PYRETHROID RESISTANCE IN *CULEX TARSALIS*

Floodwater Mosquito

January 2021

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Bonnie Ryan

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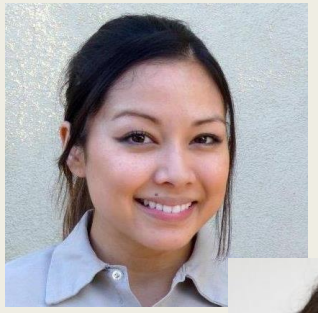
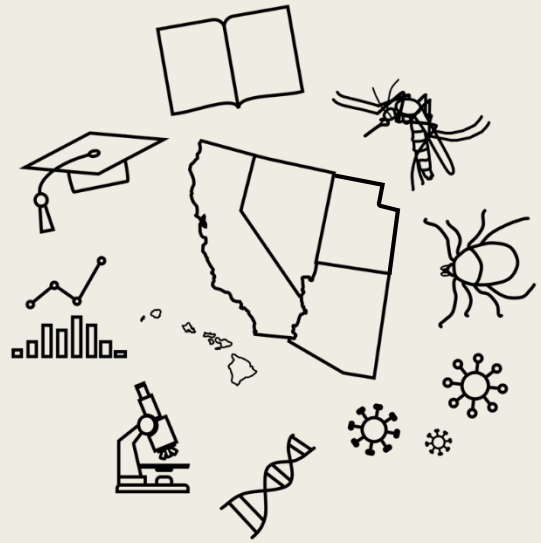


Vector Control Collaborators

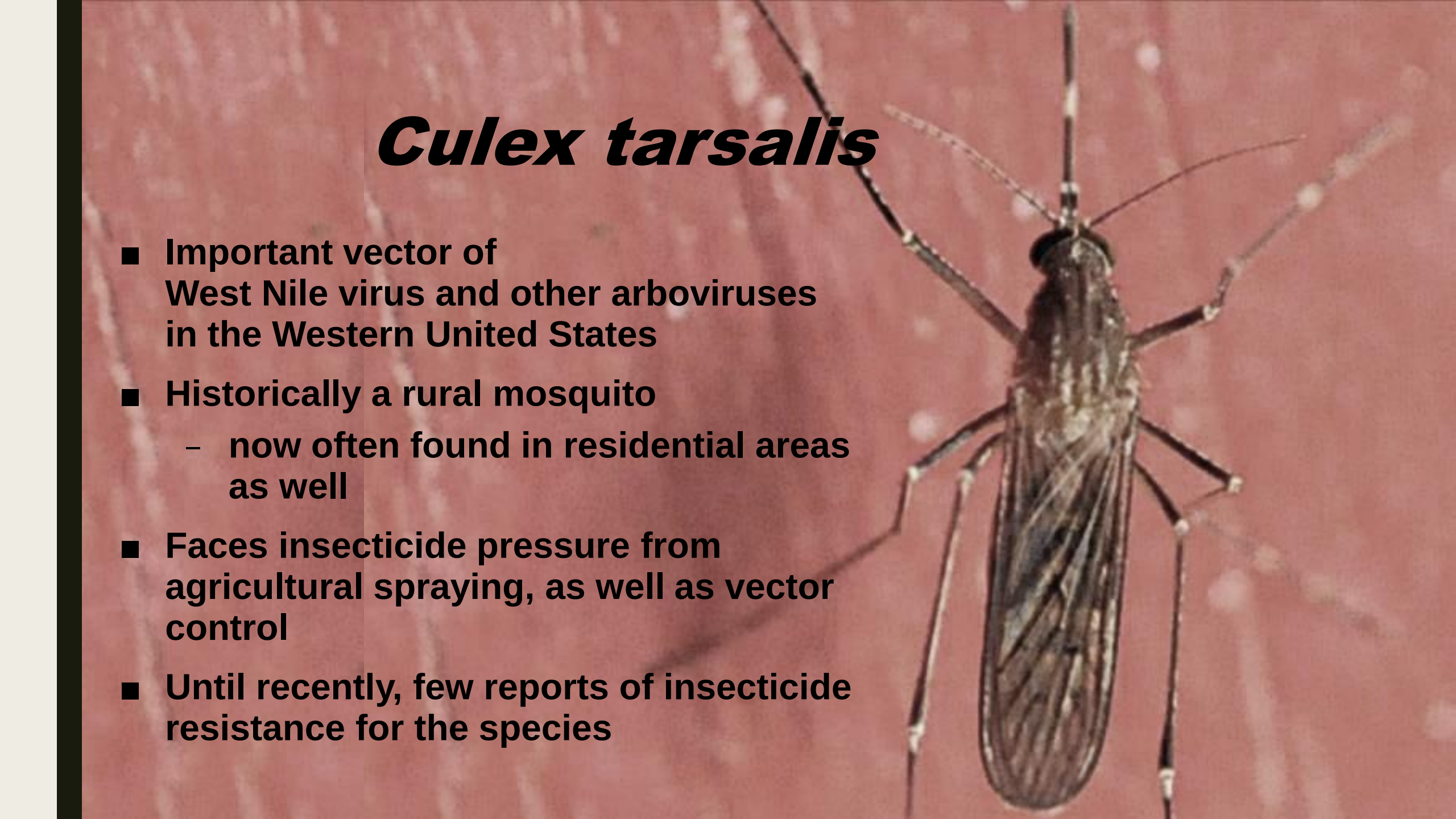
Funding

- *Pacific Southwest Regional Center of Excellence for Vector-Borne Diseases funded by the U.S. Centers for Disease Control and Prevention (Cooperative Agreement 1U01CK000516)*
- *Vector Control Collaborators*
- *University of the Pacific Graduate Program*



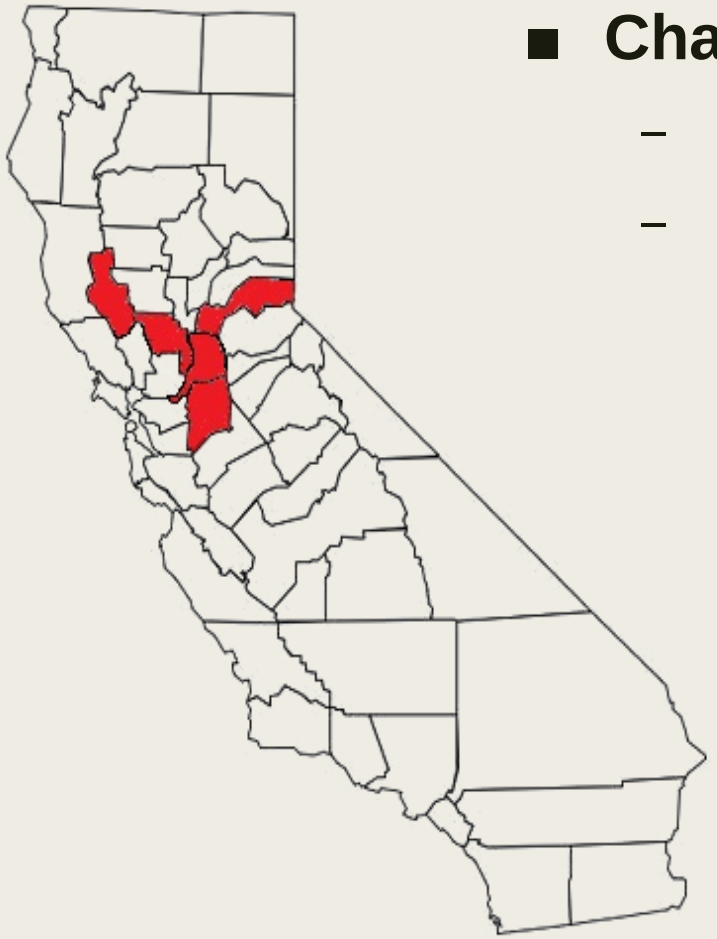
Culex tarsalis

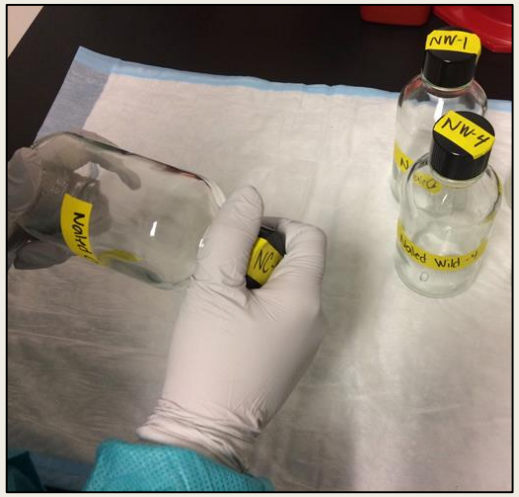
- Important vector of West Nile virus and other arboviruses in the Western United States
- Historically a rural mosquito
  - now often found in residential areas as well
- Faces insecticide pressure from agricultural spraying, as well as vector control
- Until recently, few reports of insecticide resistance for the species



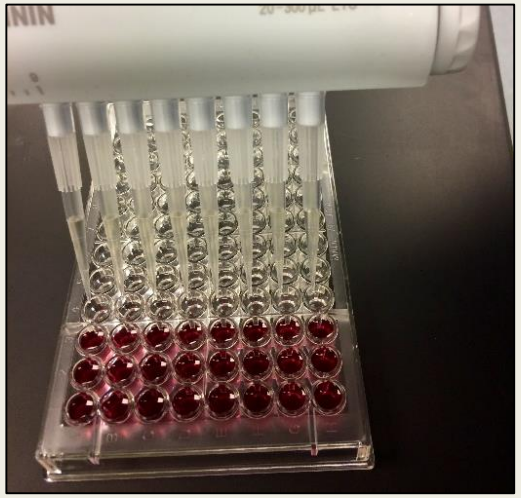
Project Goals

- Determine prevalence of pyrethroid resistance in selected study sites across 5 Northern California Counties.
- Characterize mechanisms for pyrethroid resistance
  - *enzymatic resistance*
  - *target-site mutations*

