Louisiana Agricultural Technology & Management Conference
February 12, 2026

Management of Redbanded Stink Bug: Update

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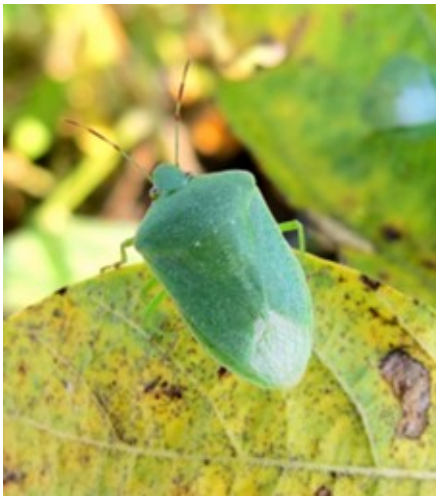
Louisiana State University AgCenter

Insecticide Resistance

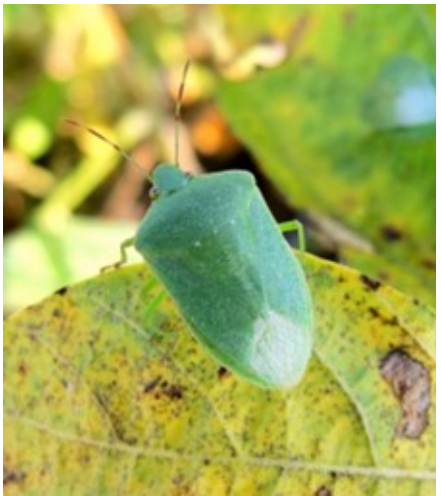


<u>Defined</u>: "a heritable, statistically defined decrease in sensitivity to a chemical in a pest population relative to the response of susceptible populations that have never been exposed to pesticides."

In the field, resistance is a "measurable reduction in the relative efficacy of a pesticide."

https://treefruit.wsu.edu/crop-protection/opm/resistance/

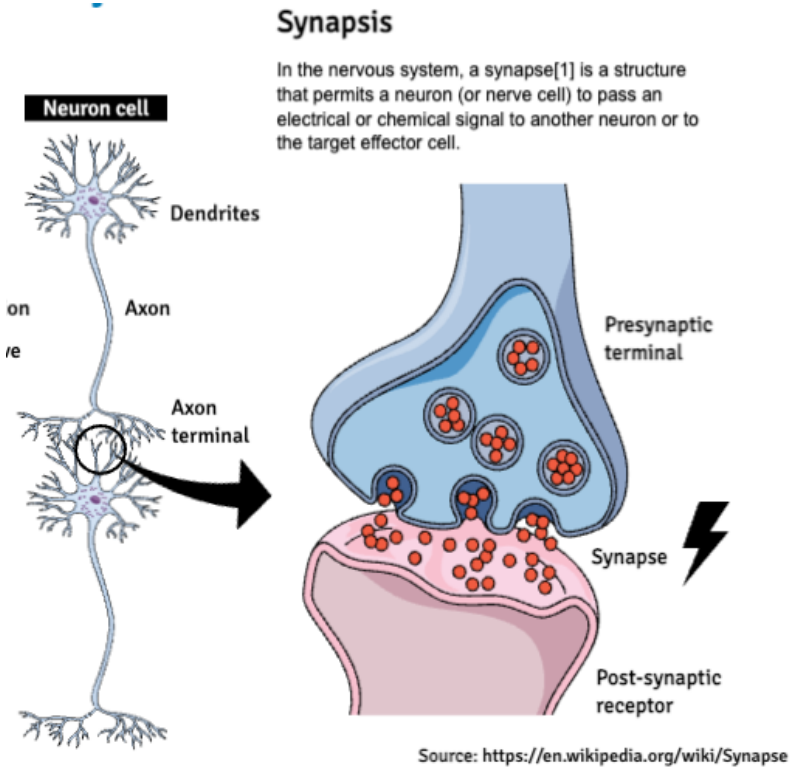
Mechanisms of Insecticide Resistance

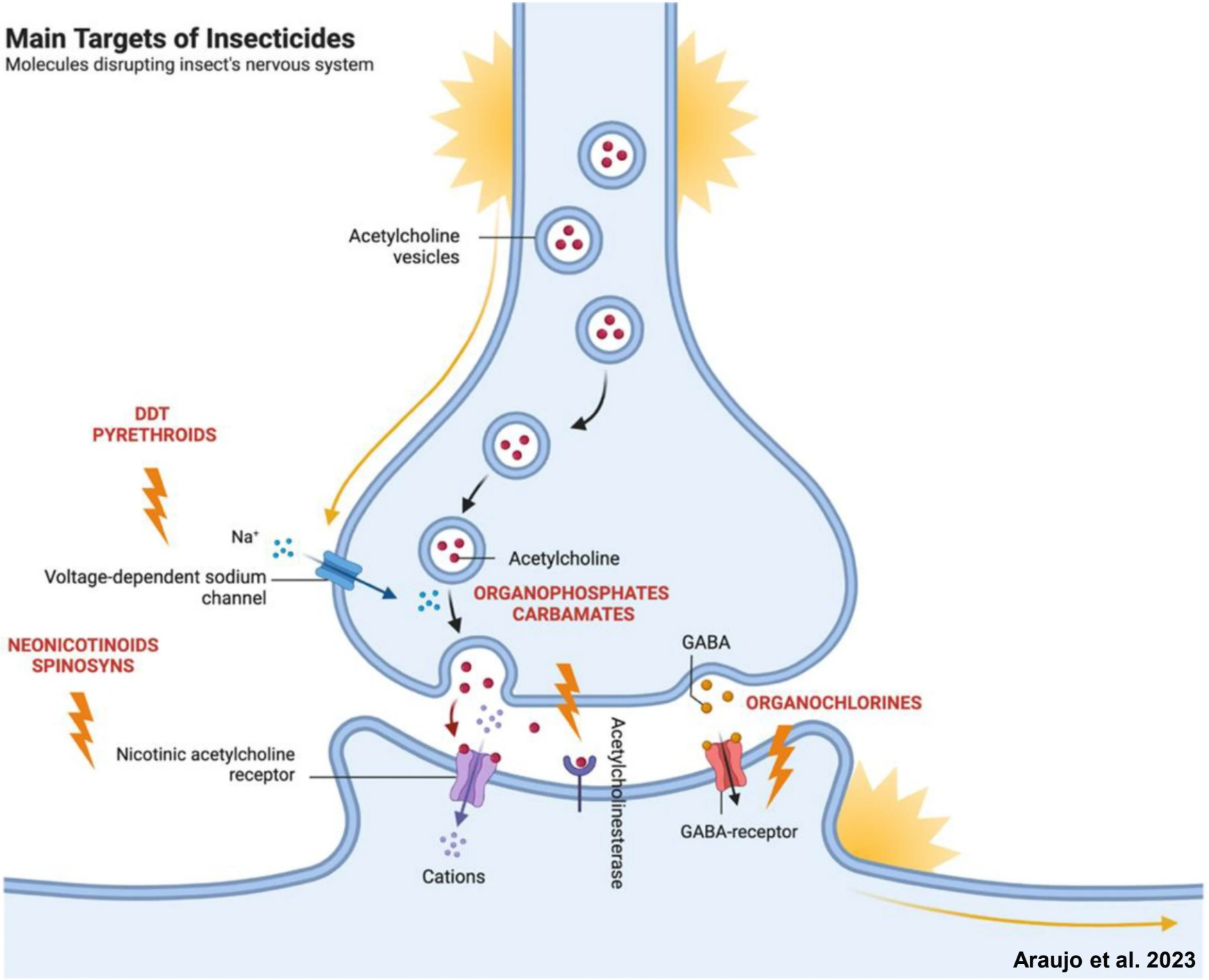


<u>Metabolic Resistance</u>: Produce higher levels of enzymes that detoxify or break down the insecticide.

