



# Insecticide Resistance Status and Mechanisms in Field Populations of the Tarnished Plant Bug *Lygus lineolaris*



*Yu Cheng Zhu*  
*Randall Luttrell*

**USDA-ARS**

**Jamie Whitten Delta States Research Center**  
Stoneville, Mississippi, USA





## Tarnished Plant Bug (TPB)

- Originally secondary pest on cotton
- Wide range of host plants species
- Local movement from one host to another host
- Extensive adoption of Bt cotton
- Reduced chemical sprays
- Insecticide resistance development
- It has become one of the most important pests on cotton

# Insecticides for Plant Bug Control

- Recommended by Delta Ag. Digest 2012
  - Organophosphates: acephate, dicrotophos, dimethoate
  - Carbamates: oxamyl,
  - Neonicotinoids: clothianidin, flonicamid, imidacloprid, thiamethoxam
  - Others: novaluron
- A cotton farmer's list:
  - Orthene (OP), Bidrin (OP), Centric (thiamethoxam),
  - Vydate: (Carb), Tombstone (cyfluthrin), Permethrin

# Multiple/Cross Resistance ???

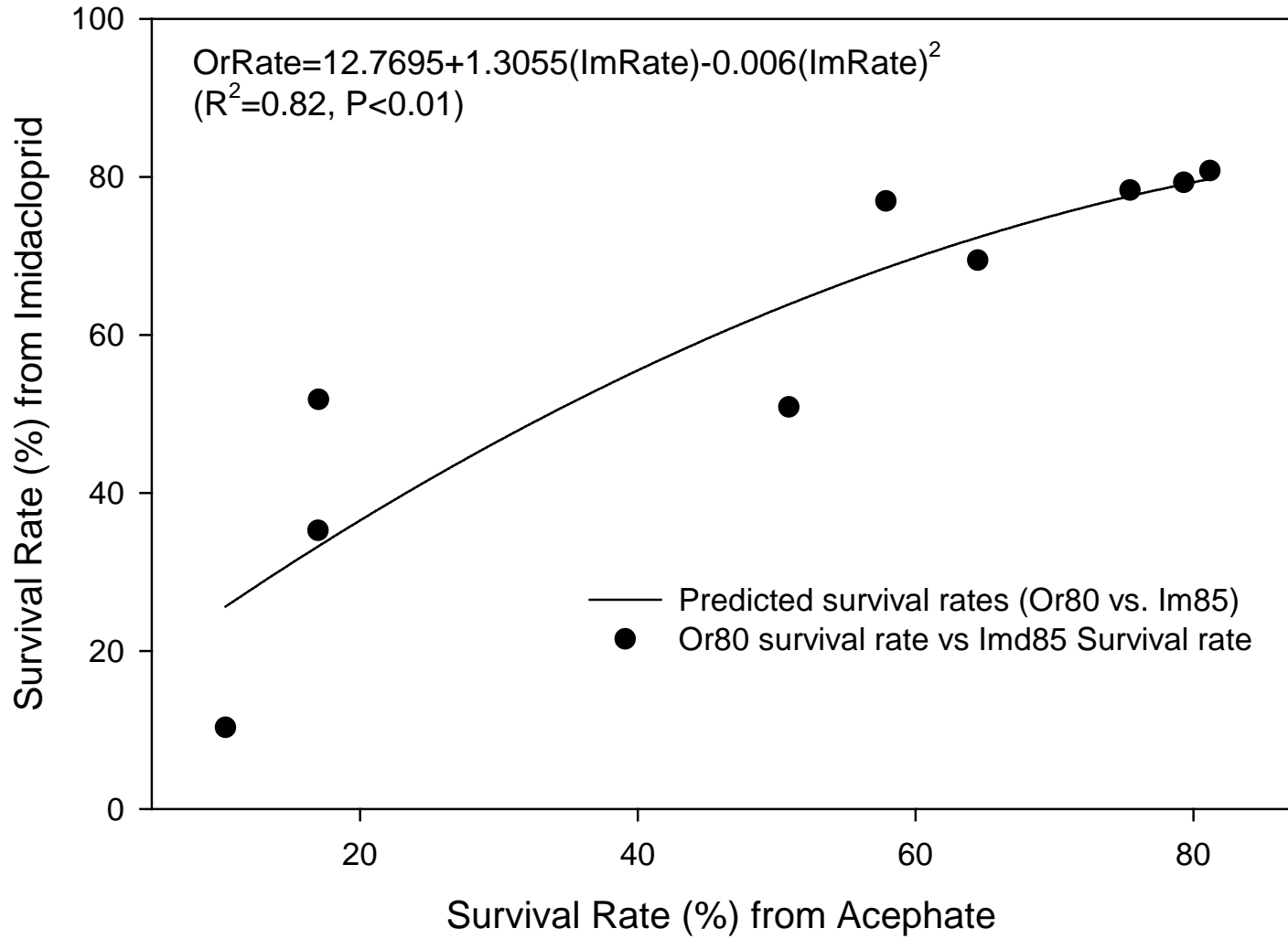
- Multiple resistance is the phenomenon in which a pest is resistant to more than one class of pesticides
- Cross-resistance is the tolerance to a usually toxic substance as a result of exposure to a similarly acting substance

# Objectives

- Investigate resistance, multiple/cross resistance in field populations of TPB
- Evaluate association of resistance development with different crop systems: (1) cotton, (2) cotton/corn/soybean, (3) non-cotton
- Examine resistance mechanisms