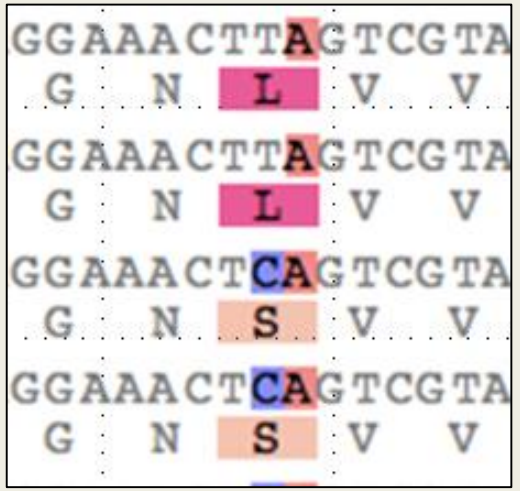




Bottle Bioassay

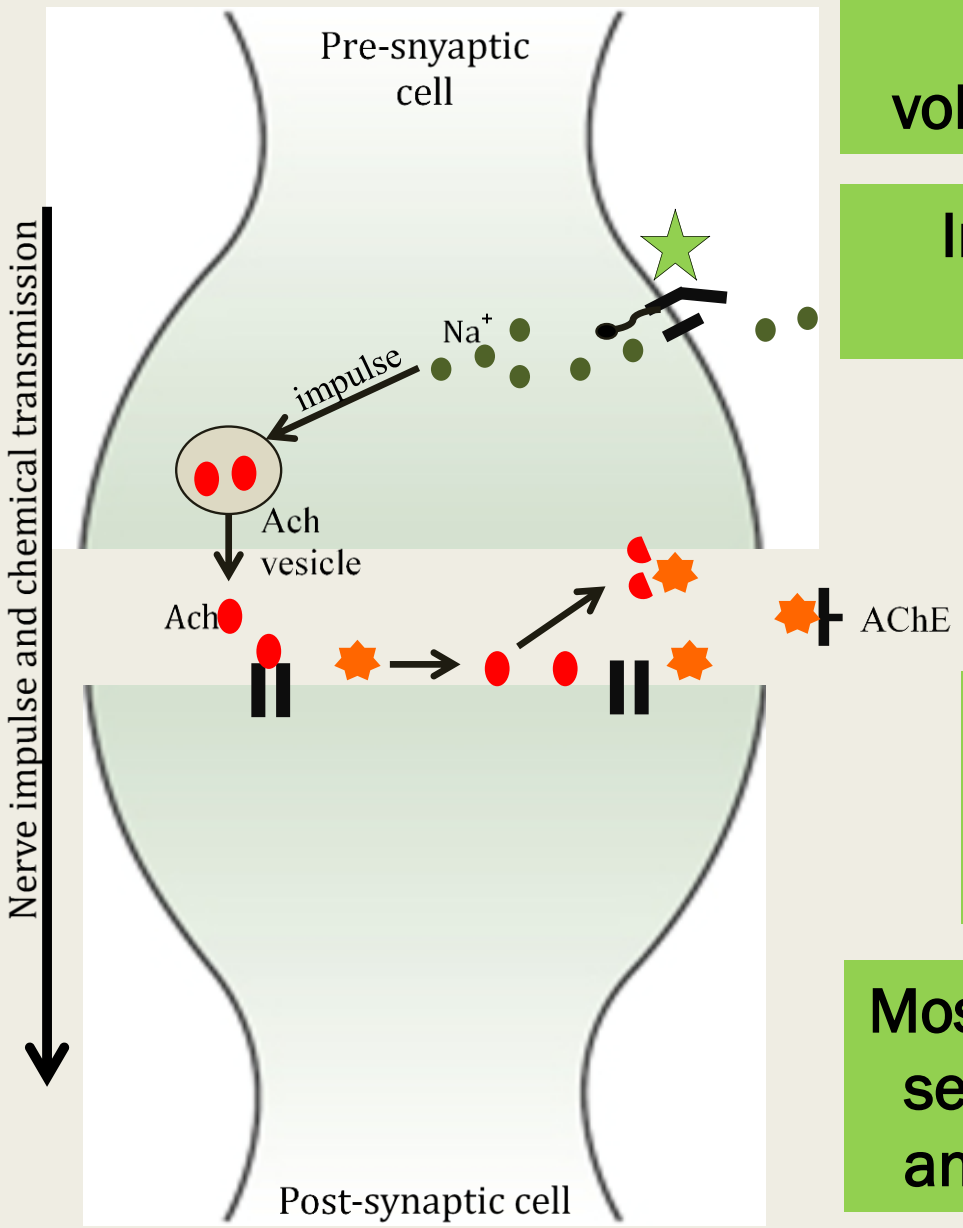


Enzyme Testing (Microplate Assay)



Target-Site Mutations (Molecular Testing)

How do pyrethroids work?



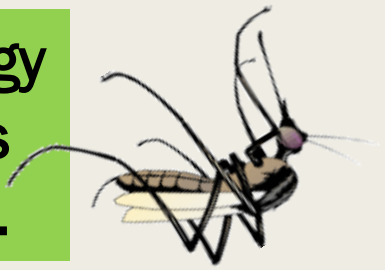
Bind to and open voltage-gated sodium channels.

Increases the influx of sodium ions (Na+).

Increases the number of action potentials in the neuron.

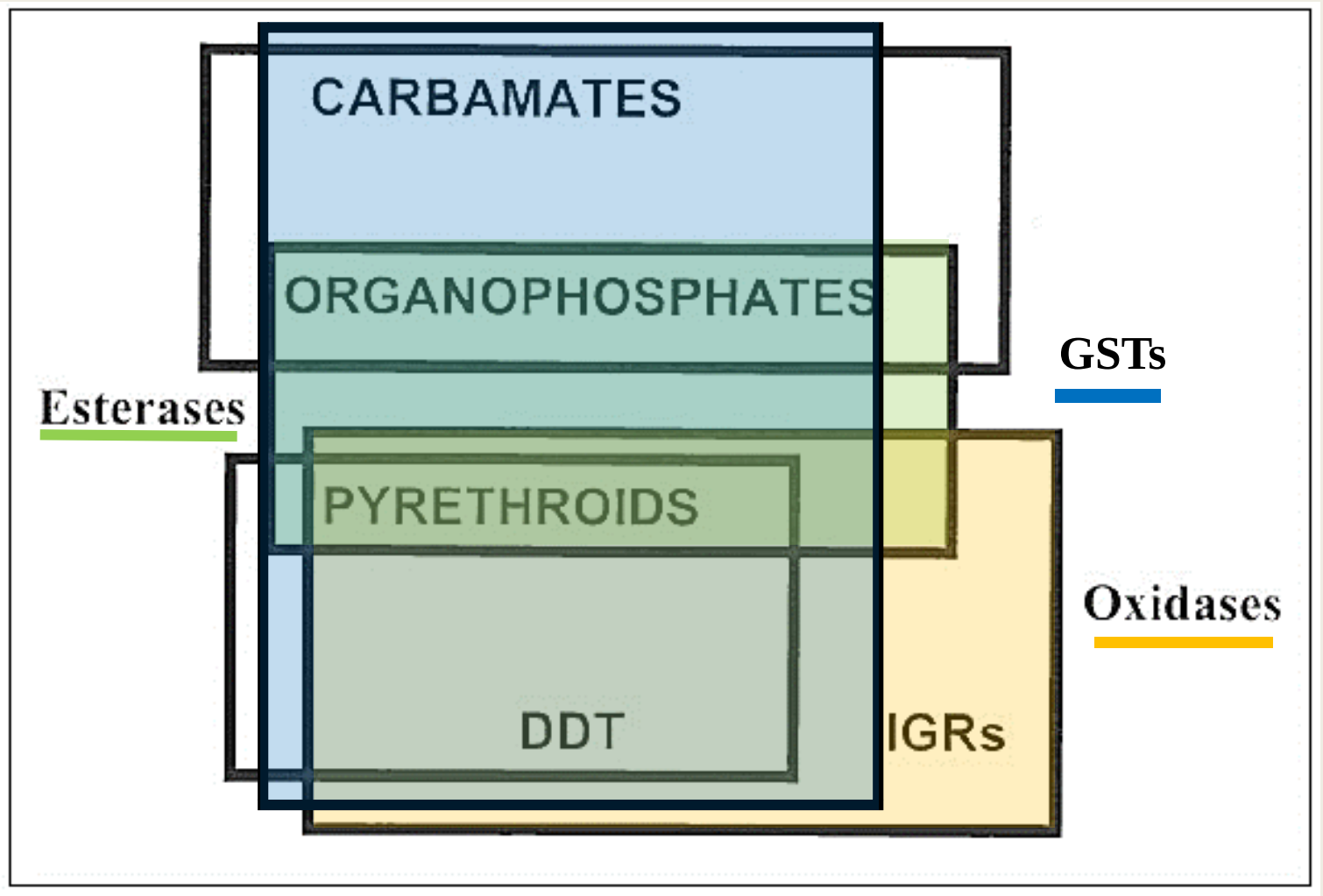
Increases the amount of acetylcholine going to other neurons and muscle cells.

Mosquito uses all its energy sending action potentials and contracting muscles.



Resistance Mechanism – Detoxifying Enzymes

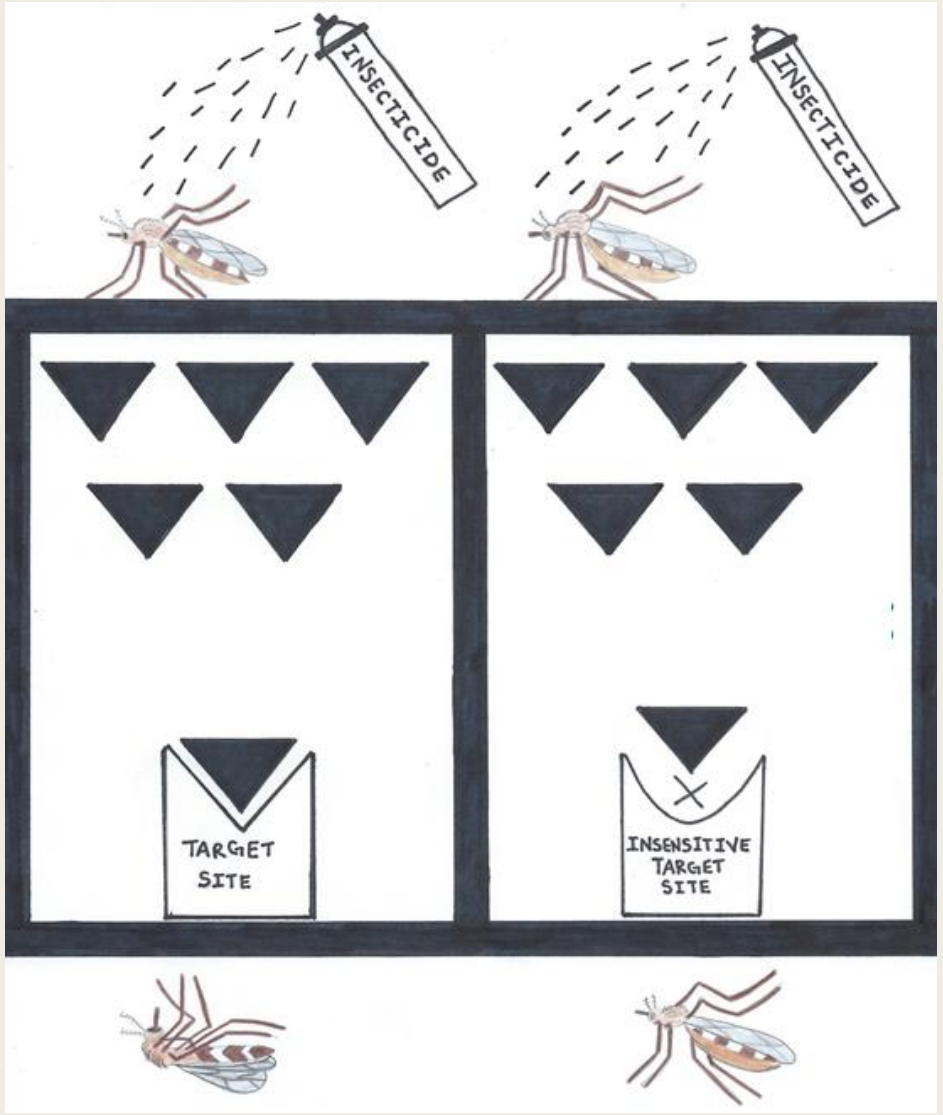
- Enzymes that breakdown/inhibit insecticides