<u>Target-Site Resistance</u>: Mutations at the site where the insecticide acts renders insecticide ineffective.

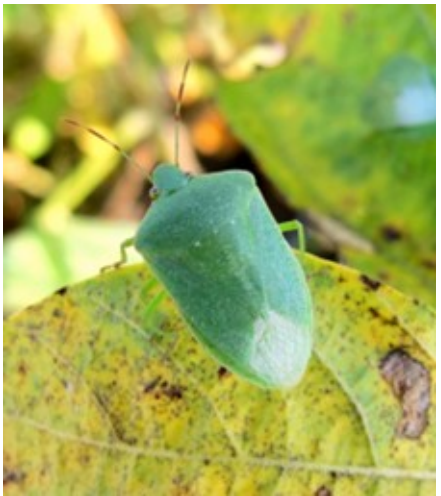
Dendrite and Axon





Araujo et al. 2023

Mechanisms of Insecticide Resistance

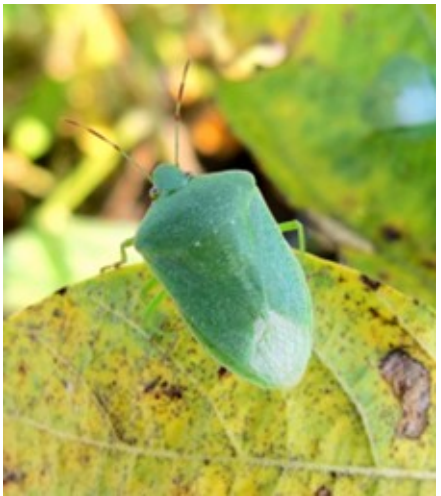


<u>Penetration Resistance</u>: Cuticle becomes thickened, slowing or stopping insecticide penetration.

<u>Behavioral Resistance</u>: Insects avoid the insecticide.

- **Altered feeding habits**
- **Avoiding treated surfaces**
- **Leaving treated surfaces when contacted**

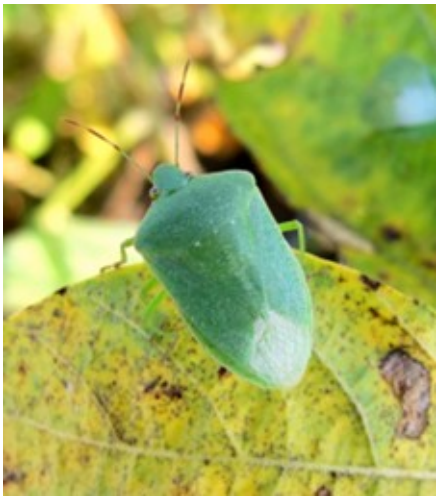
Which Mechanisms of Insecticide Resistance Does Redbanded Stink Bug Have?

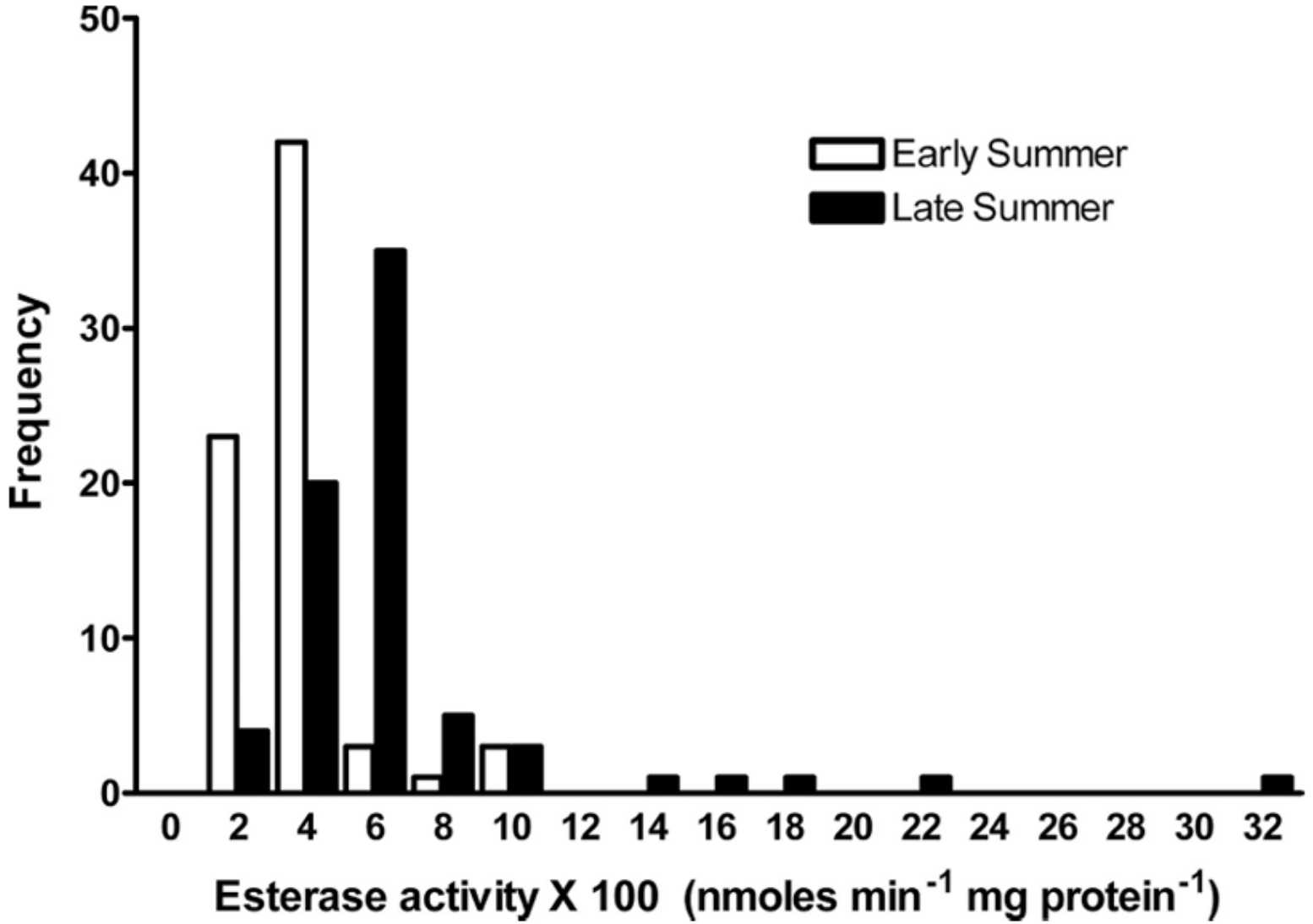


Mechanism	Yes or No
Metabolic	Yes
Target-Site	???
Penetration	???
Behavioral	Yes

Redbanded Stink Bug Esterase Levels: Metabolic Resistance

Iberville Parish, LA





Baur et al. 2010

Redbanded Stink Bug Behavioral Resistance

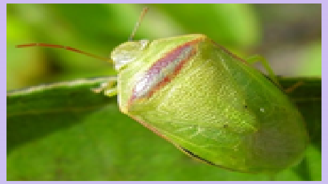
- **70% of eggs oviposited in lower two-thirds of canopy.**
- **60% of damaged seed found in lower two-thirds of canopy.**
- **With a high frequency of egg clusters and feeding damage found in the lower two-thirds of the plant canopy, redbanded stink bugs are exposed to less insecticide residues.**

