

## Abstract

Insecticide resistance has evolved in agricultural insect pests through continual misuse of synthetic chemical insecticides. Alfalfa production is a major industry in the West, covering 7.4 million acres that represents 31.2% of total US agricultural acreage. It is a highly nutritious forage for livestock and is integral in supporting the dairy, beef, wool, and other industries. Grown as extensive monocultures, alfalfa fields provide consistent climate conditions across a multitude of microhabitats that act as an insectary for hundreds of beneficial and pest insect species. Agricultural insect pests cause devastating crop loss; for example, 50-90% seed loss to *Lygus spp.* has been recorded in alfalfa. Insecticide use typically aims to eradicate pests, but chemical application must be carefully balanced to minimize damage to beneficial insect populations and maintain susceptible genes in pests. Much of the current literature on agricultural insecticide resistance focuses on field application trials using whole field population counts as a metric of efficacy. Although informative for the practical use of insecticides, this field only approach does not address the underlying mechanisms of resistance or identify toxicity endpoints. The CDC bottle assay assesses resistance by testing populations against verified diagnostic doses and times for an insecticide and is often used to define susceptibility in insect disease vectors. The purpose of this study was to apply a public health approach to insecticide resistance to advance toxicological understanding in an agricultural setting. Alfalfa weevil larvae (*Hypera postica*) and Lygus bug adults (*Lygus spp.*) were collected from Utah State University alfalfa research farms. Bioassays were performed in 1,000ml glass bottles containing approximately 25 individuals of a test insect and were coated with one of 4 insecticide active ingredients (chlorpyrifos, lambda-cyhalothrin, indoxacarb, flupyradifurone). A series of concentrations were tested and replicated three times for each insect and active ingredient. Mortality data were collected after a 24 hour observation period and used to construct survivorship curves, demonstrating the CDC bottle assay's utility for agricultural insects. This information will help insect pest managers predict efficacy and inform responsible application practices.

## Introduction

- Insecticide resistance has evolved in agricultural insect pests through continual overuse of synthetic chemical insecticides
- Alfalfa (**Figure 1**) covers 7.4 million acres in the West and supports a number of major industries, helps with environmental stability and acts as an insectary for a variety of insects.
- CDC bottle assay assesses resistance by testing populations against verified diagnostic doses and times for an insecticide and is often used to define susceptibility in insect disease vectors.



Figure 1: Alfalfa Plant

## Objectives

- **PURPOSE:** apply a public health approach to insecticide resistance to an agricultural setting
- Discover the toxicity endpoints, LD50 and LD90 of the insecticide active ingredients, chlorpyrifos, lambda-cyhalothrin, indoxacarb and flupyradifurone on alfalfa weevil larvae (*Hypera postica*) (**Figure 2**) and lygus bugs (*Lygus spp.*) (**Figure 3**).
- Build survivorship curves for each chemical for each insect and compare susceptibility and resistance of each insect in response to each chemical.



Figure 2: Alfalfa Weevil Larvae; *Hypera Postica*



Figure 3: Lygus bug; *Lygus spp.*

## Results

- Assessed toxicity endpoints for the 4 different insecticide active ingredients
- Created survivorship curves that will continue to be expanded during the next growing season
- LD50 and LD90 were higher for lygus than alfalfa weevil larvae when comparing them for both chlorpyrifos and lambda-cyhalothrin
- Chlorpyrifos (organophosphate) and Lambda-cyhalothrin (pyrethroid) modes of action are contact. Flupyradifurone (butenolide) and Indoxacarb (oxadiazine) modes of action are ingestion. This could have influenced the data collected from each chemical

Figures 6-9: Survivorship curves for Alfalfa Weevil Larvae

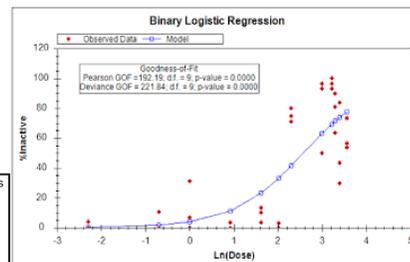


Figure 6: Alfalfa Weevil Larvae-Chlorpyrifos  
Low-0.1 µg/ml  
High-35 µg/ml  
LD50-13.06 µg/ml  
LD90-17.42 µg/ml  
p value-0.00

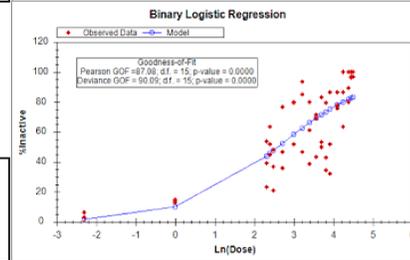


Figure 7: Alfalfa Weevil Larvae-Lambda-Cyhalothrin  
Low-0.1 µg/ml  
High-90 µg/ml  
LD50-13.45 µg/ml  
LD90-180.5 µg/ml  
p value-0.00

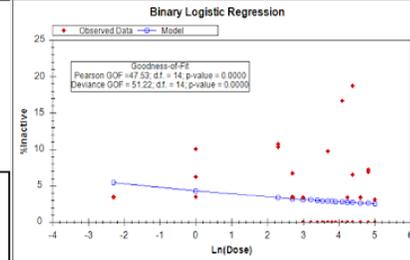


Figure 8: Alfalfa Weevil Larvae-Indoxacarb  
Low-0.1 µg/ml  
High-150 µg/ml  
LD50-0 µg/ml  
LD90-0 µg/ml  
p value-0.00