Modified from Brogdon and McAllister 1998

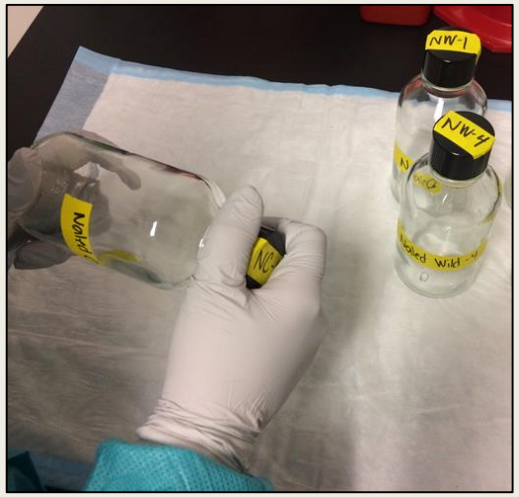
Resistance Mechanism – Target-Site Mutations

- DNA mutation
- Amino acid substitution reduces the ability of the insecticide to bind
- Mutation of Interest
  - *Knockdown resistance (kdr)*
    - Prevents pyrethroids from binding to voltage-gated sodium channels
    - Two common VGSC mutations at codon 1014 in *An. gambiae*
      - *Leucine (L) → Phenylalanine (F)*
      - *Leucine (L) → Serine (S)*



Project Goals

- **Determine prevalence of pyrethroid resistance in selected study sites across 5 Northern California Counties using Bottle Bioassays.**
- **Examine mechanisms for pyrethroid resistance**
  - ***enzymatic resistance***
  - ***target-site mutations***



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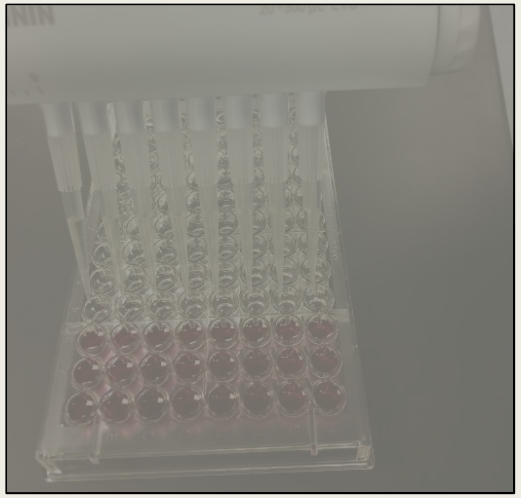

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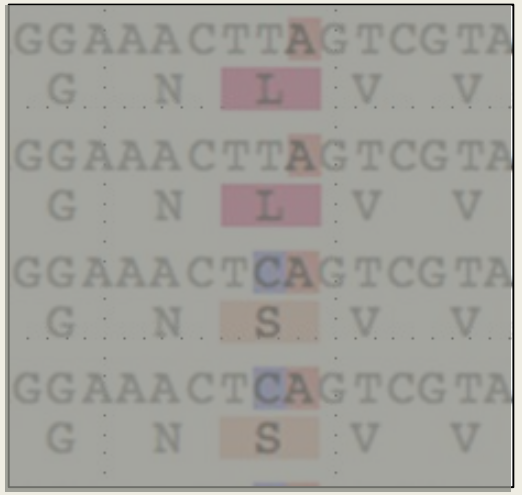
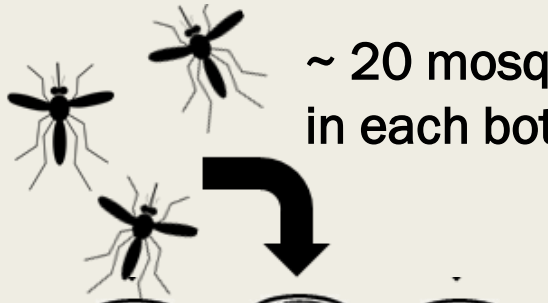


Target-Site Mutations
(Molecular Testing)

Methods – Prevalence of Resistance
Mosquito Collection – Summer 2018

- Mixed-aged female *Cx. tarsalis* were collected in CO_2-baited traps
- 3- 5 populations per district
  - San Joaquin County MVCD
  - Sac-Yolo MVCD
  - Placer County MVCD
  - Lake County VCD

Methods – Prevalence of Resistance
Bottle Bioassays



~ 20 mosq.
in each bottle

Permethrin = pyrethroid insecticide
PBO = synergist that inhibits oxidases

Control
(acetone only)

PBO Only

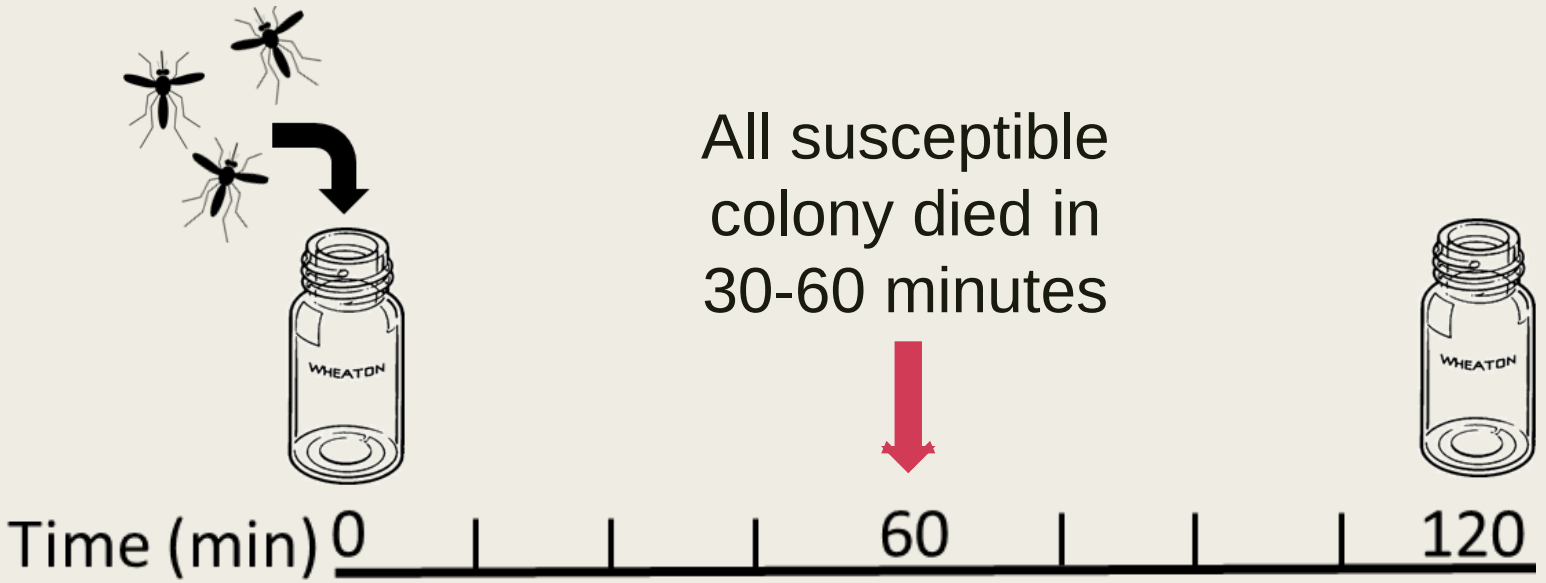
Permethrin

Permethrin
+ PBO

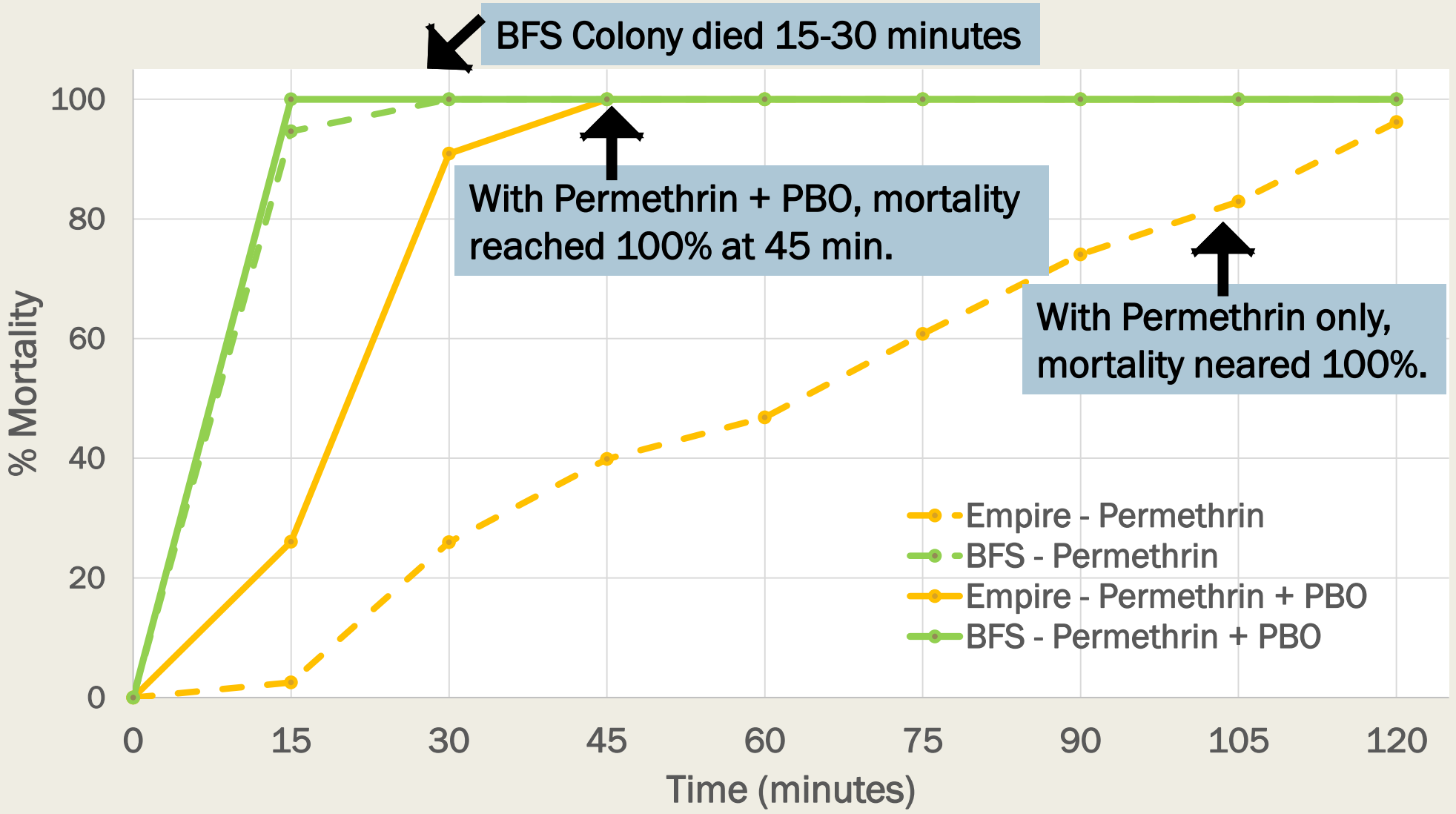
- Susceptible colony + wild-caught population
- Two biological replicates/population

