Larvicide Resistance Testing: A Tale of Trials and Tribulations

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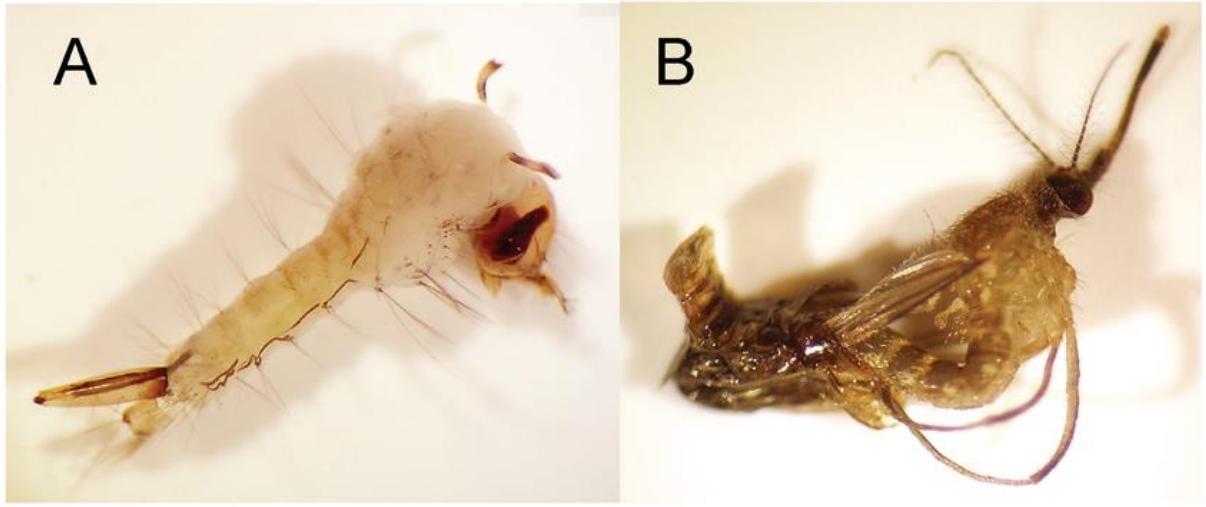
Prologue

CCMC has been using Methoprene products since at least 1985

Methoprene is an insect growth hormone

Prevents mosquito from maturing past pupal stage.

Product is mixed with sand for easier dispersal through tree canopy and other vegetation



Kamita et al. 2011

Prologue

We had some suspicious observations…



Prologue

Dredge containment area across the Savannah River

Were the mosquitoes here flying over from outside the dredge area, or were the mosquitoes emerging even after being treated?







Prologue

- Methoprene resistance has been seen across the Southeast United States in Aedes and Culex mosquitoes.
- Adult bio bottle assays are run at CCMC, but larval assays have never been done consistently.
- Trap counts being high in areas that were treated with Methoprene



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Chapter One: Practicing Protocols

