 27° C and 20.5° C L/D temperature, and 40-60% R.H. 0, 24 and 48 hr. mortality readings were recorded. Leaves from treated and control plants were fed to second instar monarch caterpillars for 4 days and then fed clean leaves thereafter until pupation. Mortality was recorded daily until adult emergence.

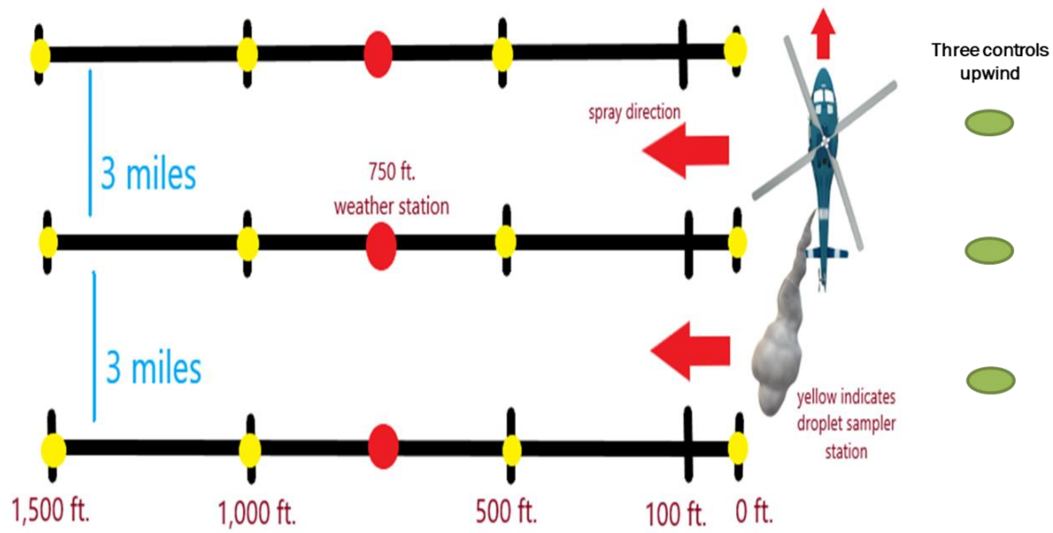

Fig. 4. Diagram of transect set up.

Fig. 5. PVC bioassay and insecticide droplet collection hangers. Milkweed plants at the time of spray test grew to the base of butterfly net.

Fig. 6. Beach Mosquito Control District Bell 206 helicopter

Fig. 7. Weather tower placed in the center of each transect.

Statistical analysis

Transect and control mortality means were analyzed with SAS PC 9.4 using PROC ANOVA and charted with standard errors. Since mortality in the control was <5%, the means did not require correction by Abbott's formula (Abbott 1925). Mortality percentages by transect and distance at 48 hrs. was charted using Microsoft EXCEL. Standard errors were not calculated because mortality readings were not averaged among the three transects due to significant differences.

Results

Weather Data:

The spray application was made over the three transects between 7:45 and 8:30 p.m. C.T. Winds were primarily from the west to southwest and varied from 2-7 mph (Fig. 8 & 9). Temperature at time of treatment was 82°F with humidity >80%. An inversion was present with temperatures at 4 ft. half a degree lower than at 35 ft. Unfortunately, all data collected at the weather towers was inadvertently lost because the Kestrel data loggers were set by default to overwrite data when the memory filled. Consequently, all weather data was taken from Weather Underground stations located adjacent to the transects.

