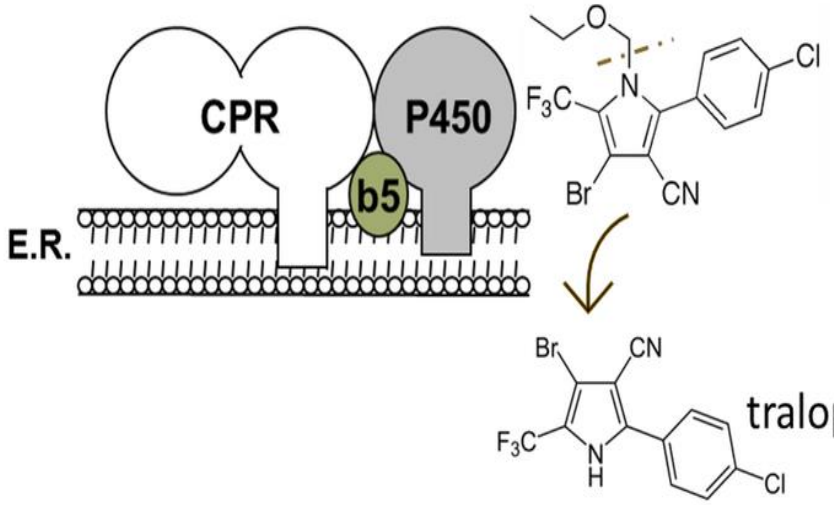
Monitoring and Addressing Growing Pyrethroid Resistance at a Global and Domestic Scale

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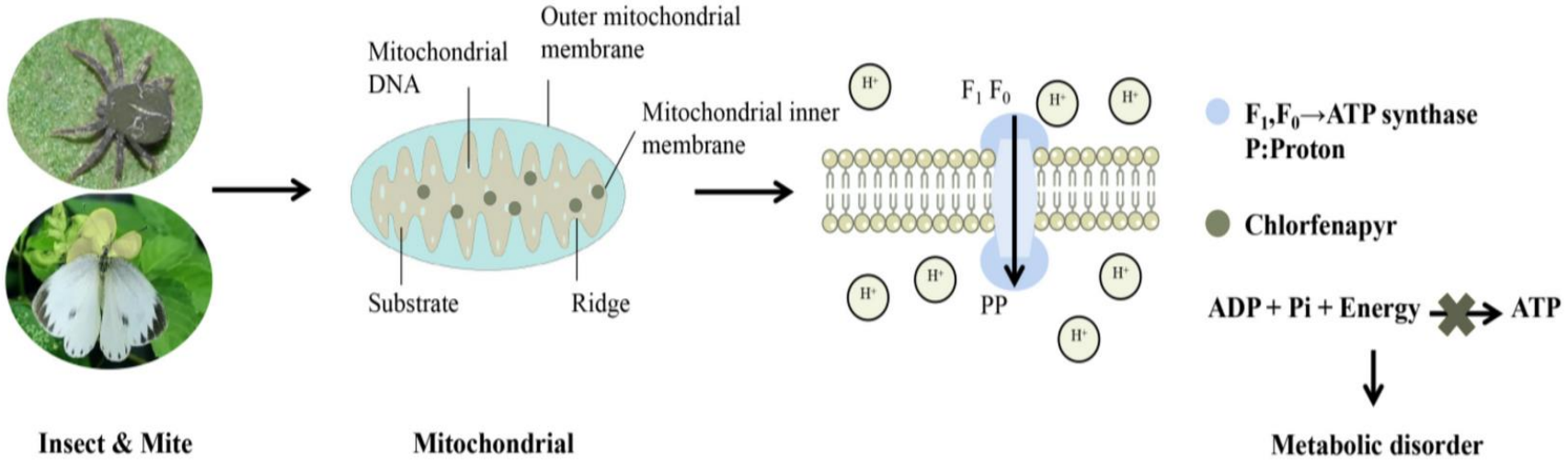
Objective

- To better understand the effect of chlorfenapyr exposure on metabolic resistant mosquitoes:
  - Transcriptomics
  - Fecundity/development
  - Changes in resistance over time
- To characterize insecticide resistance of mosquito populations in Georgia
  - CDC Bottle Assay
  - Sequencing

What Is Chlorfenapyr?