Protocols received from Dr. Eva Buckner with University of Florida FMEL

-Prepare Methoprene dilutions

-Place 3rd/4th instar *Cx. quinquefasciatus* larvae in cups with 100 mL of water

-Treat cups with increasing doses of Methoprene

-Feed larvae hog pellets

-Read results after 7 days







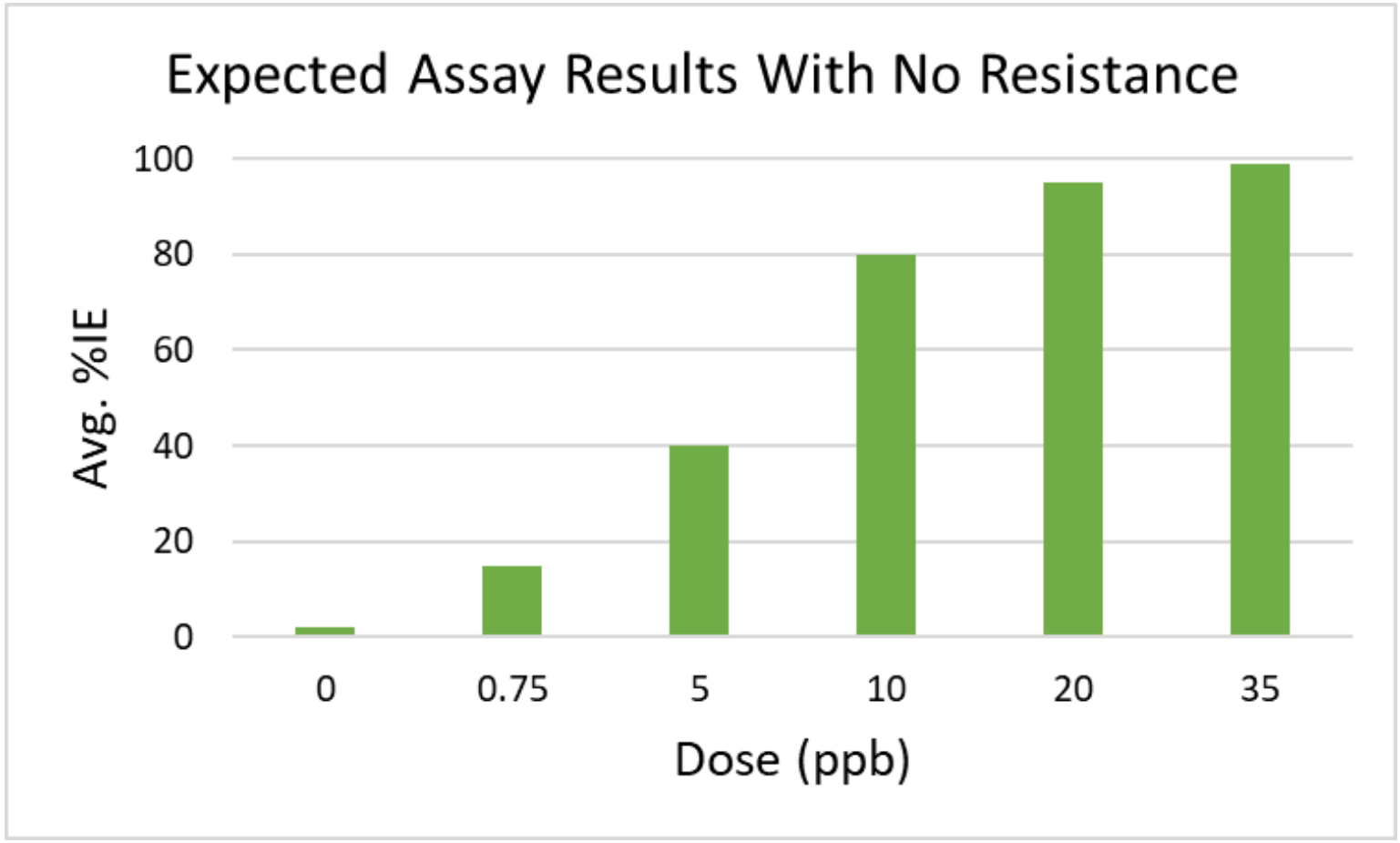
Chapter One: Practicing Protocols



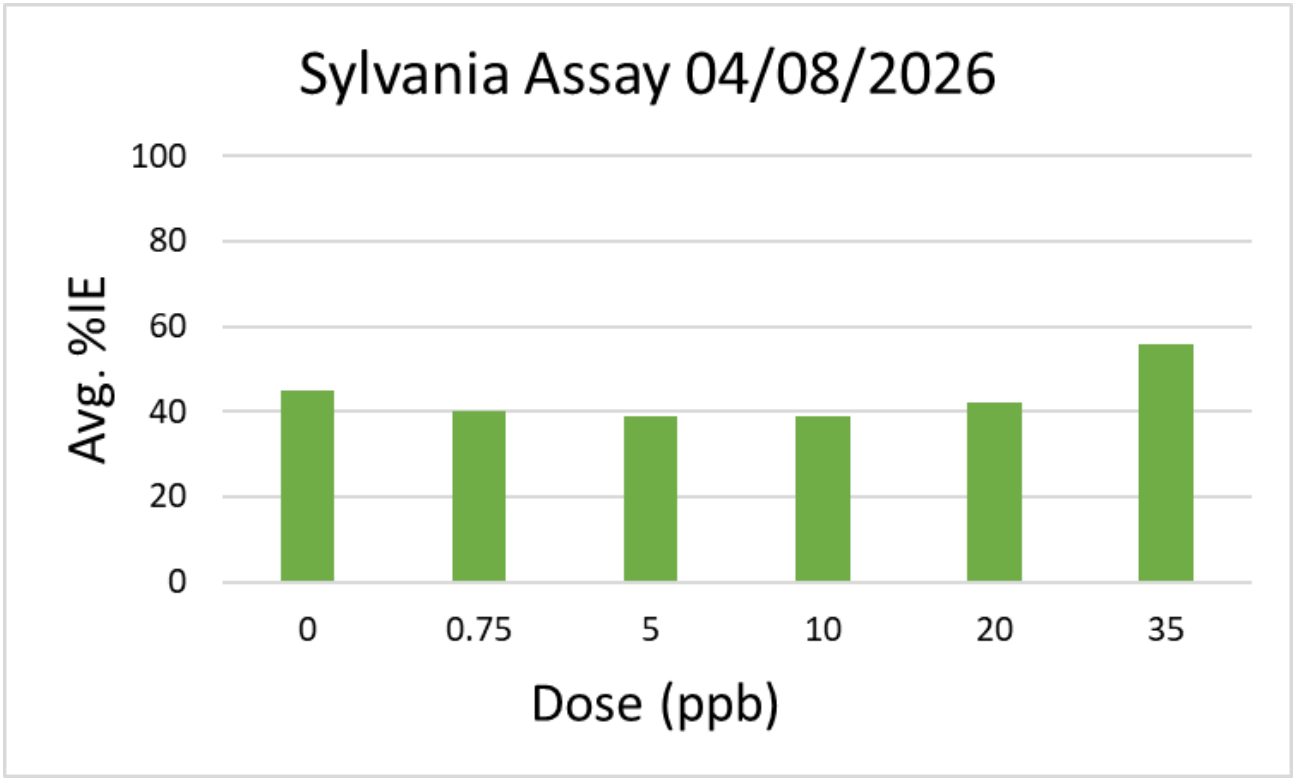
Chapter One: Practicing Protocols

Expected Results:

- Control cups should have < 20% inhibited emergence
- Higher doses should have > 90% inhibited emergence



Chapter One: Practicing Protocols



What actually happened

Chapter One: Practicing Protocols

Early testing, mistakes happen!

Adjustments made to rearing conditions, no more glass or plastic!

Food assay to ensure larvae were getting the right amount of food

Something was in the works that would make it much easier to do more assays…





Chapter Two: Extra Hands

- Our summer intern, Jordan, came to Chatham County Mosquito Control!
- Her main project was running larval assays
- Results became much more frequent and consistent!