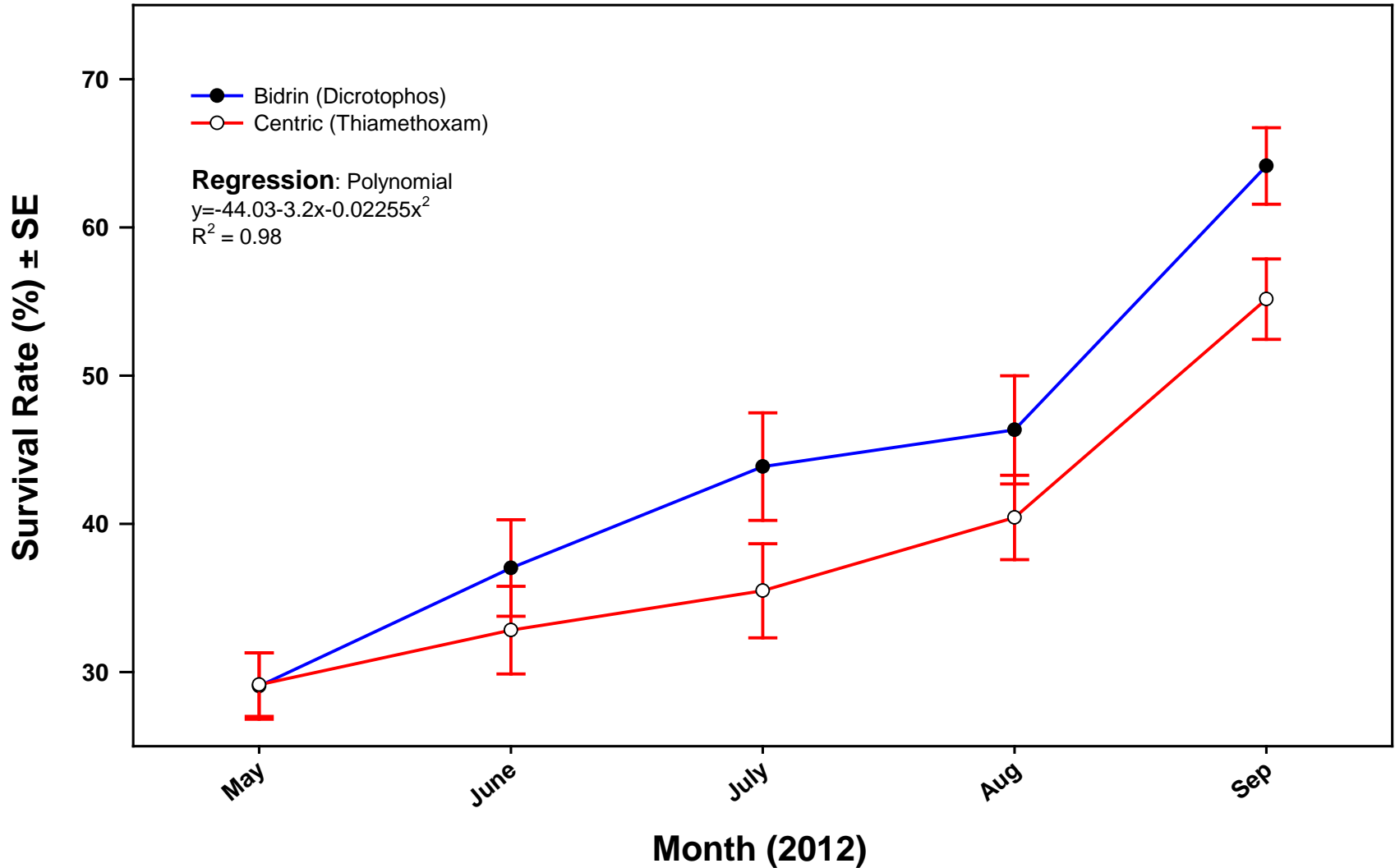
# Bioassay: Precise Spray Using Modified Potter Spray Tower





Relationship between OrRate and ImRate

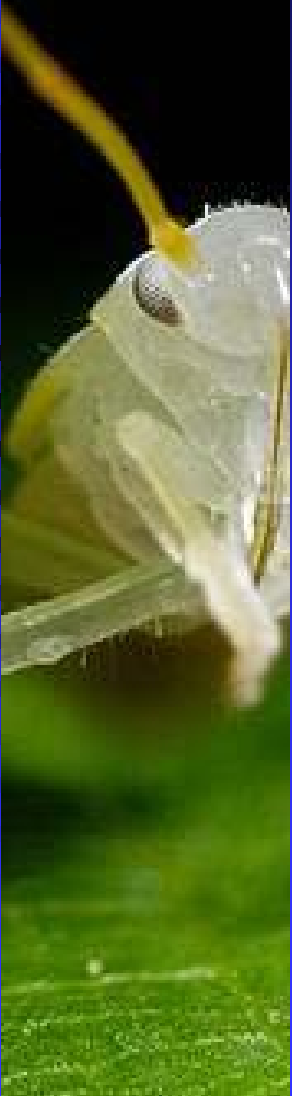
# Dose Response of TPB to Bidrin and Centric in 2012