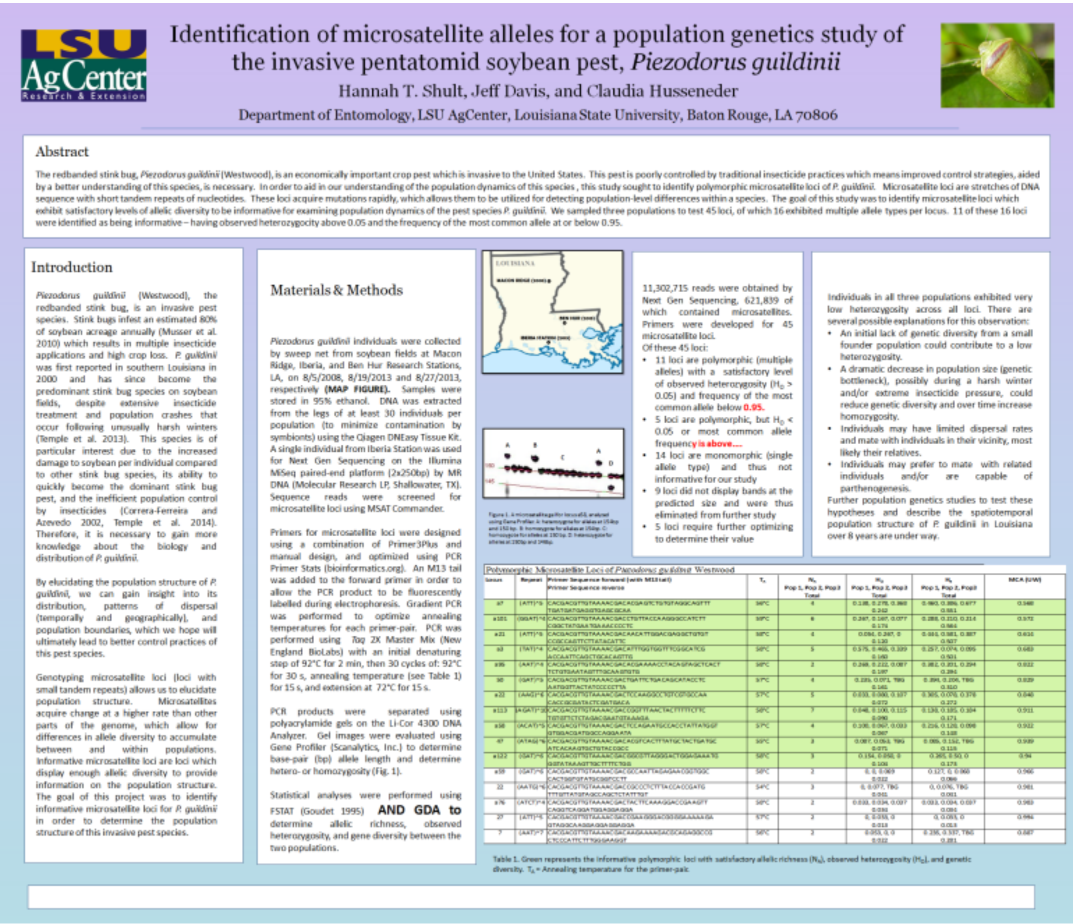
Temple et al. 2016

Identification of microsatellite alleles for a population genetics study of the invasive pentatomid soybean pest, *Piezodorus guildinii*

Hannah T. Shult, Jeff Davis, and Claudia Husseneder

Department of Entomology, LSU AgCenter, Louisiana State University, Baton Rouge, LA 70806





Identification of microsatellite alleles for a population genetics study of the invasive pentatomid soybean pest, *Piezodorus guildinii*

Hannah T. Shult, Jeff Davis, and Claudia Husseneder

Department of Entomology, LSU AgCenter, Louisiana State University, Baton Rouge, LA 70806

Abstract

The redbanded stink bug, *Piezodorus guildinii* (Westwood), is an economically important crop pest which is invasive to the United States. This pest is poorly controlled by traditional insecticide practices which means improved control strategies, aided by a better understanding of this species, is necessary. In order to aid in our understanding of the population dynamics of this species, this study sought to identify polymorphic microsatellite loci of *P. guildinii*. Microsatellite loci are stretches of DNA sequence with short tandem repeats of nucleotides. These loci acquire mutations rapidly, which allows them to be utilized for detecting population-level differences within a species. The goal of this study was to identify microsatellite loci which exhibit satisfactory levels of allelic diversity to be informative for examining population dynamics of the pest species *P. guildinii*. We sampled three populations to test 45 loci, of which 16 exhibited multiple allele types per locus. 11 of these 16 loci were identified as being informative – having observed heterozygocity above 0.05 and the frequency of the most common allele at or below 0.95.

Introduction

Piezodorus guildinii (Westwood), the redbanded stink bug, is an invasive pest species. Stink bugs infest an estimated 80% of soybean acreage annually (Musser et al. 2010) which results in multiple insecticide applications and high crop loss. *P. guildinii* was first reported in southern Louisiana in 2000 and has since become the predominant stink bug species on soybean fields, despite extensive insecticide treatment and population crashes that occur following unusually harsh winters (Temple et al. 2013). This species is of particular interest due to the increased damage to soybean per individual compared to other stink bug species, its ability to quickly become the dominant stink bug pest, and the inefficient population control by insecticides (Correra-Ferreira and Azevedo 2002, Temple et al. 2014). Therefore, it is necessary to gain more knowledge about the biology and distribution of *P. guildinii*.

By elucidating the population structure of *P. guildinii*, we can gain insight into its distribution, patterns of dispersal (temporally and geographically), and population boundaries, which we hope will ultimately lead to better control practices of this pest species.

Genotyping microsatellite loci (loci with small tandem repeats) allows us to elucidate population structure. Microsatellites acquire change at a higher rate than other parts of the genome, which allow for differences in allele diversity to accumulate between and within populations. Informative microsatellite loci are loci which display enough allelic diversity to provide information on the population structure. The goal of this project was to identify informative microsatellite loci for *P. guildinii* in order to determine the population structure of this invasive pest species.

Materials & Methods

Piezodorus guildinii individuals were collected by sweep net from soybean fields at Macon Ridge, Iberia, and Ben Hur Research Stations, LA, on 8/5/2008, 8/19/2013 and 8/27/2013, respectively **(MAP FIGURE).** Samples were stored in 95% ethanol. DNA was extracted from the legs of at least 30 individuals per population (to minimize contamination by symbionts) using the Qiagen DNEasy Tissue Kit. A single individual from Iberia Station was used for Next Gen Sequencing on the Illumina MiSeq paired-end platform (2x250bp) by MR DNA (Molecular Research LP, Shallowater, TX). Sequence reads were screened for microsatellite loci using MSAT Commander.

Primers for microsatellite loci were designed using a combination of Primer3Plus and manual design, and optimized using PCR Primer Stats (bioinformatics.org). An M13 tail was added to the forward primer in order to allow the PCR product to be fluorescently labelled during electrophoresis. Gradient PCR was performed to optimize annealing temperatures for each primer-pair. PCR was performed using *Taq* 2X Master Mix (New England BioLabs) with an initial denaturing step of 92°C for 2 min, then 30 cycles of: 92°C for 30 s, annealing temperature (see Table 1) for 15 s, and extension at 72°C for 15 s.

