

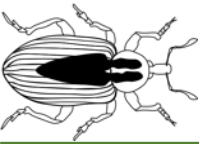
# Alfalfa Weevils Across the Western United States are Resistant to Multiple Type II Pyrethroid Insecticides



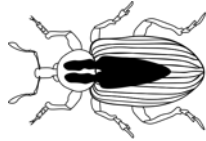
**Erika A. Rodbell, PhD**

Western Alfalfa & Forage  
Symposium

December 13, 2023



# Alfalfa weevil, an economic pest



## Larvae cause economic injury

- Feeding reduces leaf to stem ratio (skeletonization)
- Yield loss of 10-15% annually
- Drought conditions yield loss can approach 100%
- Reduces overall forage protein content

## Adults cause injury by chewing stems

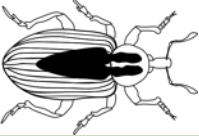
Does not cause economic injury

NDSU 2013

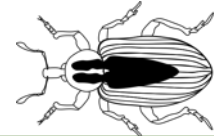
Pellissier et al. 2017

University of Illinois 2023

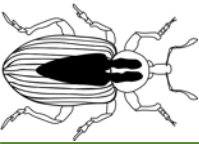




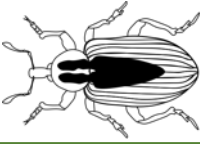
# Insecticides for alfalfa weevil control



Active Ingredient	Mode of Action
Carbaryl	1A
Methomyl	1A
Dimethoate	1B
Malathion	1B
Phosmet	1B
Permethrin	3A, Type I
Alpha-cypermethrin	3A, Type II
Beta-cyfluthrin	3A, Type II
Cyfluthrin	3A, Type II
Gamma-cyhalothrin	3A, Type II
Lambda-cyhalothrin	3A, Type II
Zeta-cypermethrin	3A, Type II
Spinosad	5
Indoxacarb	22A

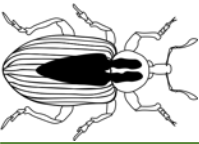


# Objectives

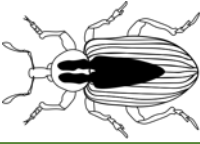


- 1. Evaluate the geographic extent and intensity of lambda-cyhalothrin resistance in the Western US**
- 2. Evaluating resistance to multiple pyrethroid active ingredients in three production zones in the Western US**



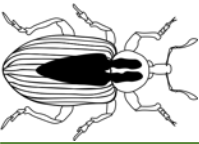


# Objective 1

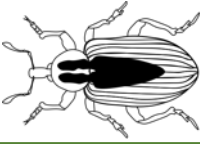


**Evaluate the geographic extent and intensity of lambda-cyhalothrin resistance in the Western US**





# Methods: Weevil collection



## 1. Field collection

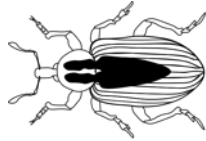
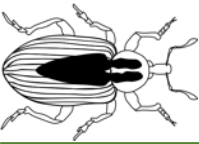


## 2. Contact Bioassays



## 3. Mortality evaluation





## Probit analysis Polo PC

LC<sub>50</sub>- lethal concentration

Results

Padlock.bt

3 Lambda6/...

	Parameter	Standard Error	T Ratio
Intercept	-0.896932	0.108790	-8.244604
Slope	0.398628	0.097775	4.076987

Chi square goodness of fit test

X	N	Respond	Expected	Residual	Probability	Standard ...
0.003300	51	1	1.511602	-0.511602	0.029639	-0.422422
0.010000	51	3	2.300853	0.699147	0.045115	0.471681
0.033000	48	1	3.285225	-2.285225	0.068442	-1.306296
0.100000	50	12	4.878176	7.121824	0.097564	3.394331
0.330000	52	2	7.181537	-5.181537	0.138106	-2.082683

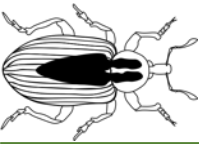
Variance covariance matrix

	Intercept	Slope	Natural
Intercept	0.011835	0.005855	
Slope	0.005855	0.009560	

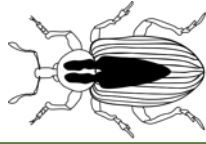
Lethal dose matrix

LD	Lethal dose	Limit	90%	95%	99%
50	177.85	lower	4.850000	2.941000	1.078000
		upper	1638035226...	0.000000	0.001000

Chi-square 19.6551    Degrees of freedom 5    Heterogeneity 3.931



# Objective 1: Statistical analysis

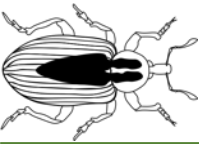


## 1: LC<sub>50</sub>

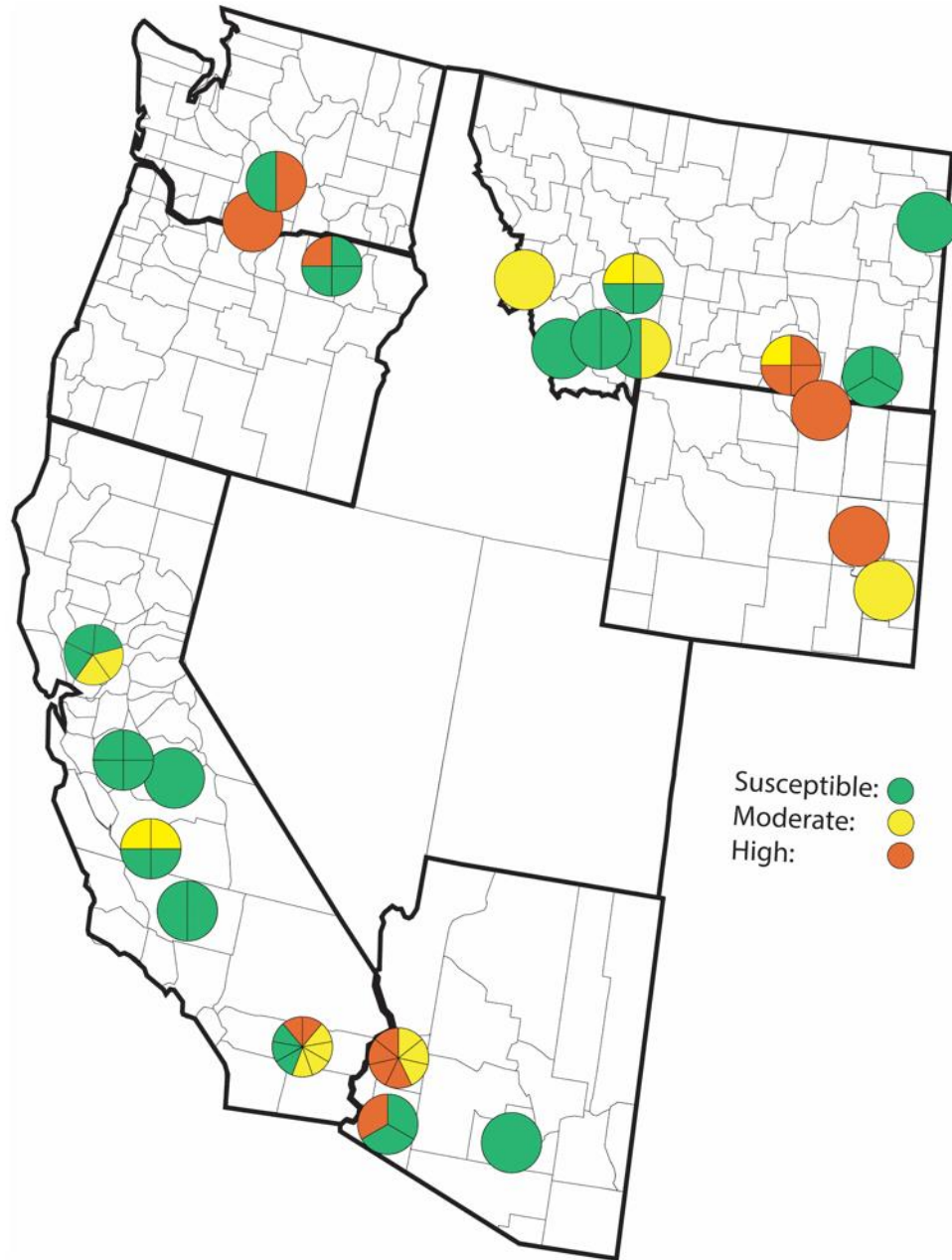
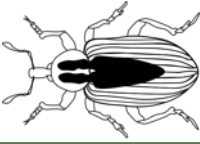
	$\lambda$ -cyhalothrin
<b>AZ-1</b>	$>10\mu\text{g}/\text{cm}^2$
<b>MT-1</b>	$4.50\mu\text{g}/\text{cm}^2$
<b>WA-1</b>	$1.32\mu\text{g}/\text{cm}^2$
<b>AZ-2</b>	$0.93\mu\text{g}/\text{cm}^2$
<b>MT-2</b>	$0.26\mu\text{g}/\text{cm}^2$