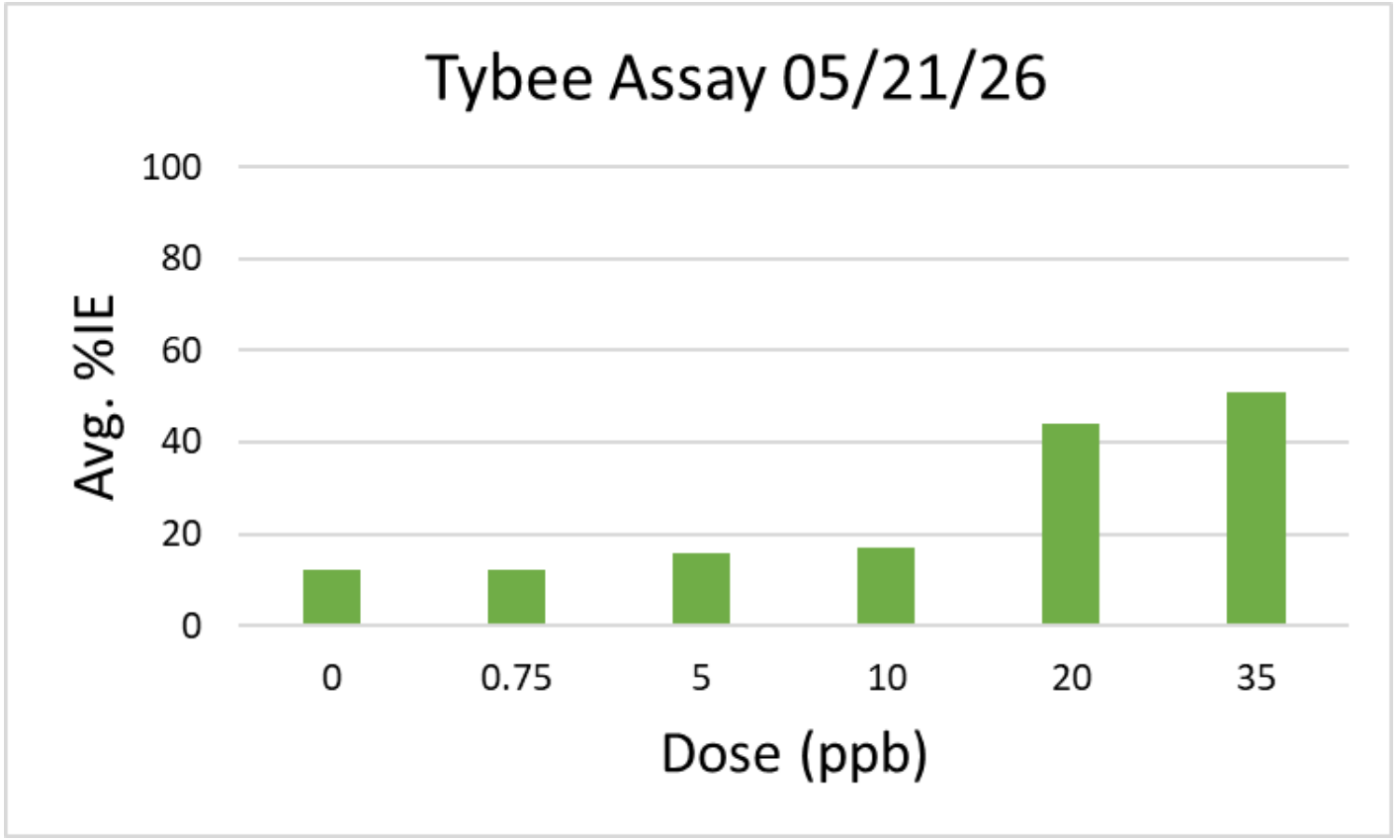
Chapter Two: Extra Hands

Very high survivability in the control treatment!