PCR products were separated using polyacrylamide gels on the Li-Cor 4300 DNA Analyzer. Gel images were evaluated using Gene Profiler (Scanalytics, Inc.) to determine base-pair (bp) allele length and determine hetero- or homozygosity (Fig. 1).

Statistical analyses were performed using FSTAT (Goudet 1995) **AND GDA to** determine allelic richness, observed heterozygosity, and gene diversity between the two populations.

LOUISIANA

11,302,715 reads were obtained by Next Gen Sequencing, 621,839 of which contained microsatellites. Primers were developed for 45 microsatellite loci.

Of these 45 loci:

- 11 loci are polymorphic (multiple alleles) with a satisfactory level of observed heterozygosity (H_O > 0.05) and frequency of the most common allele below **0.95.**
- 5 loci are polymorphic, but H_O < 0.05 or most common allele frequency **is above….**
- 14 loci are monomorphic (single allele type) and thus not informative for our study
- 9 loci did not display bands at the predicted size and were thus eliminated from further study
- 5 loci require further optimizing to determine their value

Individuals in all three populations exhibited very low heterozygosity across all loci. There are several possible explanations for this observation:

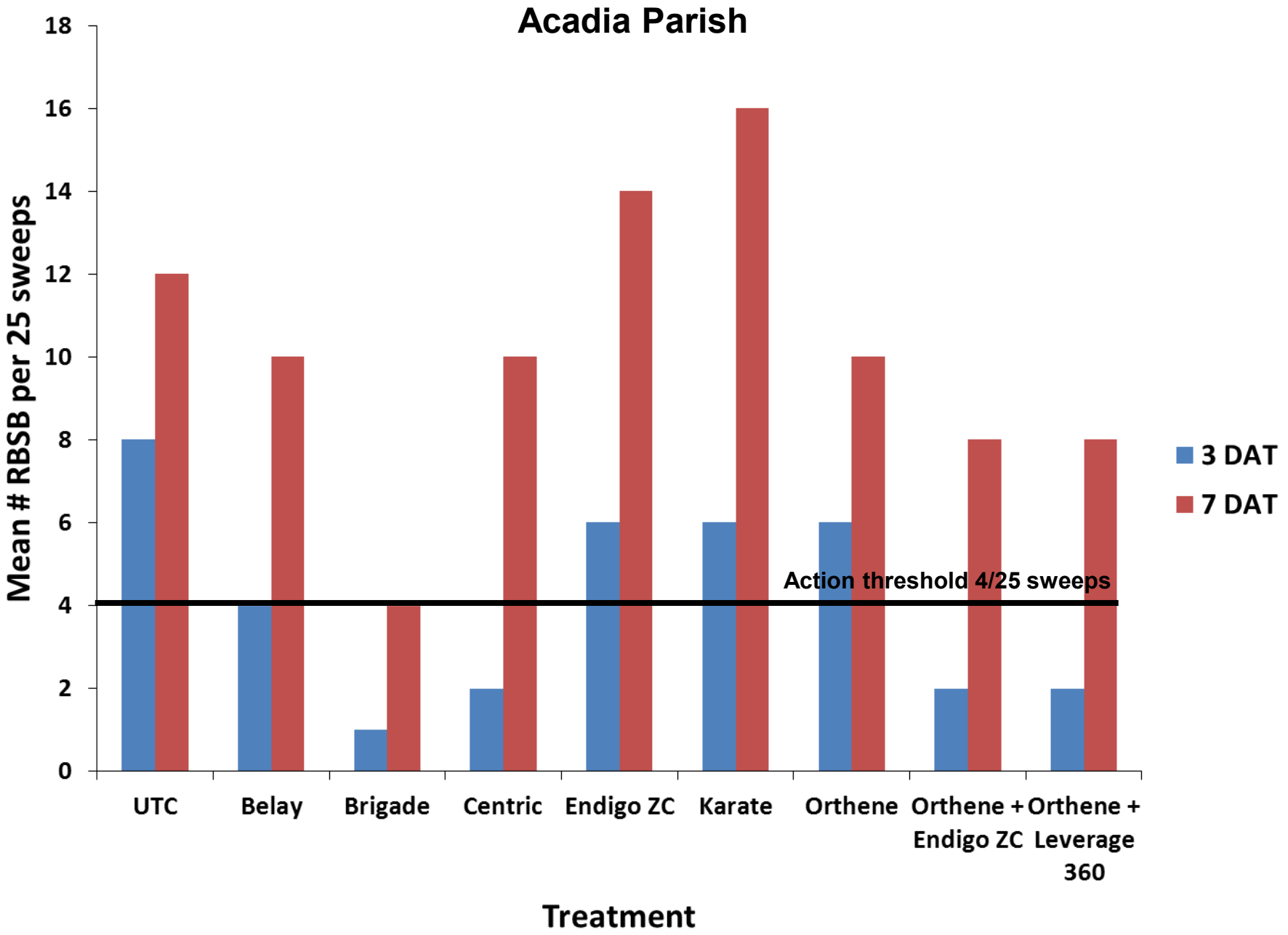
- An initial lack of genetic diversity from a small founder population could contribute to a low heterozygosity.
- A dramatic decrease in population size (genetic bottleneck), possibly during a harsh winter and/or extreme insecticide pressure, could reduce genetic diversity and over time increase homozygosity.
- Individuals may have limited dispersal rates and mate with individuals in their vicinity, most likely their relatives.
- Individuals may prefer to mate with related individuals and/or are capable of parthenogenesis.

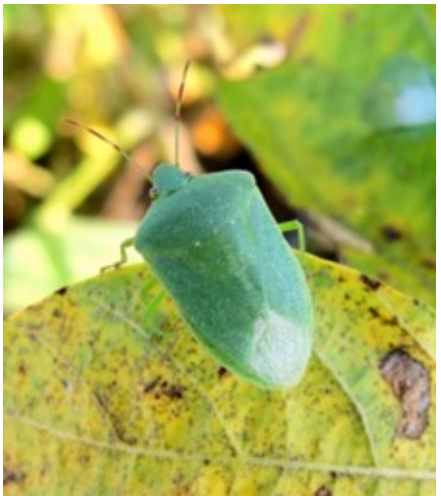
Further population genetics studies to test these hypotheses and describe the spatiotemporal population structure of *P. guildinii* in Louisiana over 8 years are under way.

Polymorphic Microsatellite Loci of *Piezodorus guildinii* Westwood

Table 1. Green represents the informative polymorphic loci with satisfactory allelic richness (N_A), observed heterozygosity (H_O), and genetic diversity. T_a = Annealing temperature for the primer-pair.

- **Small founder population with limited genetic diversity.**
- **Harsh winters and insecticide pressure are reducing genetic diversity.**
- **RBSB has limited dispersal rates and mate with individuals in their vicinity, most likely their relatives.**
- **Resistance will be localized but will be permanent**


Acadia Parish
Mean # RBSB per 25 sweeps
18
16
14
12
10
8
6
4
2
0
Action threshold 4/25 sweeps
3 DAT
7 DAT
UTC
Belay
Brigade
Centric
Endigo ZC
Karate
Orthene
Orthene + Endigo ZC
Orthene + Leverage 360
Treatment



New Toxicologist Hired!