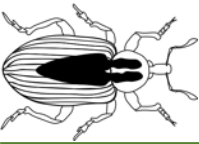
## 2: Categories

Resistance Category	LC <sub>50</sub> ( $\mu\text{g}/\text{cm}^2$ )	Resistance Ratio (LC <sub>50</sub> /0.013)	Times (X) Higher Label Rate of 0.34 $\mu\text{g}/\text{cm}^2$
<b>Susceptible</b>	$<0.30$	$<23.08$	$< 0.9\text{X}$
<b>Moderate</b>	$0.30 - 1.0$	$23.08 - 76.9$	$0.9\text{X} - 2.9\text{X}$
<b>High</b>	$> 1.0$	$>76.9$	$> 2.9\text{X}$

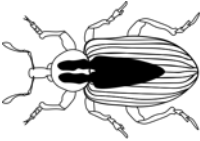


# Objective 1: Results-lambda-cyhalothrin



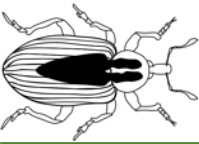


# Objective 1: Conclusions

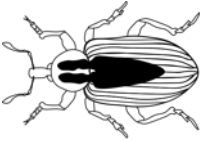


- **Highly resistant field sites in each state tested**
  - Characterized by  $LC_{50}$  values
- **Many susceptible locations remain, IRM needed**
- **In some cases, susceptible locations occurred within the same county as highly resistant locations**
- **Others, entire valleys contained high degrees of resistance**



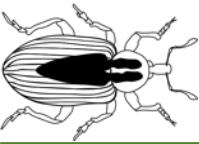


## Objective 2

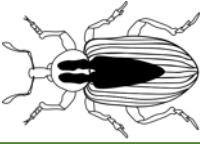


**Evaluating resistance to multiple pyrethroid active ingredients in three production zones in the Western US**

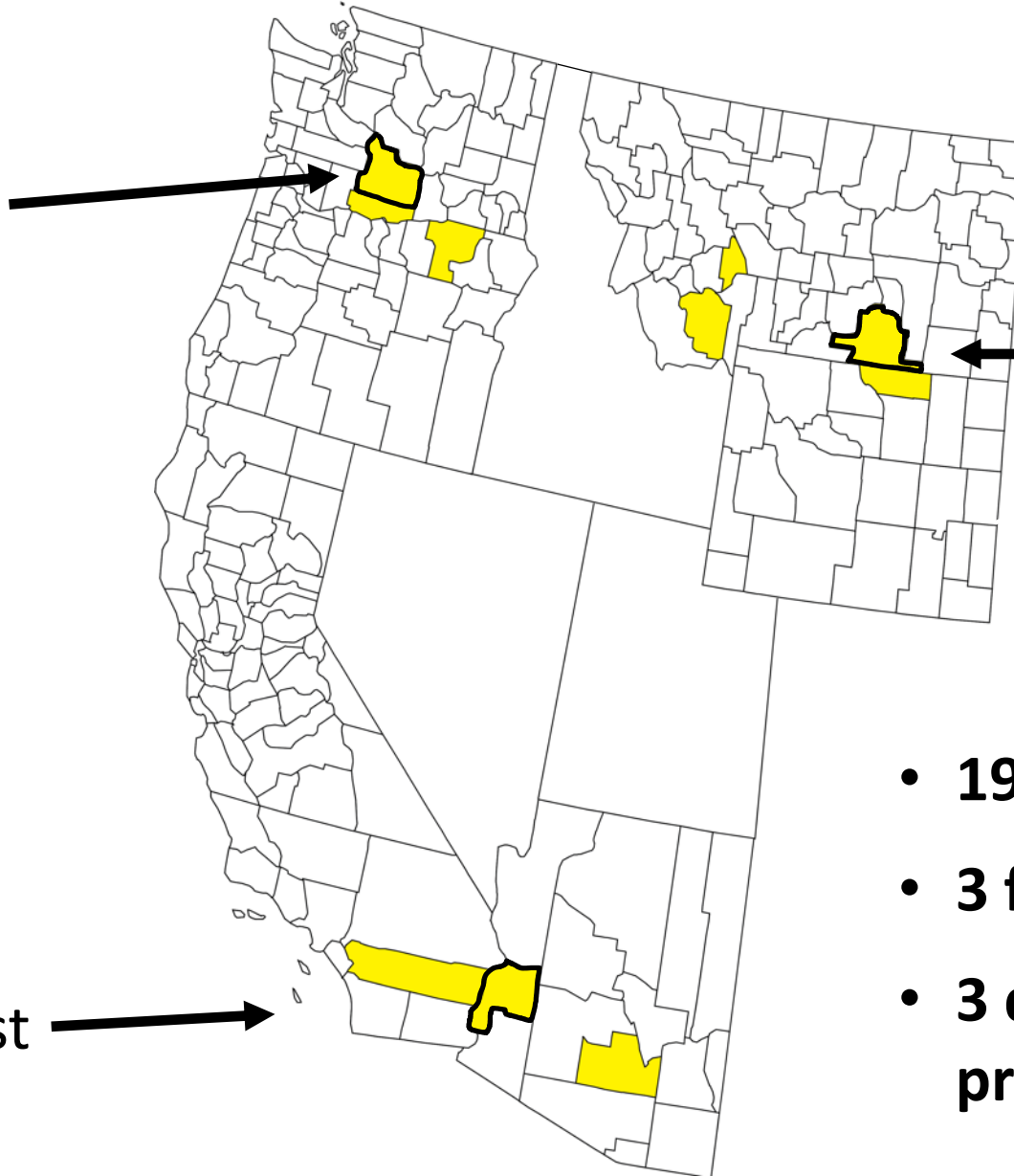




## Objective 2: Locations



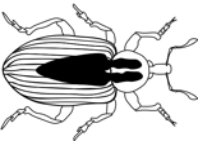
Pacific  
Northwest



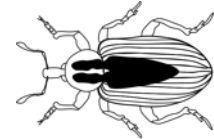
Intermountain  
West

Southwest

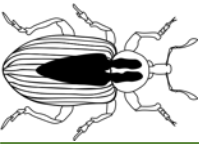
- **19 field sites tested**
- **3 field trials**
- **3 distinct production zones**