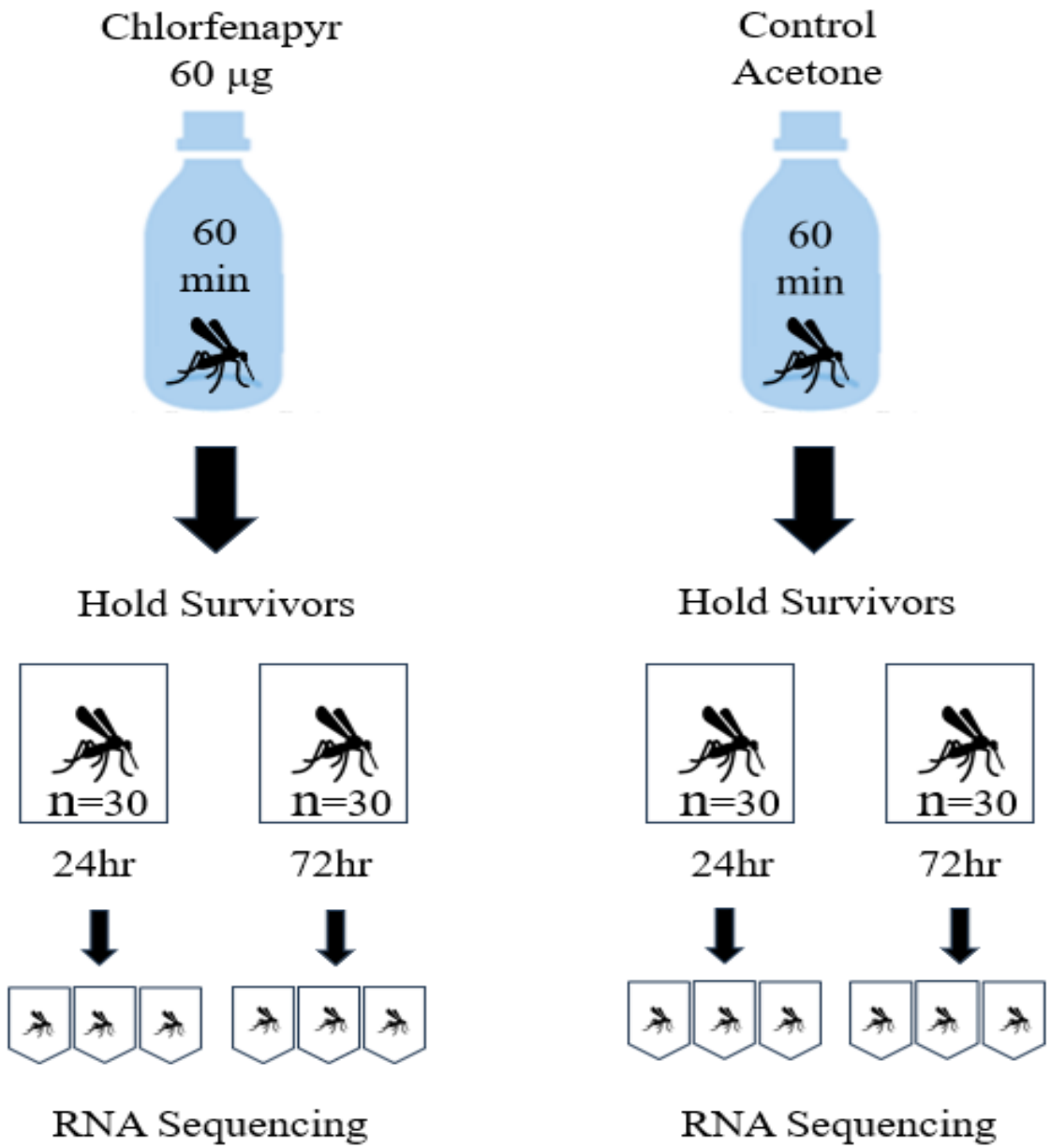


- Chlorfenapyr is a pyrrole-based pro-insecticide, introduced to target pyrethroid-resistant mosquitoes due to its unique mode of action utilizing P450s. It is metabolized into tralopryril which inhibits the mitochondrial electron transport chain interrupting the production of Adenosine triphosphate (ATP).



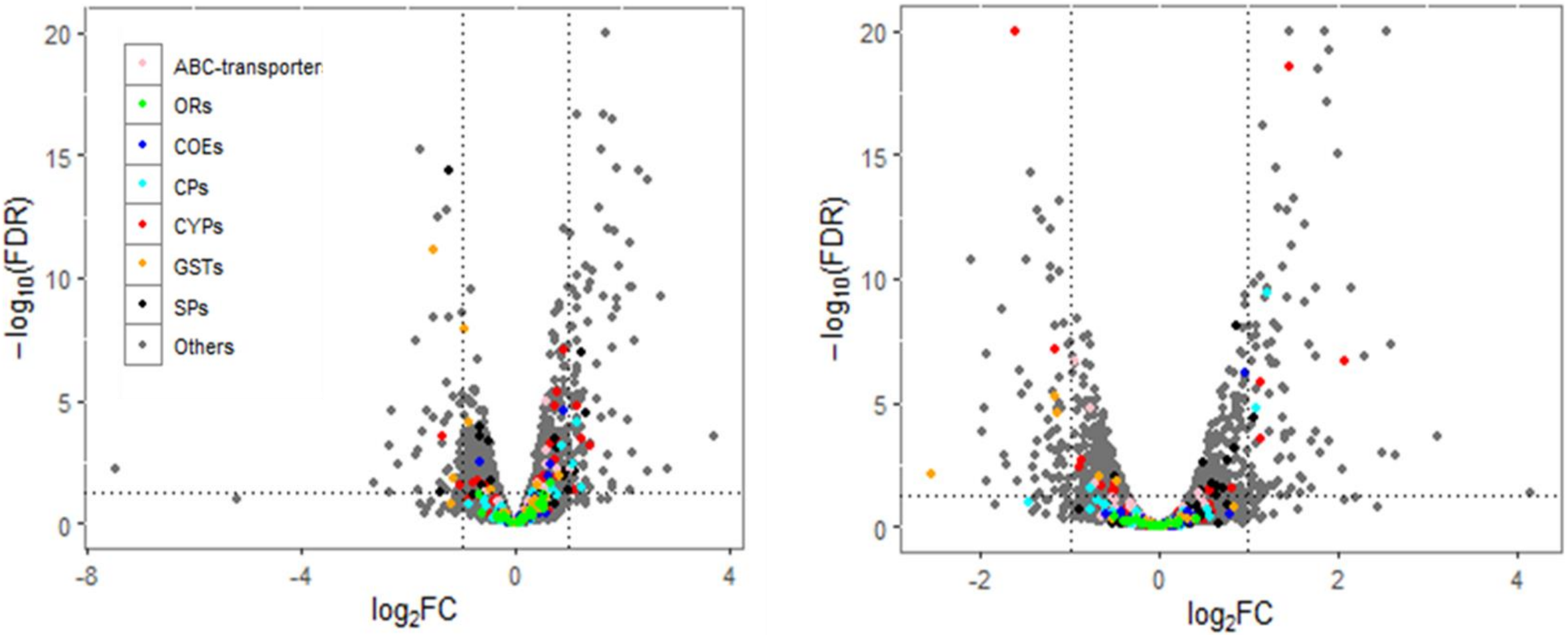
How Does Chlorfenapyr Exposure Affect The Transcriptome of Metabolic Resistant Mosquitoes (24 vs 72 Hours)?