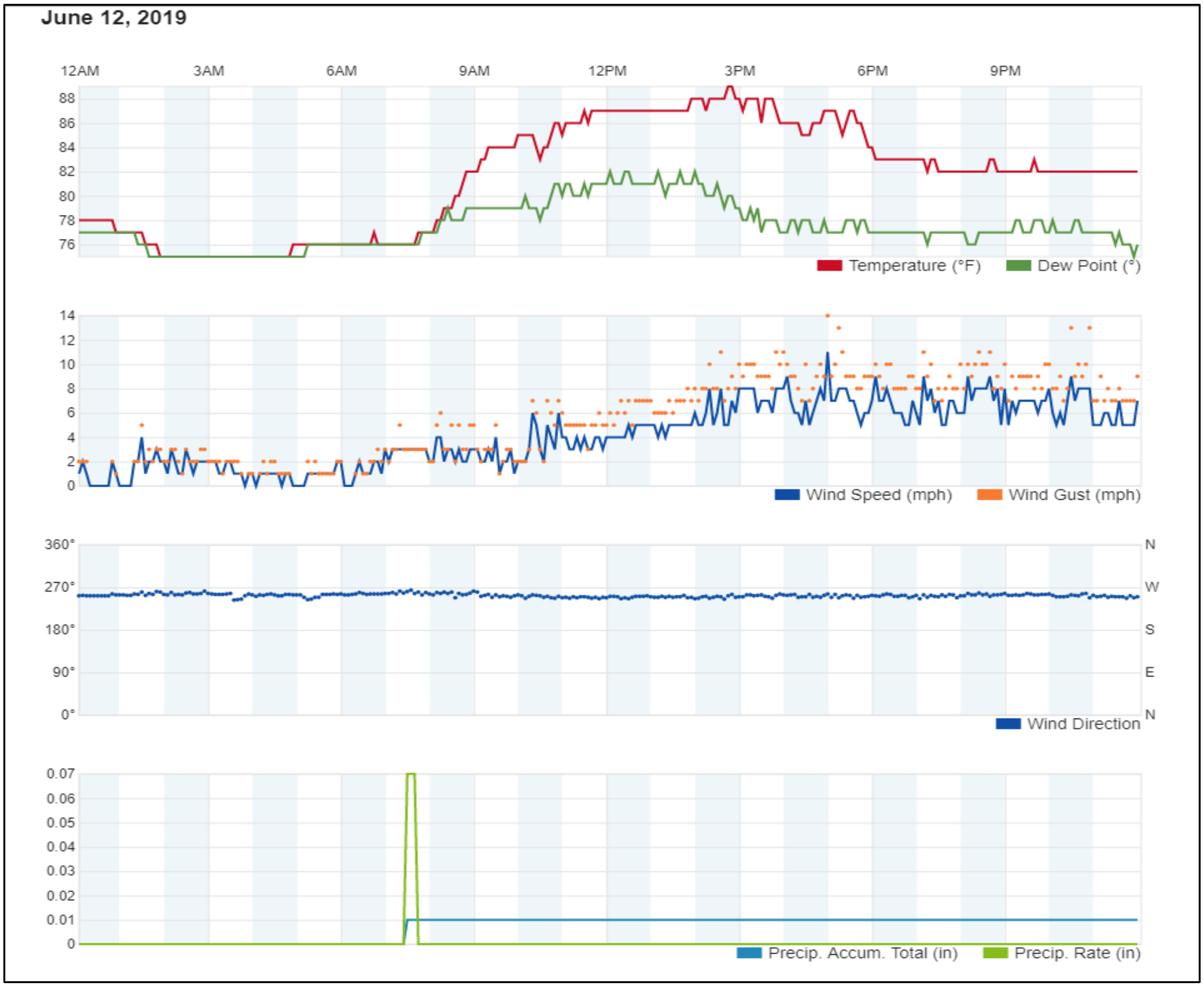

Fig. 8. Weather data from the nearest weather station to Transect X.