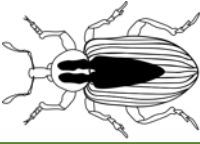
## Objective 2: Active ingredients



<b>Name</b>	<b>Product Name</b>	<b>Type</b>
<b>Alpha-cypermethrin</b>	<b>Fastac<sup>®</sup> (BASF)</b>	<b>Type II</b>
<b>Beta-cyfluthrin</b>	<b>Baythroid XL<sup>®</sup> (Bayer)</b>	<b>Type II</b>
<b>Lambda-cyhalothrin</b>	<b>Warrior II<sup>®</sup> (Syngenta)</b>	<b>Type II</b>
<b>Zeta-cypermethrin</b>	<b>Mustang Maxx<sup>®</sup> (FMC)</b>	<b>Type II</b>
<b>Bifenthrin</b>	<b>Brigade<sup>®</sup> (FMC)</b>	<b>Type I</b>
<b>Permethrin</b>	<b>Ambush<sup>®</sup> (AgNova)</b>	<b>Type I</b>



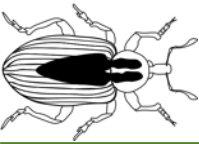
## Objective 2: Approach



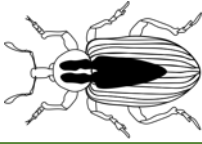
### Methods for testing multiple resistance:

1. Correlations with  $LC_{50}$  values (e.g., Vassiliou et al. 2010)
2. Percentage mortality at diagnostic concentration ( $3.3\mu\text{g}/\text{cm}^2$ ) (e.g., Menger et al. 2020)
3. Field trials





# Objective 2: Statistical analysis



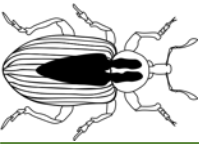
## Method #1: Correlation

1. Prism Graphical Statistics Software
2. Correlation of  $LC_{50}$  values
3. Line best fit;  $R^2$  value goodness of fit

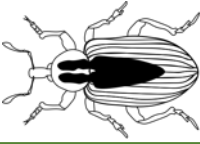
Smirle et al. 2002 , Vassiliou et al. 2010, Min et al. 2014

## How does a correlation determine multiple resistance?

P-values determines if the slope is significantly different from zero

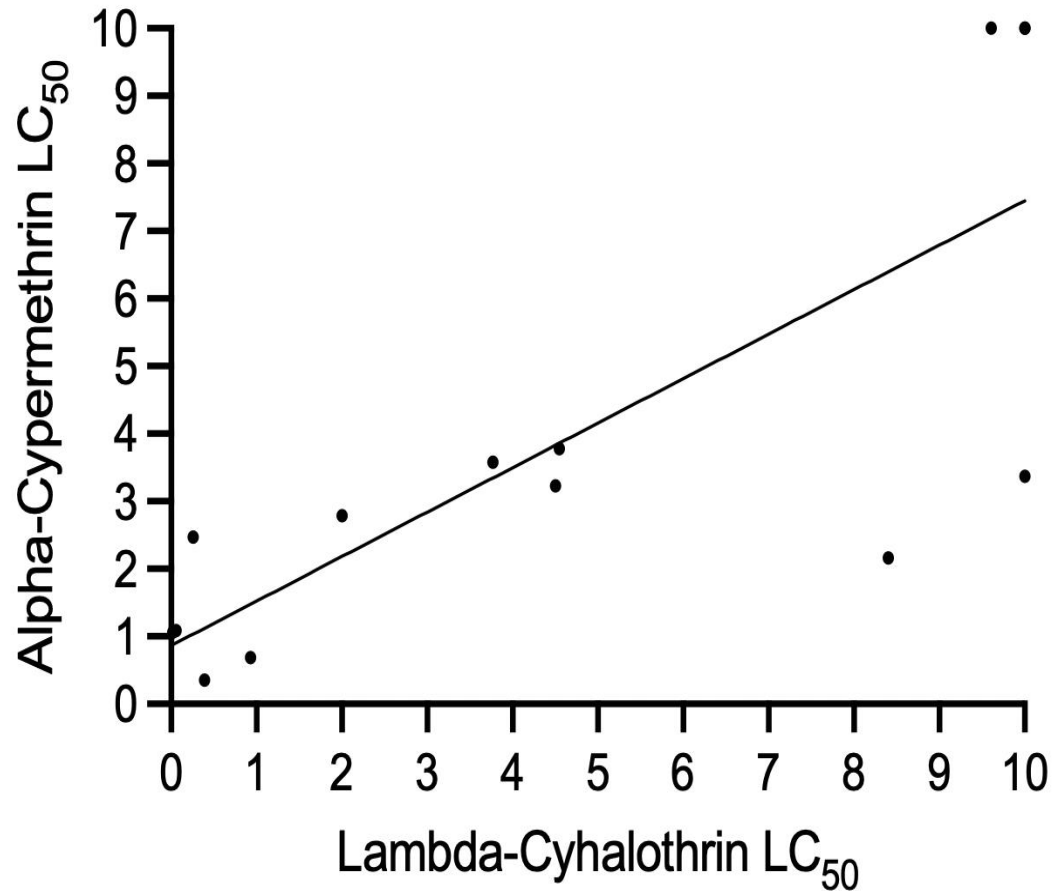


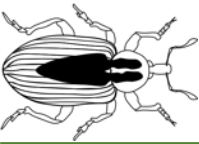
## Objective 2: Correlation results



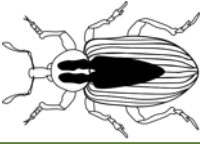
### Lambda-cyhalothrin (Type II) & Alpha-cypermethrin (Type II)