Methods – Prevalence of Resistance
Bottle Bioassays

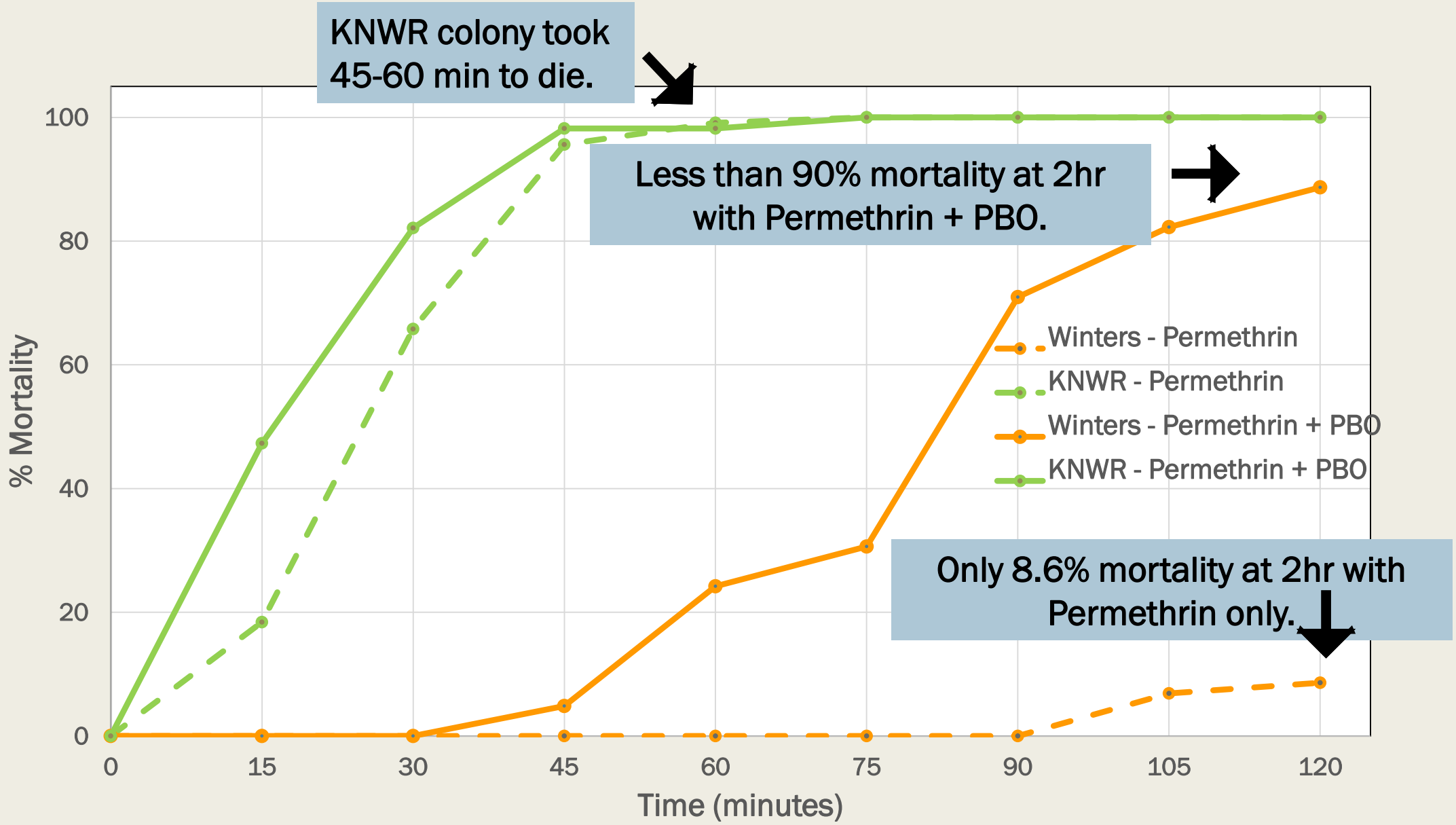
- Recorded mortality every 15 minutes



Empire in San Joaquin County



Winters in Yolo County



Summary of 17 Study Sites

Percent Mortality after 2-hr Bottle Bioassay			
		Permethrin	Permethrin + PBO
Lake	Starke Ranch	12.7	97.3
	Anderson Marsh	25.4	92.8
Placer	Phillip	22.8	100
	Locust	49.5	98.1
	Amorosa	64.2	100
San Joaquin	Vine	1.5	100
	Escalon	11.4	100
	Wright	38.7	100
	McMullin	40.4	100
	Canal Ranch	52.8	100
	Empire	96.2	100
Sacramento-Yolo	Winters	8.6	88.7
	Conaway	9.1	100
	Natomas	12.4	92.1
	District 108	15.2	94.3
	Vic Fazio	17.9	91.1
	Brannon Island	52.5	98.3

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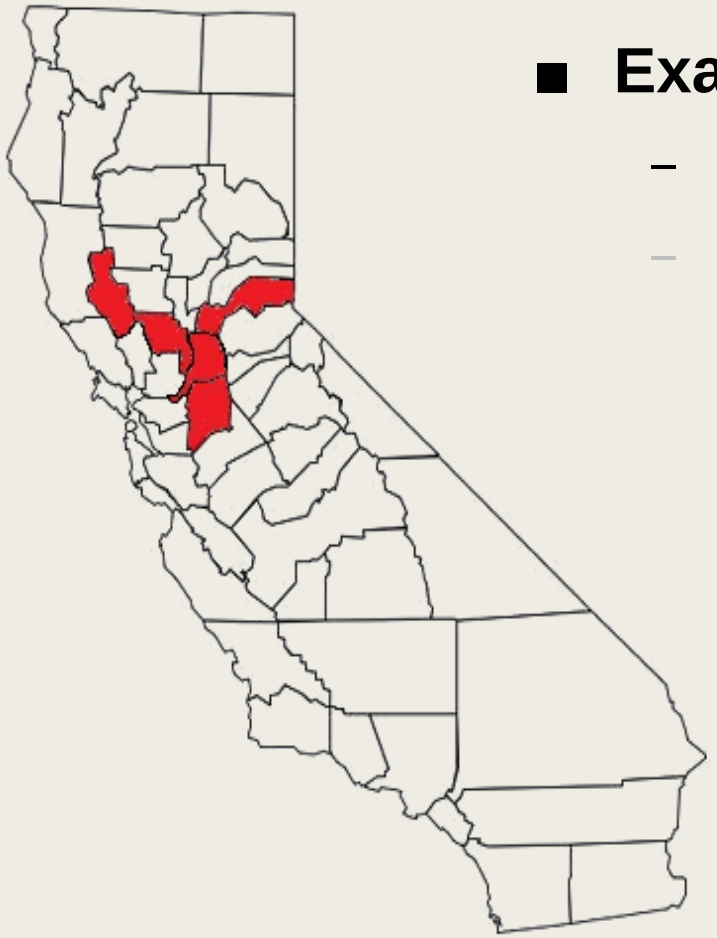
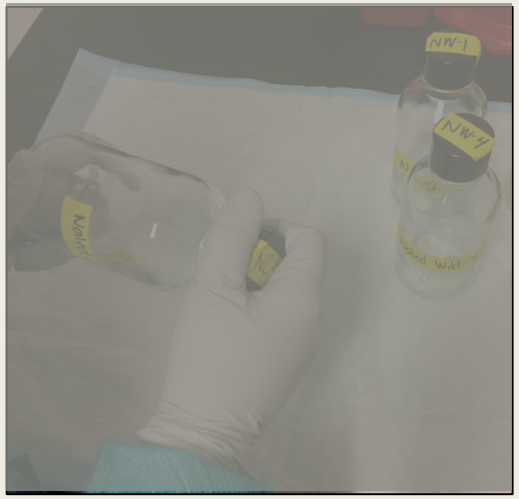
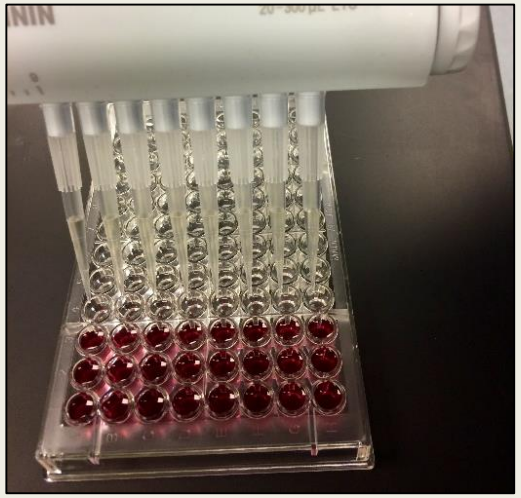


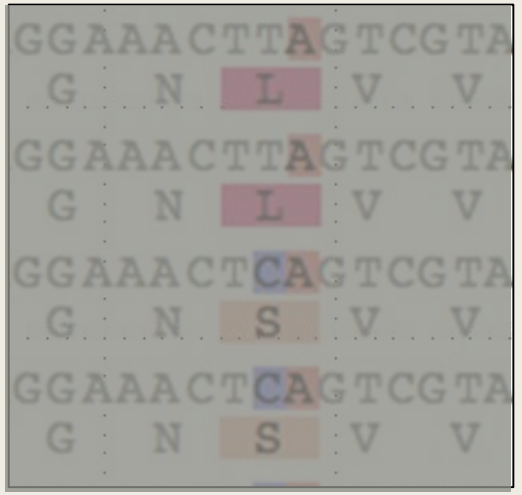
- **Examine mechanisms for pyrethroid resistance**
  - ***enzymatic resistance***
  - *target-site mutations*



Bottle Bioassay

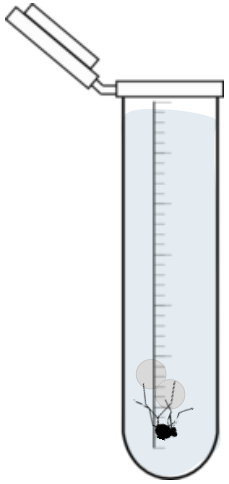


Enzyme Testing (Microplate Assay)

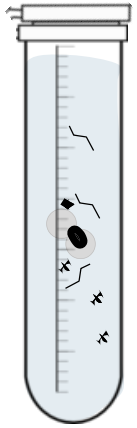


Target-Site Mutations (Molecular Testing)