Experimental Outline

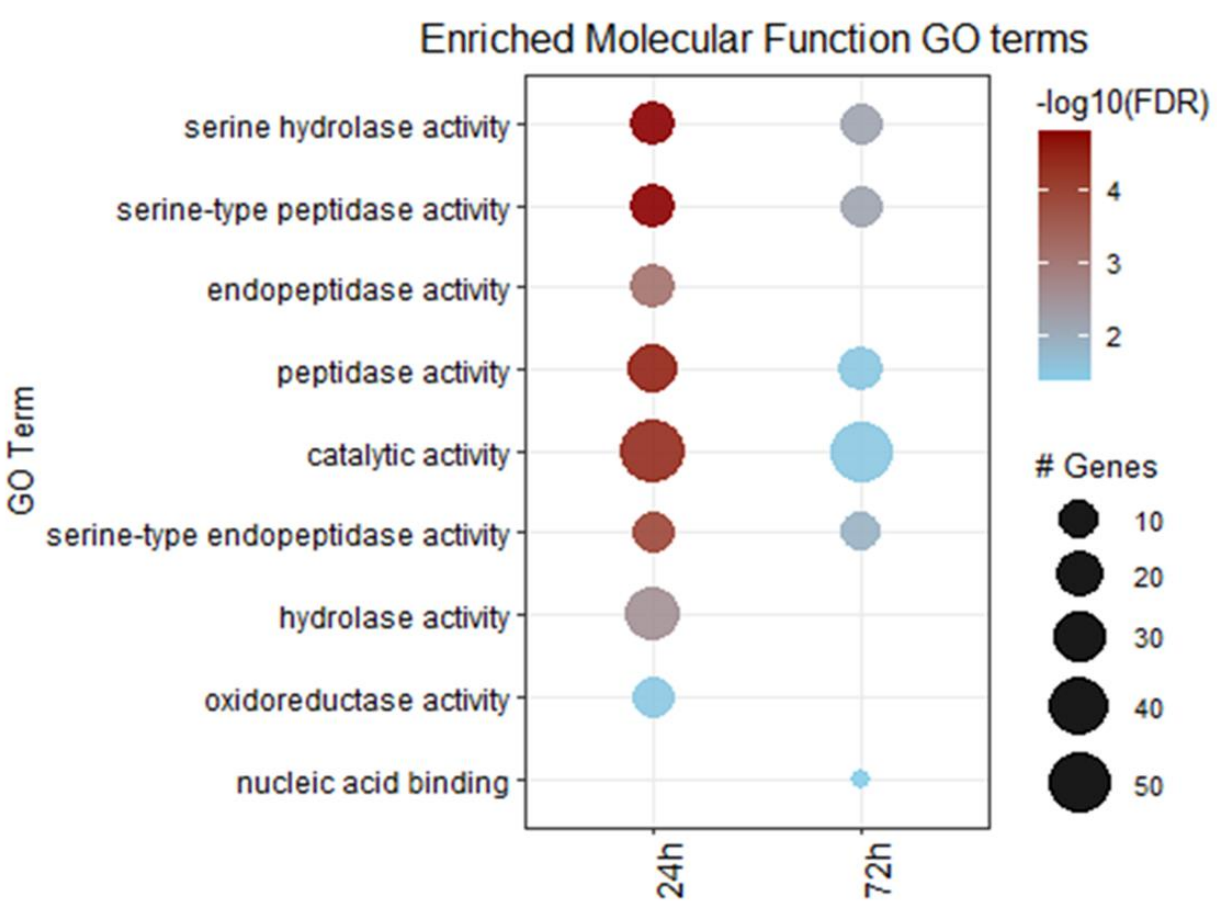


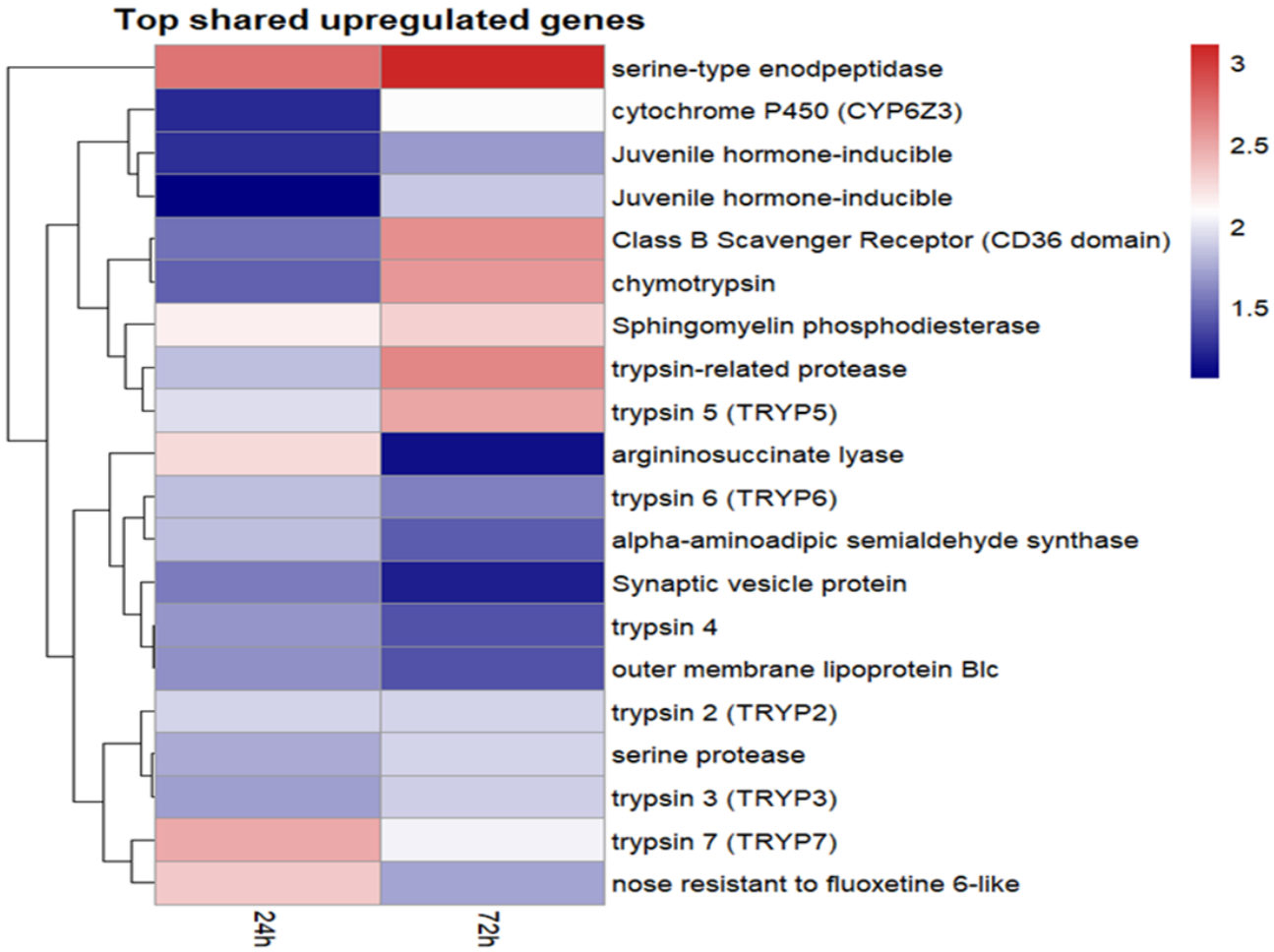
24 Hours

72 Hours



Volcano Plots of Differentially Expressed Genes Between Chlorfenapyr Treated Mosquitoes (60µg) and Untreated Mosquitoes at 24 and 72 hours.





Core transcriptional response to chlorfenapyr and top shared upregulated genes at 24 and 72 hours.