14 locations  
 $R^2$ : 0.62  
P: 0.0008



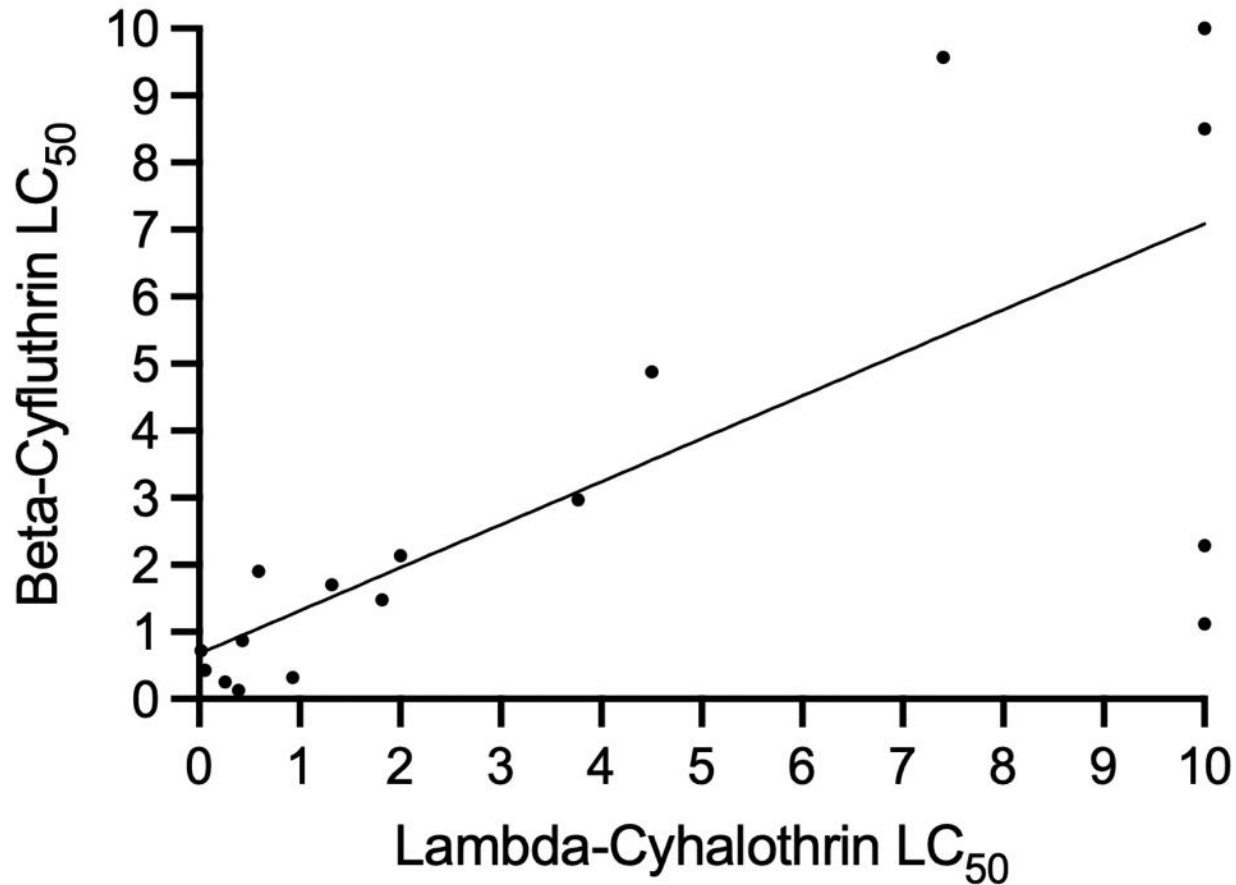


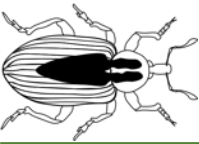
## Objective 2: Correlation results



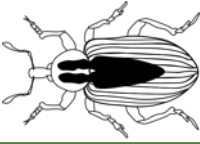
### Lambda-cyhalothrin (Type II) & Beta-cyfluthrin (Type II)

18 locations  
 $R^2: 0.55$   
 $P: 0.0004$



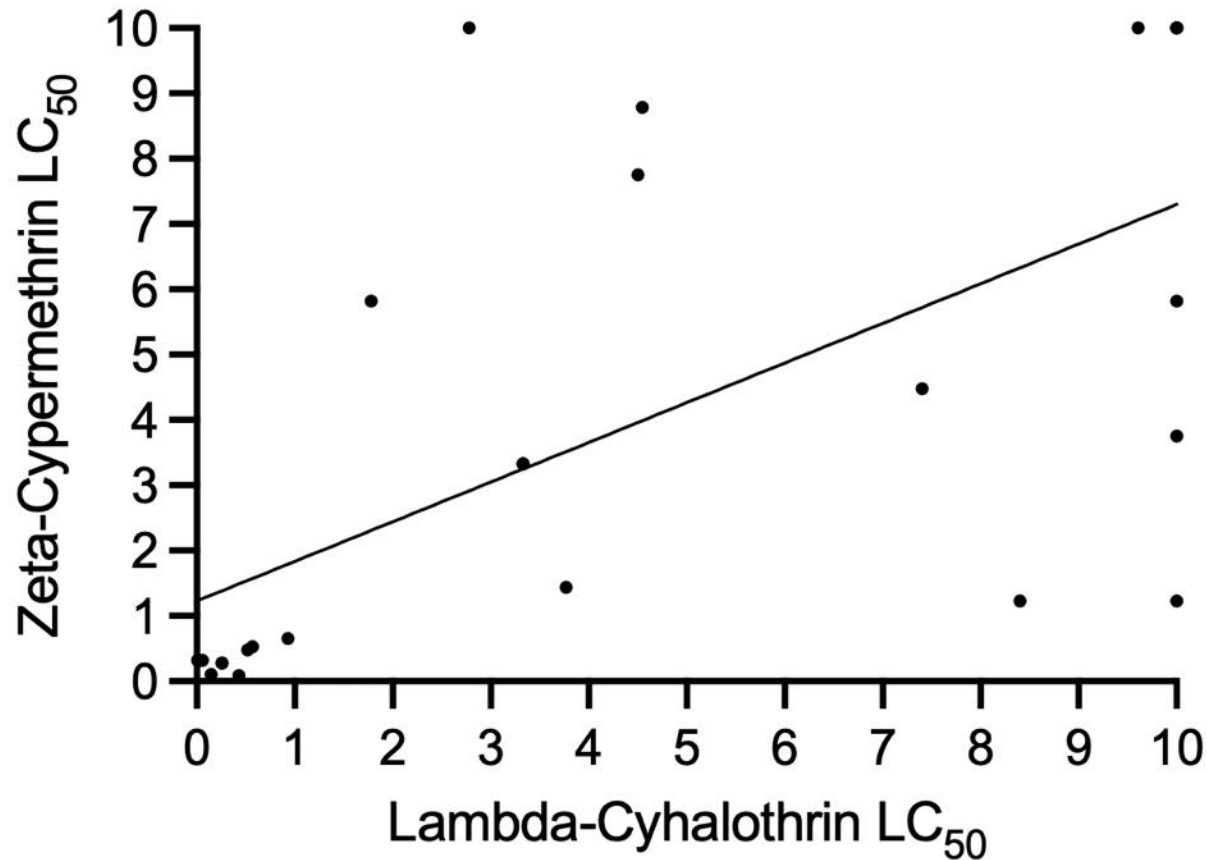


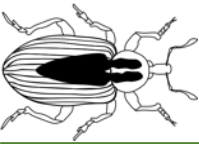
## Objective 2: Correlation results



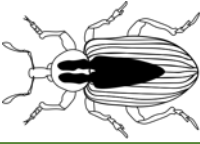
### Lambda-cyhalothrin (Type II) & Zeta-cypermethrin (Type II)