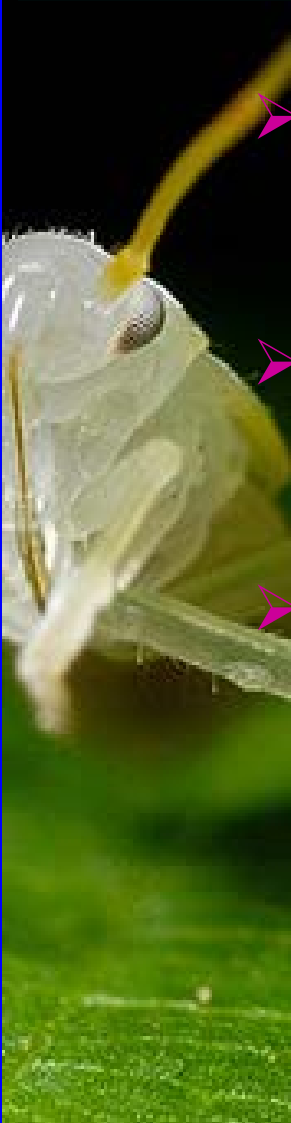


# Summary: Resistance Levels

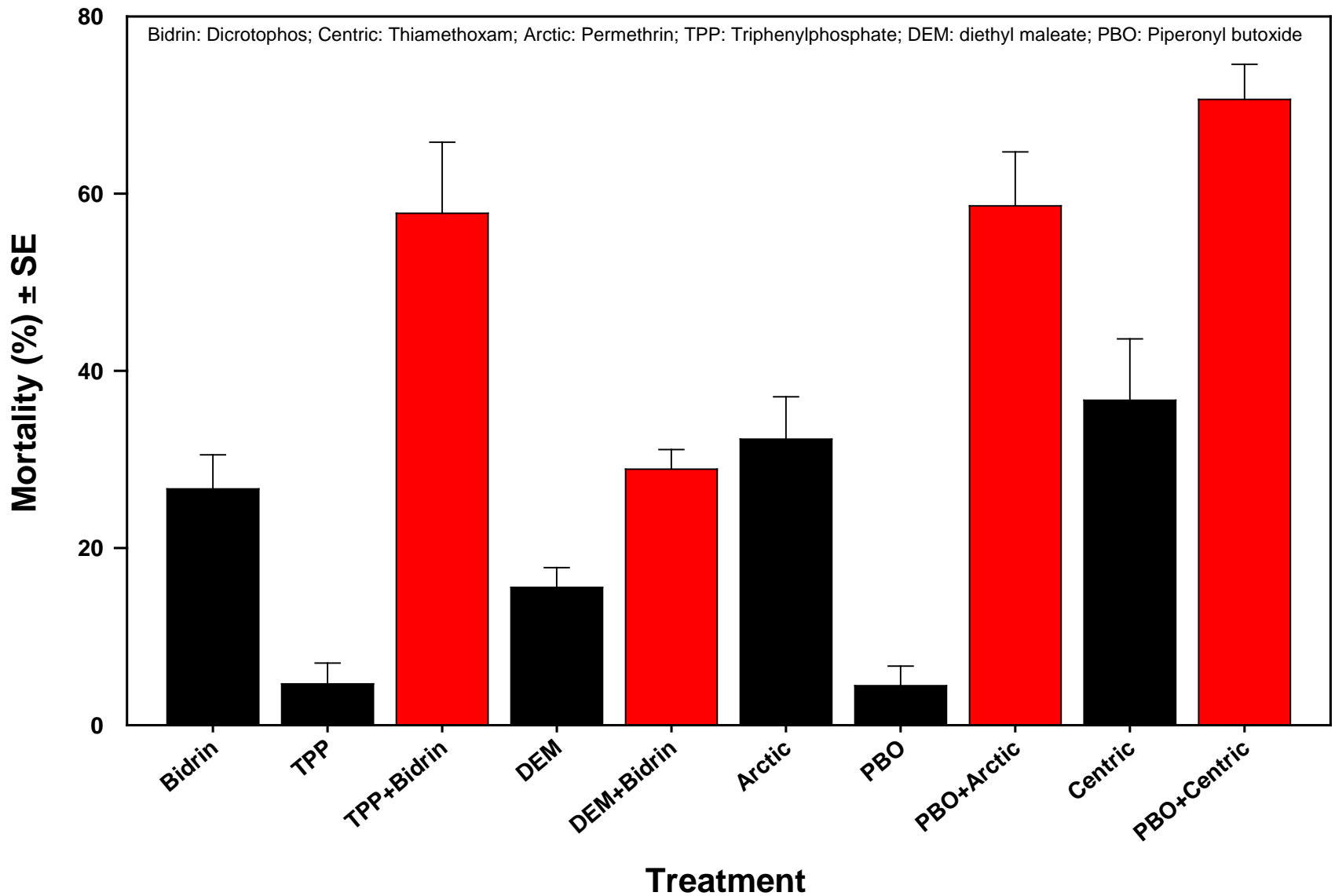
- Change over season
- Vary from farm to farm, from field to field
- Associated with crop system and spray intensity
- Associated with population density



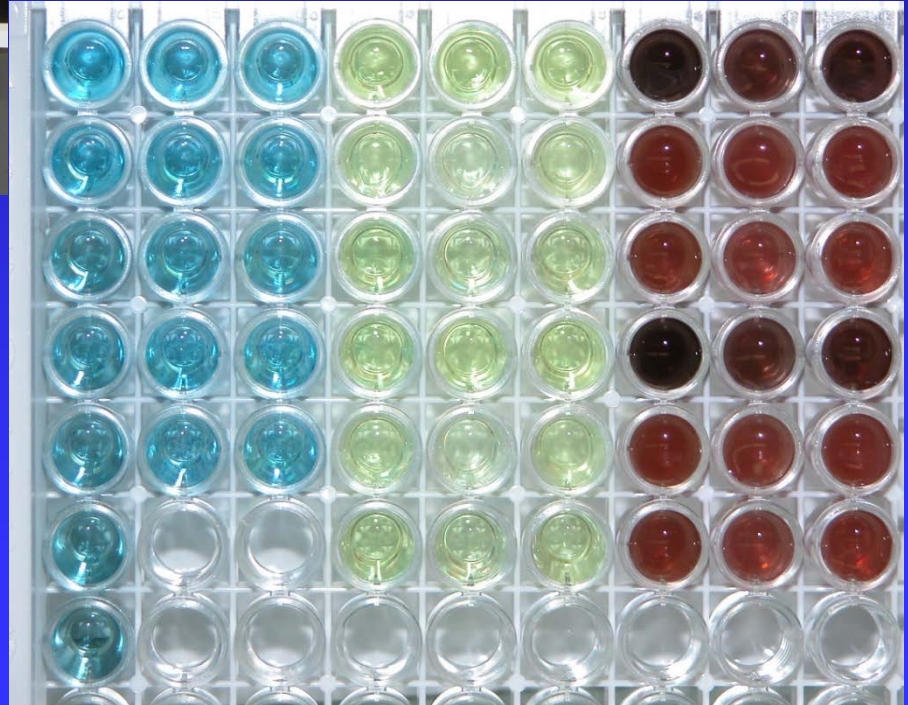
# Examination of Insecticide Resistance Mechanisms