Conclusion

- Two p450s, CYP6Z3 and CYP6M2 were consistently upregulated between the 24 hours and 72 hours. Both are associated with pyrethroid resistance.
- Downregulation of Glutathione S-transferaces (GST)
- At 72 hours CYP6Z3 log fold change Increased, indicating a potential increase in chlorfenapyr metabolism after 24 hours.
- Exposure induced a sustained coordinated transcriptional response with the upregulation of genes associated with proteolytic activity such as Chymotrypsin-1 and multiple trypsin genes including TRYP2,TRYP3,TRYP4,TRYP5,TRYP6, and TRYP7.

Will Repeated Chlorfenapyr Exposure Over Generations Affect Pyrethroid Resistance ?

Experimental Outline

Siaya
(Resistant *An. gambiae*)
4-6 day old



Chlorfenapyr Exposure
(60 µg)

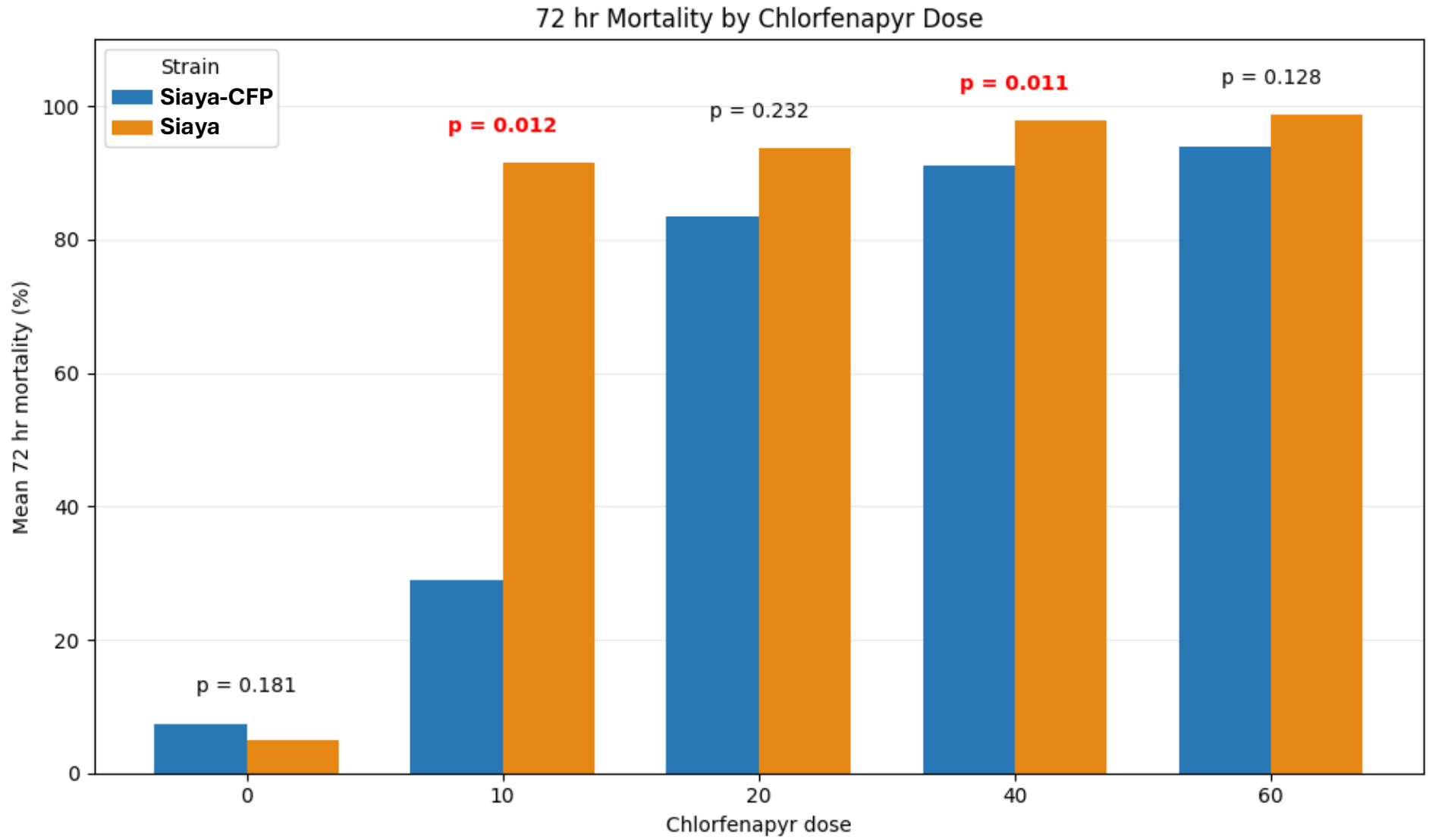
Survivors Blood Fed

Exposed Females Lay Eggs

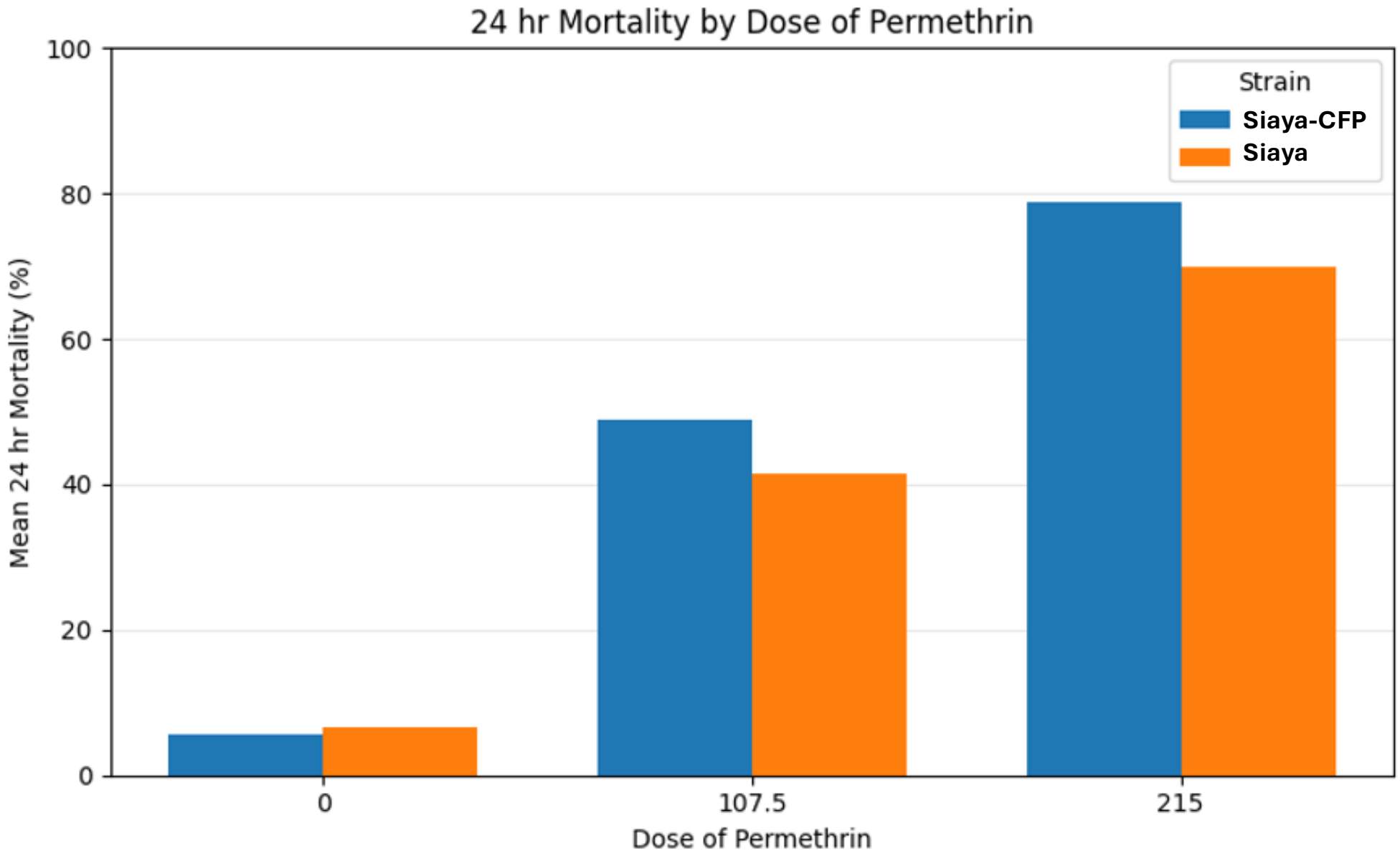
Recovery Generation

Repeat with 1-2 recovery generations between each selection

Chlorfenapyr Mortality of Siaya after 10 generations (6 exposures)