28 locations  
 $R^2: 0.41$   
 $P: 0.0005$



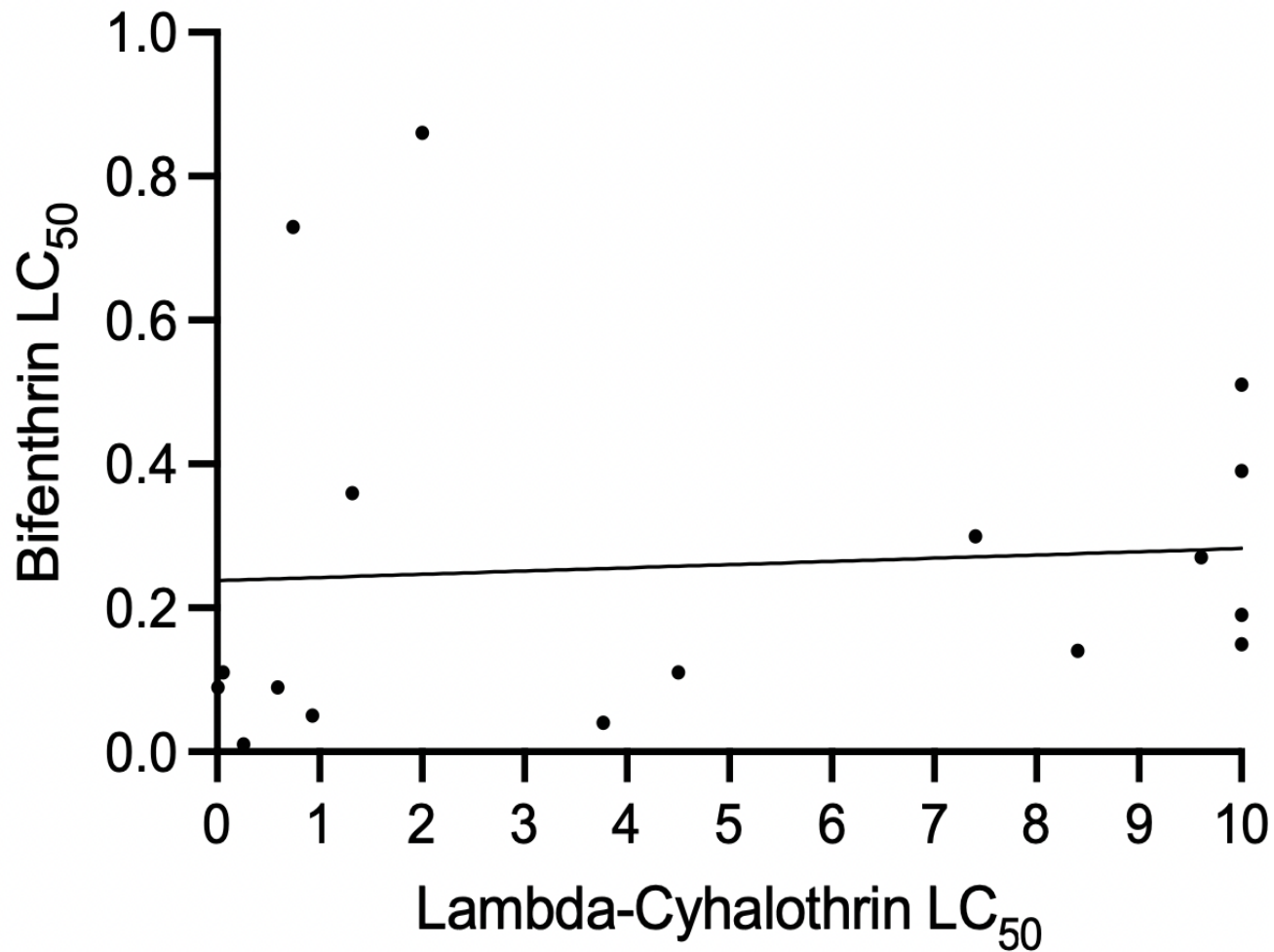


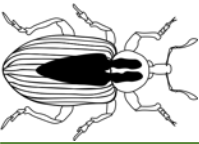
## Objective 2: Correlation results



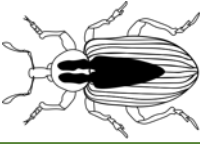
### Lambda-cyhalothrin (Type II) & Bifenthrin (Type I)

17 locations  
 $R^2: 0.006$   
 $P: 0.77$



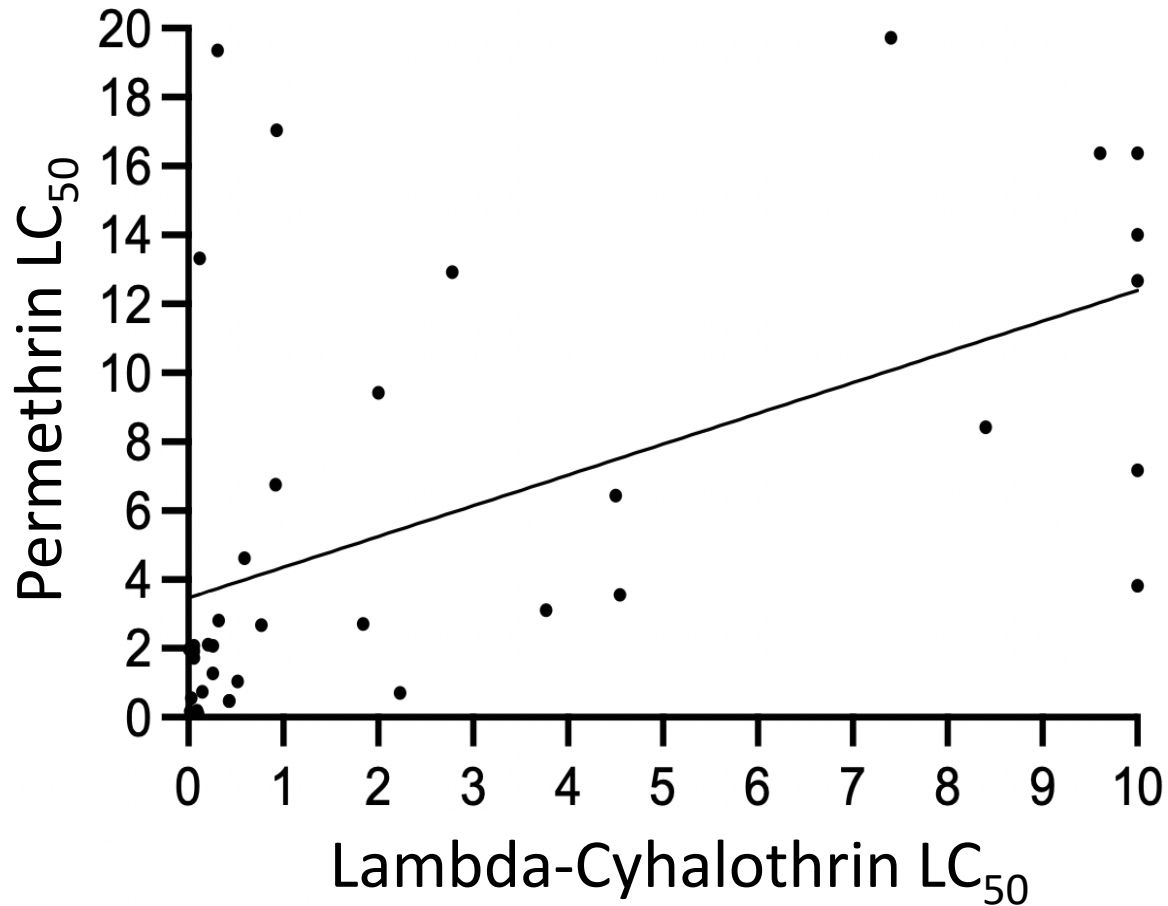


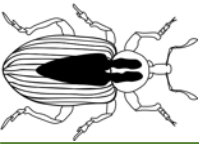
# Objective 2: Correlation results



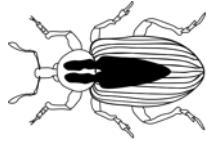
## Lambda-cyhalothrin (Type II) & Permethrin (Type I)