Jonathan Hernandez

jrhernandez@agcenter.lsu.edu

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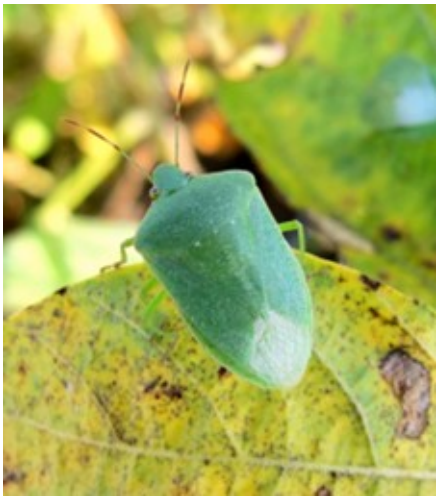
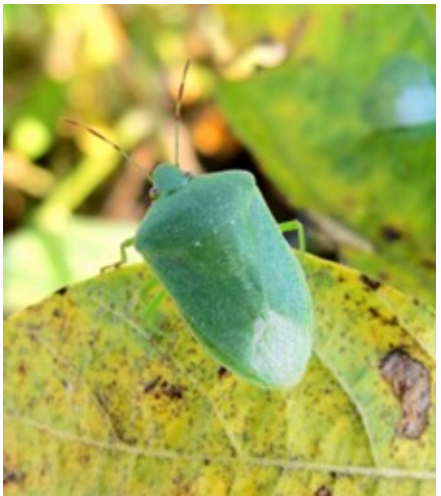
Monitoring for Insecticide Resistance

Stink Bug Insecticide Resistance Test Kits



- Proactive approach
- Provide “diagnostic dose kits”
- Test and report
- If a population is found to be resistant, follow-up collections made for further testing

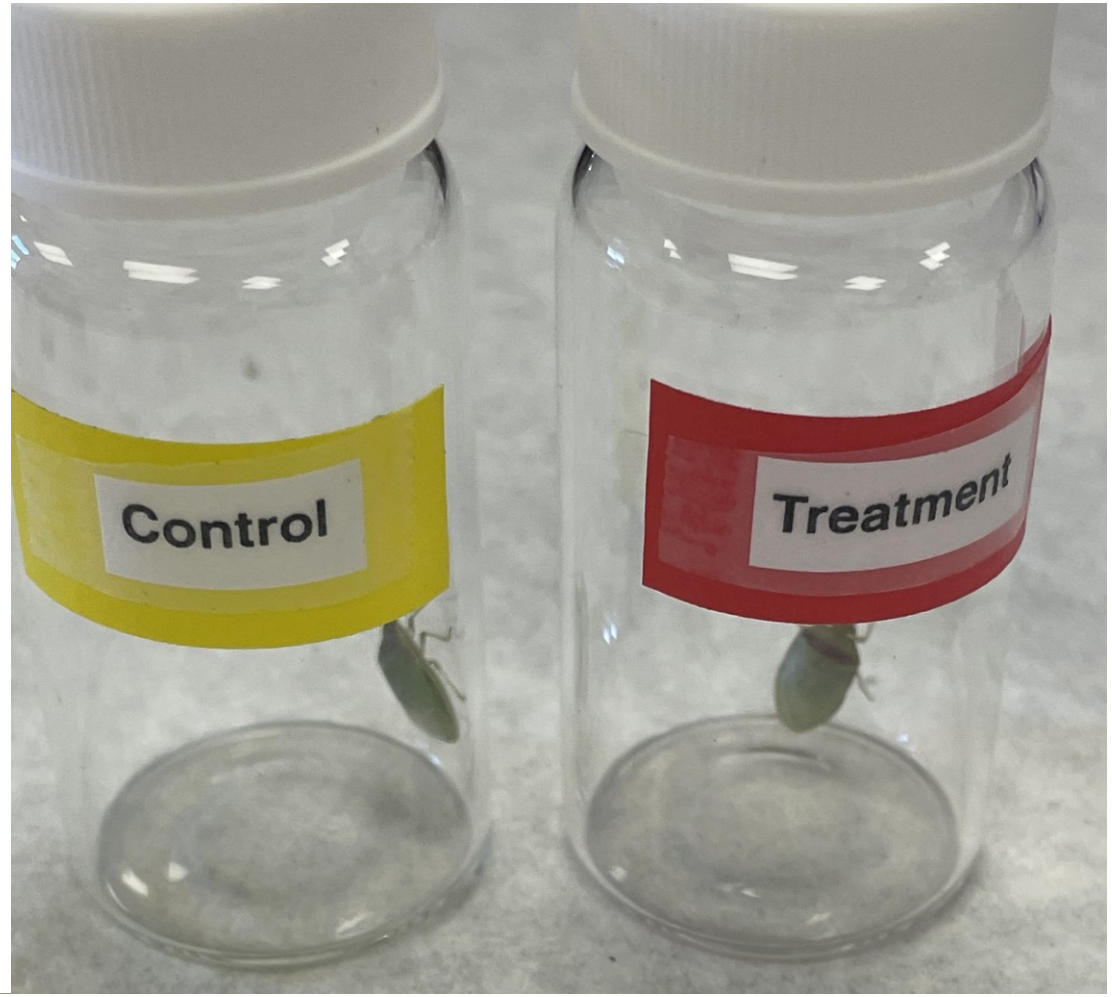
Stink Bug Insecticide Resistance Test Kits