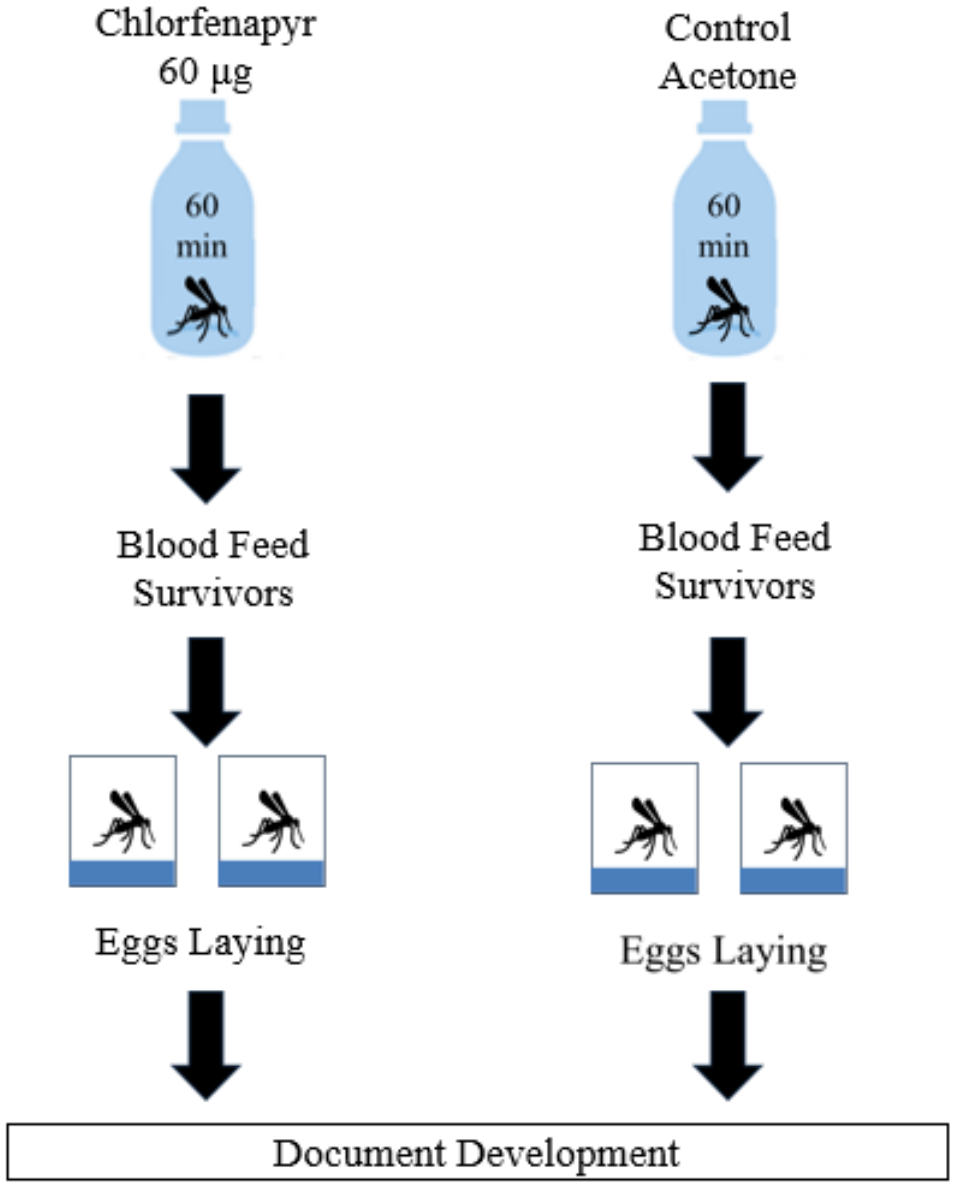


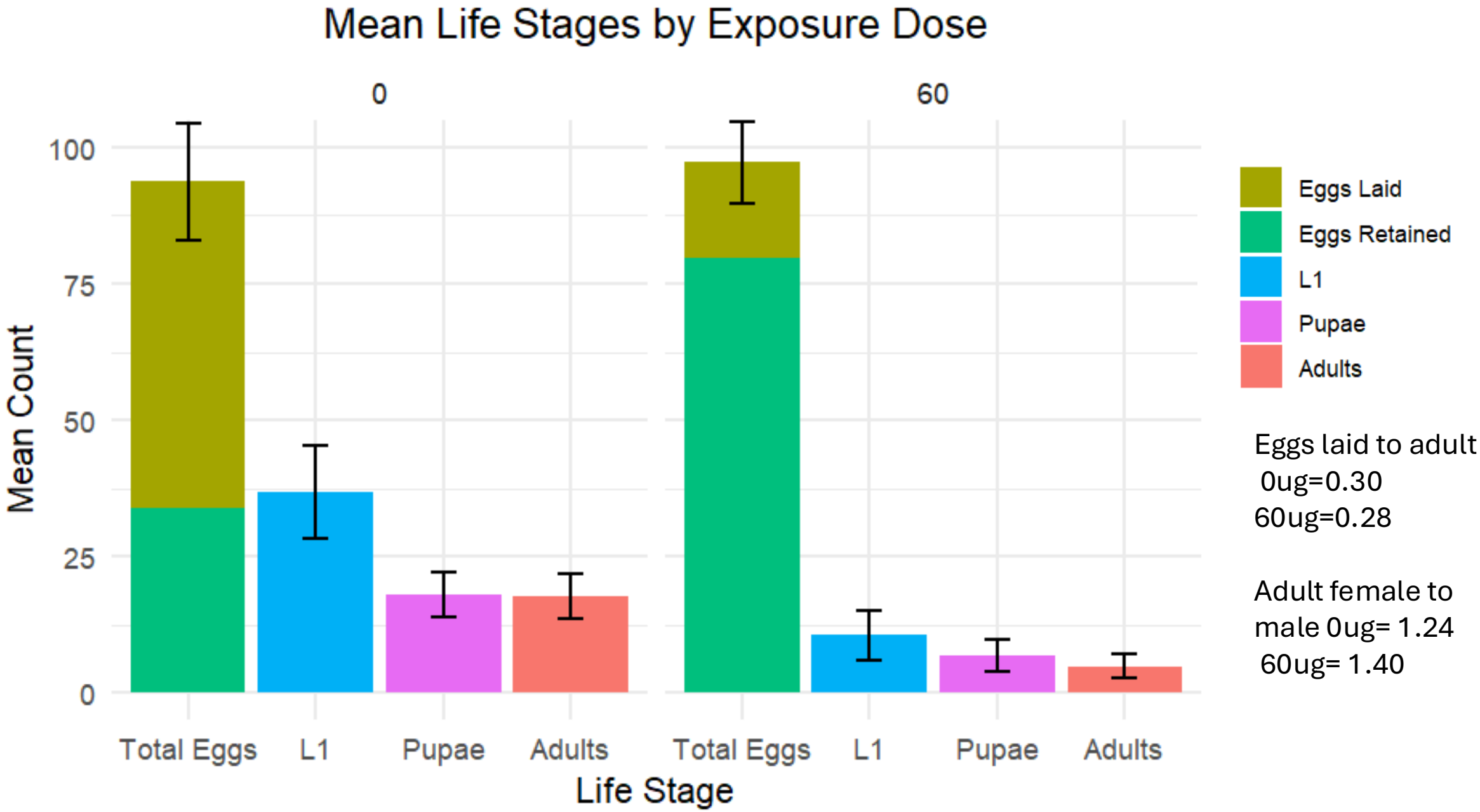
Permethrin Mortality of Siaya after 10 generations (6 exposures)



Will Chlorfenapyr Exposure Affect Fecundity and Development?

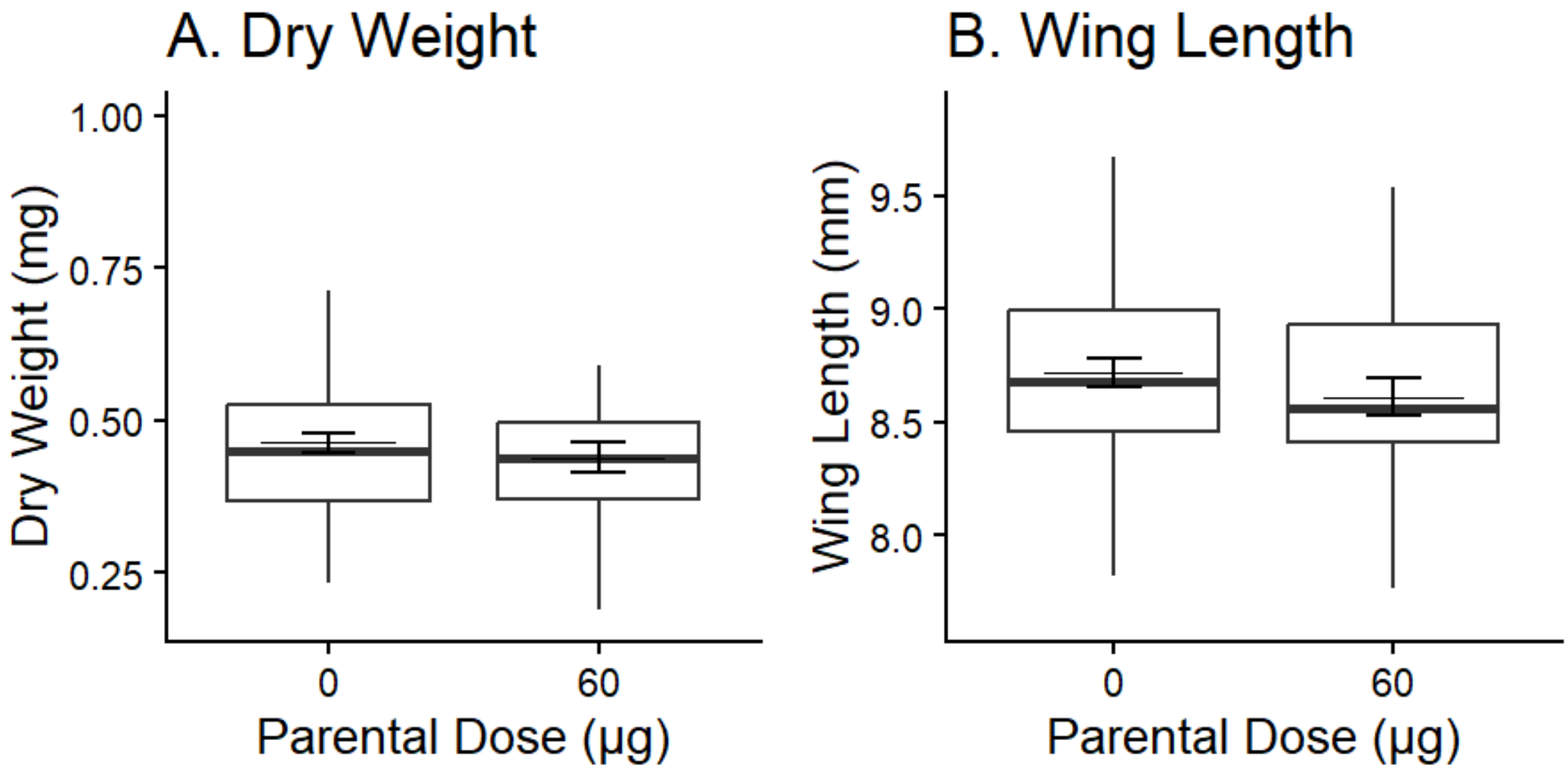
Experimental Outline





Eggs laid to adult
0ug=0.30
60ug=0.28

Adult female to male 0ug= 1.24
60ug= 1.40


A. Dry Weight
Dry Weight (mg)
1.00
0.75
0.50
0.25
0
60
Parental Dose (µg)
B. Wing Length
Wing Length (mm)
9.5
9.0
8.5
8.0
0
60
Parental Dose (µg)