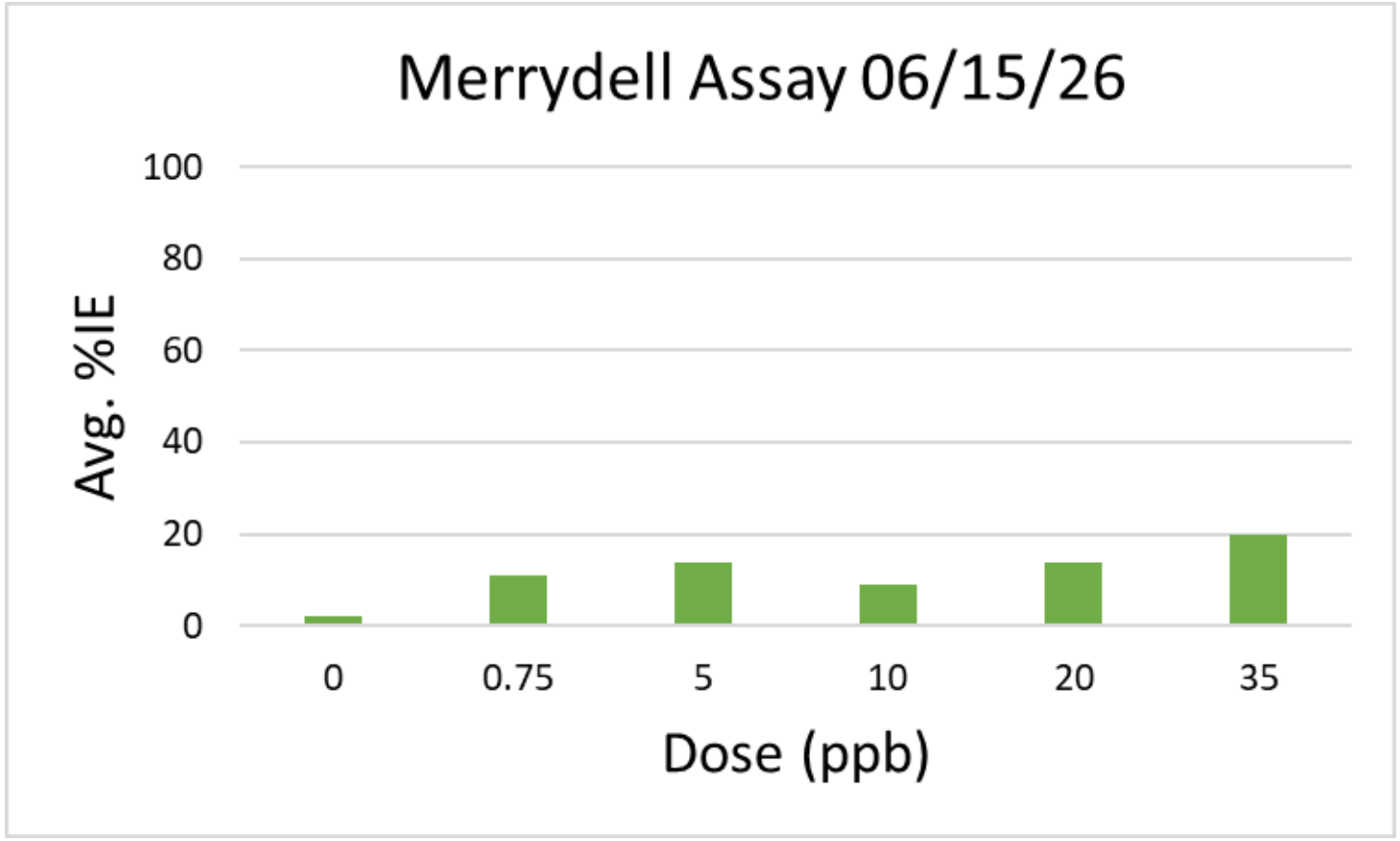
Very high survivability in the higher doses!