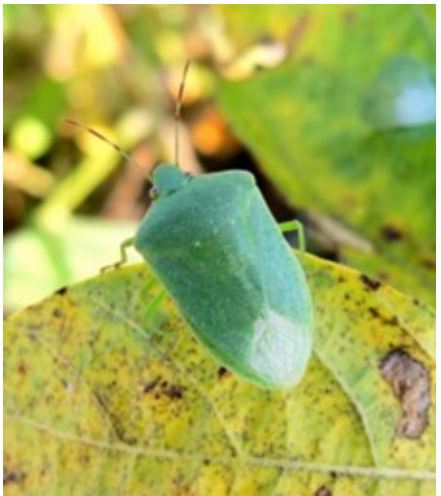
Adult·Vial·Test·protocol·(AVT)¶

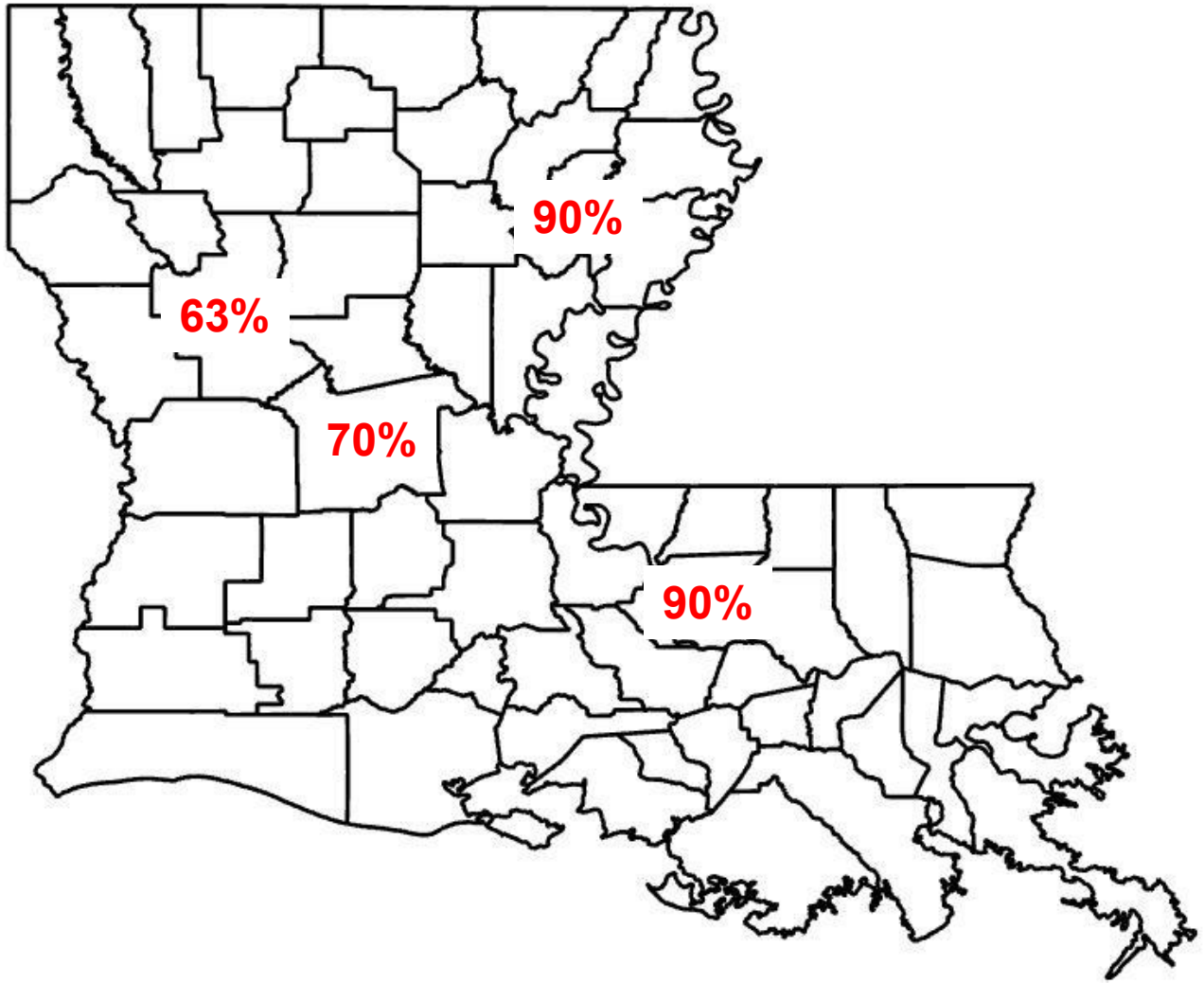
¶

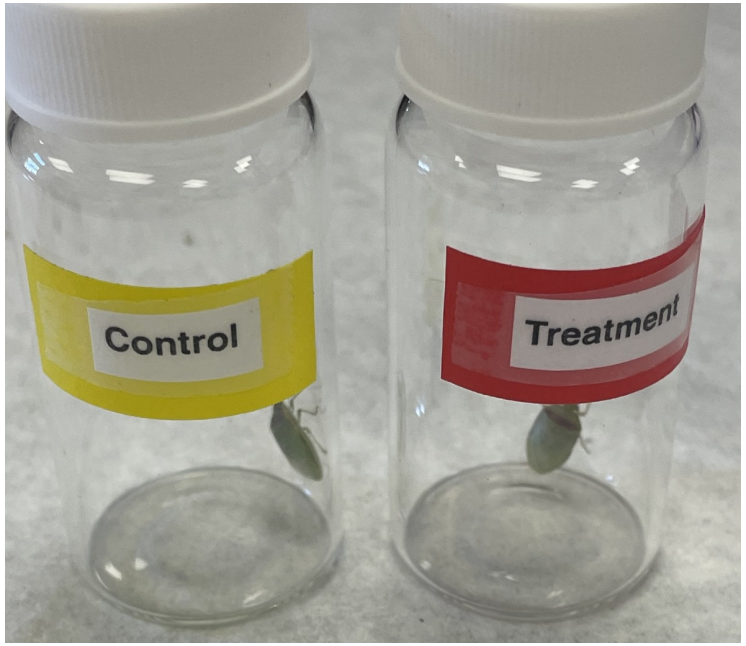
1. → Open·kit·and·check·contents.·It·should·include·20·vials·(10·labelled·control·and·10·labelled·treatment)·plus·a·pair·of·gloves.¶
2. → Be·sure·insects·used·in·the·test·are·active.·Discard·any·dead·or·inactive·insects.¶
3. → Wear·gloves·before·opening·vials.¶
4. → Start·with·control·vials.·¶
5. → Carefully,·place·one·live·insect·in·each·vial.¶
6. → Close·vial·tightly·and·then·loosen·lid·¼·turn.¶
7. → Check·that·insects·are·over·the·glass·and·not·hiding·inside·the·lid.·They·must·walk·over·interior·or·bottom·of·the·vials.·If·they·are·hiding·in·the·lid,·remove·them·by·softly·tapping·the·vials.¶
8. → Place·vials·on·a·flat·surface·for·4·hours·at·room·temperature.·Vials·should·not·be·under·direct·sun.¶
9. → Wait·4·hours·to·record·insect·mortality.¶
10. → Check·one·vial·at·the·time·on·a·flat·and·clean·surface.·Start·with·control·vials.·Remove·each·insect·from·the·vial·and·proceed·to·confirm·if·is·alive·(A)·or·dead·(D).·Insects·flipped·upside·down·no·turning·in·more·than·5·seconds·are·considered·dead,·as·well·as·insects·showing·slow·or·uncoordinated·motions.¶
11. → Record·mortality·results.·Every·dead·insect·will·represent·10%·mortality.¶



Initial Monitoring Revealed Reduced Efficacy







Insecticide Resistance Monitoring:

- **Redbanded stink bug**
- ***E. quadrator***

Acephate

2025: LC_{50} 15.0 ppm
2024: LC_{50} 10.0 ppm
2023: LC_{50} 10.0 ppm
2022: LC_{50} 10.0 ppm
2021: LC_{50} 10.0 ppm
2020: LC_{50} 10.0 ppm
2015: LC_{50} 10.0 ppm
2011: LC_{50} 6.0 ppm
2010: LC_{50} 3.5 ppm

Increased last year

Bifenthrin

2025: LC_{50} 5.0 ppm
2024: LC_{50} 5.0 ppm
2023: LC_{50} 2.5 ppm
2022: LC_{50} 2.5 ppm
2020: LC_{50} 2.5 ppm
2010: LC_{50} 0.5 ppm