Conclusion

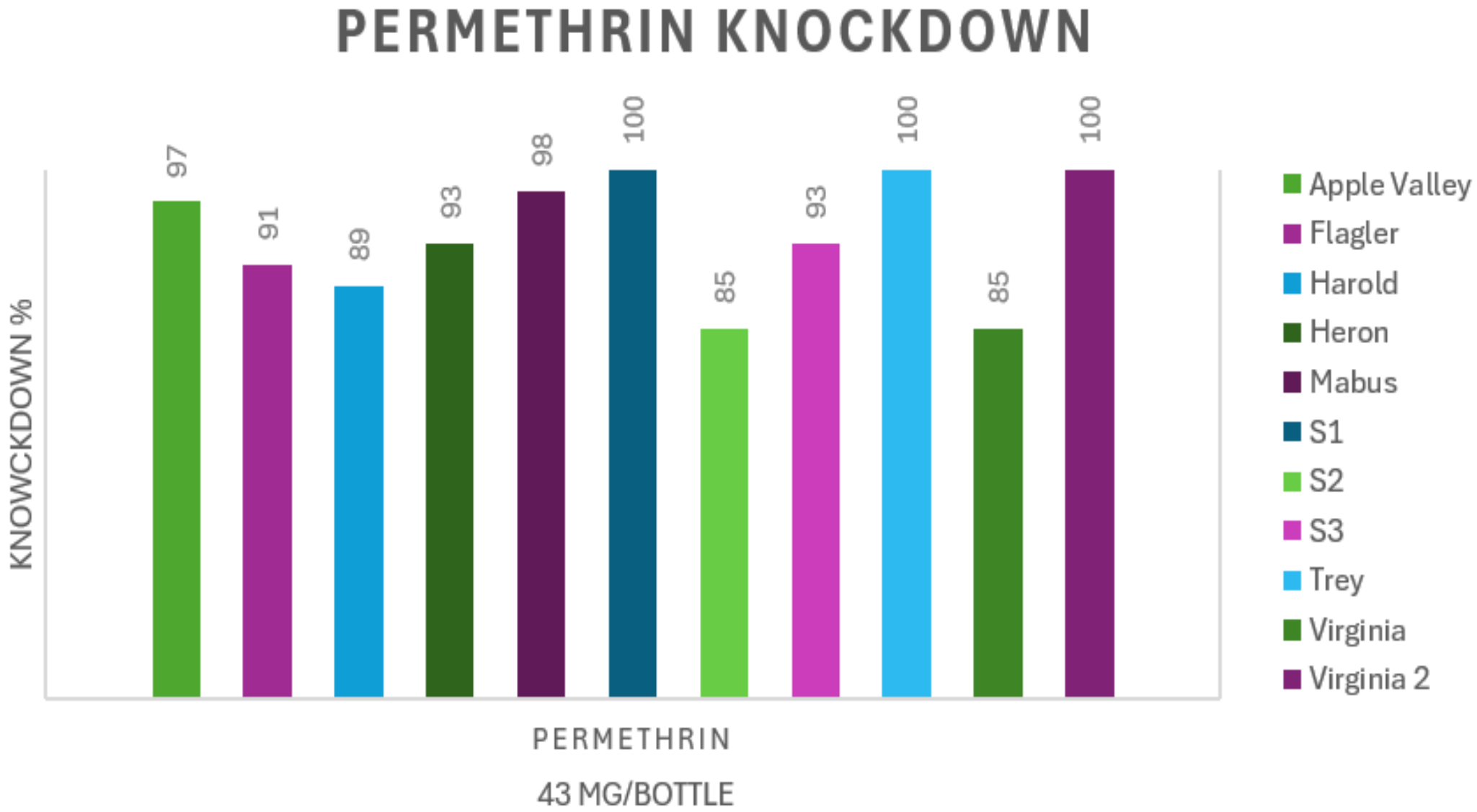
- Chlorfenapyr
  - Induced a unique transcriptional response consisting of an upregulation of CYP6Z3 and CYP6M2, upregulation of serine proteases, and down regulation of GSTs.
  - Repeated exposure of chlorfenapyr over 10 generations of a permethrin resistant strain had statistically significant lower rates of mortality at low doses (10% and 40% of diagnostic dose) of Chlorfenapyr while maintaining a high level of resistance of permethrin.
  - No significant changes in exposed female mosquitoes offspring's size and wing length and eggs laid to adult ratio.
  - Exposed mosquitoes retained eggs at a high rate but was not repeatable.