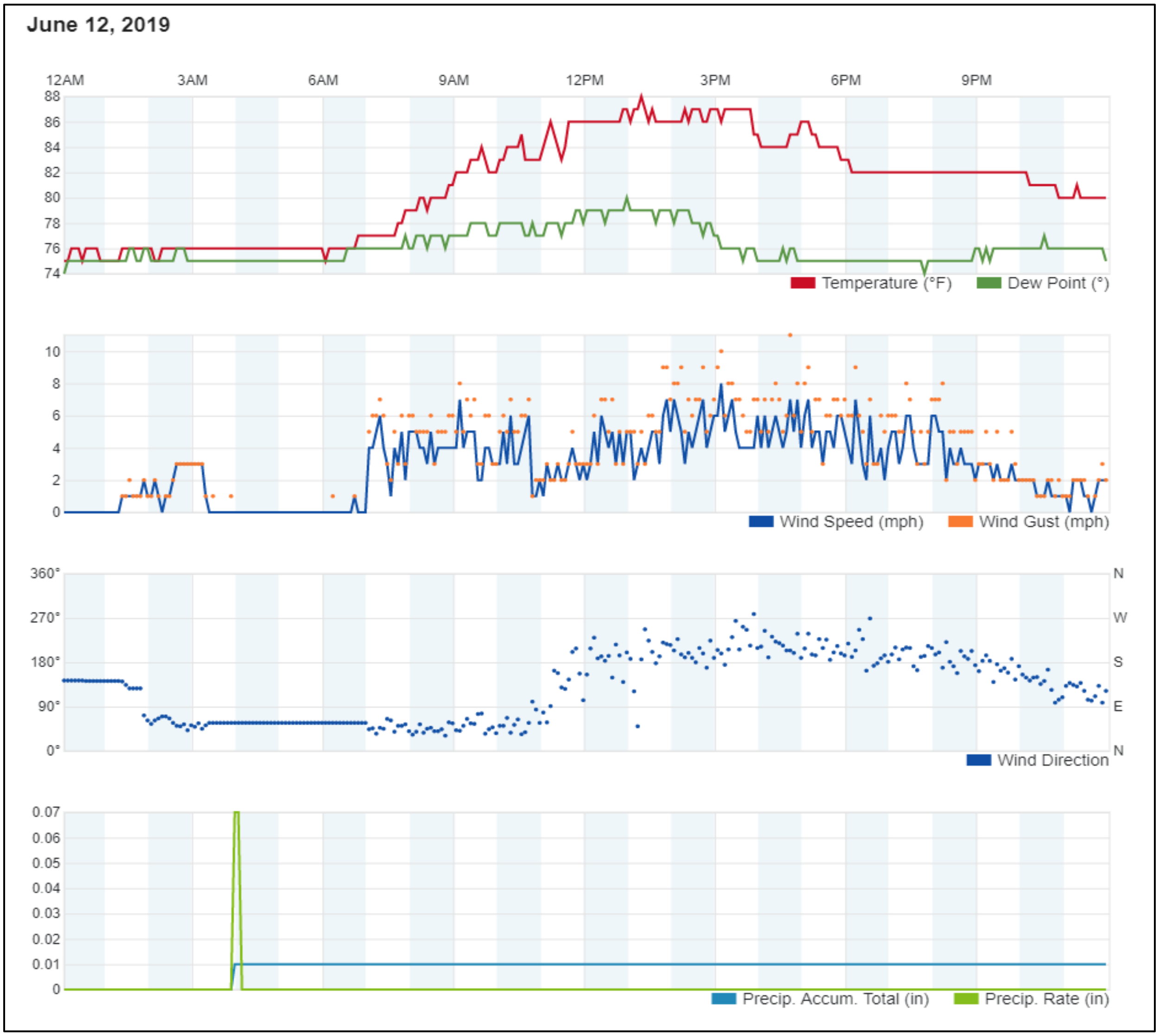

Fig. 9. Weather data from the nearest station to Transect Y & Z.

Spray Application

Onboard equipment for targeting the spray was not working properly, so the pilot had to manually apply the swath based on his experience, taking into account wind and offset distance. Droplets were small at all transects and distances (Table 1). Relatively few droplets were collected on the slides at each location. This may explain why mosquito mortality was lower than expected.

VMD Dv0.5				
Transect	**Feet**			
	0	**500**	**1000**	**1500**
X	9.19	8.54	9.95	8.29
Y	9.74	8.8	8.54	8.05
Z	7.75	7.46	0*	7.69

*Slides were smudge and could not be read properly.

Table 1. Droplet sizes out of 200 counted per collection spinner.

Mosquitoes

Significant differences were found among transect mortality means, thus data from each transect were viewed separately at 0, 24, and 48 hr. post-treatment readings (Table 2 & Fig. 10).

Number of Observations Read	18
Number of Observations Used	18

The SAS System

The ANOVA Procedure

Dependent Variable: PCDEADC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	10	16977.55556	1697.75556	5.63	0.0158
Error	7	2112.22222	301.74603		
Corrected Total	17	19089.77778			

R-Square	Coeff Var	Root MSE	PCDEADC Mean
0.889353	52.46226	17.37084	33.11111

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TRANSECT	3	11275.11111	3758.37037	12.46	0.0034
FEET	7	5702.44444	814.63492	2.70	0.1067

Table 2. Analysis of variance of transect and distance mortality means at 48 hrs. posttreatment.

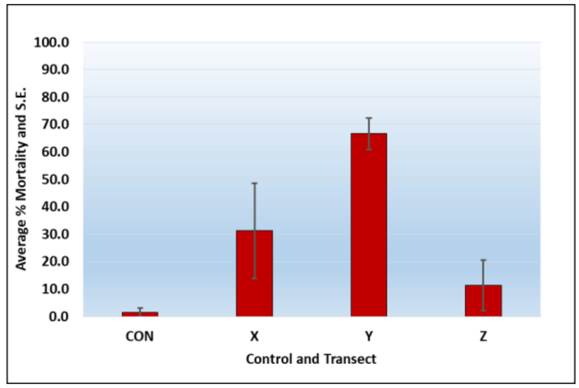

Fig. 10. Mean mortality percentage by transect and control.

Mortality in Transect Y at 0, 24, and 48 hrs. posttreatment was significantly greater and more widespread over distance downwind compared to Transects X and Z (Fig. 11). In the 48 hr. readings, percent mortality ranged from 71-77% out to 1000 ft. and dropped to 55% at 1500 ft. Mortality in the other two transects ranged widely with 100% mortality at 100 ft. and 48% at 1000 ft., while all the other distances did not exceed 20%. The control did not exceed 5% mortality. Differences in tree and brush coverage surrounding the three transects could have contributed to poor penetration.