Chapter Two: Extra Hands

Having such a low kill leaves us unable to calculate an accurate LC50, the lethal concentration to kill 50% of the test population.



Chapter Two: Extra Hands

Thank you Jordan!

- Consistent rearing conditions and schedule
- More larvae surviving to adulthood
- Data from different parts of the county

Still one major obstacle…

Chapter Three: Sebring Shenanigans

In order to fully trust our field results, we needed to compare our assays to a strain of mosquito larvae that is susceptible to Methoprene.

Sebring colony originally from Sebring, Florida has been lab reared for over 40 years.

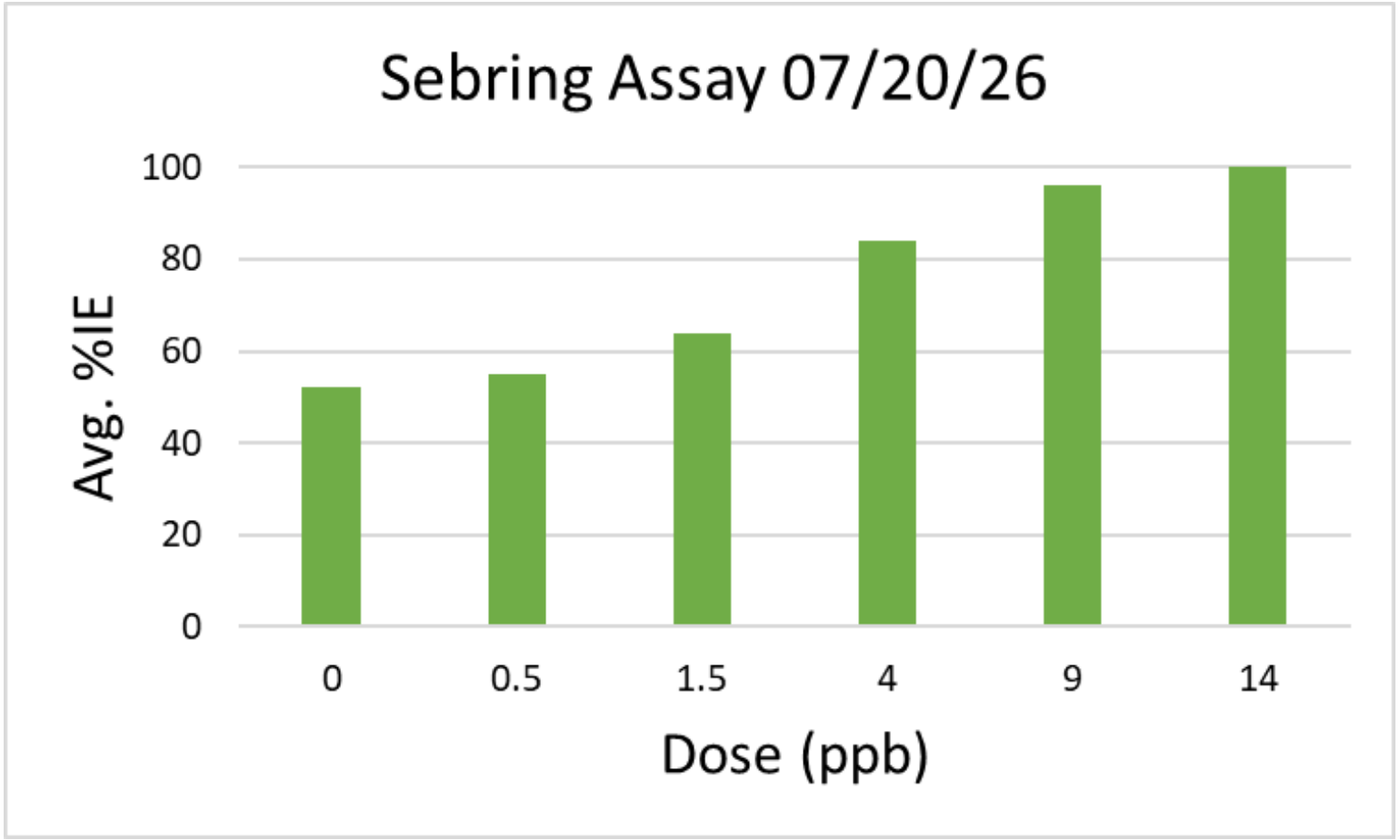
Lab conditions have made them wimpy and inbred, which led us to some poor results…



Chapter Three: Sebring Shenanigans

Was the high inhibited emergence from the Methoprene or something else?

Sebring larvae were also struggling to mature in the paper cups they were being reared in.



Chapter Three: Sebring Shenanigans

Thankfully, other people have a lot more experience with these larvae!

Zoom meeting with Nicole Foley and Casey Crockett from the CDC.

Shared their knowledge on rearing mosquito larvae:

- Sebring need plenty of space
- Quantity of food is very important

Added that we were not alone in our suspicion of Methoprene resistance.

New and improved rearing protocol!