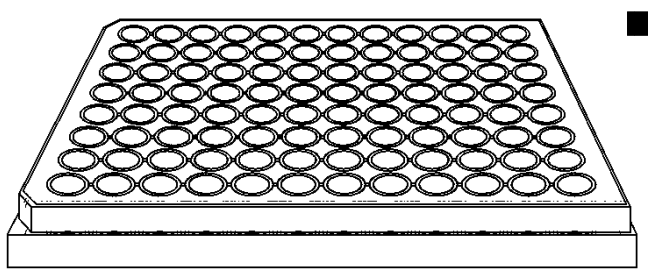
Microplate Enzyme Assay Procedure



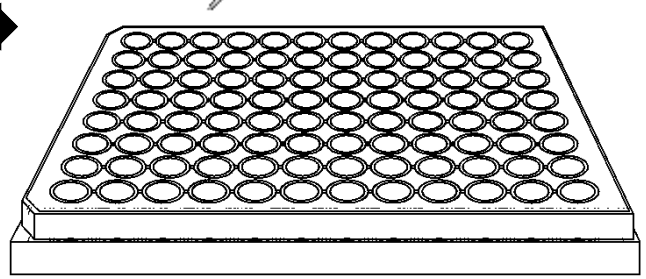
Mosquitoes ground in buffer with beads



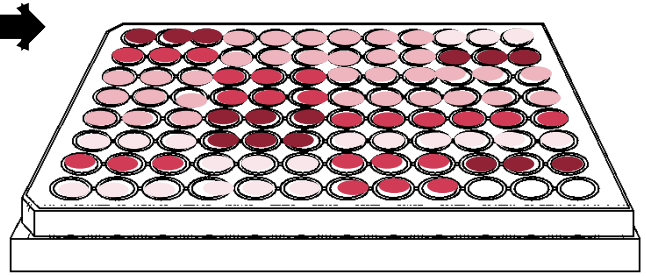
Mosquito homogenate (containing enzymes) added in triplicate



Substrate and other reactants added



Enzyme activity measured by absorbance of the product

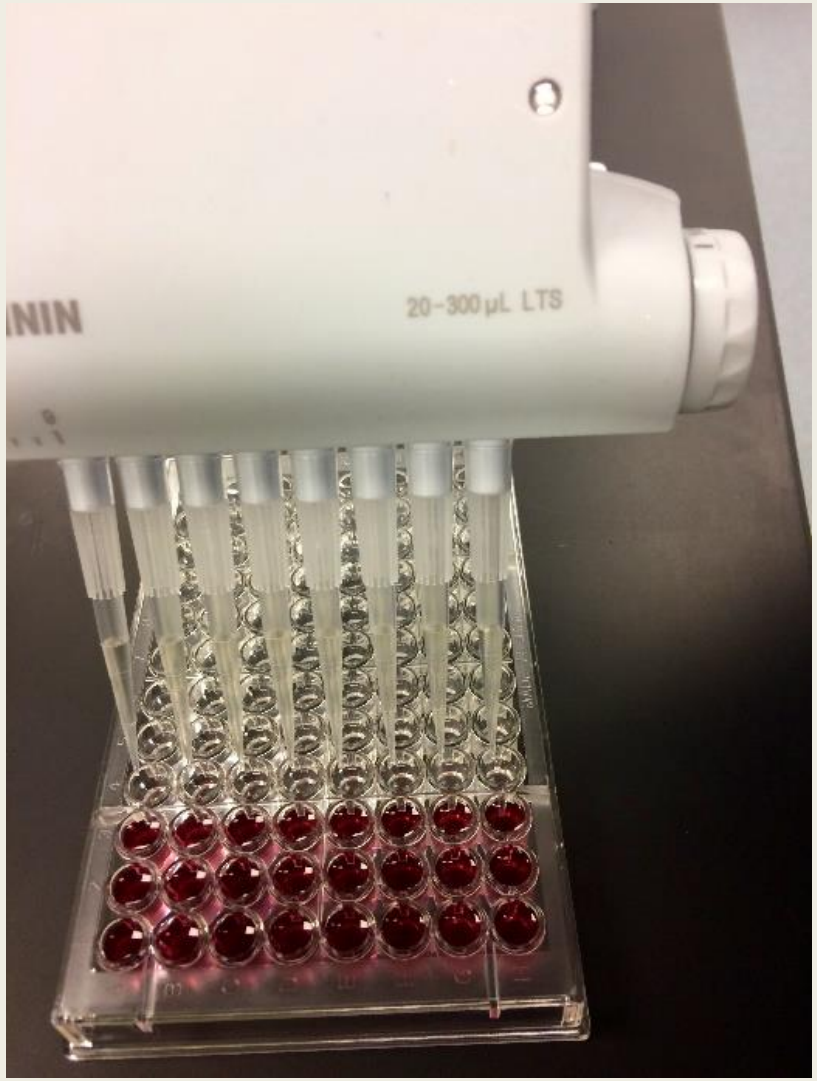


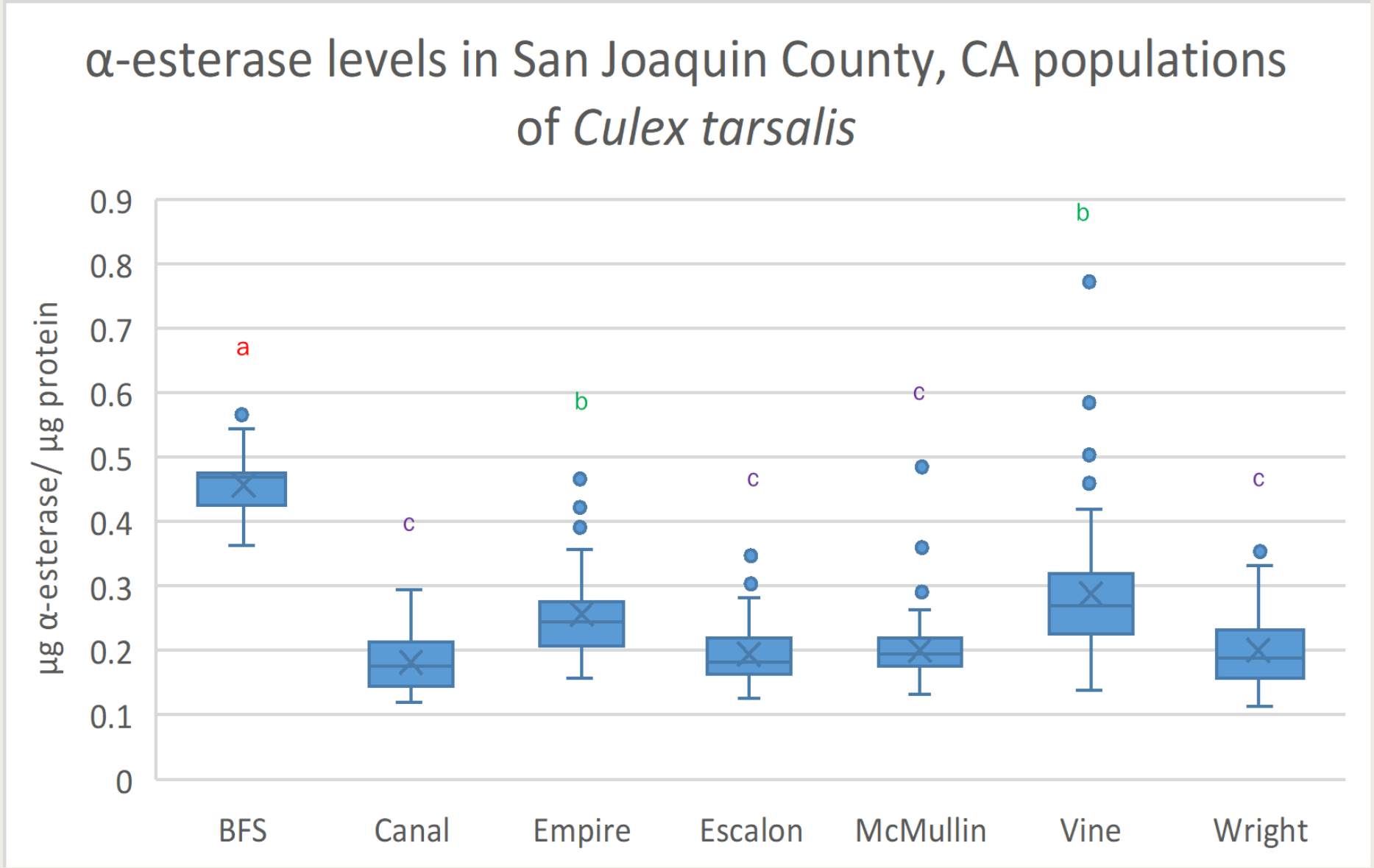
Enzyme Assays

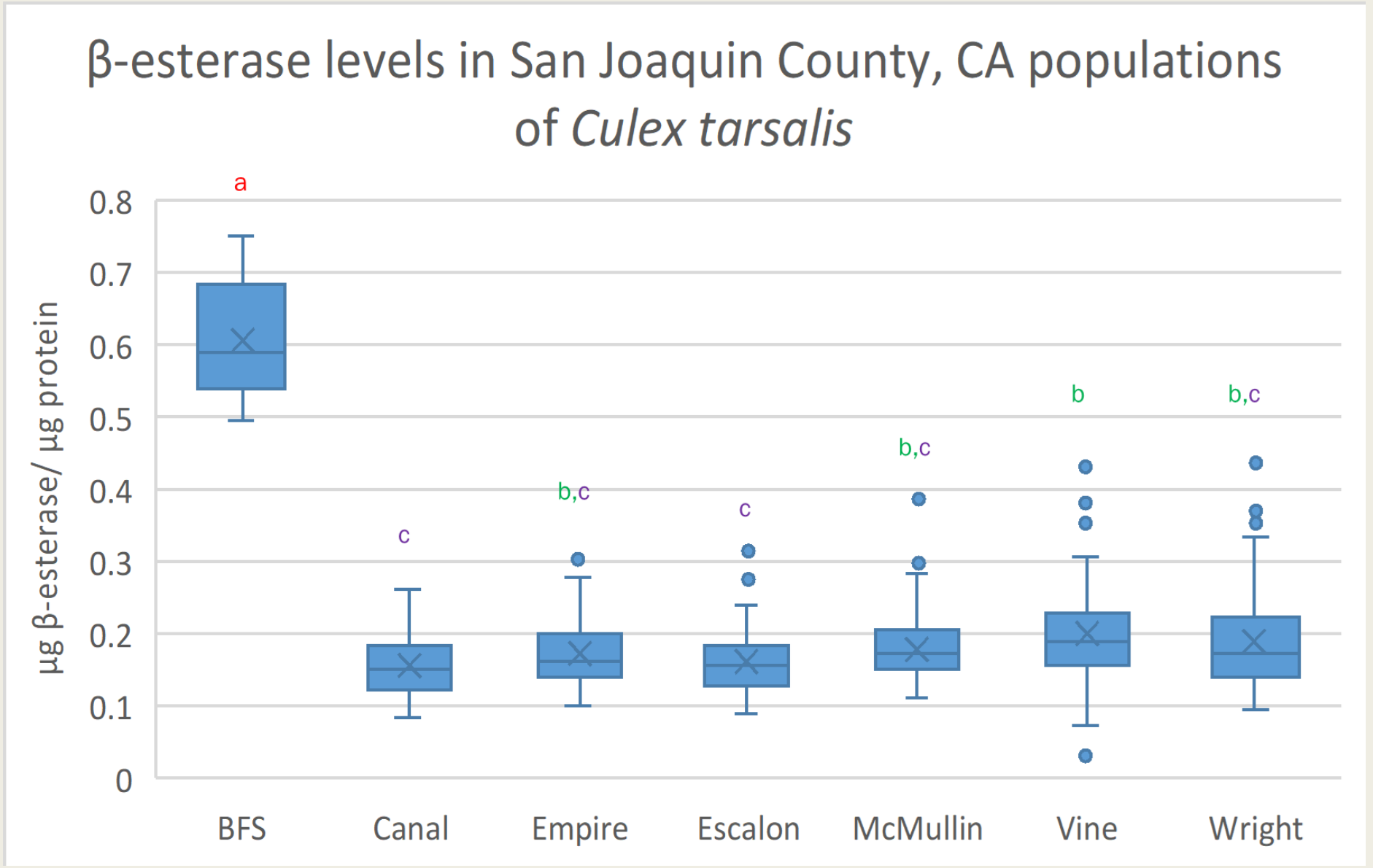
Enzyme levels tested for:

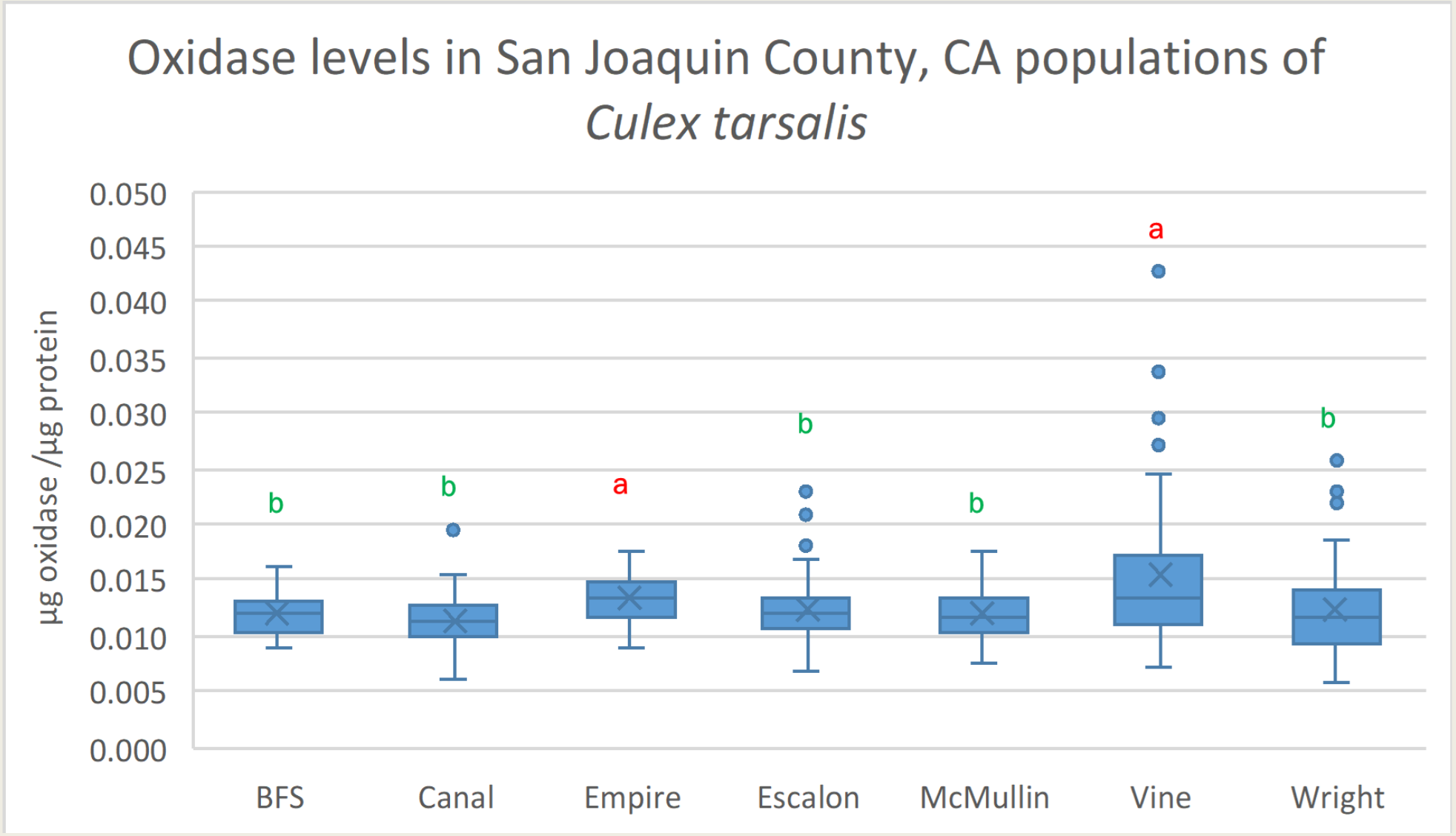
- Oxidases
- Alpha-esterases
- Beta-esterases
- Glutathione-S-transferases (GSTs)
- Acetylcholinesterase (ACE)

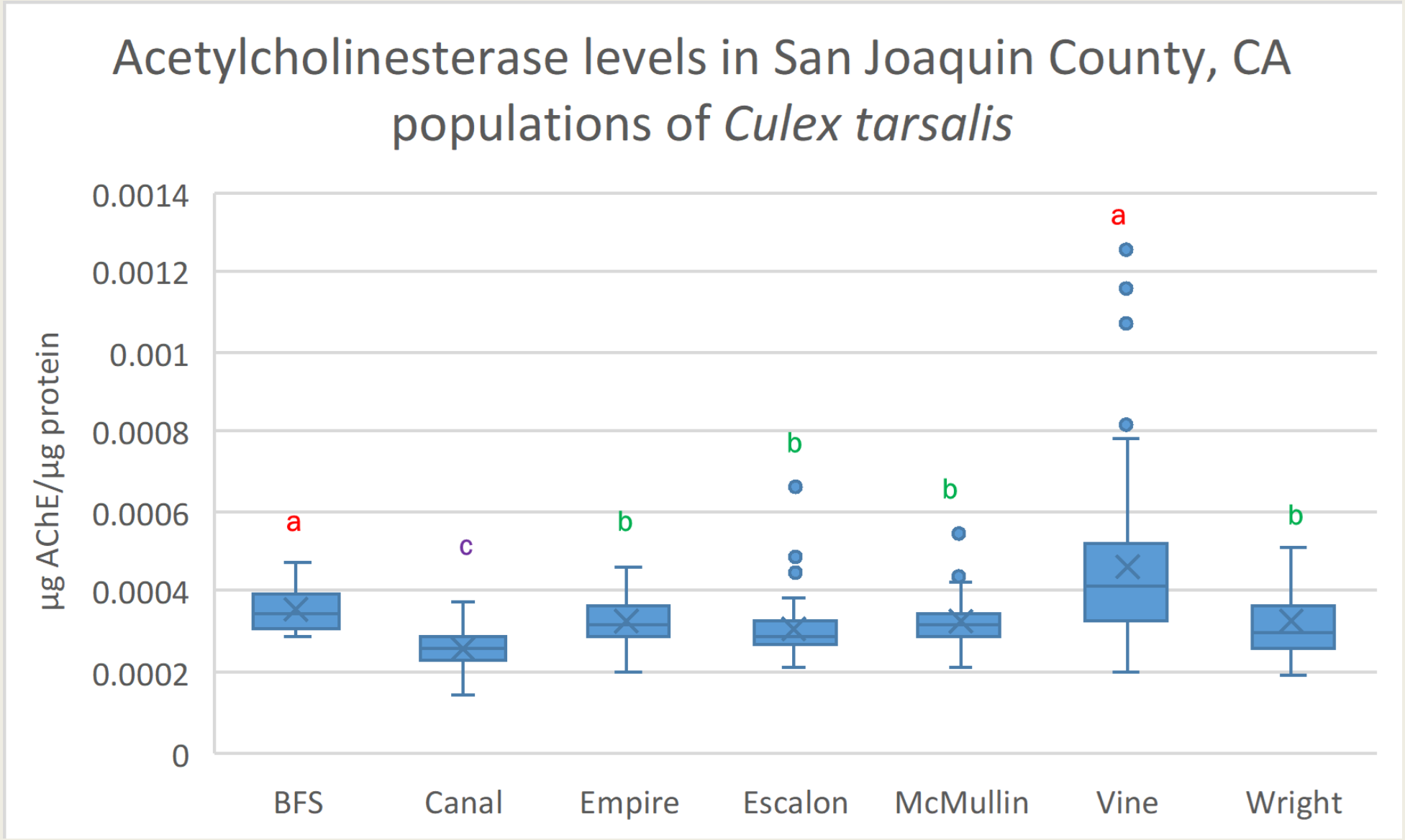
- Protein (for normalizing for mosquito size)




α-esterase levels in San Joaquin County, CA populations of Culex tarsalis
µg α-esterase/ µg protein
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
a
c
b
c
c
b
c
c
BFS
Canal
Empire
Escalon
McMullin
Vine
Wright

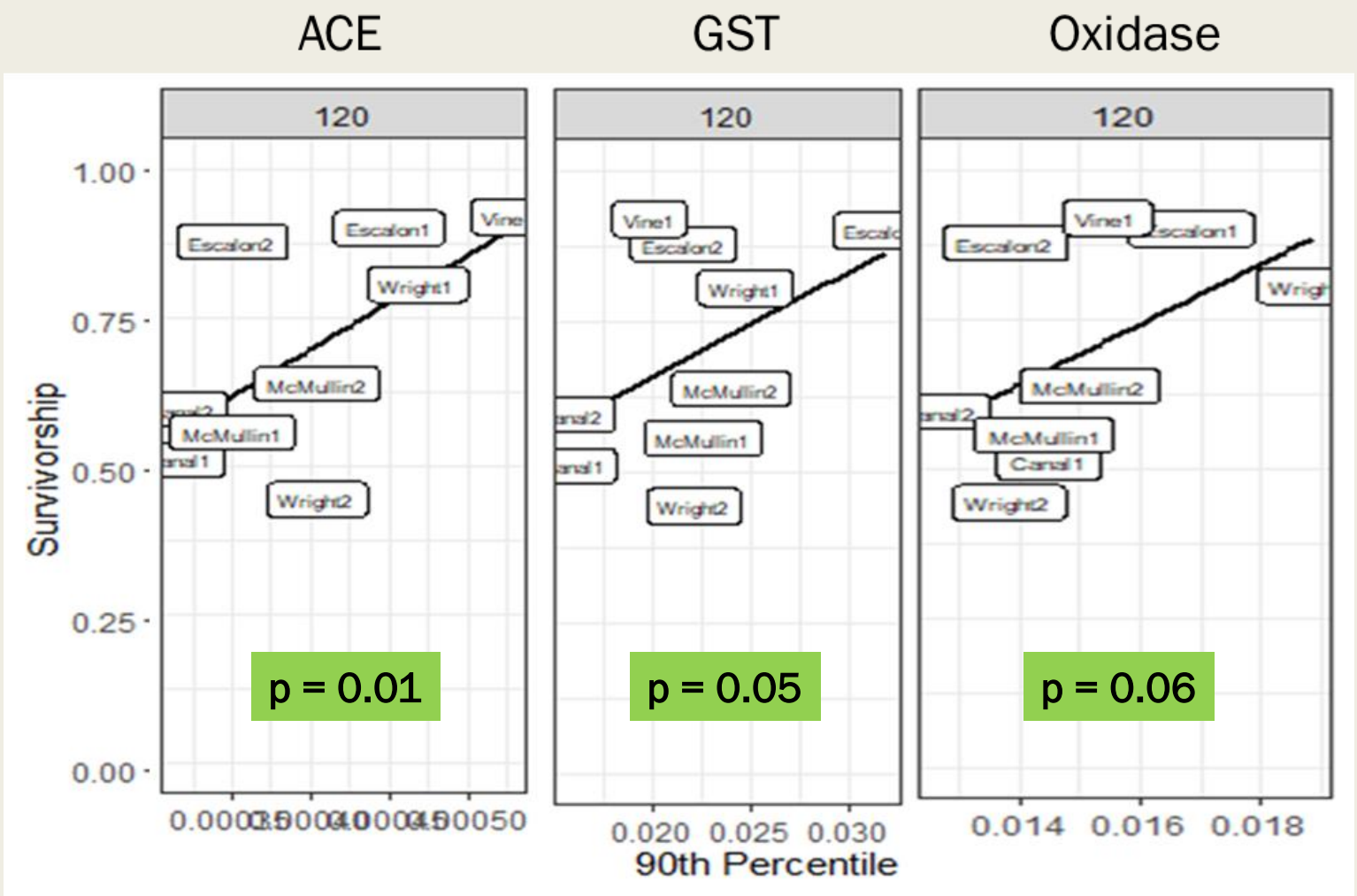

β-esterase levels in San Joaquin County, CA populations of Culex tarsalis
µg β-esterase/ µg protein
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
a
c
b,c
c
b,c
b
b,c
BFS
Canal
Empire
Escalon
McMullin
Vine
Wright