39 locations  
 $R^2: 0.29$   
 $P: 0.006$





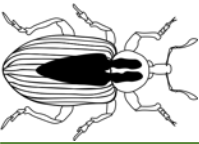
## Objective 2: Statistical analysis



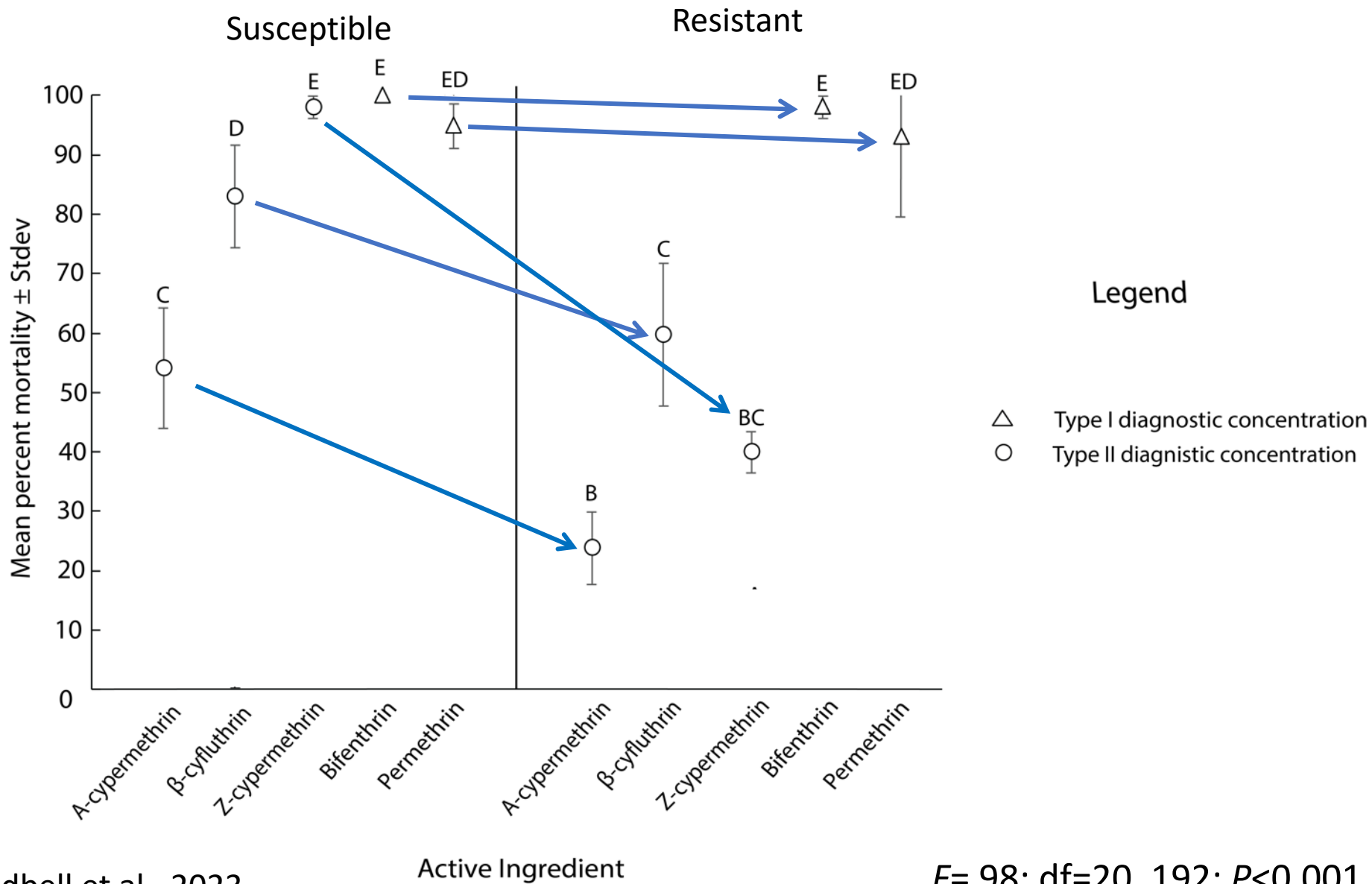
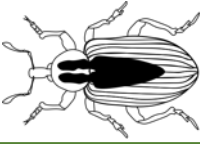
### Method 2: Percentage Mortality

- Post-hoc; raw mortality data from bioassays
- Divided locations based on lambda-cyhalothrin resistance levels: Susceptible & High
- Percentage mortality at a diagnostic concentration for all active ingredients ( $3.3\mu\text{g}/\text{cm}^2$ )
- R-Studio: ANOVA; Tukey HSD

Menger et al. 2020



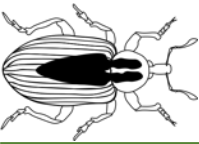
# Objective 2: Results- percentage mortality



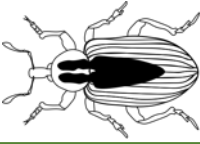


**Can laboratory results be corroborated with field trials?**

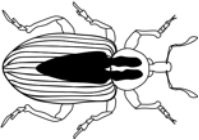




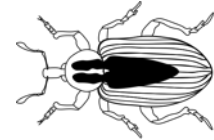
# Objective 2: Field trial



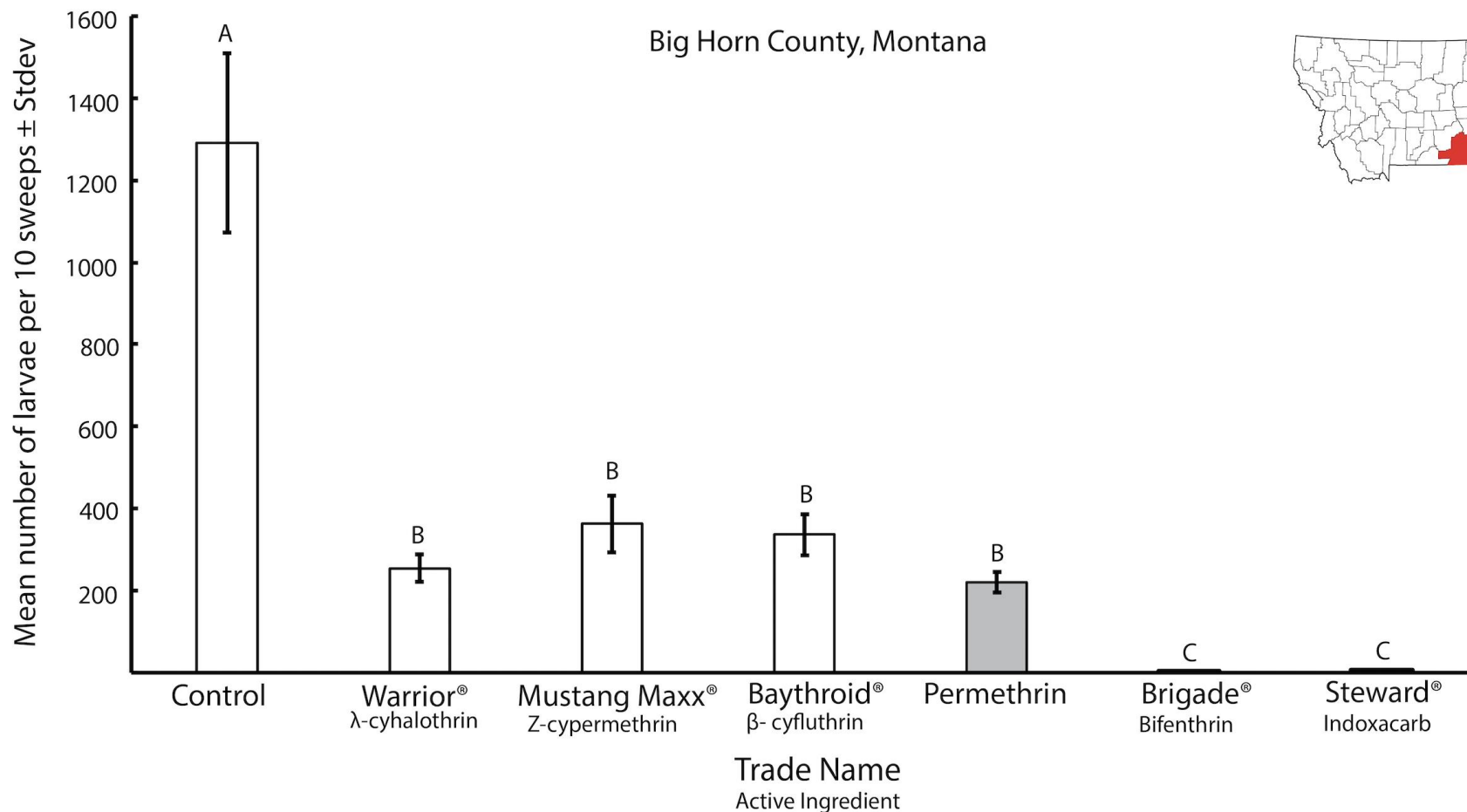
- **Treatment:**
  1. Untreated check
  2. WarriorII®
  3. Mustang Maxx®
  4. Baythroid®
  5. Permethrin
  6. Brigade®
  7. Steward EC®, Indoxacarb (MoA 22A)
- **Maximum label rate**
- **Randomized complete block design (n= 5 blocks)**
- **10-sweep samples 6-days post application**
- **R-Studio: ANOVA, Tukey HSD Test**