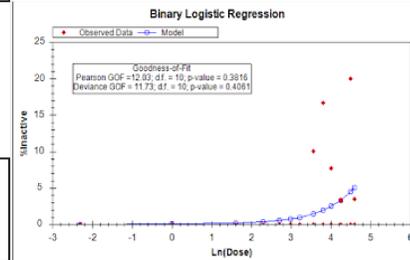


Figure 9: Alfalfa Weevil Larvae-Flupyradifurone  
Low-0.1 µg/ml  
High-100 µg/ml  
LD50-1071 µg/ml  
LD90-6314 µg/ml  
p value-0.38

Figures 10-13: Survivorship curves for Lygus

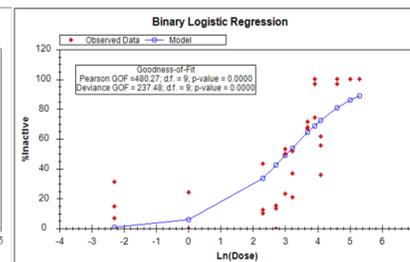


Figure 10: Lygus- Chlorpyrifos  
Low-0.1 µg/ml  
High-200 µg/ml  
LD50-21.08 µg/ml  
LD90-230.2 µg/ml  
p value-0.00

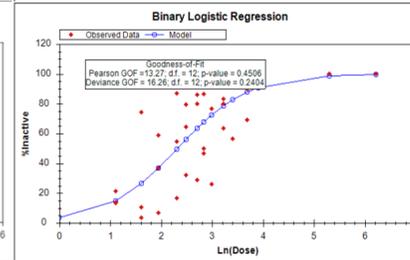


Figure 11: Lygus-Lambda-cyhalothrin  
Low-1 µg/ml  
High-500 µg/ml  
LD50-10.17 µg/ml  
LD90-46.89 µg/ml  
p value-0.45

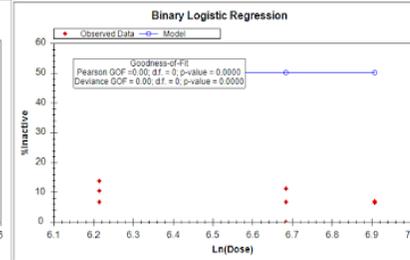


Figure 12: Lygus-Indoxacarb  
Low-500 µg/ml  
High-1000 µg/ml  
LD50-N/A  
LD90-N/A  
p value-0.00

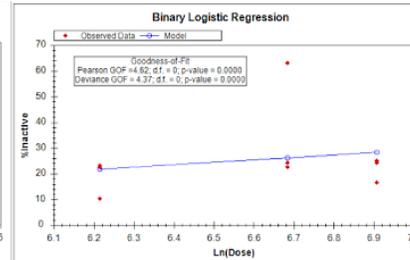


Figure 13: Lygus-Flupyradifurone  
Low-500 µg/ml  
High-1000 µg/ml  
LD50-6171 µg/ml  
LD90-457897 µg/ml  
p value-0.00

## Methods

- Adapted the CDC bottle assay for arthropod disease vectors to an agricultural setting.
- Insects were collected via field sweeping at the Utah State University alfalfa fields. Fields were within 0.25 mile of each other. (**Figure 4**)
- Bioassays were performed in 1,000 ml glass bottles containing approximately 25 individuals of a test insect and were coated with one of 4 insecticide active ingredients (chlorpyrifos, lambda-cyhalothrin, indoxacarb, flupyradifurone) (**Figure 5**)
- A series of concentrations were tested and replicated three times for each insect and active ingredient
- Mortality data were collected after a 24 hour observation period and used to construct survivorship curves, demonstrating the CDC bottle assay's utility for agricultural insects.

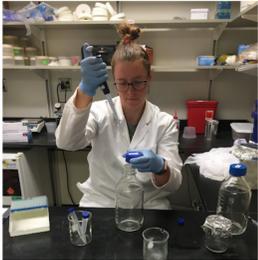


Figure 5: Undergraduate researcher, Rebecca Bernhardt preparing bottles for adapted CDC bottle assays



Figure 4: Utah State University research alfalfa fields location and proximity.

## Conclusion

- Lygus required higher concentrations of chlorpyrifos and lambda-cyhalothrin to reach LD50 and LD90 when compared to the alfalfa weevil larvae. This could be influenced by size differences (i.e. lygus are larger), life stage (adult vs. larva) or body type (lygus are hard bodied and alfalfa weevil larvae are soft bodied).
- Indoxacarb and Flupyradifurone require much higher concentrations to create a curve and have significant data. This could be influenced by mode of action of each insecticide (contact vs. ingestion).
- Adjustments need to be made to the experimental methods and further trials performed in order to obtain significant results for flupyradifurone and indoxacarb. Additional trials will also be performed to refine all survivorship curves and obtain statistically significant data.
- Future trials will include other pest insects, beneficiaries such as pollinators and other natural enemies in the alfalfa agroecosystems as well as a molecular examination of resistant genes.

## Acknowledgements

Theresa Pitts-singer, USU, USDA bee lab; Ricardo Ramirez, USU dept biology; Kimberly Hageman, USU dept chemistry; Randa Jabour, UWYO dept plant sciences; Keith Wilson.  
Funded by USDA NIFA

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