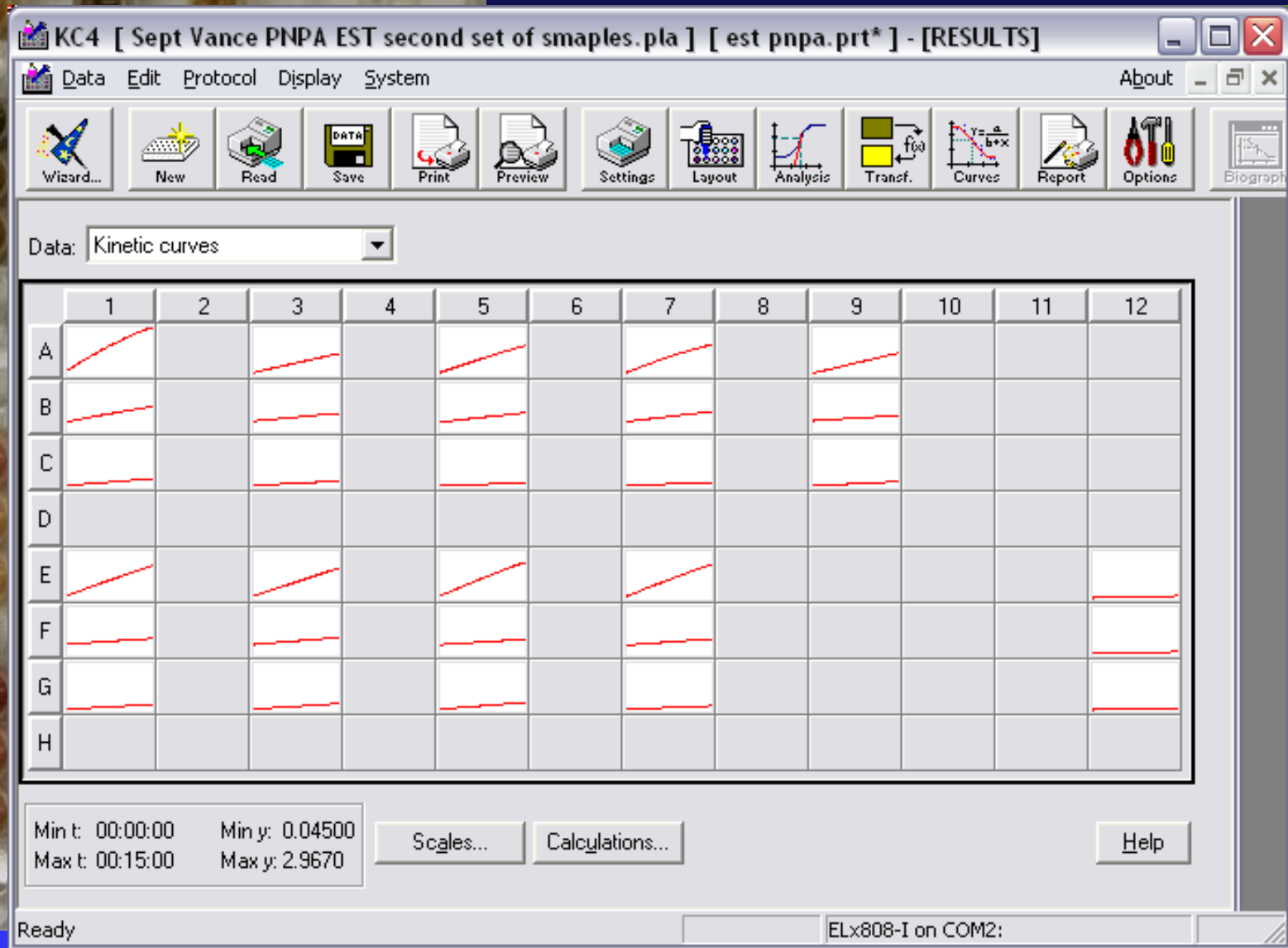
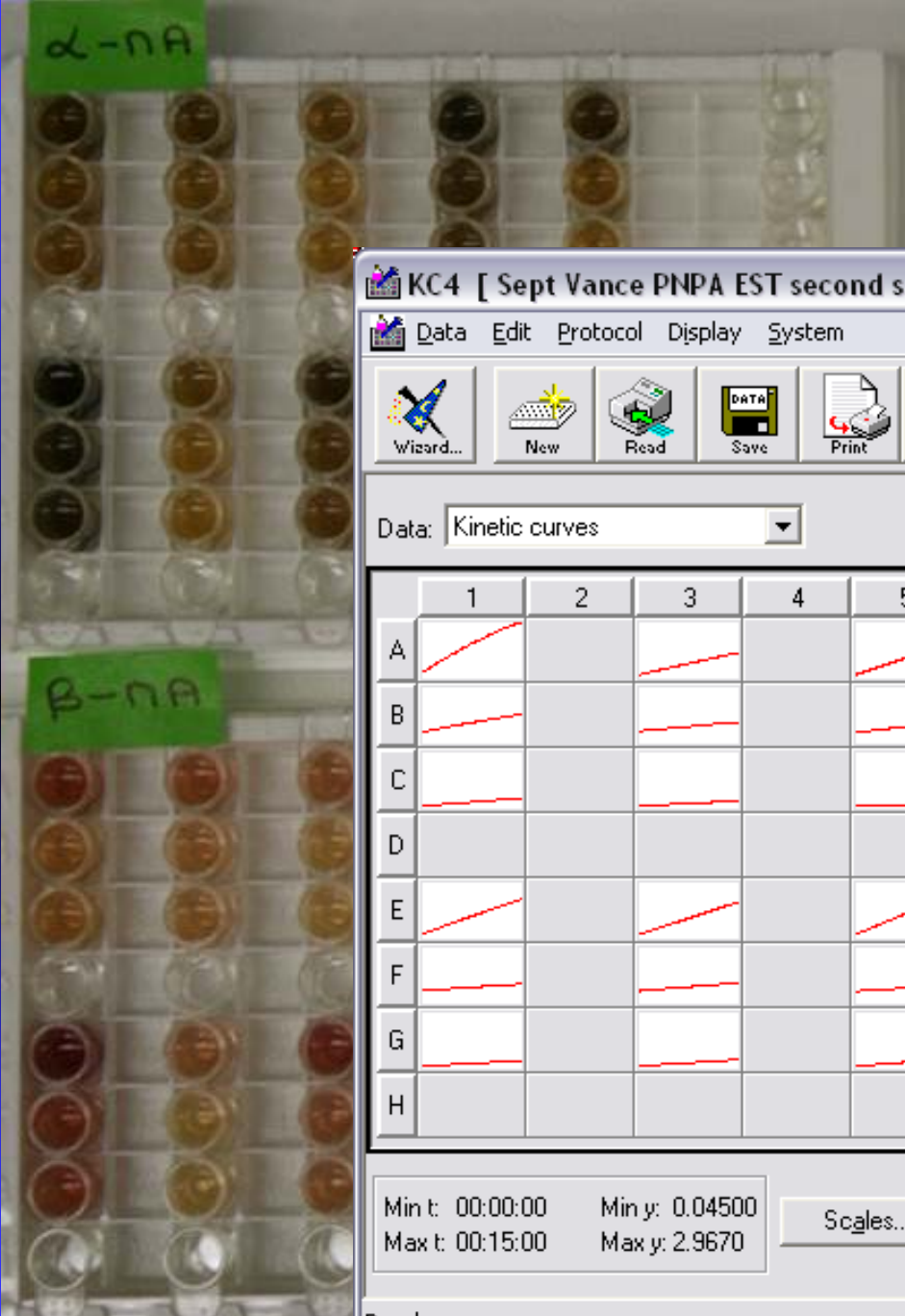
- 
- Synergism with detoxification enzyme inhibitors
    - Bioassay
    - Enzyme activity assay
  - Gene regulation profile
    - Microarray analysis of 7,000+ genes
    - Real-time PCR: detoxification candidate genes
  - Gene structure/sequence analyses
    - Esterases
    - Cytochrome P50 oxidases
    - Glutathione S-transferase