# Objective 2: Field trial results

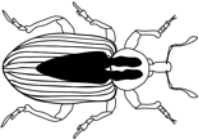


Big Horn County, Montana

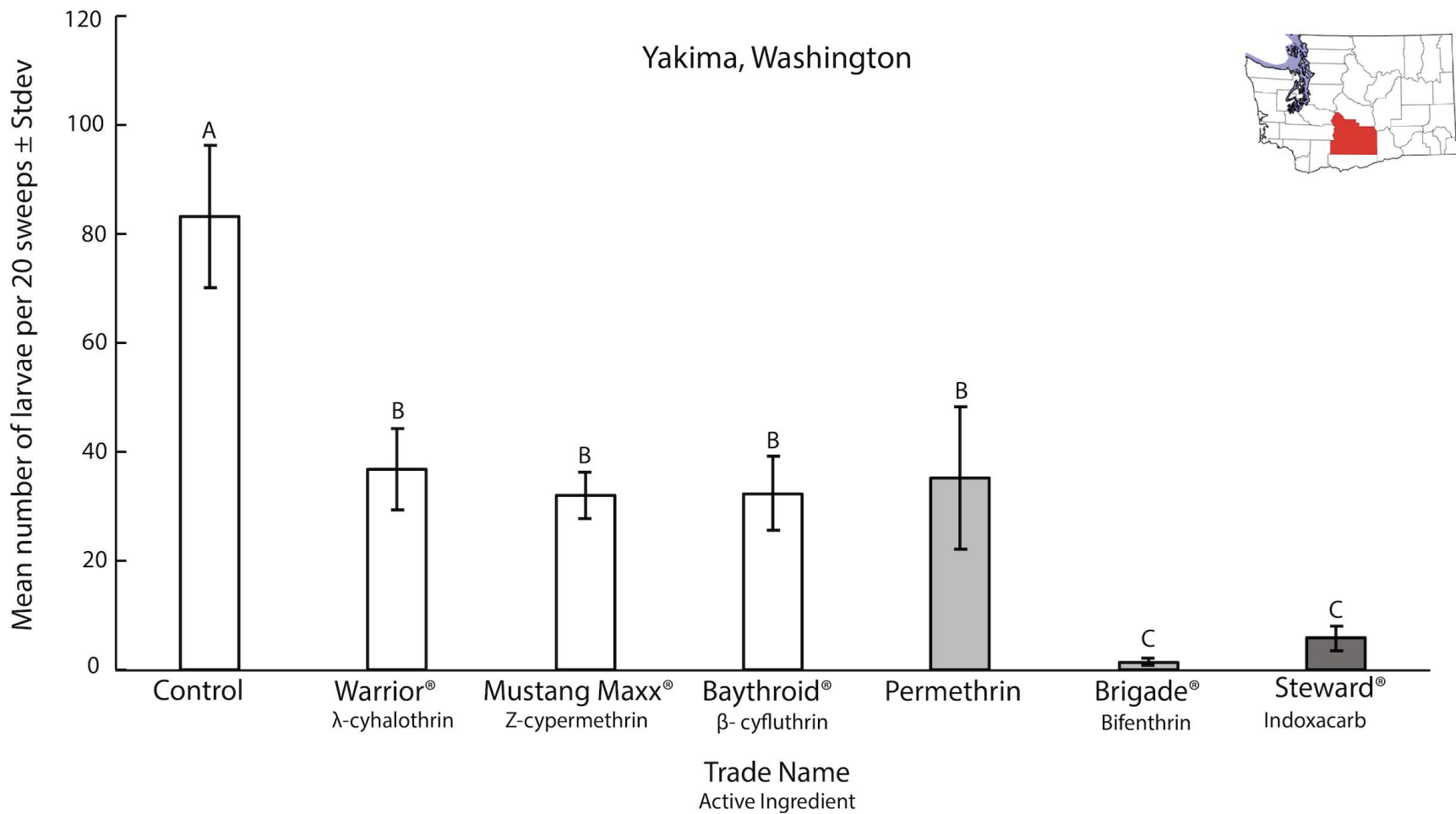
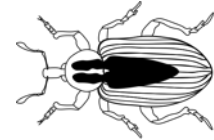