Oxidase levels in San Joaquin County, CA populations of
Culex tarsalis
µg oxidase /µg protein
0.050
0.045
0.040
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
b
b
a
b
b
a
b
BFS
Canal
Empire
Escalon
McMullin
Vine
Wright


Acetylcholinesterase levels in San Joaquin County, CA populations of *Culex tarsalis*
µg AChE/µg protein
0.0014
0.0012
0.001
0.0008
0.0006
0.0004
0.0002
0
a
c
b
b
b
a
b
BFS
Canal
Empire
Escalon
McMullin
Vine
Wright

Preliminary Regressions

Survivorship vs. 90th Percentile Enzyme Levels



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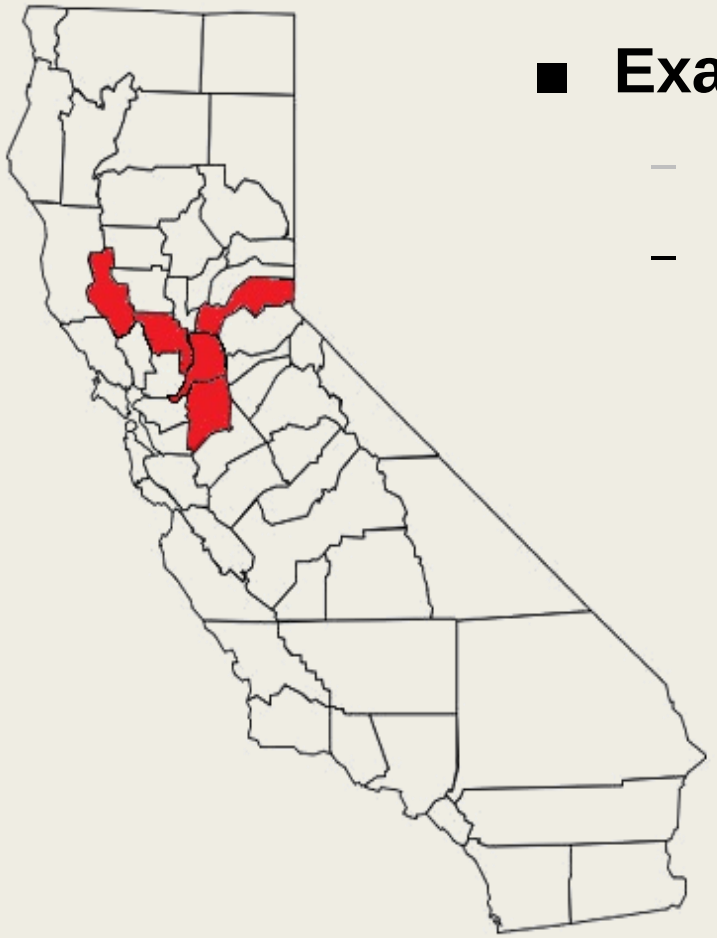

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  - *enzymatic resistance*
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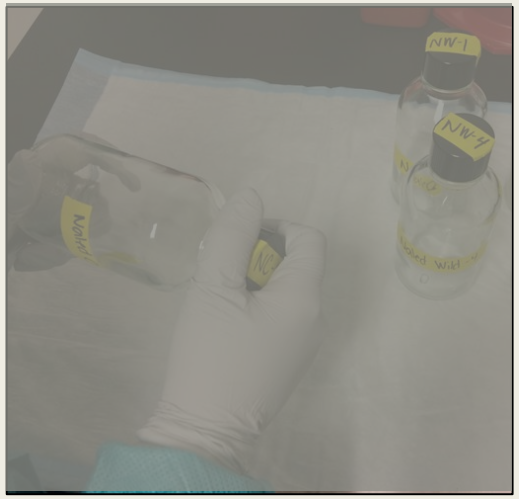

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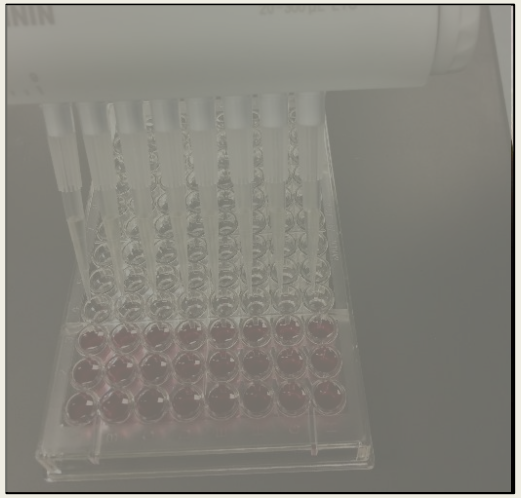

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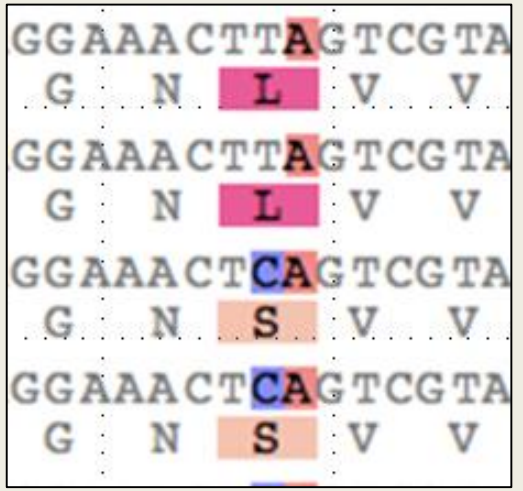


Target-Site Mutations (Molecular Testing)

Detection of *kdr* mutation

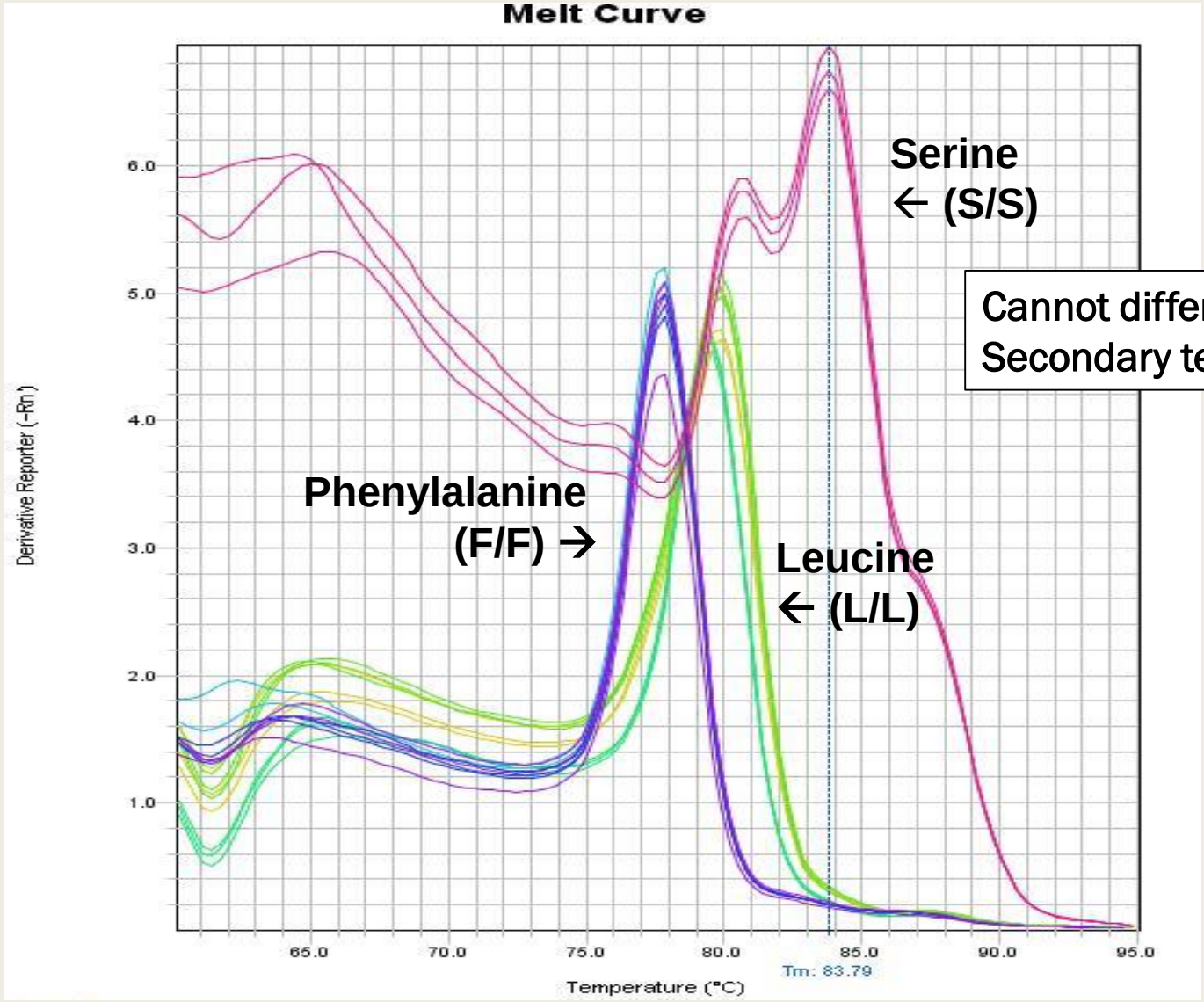
- Methods:
  - *SYBR green qPCR assay using primers with allele-specific GC rich tails*

GGGGCGGGGCCAC.....TTA — Leucine (L) (Melt Temp ~ 80°)

CCAC.....TTT — Phenylalanine (F) (Melt Temp ~77°)