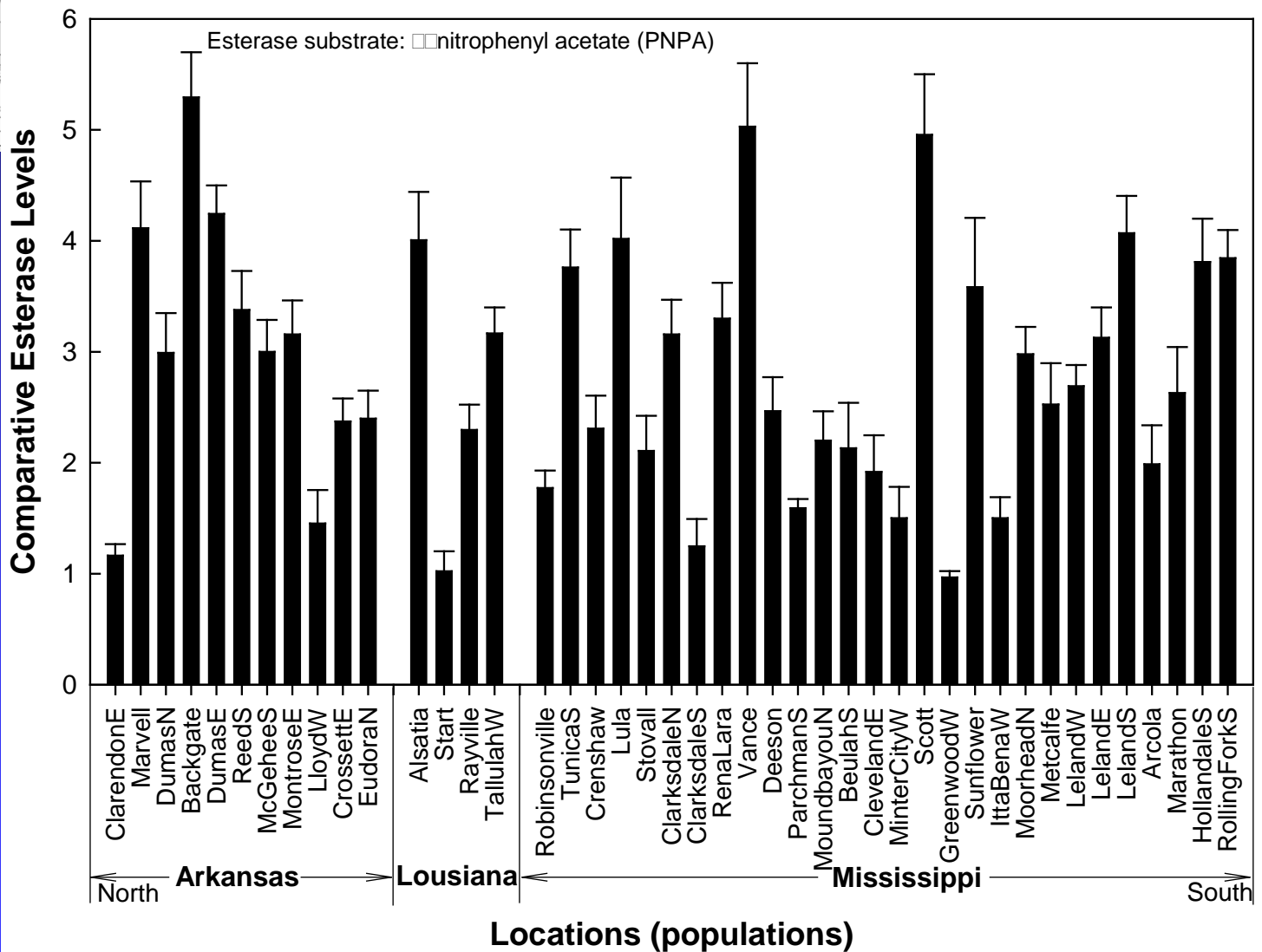
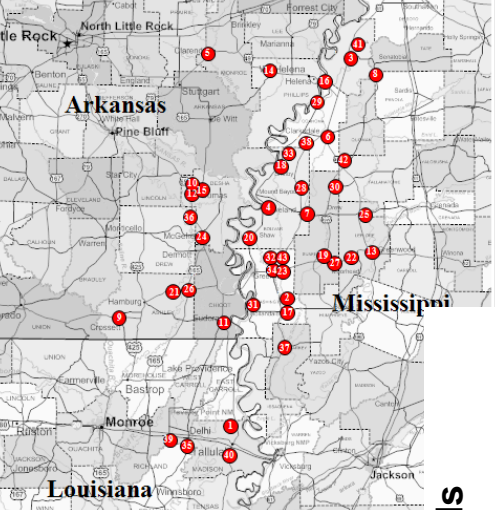
# Synergism of esterase, GST, and P450 inhibitors against Bidrin-selected TPB