CDC Bottle Assay Resistance Testing

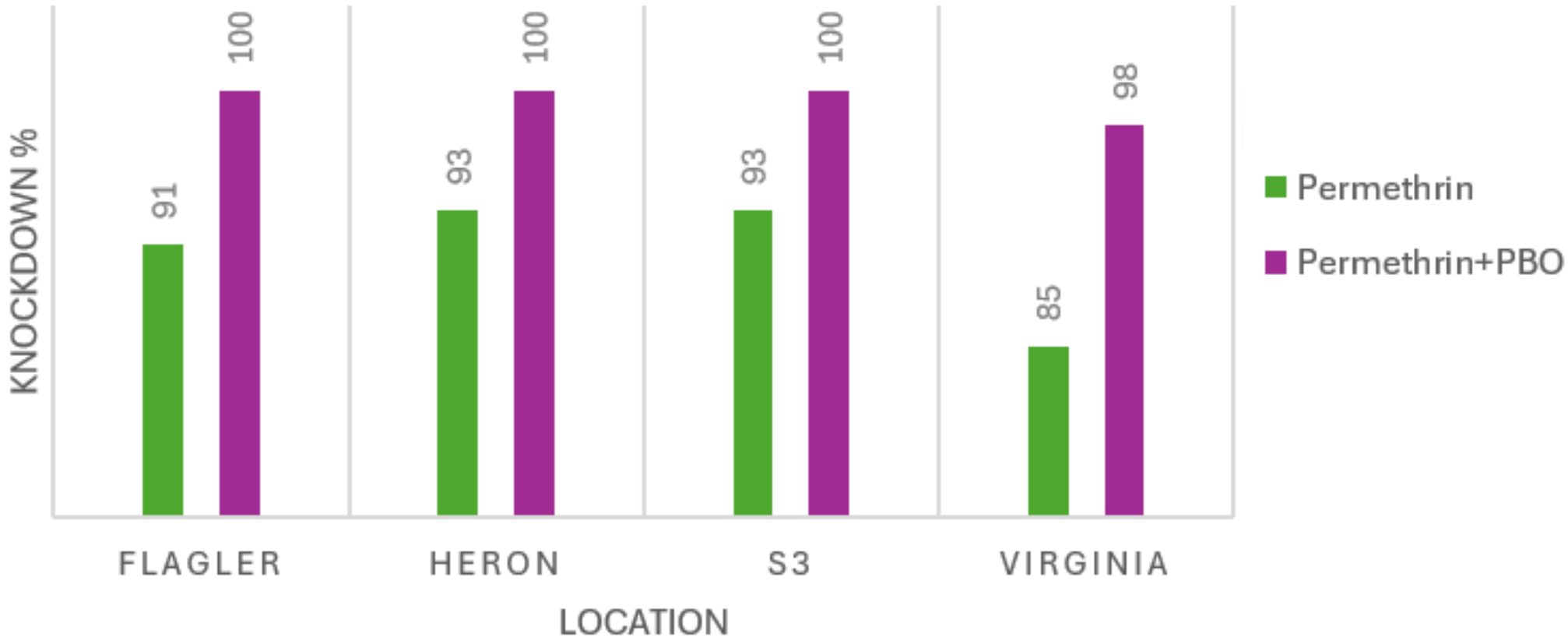




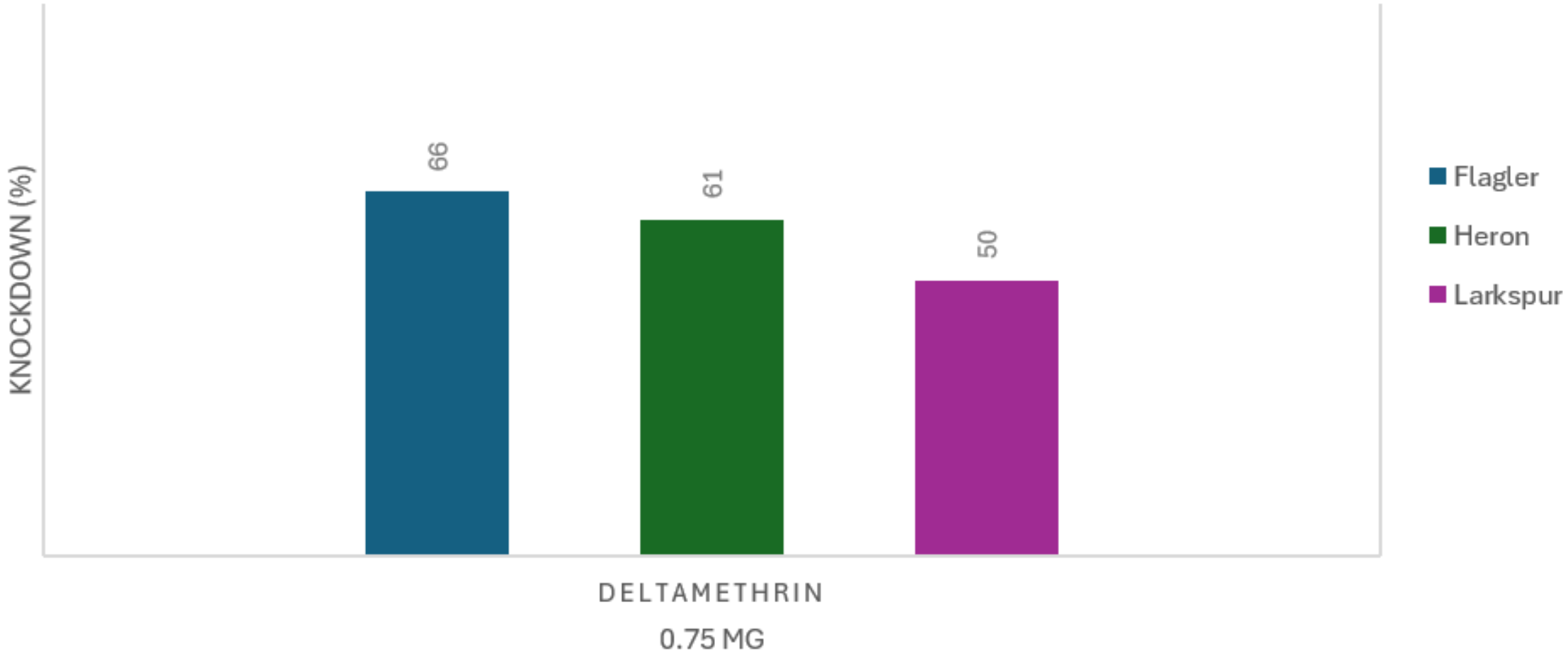



PERMETHRIN KNOCKDOWN
KNOWCKDOWN %
97
91
89
93
98
100
85
93
100
85
100
PERMETHRIN
43 MG/BOTTLE
Apple Valley
Flagler
Harold
Heron
Mabus
S1
S2
S3
Trey
Virginia
Virginia 2

PERMETHRIN AND PERMETHRIN + PBO KNOCKDOWN



DELTAMETHRIN KNOCKDOWN



Sequencing Resistance

Culex quinquefasciatus



Insecticide Exposure



24 Hour Recovery Period



DNA Extraction & PCR



Voltage Gate Sodium Channel
Amplicon Sequencing

Conclusion

- Permethrin and Deltamethrin resistance is observed in several locations.
- PBO is an affective synergist for permethrin resistant populations.

Future Directions

- Amplicon Sequencing Voltage Gate Sodium Channel of exposed survivors.
- Further investigate P450 expression in resistant *Culex* mosquitoes.

Acknowledgments

I would like to acknowledge the Insecticide Resistance Vector Control team at the Division of Parasitic Diseases and Malaria (Lucy Mackenzie Impoinvil PHD, Daniel Hartman PHD, Dieunel Derilus PHD, Lisa J Reimer PHD) at CDC.

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