Chapter Three: Sebring Shenanigans

Paper cups → Metal trays





Chapter Three: Sebring Shenanigans

Hog pellets → Liver powder



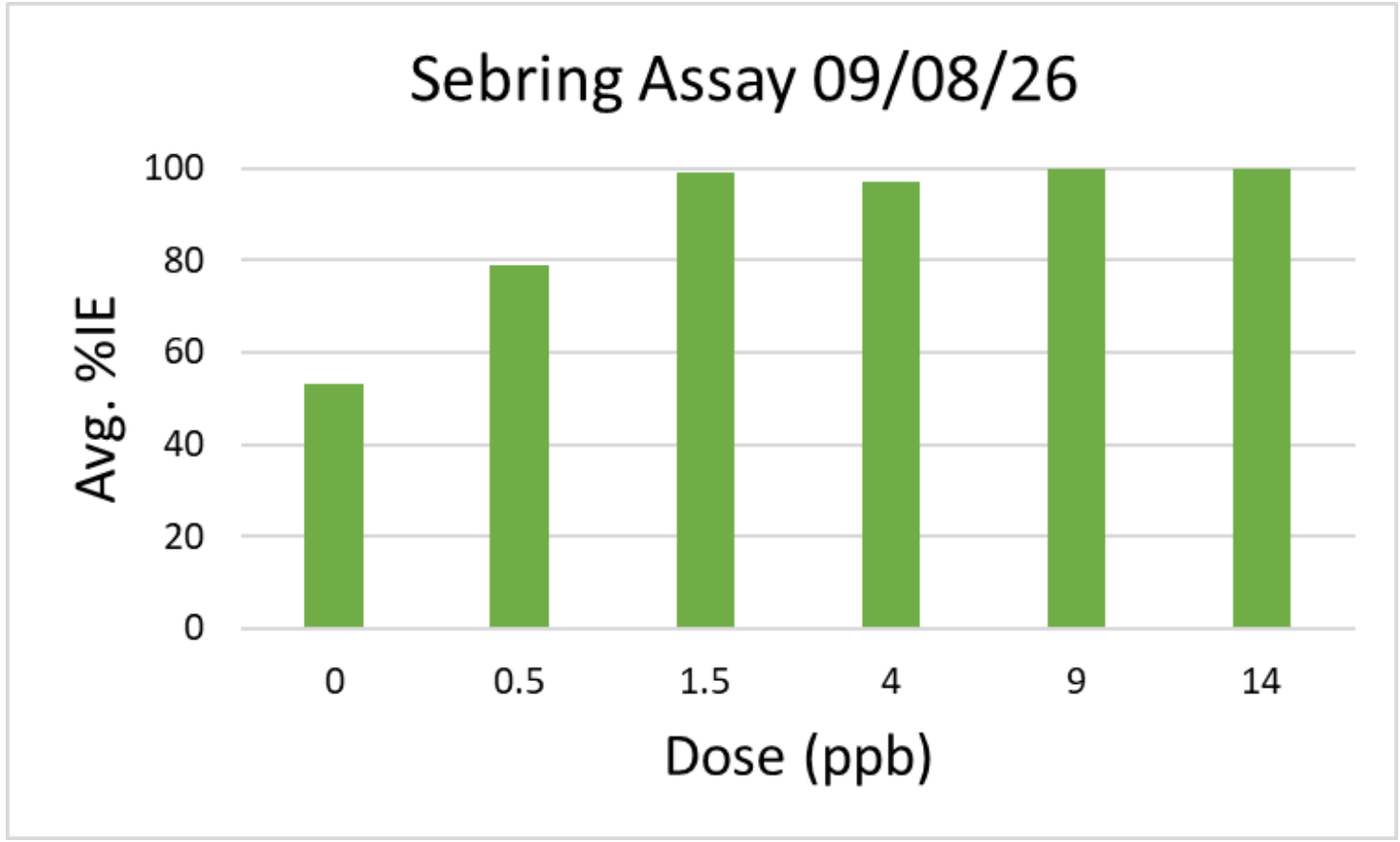


Chapter Three: Sebring Shenanigans

Still way too high of a kill in the control treatment.

Some good news!

Larvae being reared in metals pans had more adults emerging than they did being reared in the paper cups!



Future Chapters

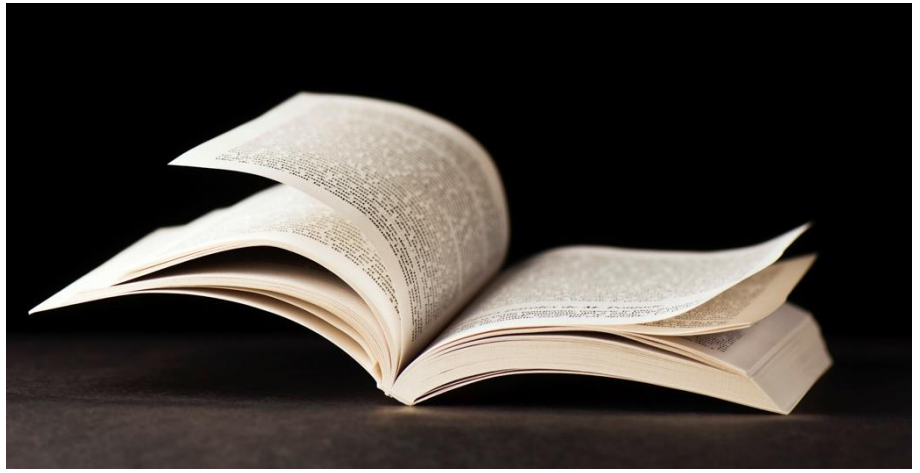
The work is never done!

- More Sebring assays
- Assays with other active ingredients
- Increasing doses in cups
- Calculating resistance ratios and LC_{50} values
- Assay data from every part of the county
- Seasonality of resistance?
- Testing resistance in other species like *Ae. taeniorhynchus*



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The Rest is Still Unwritten!



Thank you for your time!

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Questions?