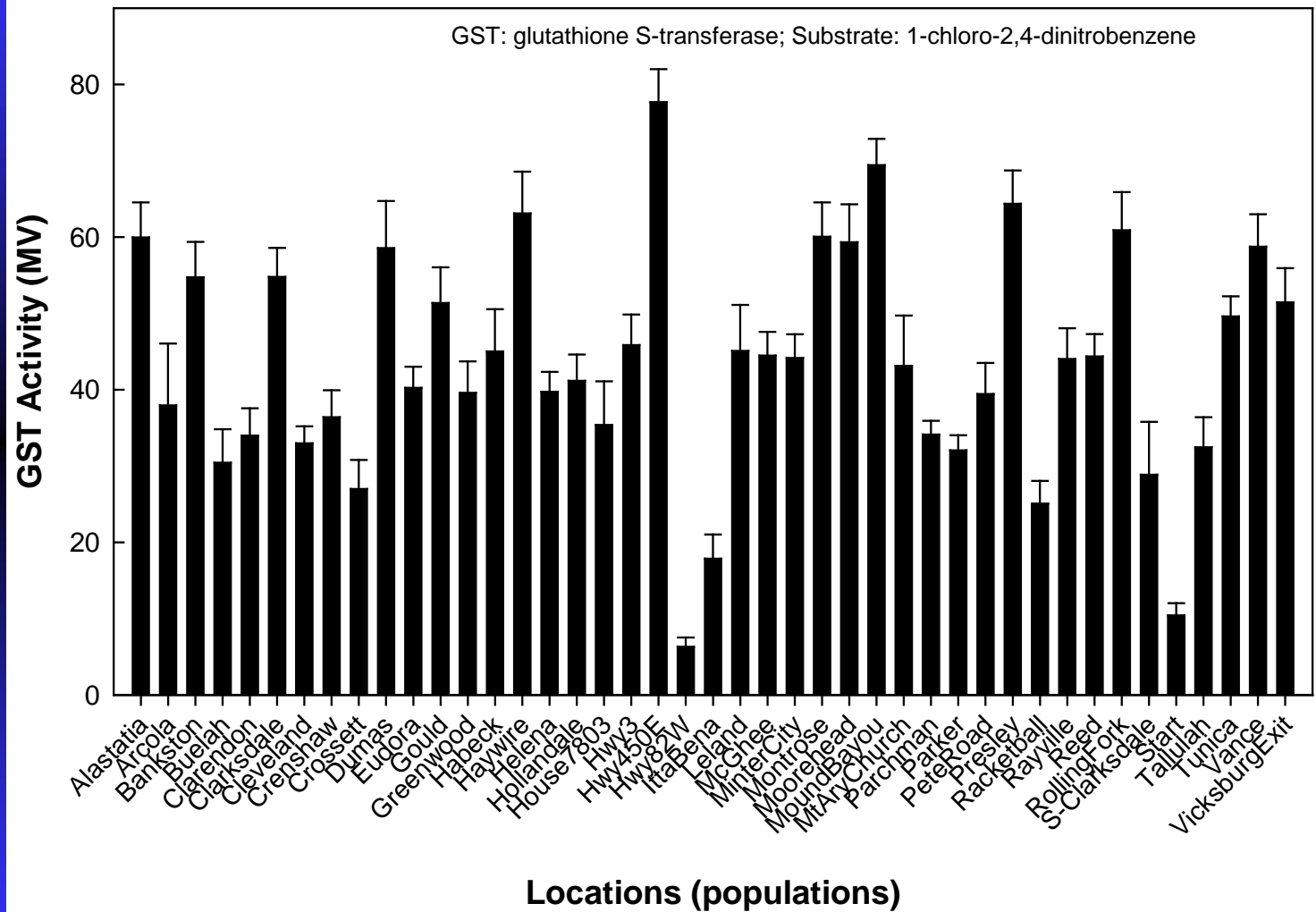


# Examine and Compare Detoxification Enzyme Activity

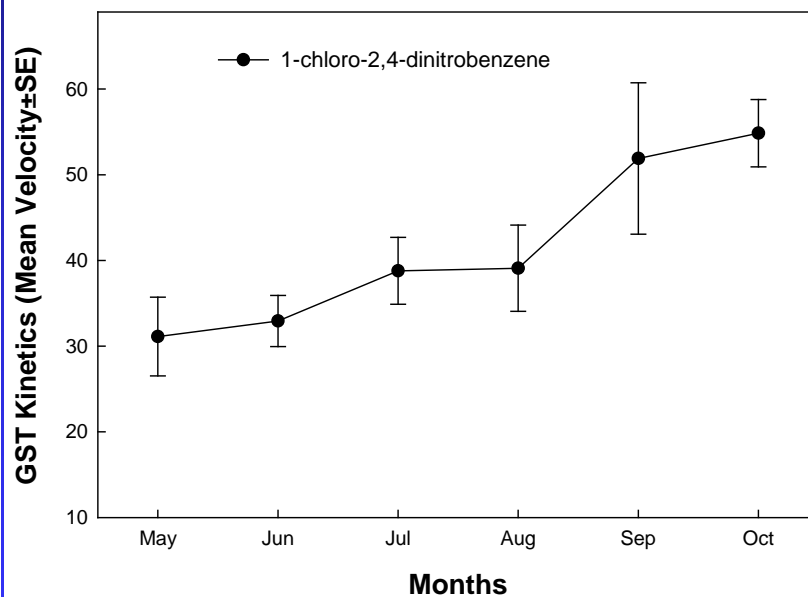
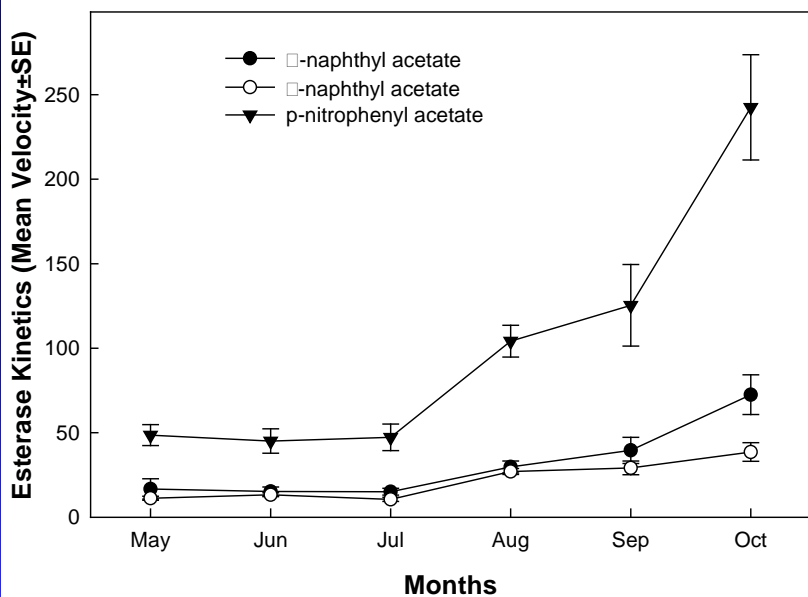