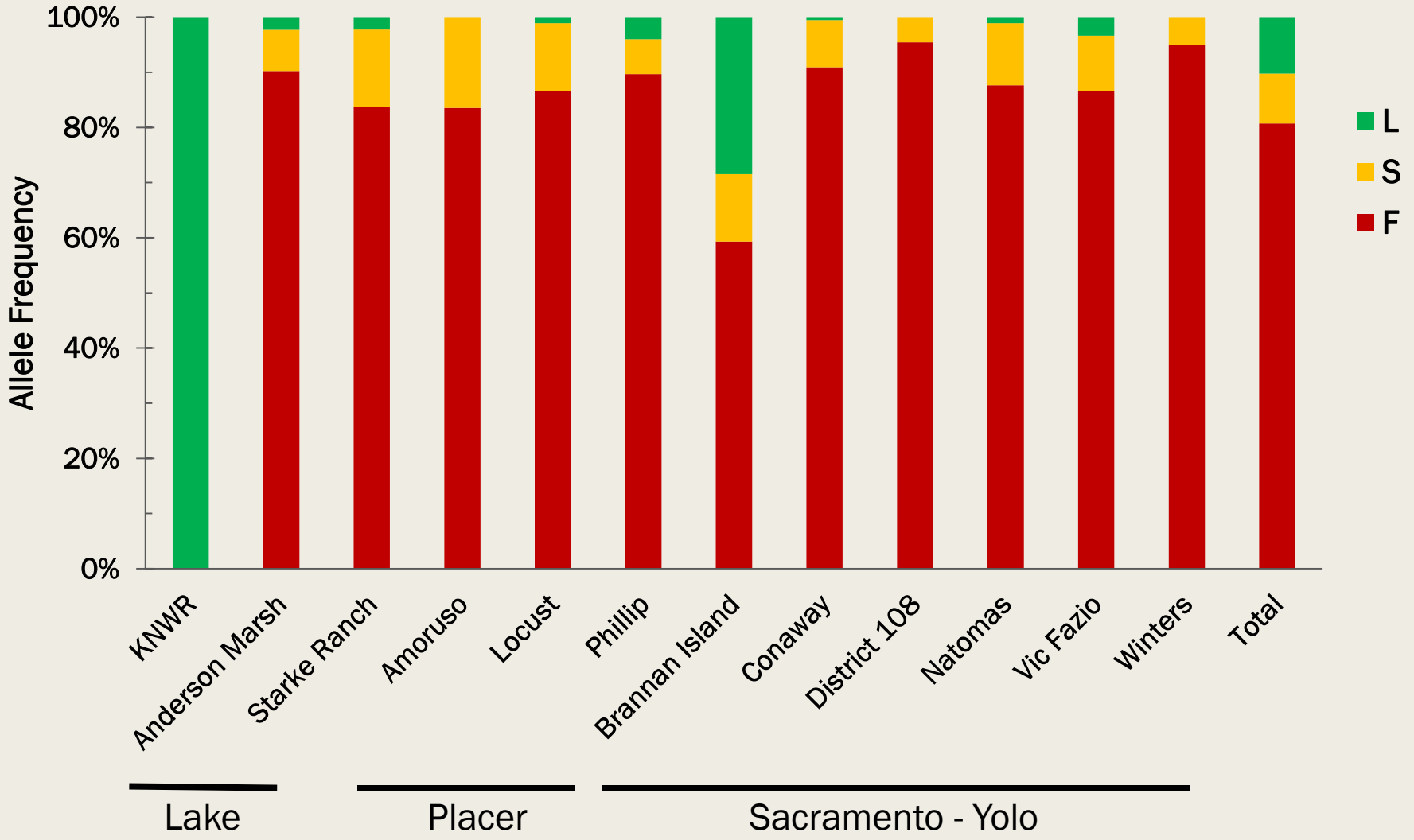
GGGGCGGGCAGGGGGGCGCGGGCGAGGCCAC.....TC — Serine (S) (Melt Temp ~ 83°)

Detection of *kdr* mutation

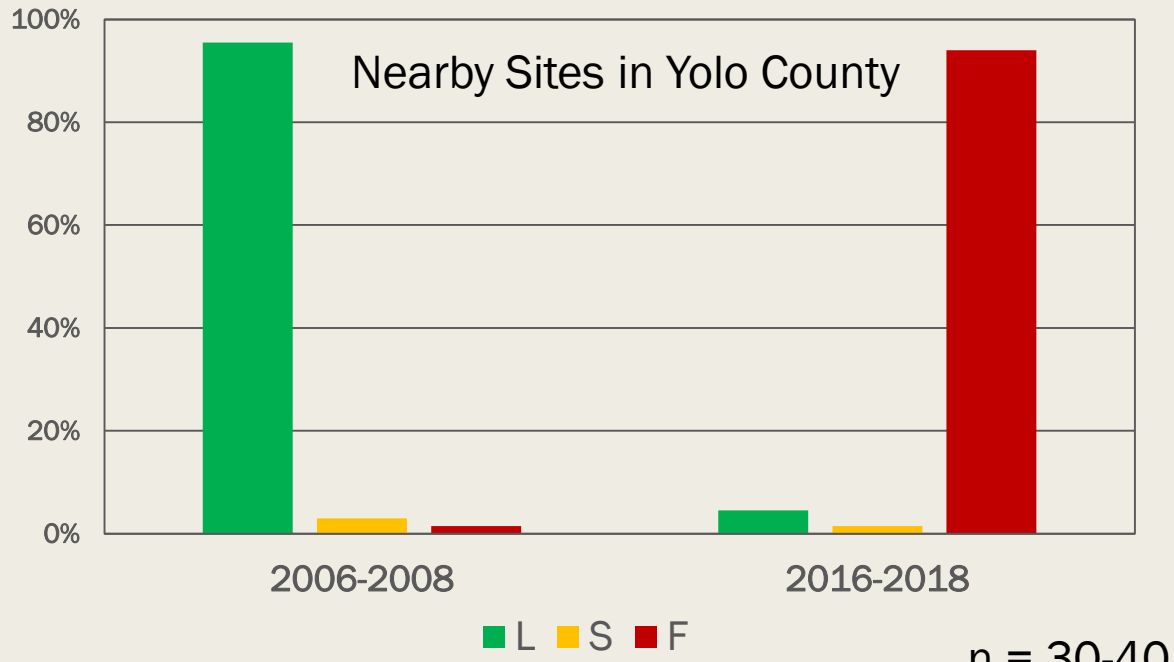


Cannot differentiate L/S from S/S. Secondary test required.

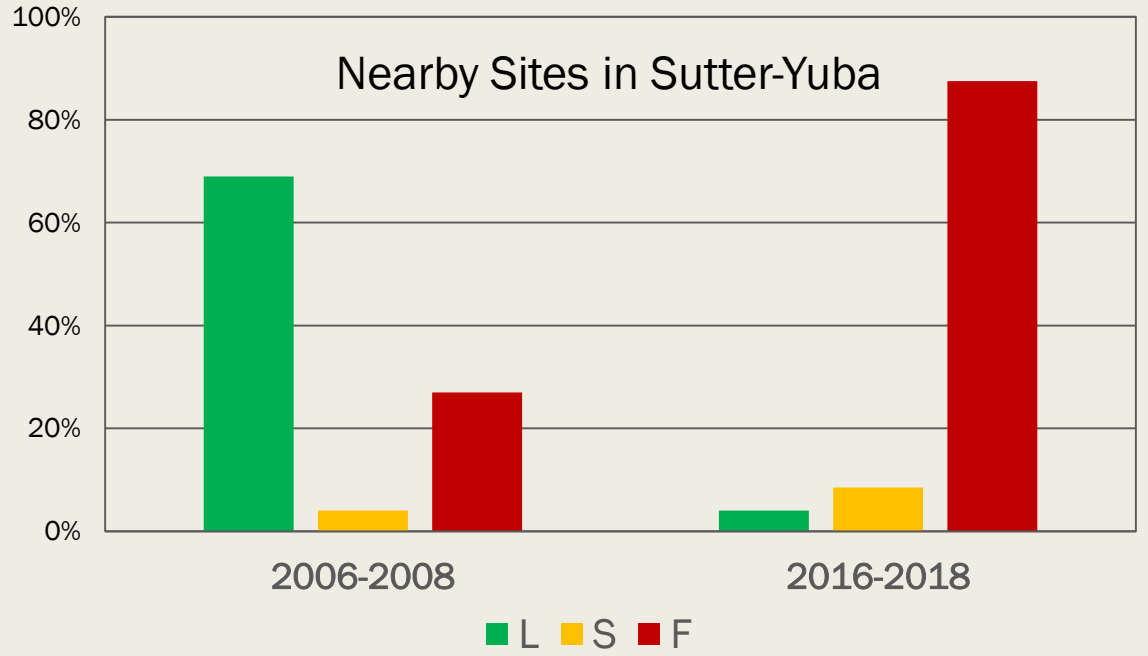
kdr Allele Prevalence – Lake, Placer, and Sacramento-Yolo



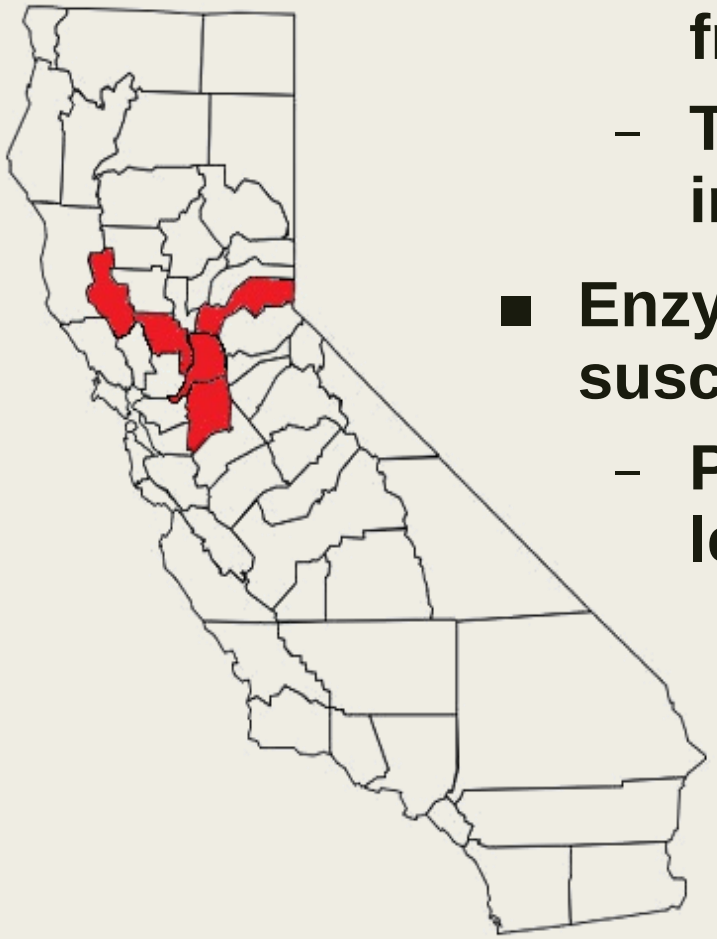
kdr Allele Prevalence Change Over Time



n = 30-40



Summary



- Substantial resistant to pyrethroids in *Cx. tarsalis* in our 5 study counties.
  - Mortality in bottle bioassays ranged from 1.5% to 96.2% at 120 minutes.
  - The addition of PBO brought mortality above 90% in most areas.
- Enzyme data is a little cloudy since the current susceptible colonies are not good references.
  - Potential of increased oxidases, GSTs, and ACE leading to resistance.
    - High prevalence of L → F kdr mutation in many study sites. (Some L → S mutations as well.)
      - Prevalence has increased greatly in the last 10 years.


Thank you!
tthiemann@pacific.edu