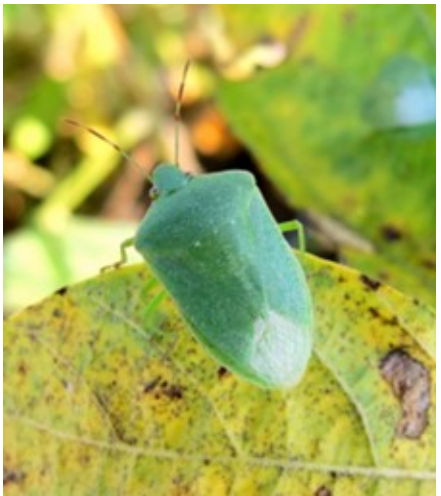
No change last year

Acephate

2025: LC_{50} 0.3 ppm
2024: LC_{50} 0.3 ppm
2023: LC_{50} 0.3 ppm

No change last year

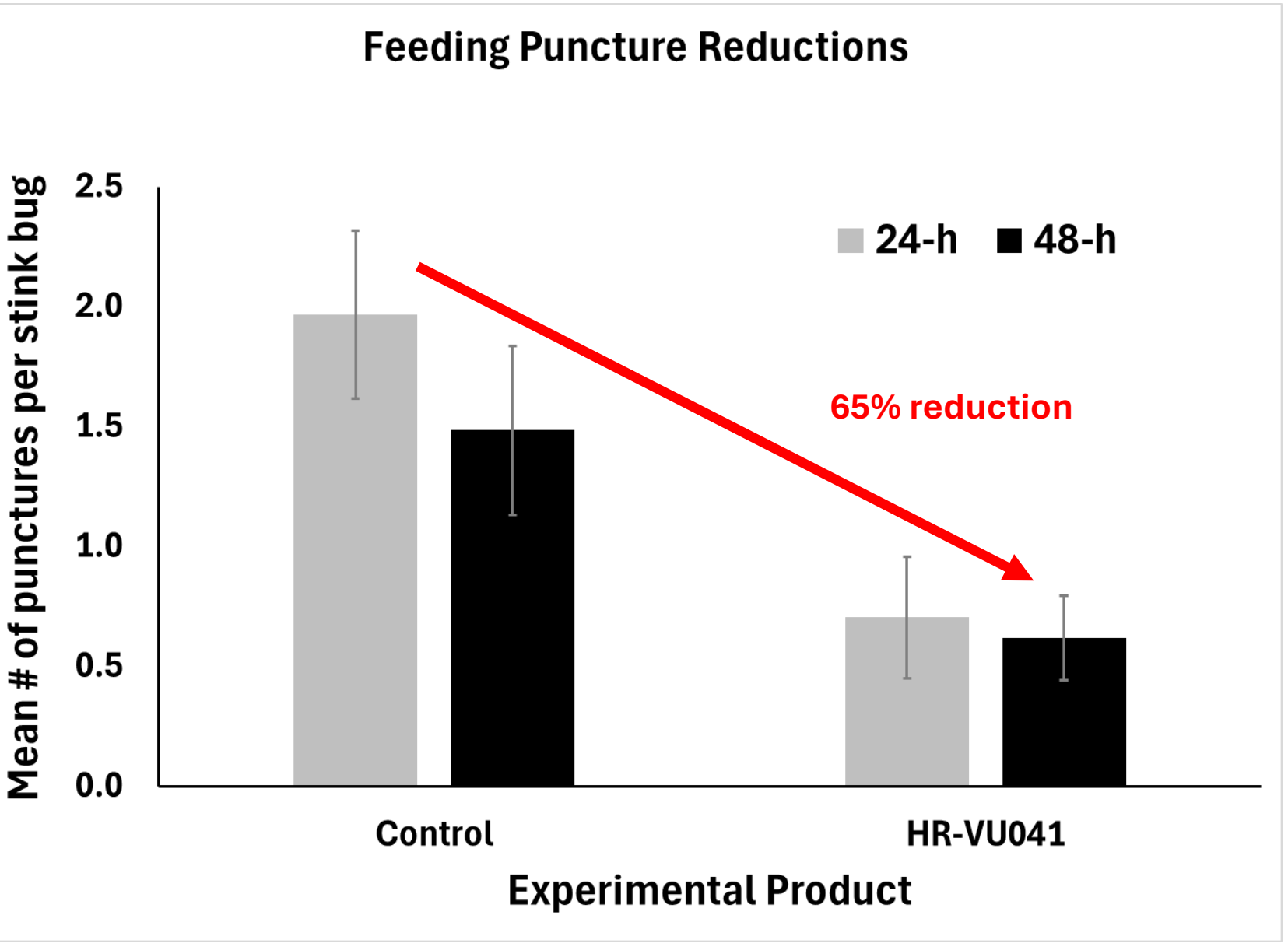
Lessons Learned from 2025

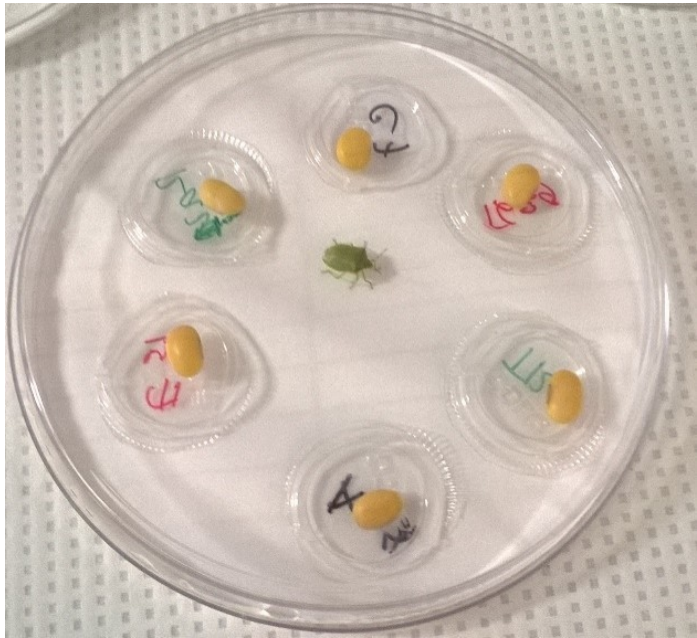


- **Test early and often (cover crops and soybeans)**
- **Prepare kits a week in advance of use**
- **Expand testing network: we need many more samples**

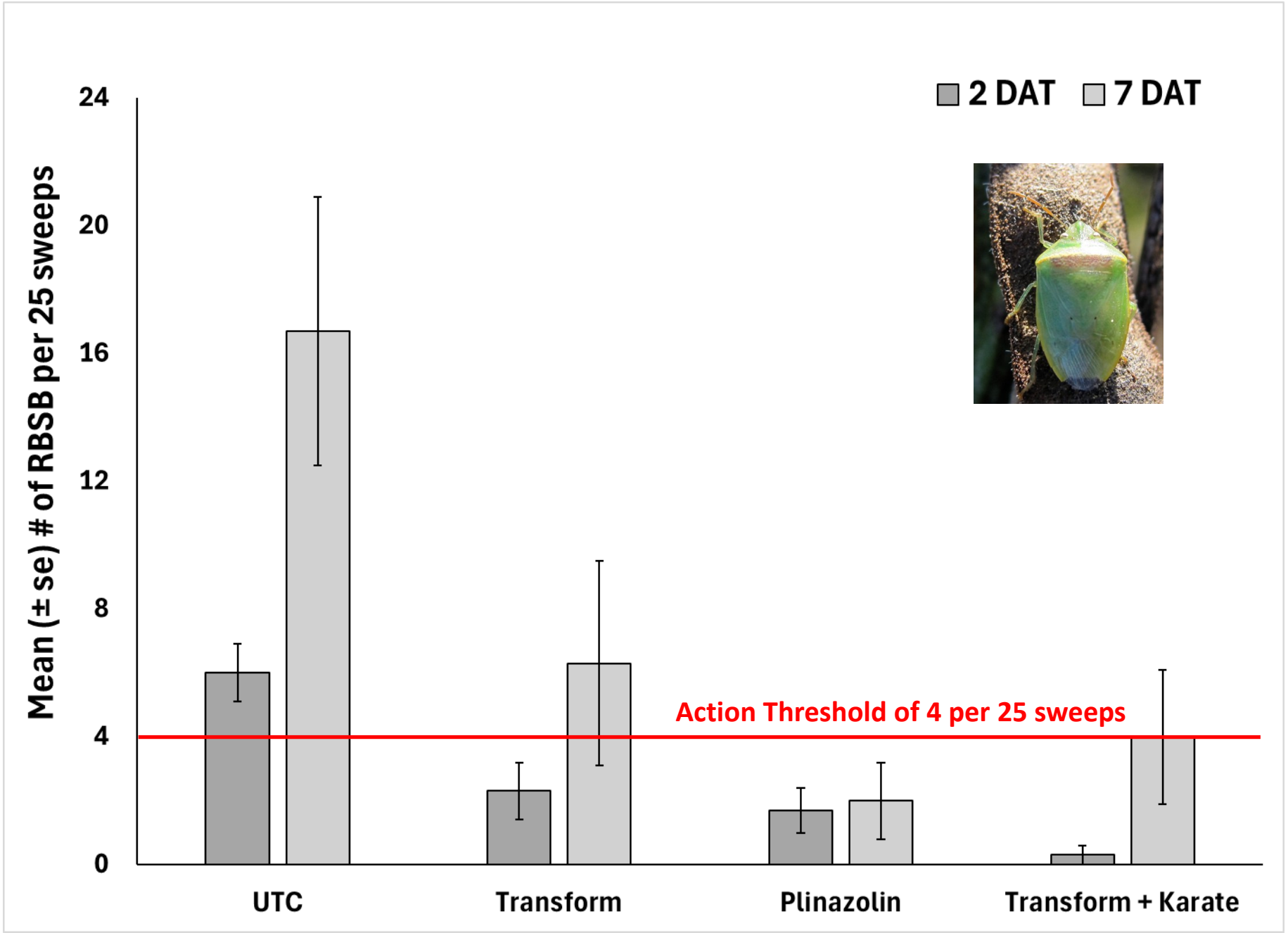
Kir inhibitor effects on stylet sheath formation and mortality