# Changes of Esterase and Glutathione S-transferase Activities

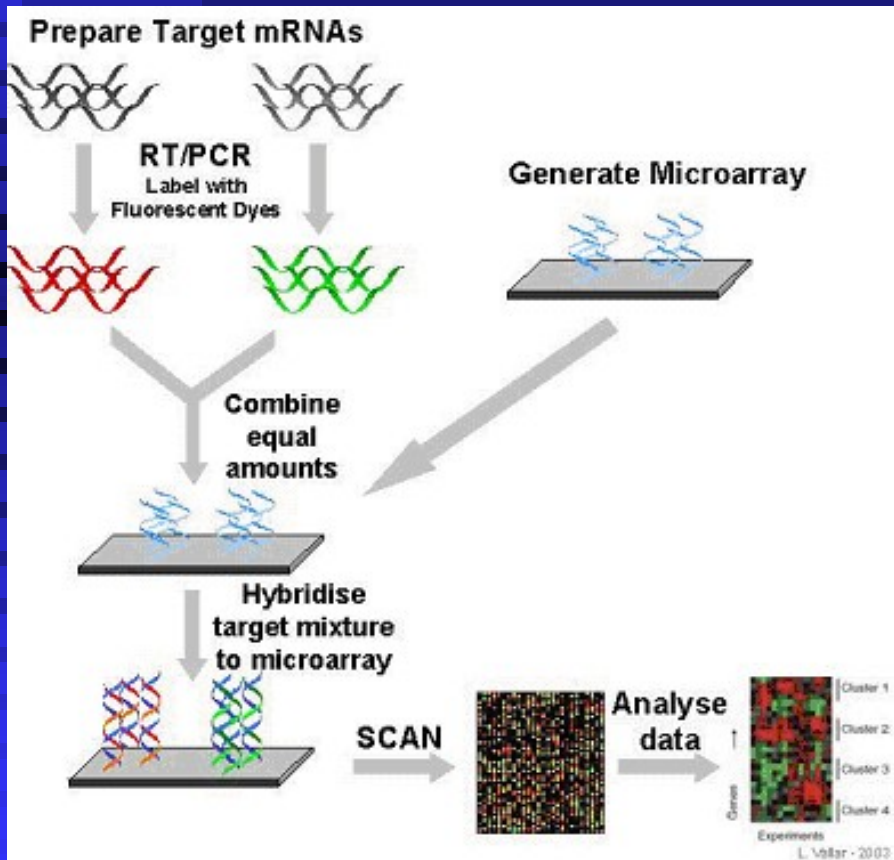




## Biological Response and Enzymatic Activities in Acephate-treated Tarnished Plant Bug

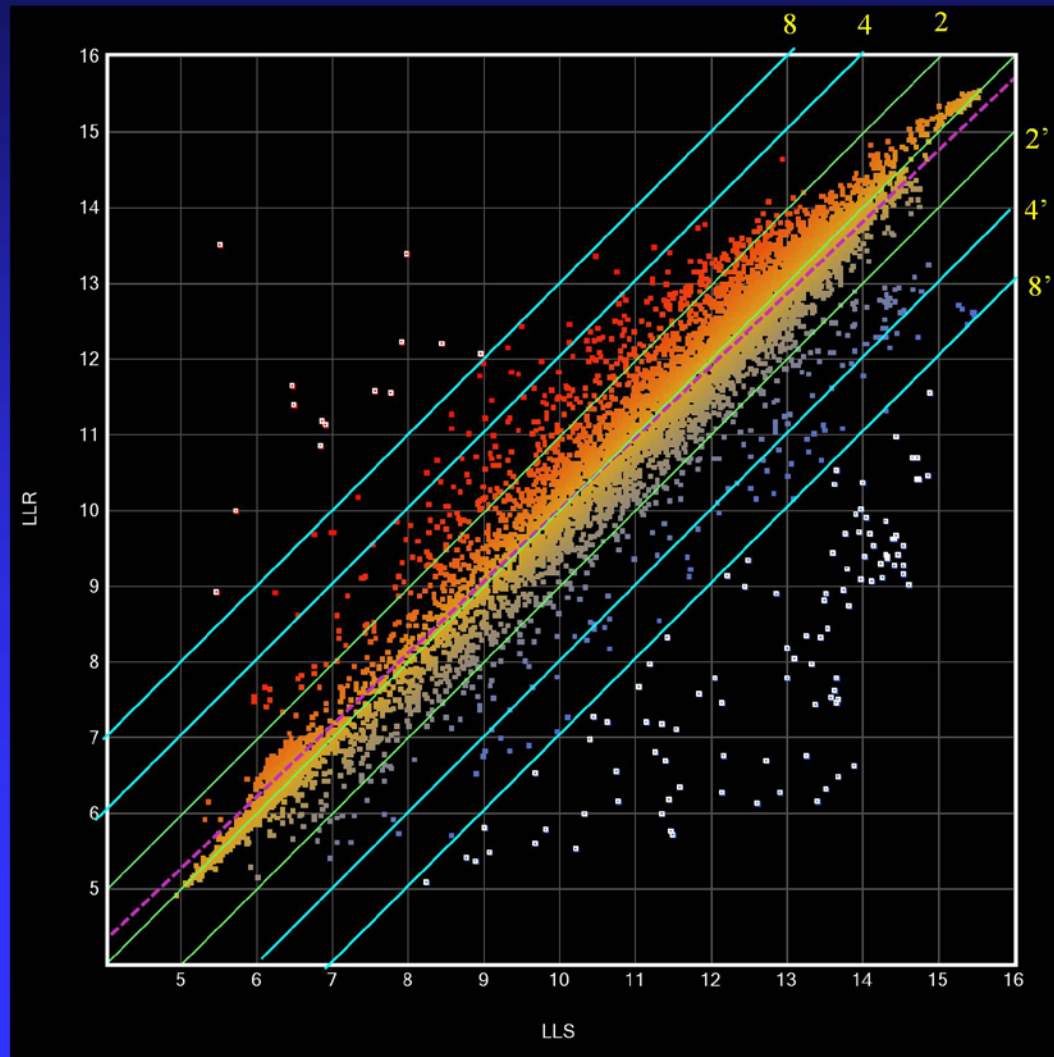
Insect	Bioassay		Esterase: $\alpha$ -NA		Esterase: $\beta$ -NA		Esterase: PNPA		GST: CDNB	
	LC <sub>50</sub> a.i. (95%FL)	Ratio	MV $\pm$ SE	Ratio	MV $\pm$ SE	Ratio	MV $\pm$ SE	Ratio	MV $\pm$ SE	Ratio
LLS	148.3(122.9-174.8)	1	21.6 $\pm$ 2	1	19.4 $\pm$ 1.5	1	29.7 $\pm$ 3.5	1	39.2 $\pm$ 1.6	1
Lula	321.1(257.8-402.5)	2.2	54.3 $\pm$ 6.3	2.5	27.3 $\pm$ 2.4	1.4	39.2 $\pm$ 3.9	1.3	27.7 $\pm$ 1.9	0.7
Lula600	874.4(742.7-1042)	5.9	97.7 $\pm$ 14.3	4.5	55.8 $\pm$ 9.1	2.9	81.7 $\pm$ 8.2	2.8	35.9 $\pm$ 3.1	0.9