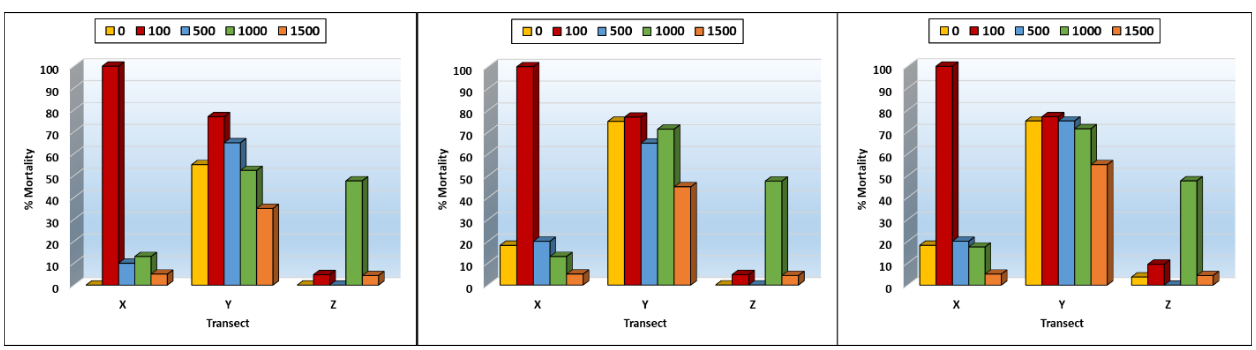

Fig. 11. 0, 24, and 48 hr. mortality for *Aedes taeniorhynchus* at three transects (X, Y, & Z) downwind 0, 100, 500, 1000 and 1500 ft. from naled application.

Butterflies

Very little mortality was observed at the 0, 24, and 48 hr. posttreatment periods (Fig. 12). That which did occur was likely associated with handling in the case of the one dead butterfly in Transect X at 1500 ft. In Transect Y at the 0 ft. interval, 1 cage of five butterflies fell off the hanger during the test and was found on the ground with ants covering the butterflies. No other mortality was observed in the treatments or controls.

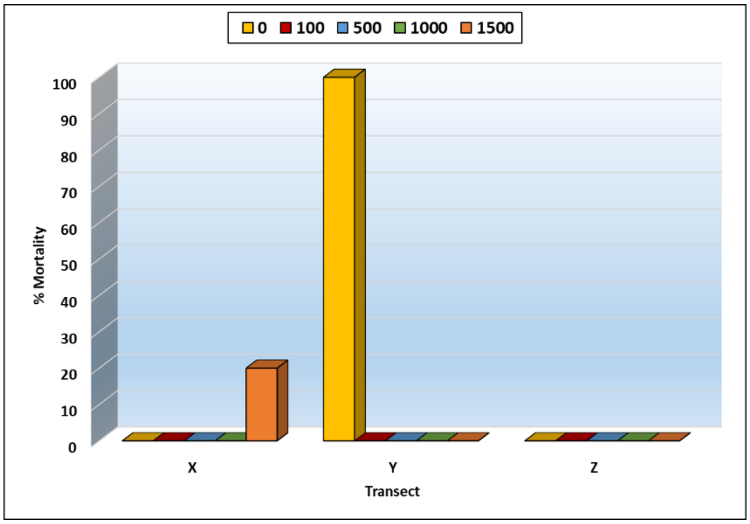

Fig. 12. Monarch butterfly mortality at three transects.

Caterpillar leaf feeding bioassays

Second instar caterpillars fed leaves from plants exposed at each of the bioassay hangers developed without any more mortality than appeared in the controls. In fact, one of the controls was the only treatment that had 100% mortality. Most developed through to chrysalis without mortality. Intermittent mortality that was observed usually involved one or two and rarely three of the developing five caterpillars and was attributed to disease or parasitism.

Discussion

It is very difficult to expect consistent data from one spray trial, even though this study had replicated transects. It normally takes several trials to obtain repeatable, reliable data. Our research team and cooperators put their best efforts forward within the given time constraints. The setbacks inherited from the impacts of Hurricane Michael and other uncontrollable factors were too much to overcome. That being said, several valuable lessons were learned on what it will take to properly complete this study. Additional spray trials are definitely warranted. Much of the equipment to conduct further tests (e.g., specialized cages, weather towers, droplet collectors, etc.) developed through this project have been given to Beach Mosquito Control District so that continued studies may be possible.

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