- Southern green stink bug





Evaluate untested commercially available insecticides for stink bug control



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PULL HERE TO OPEN

ISOCYCLOSERAM | GROUP 30 | INSECTICIDE

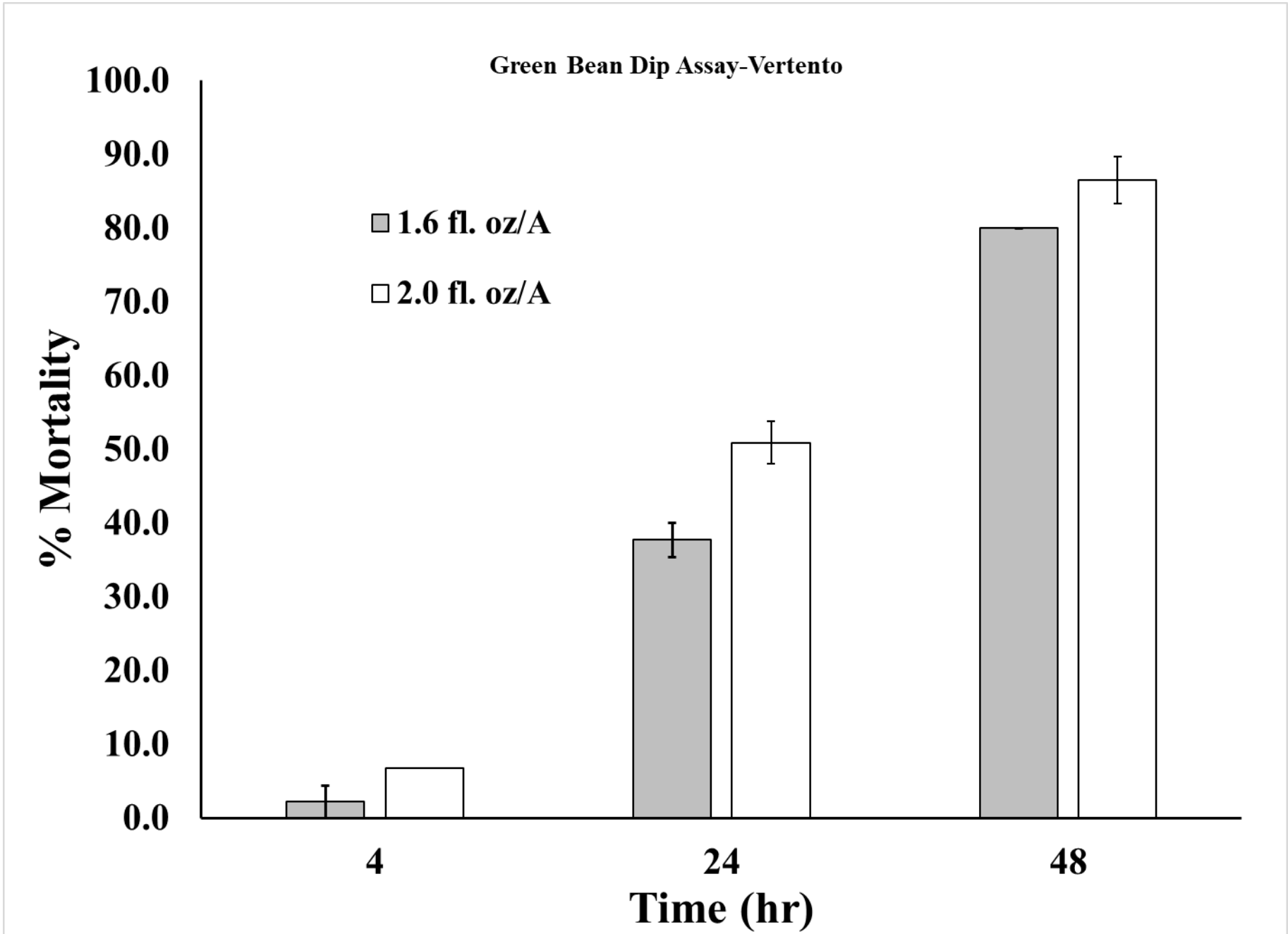
Vertento®

Escanee QR para Español

syngenta.

Insecticide

PLINAZOLIN® technology*



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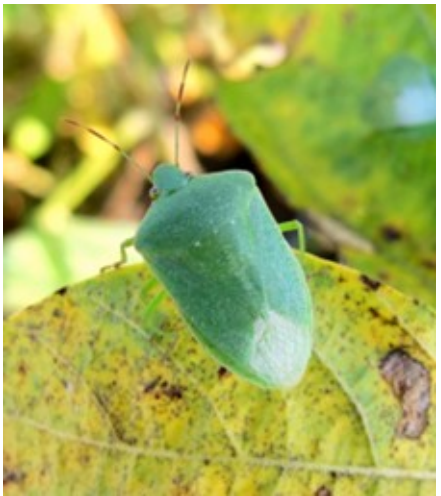
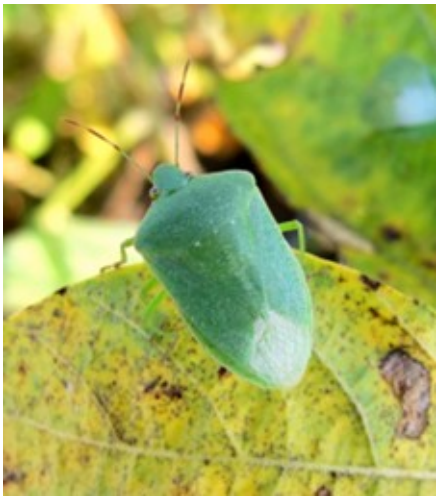


Summary

- Redbanded stink bug is resistant to insecticides
- Mechanisms of resistance involve metabolism and behavior
- Utilizing monitoring kits detected a change in resistance
- This change was confirmed via laboratory testing
- We continue to monitor for resistance, and we have test kits available
- Vertento (isocycloseram) is labeled for soybean and the use rate for redbanded stink bug is 2.0 fl. oz/A.
- Vertento kills slower than acephate or a pyrethroid



Thank you

Questions?

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jeffdavis@agcenter.lsu.edu