# Resistance Gene Expression: Microarray

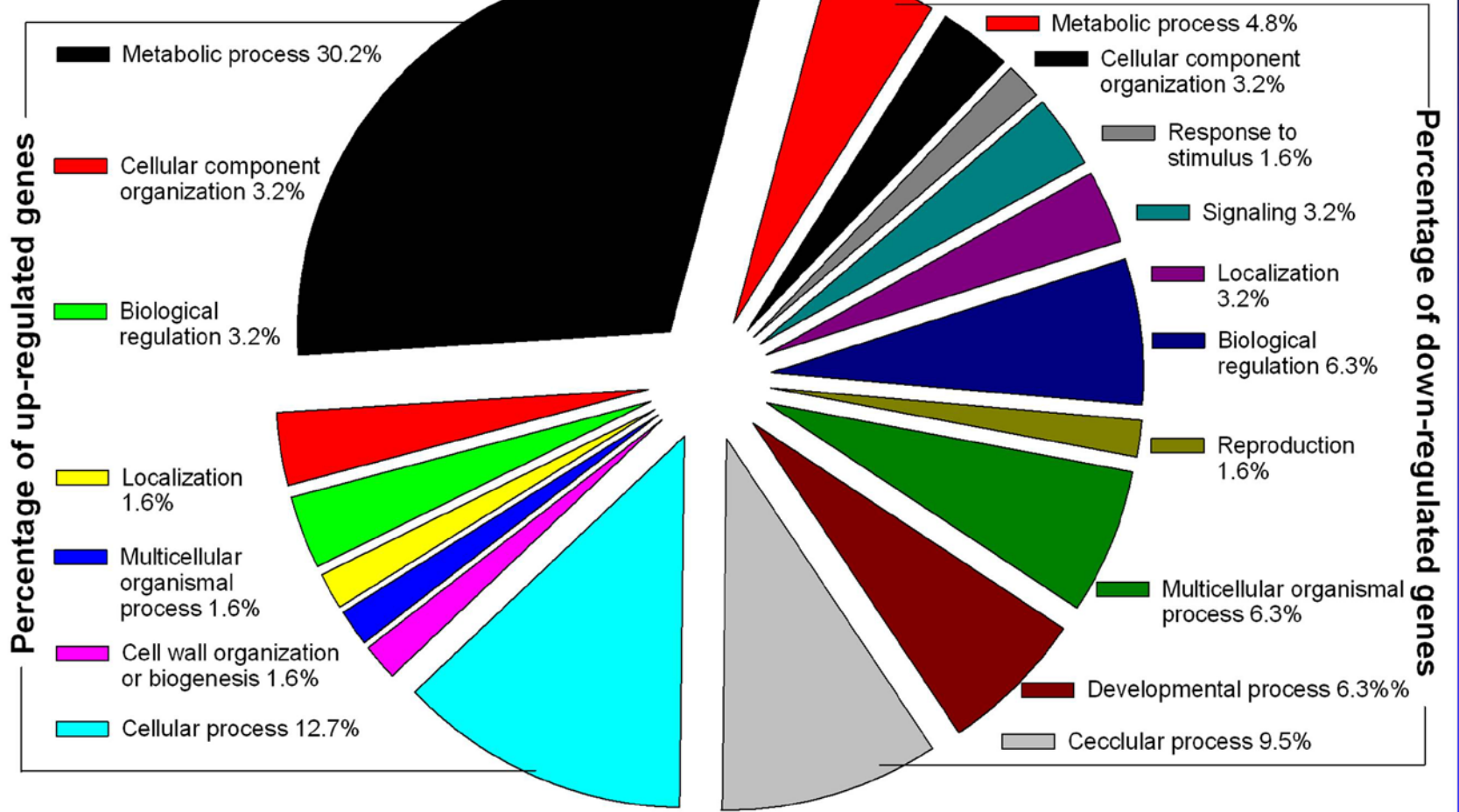


- Roche-Nimblegen 4-plex 72k expression DNA chips
- 60-bp probe
- ~5 repeats per sequence
- Tarnished plant bug samples: OP-S and OP-R
- Run 4 DNA chips (repeats) per sample
- Prepare total RNA and ds-cDNA
- Labeling, hybridization, scanning
- Data collecting and processing with ArrayStar.

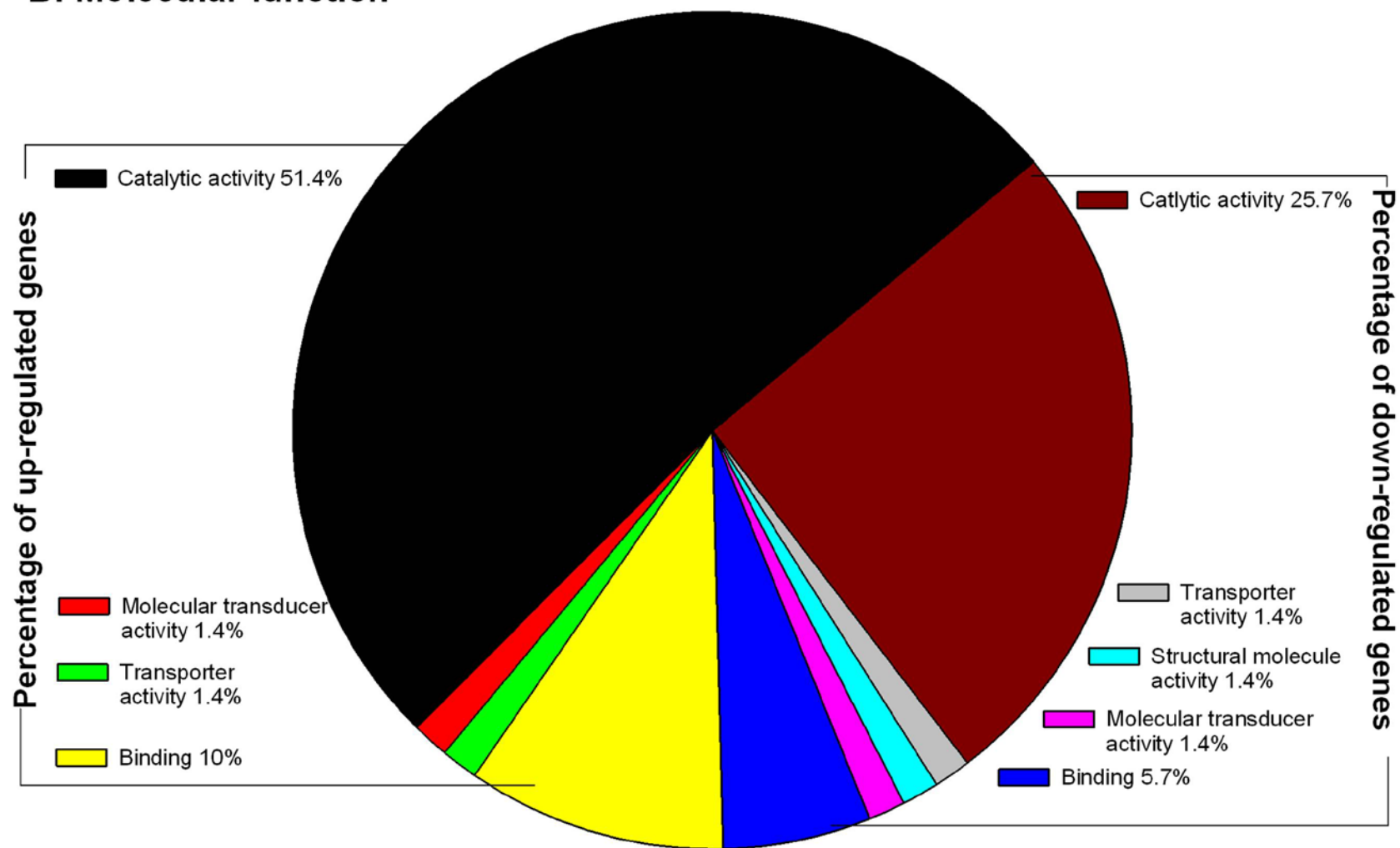
# 635, 186, and 113 Genes Were Up- and Down-regulated with 2-, 4-, 8-fold Changes in OP-R Strain



# A: Biological process



## B: Molecular function



# Summary: Insecticide Resistance Mechanisms



- Many TPB field populations have developed multiple/cross resistance to different classes of insecticides.
- Elevated metabolic detoxification is the major insecticide resistance mechanism.
- Esterase and cytochrome P450 oxidase genes are mainly responsible for the resistance development.
- Down-regulation of major reproductive and digestive related genes are associated with fitness cost of the resistance.
- Gene structure modifications are minor.



**Thank You Very Much  
for Your Attention!**

Contact me at 662-686-5360  
[yc.zhu@ars.usda.gov](mailto:yc.zhu@ars.usda.gov)