$F= 25.48; df=6, 28; P<0.0001$

Rodbell et al., 2023

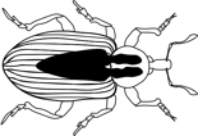


# Objective 2: Field trial results

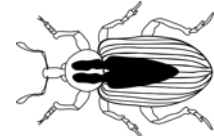


$F = 10.65; df = 6, 28; P < 0.0001$

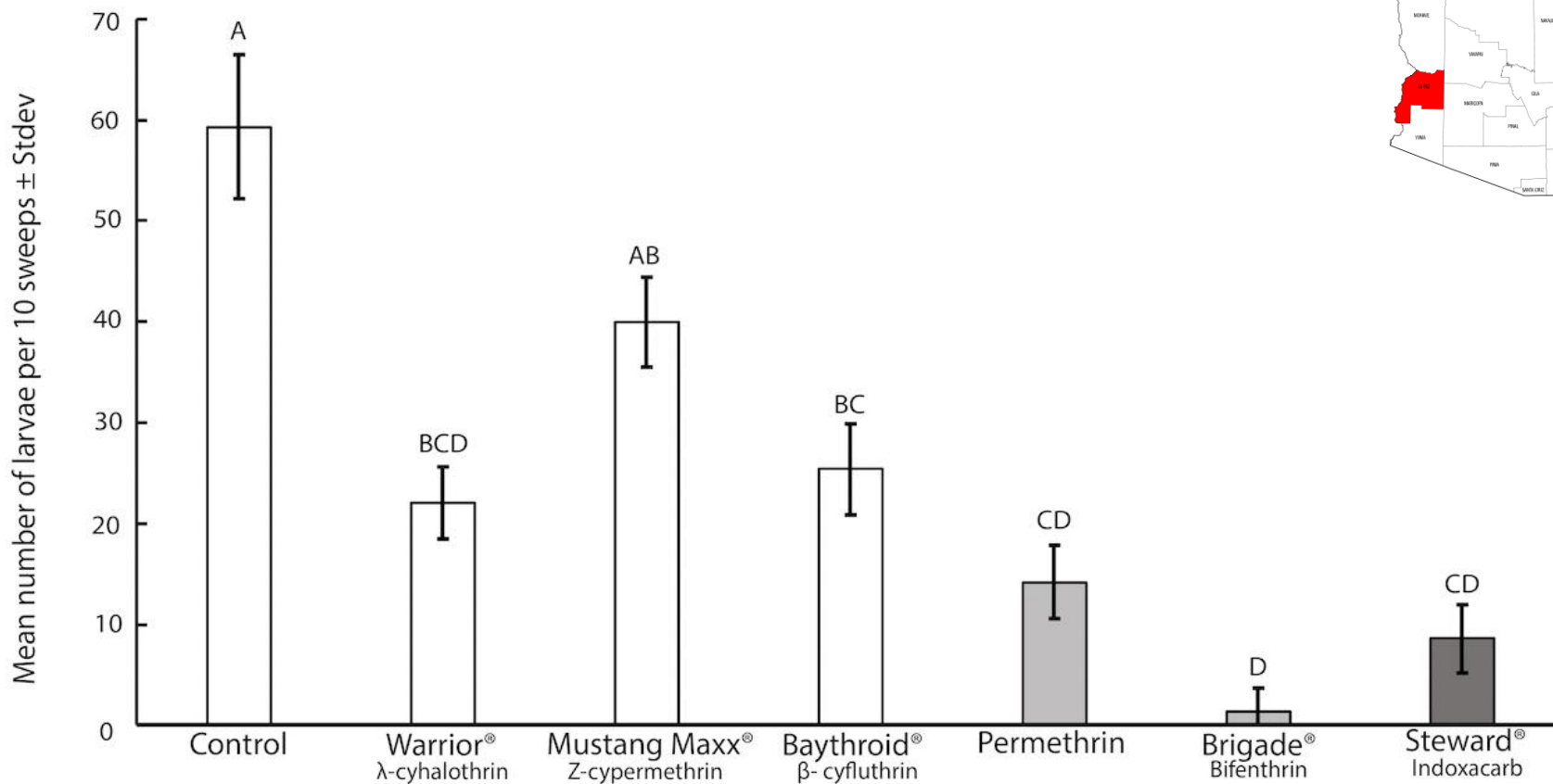
Rodbell et al., 2023



# Objective 2: Field trial results

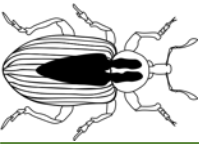


La Paz County, AZ

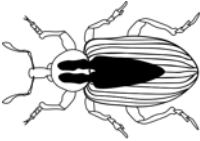


F= 15.92; df=6,28; P< 0.0001

Rodbell et al., 2023



## Objective 2: General conclusions



- Multiple resistance confirmed among pyrethroid type II active ingredients tested, across western production zones
  - **If you have resistance:** All commercial MoA 3A products for forage alfalfa production will not be effective and cannot be interchanged
- Field and laboratory bioassays corroborate each other
- Incomplete multiple resistance between lambda-cyhalothrin and permethrin
- Bifenthrin and indoxacarb were highly efficacious



# Acknowledgements



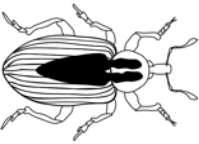
Kevin Wanner, Ian Grettenberger, Madi Hendrick, Moneen Jones, Ruth O'Neil, Chris Caron, Silvia Rondon, Loren Meagher, Michael Rethwisch

Contributing producers and MSU Extension agents for  
their support

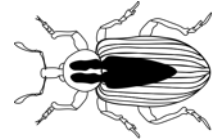
Wanner Lab Undergraduate and Graduate Students

USDA NIFA Alfalfa Seed and Alfalfa Forage Systems Program (2019-70005-30286) and  
the National Alfalfa Forage Alliance

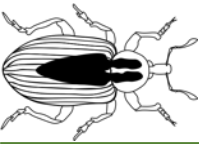




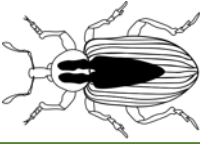
# Questions?



Parker AZ, 2021



# General Recommendations



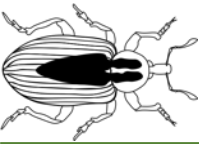
## High Resistance:

- Utilize indoxacarb (MoA 22A) to limit endemic levels
- After 4-5 years test for improved pyrethroid efficacy

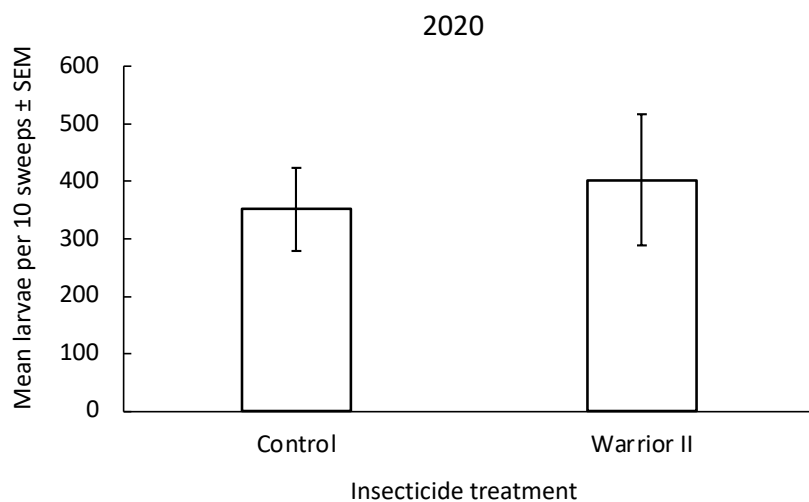
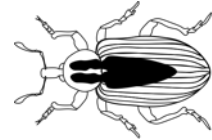
## Susceptible or Moderate Resistance:

- Use pyrethroids and indoxacarb no more than once every three years
- Utilize non-chemical control options to limit selection pressure

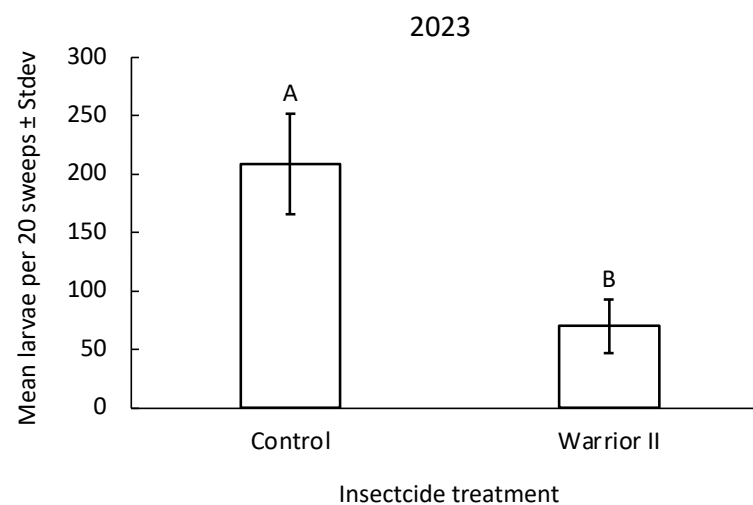




# Improved lambda-cyhalothrin efficacy



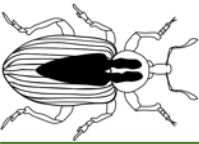
F= 0.14; df= 1, 6; P= 0.72



F= 8.09; df= 1, 6; P= 0.03

Resistance Category	Times (x) Higher label rate
Susceptible	<1.0x
Moderate	1.0-3.0x
High	>3.0x

Year	LC <sub>50</sub> µg/cm <sup>2</sup>
2020	>3.3
2023	0.34



# Alfalfa weevil life cycle

