Spatial and temporal diversity of polyphagous pests: corn earworm or bollworm (*Helicoverpa zea)*

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Entomological Society of America Annual Meeting

December 14, 2010

Acknowledgments

All my graduate students.

- Dr. Michael Longnecker Prof. of Statistics, TAMU.

Research Assistants

- Lori Nemec
- Jessica Moore (now LSU)
- Terry Junek
- AgriLife Extension Collaborators
- *H. zea* experts: S. Fleischer, B. Hutchison, J. Westbrook.

Funding Provided By:

- IRAC (Savinelli, Head)
- Cotton, Inc. (O'Leary)
- C. Everette Salyer Fellowship in Cotton Research (TAMU) to B.W. Hopkins
- Texas AgriLife Research

Introduction: *Helicoverpa zea*

- Highly polyphagous (>100 plant spp.)
- Migratory
- Larvae are important pest of cotton (bollworm), corn (earworm), and grain sorghum (headworm)
- Multivoltine species (5-7 generations per year in Texas)
- Feeds on uncultivated hosts

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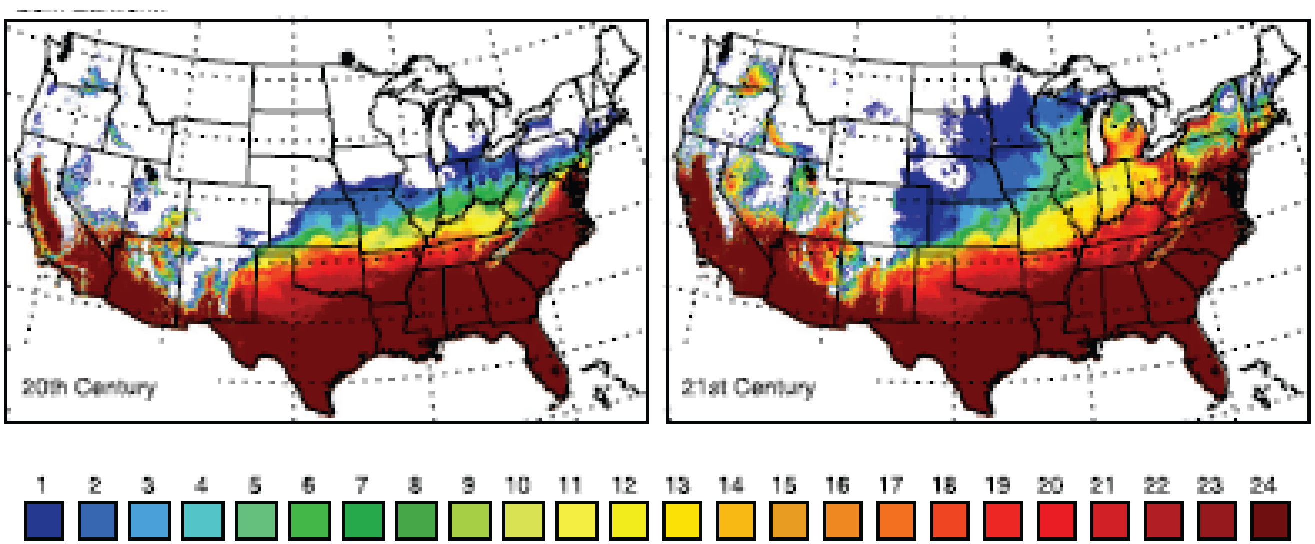
Economic Impact: only in 2009 second to hemipterans in damage to cotton

- Estimated annual damage of *H. virescens* and *Helicoverpa zea* on all US crops was $1 billion (Fitt, 1989)
  - After $250 million on insecticide control
- Currently majority of heliothine complex is represented by *Helicoverpa zea* individuals
- 125,000 to 520,000 bales lost per year (Williams, 2005-2009)

Challenge: The suitable area for corn earworm is expected to expand with climate change

Current and future temperature envelope for a migratory, cosmopolitan taxon.

Color contours show the number of years that are suitable (out of a maximum of 24)

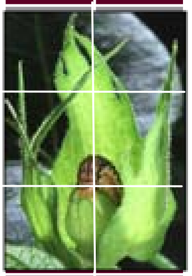


20th century distribution (left panel)

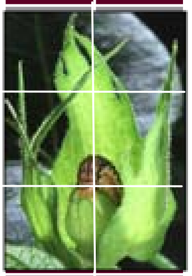
21st century distribution (right panel)

N.S. Diffenbaugh et al., Environ. Res. Lett 3 (2008)

Pyrethroids: effective and economical to control *H. zea*



- Pyrethroid resistance had been reported in Arkansas, Texas 1988, Illinois 1991; Cypermethrin 3-18 fold (Abd-Elghafar et al., 1993; *Kanga et al., 1996).*
- Resistance to cyhalothrin and cypermethrin in South Carolina in 1996 *(Brown et al., 1998, Crop protection 17:441-445; Walker et al., 1998).*
- Wide adoption of *Bt* cotton after 1996 controlled budworm (*H. virescens)* populations but *H. zea* required 1-2 insecticide applications in *Bt* cotton (Cry1Ac).
- In Texas, re-initiated pyrethroid monitoring in 1997.



The story:

How pyrethroid resistance research was adapted to address

Evolution of Resistance on a Variable Landscape and

with a Migratory Pest

Outline

- Cypermethrin resistance monitoring with Adult Vial Test began in 1997 in Burleson Co.

- Dosage validation 2 concentrations 3 µg/vial (LC_{99}) and 10 µg/vial (used previously for *H. virescens*)

- Statewide since 2003 using probit analysis (many concentrations): level and frequency of resistance

- Observed spatial patterns of resistance in Texas

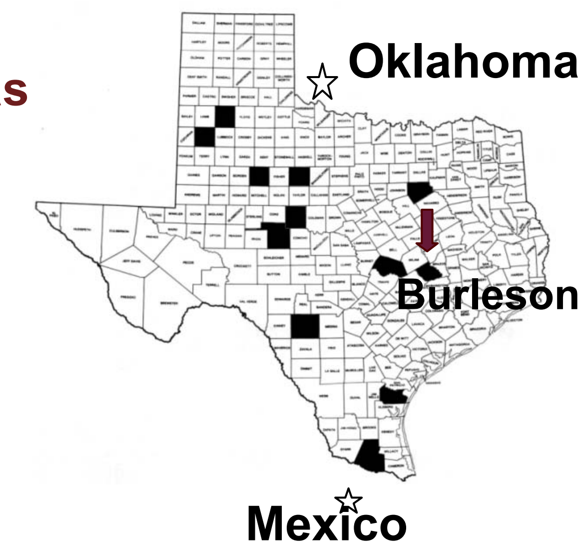
- Identify continuous source populations

- Investigated wind trajectories in relationship to patterns of resistance

- Expanded surveys to Oklahoma and MX

- Validated the AVT for *H. zea*

- Elucidated mechanisms of resistance present



Method: Adult vial test

- 1 larva or moth per vial: initially 2 dosages; evolved up to 9 dosages
- Mortality counts at 24 h of exposure
- Data analysis with PoloPlus
  - Likelihood ratio tests of equality and parallelism
- Probit graphs in SigmaPlot

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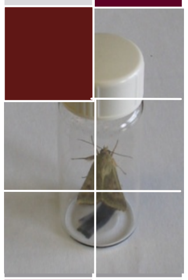
Dosage validation: Resistance to λ-cyhalothrin in adult *H. zea* is incompletely dominant



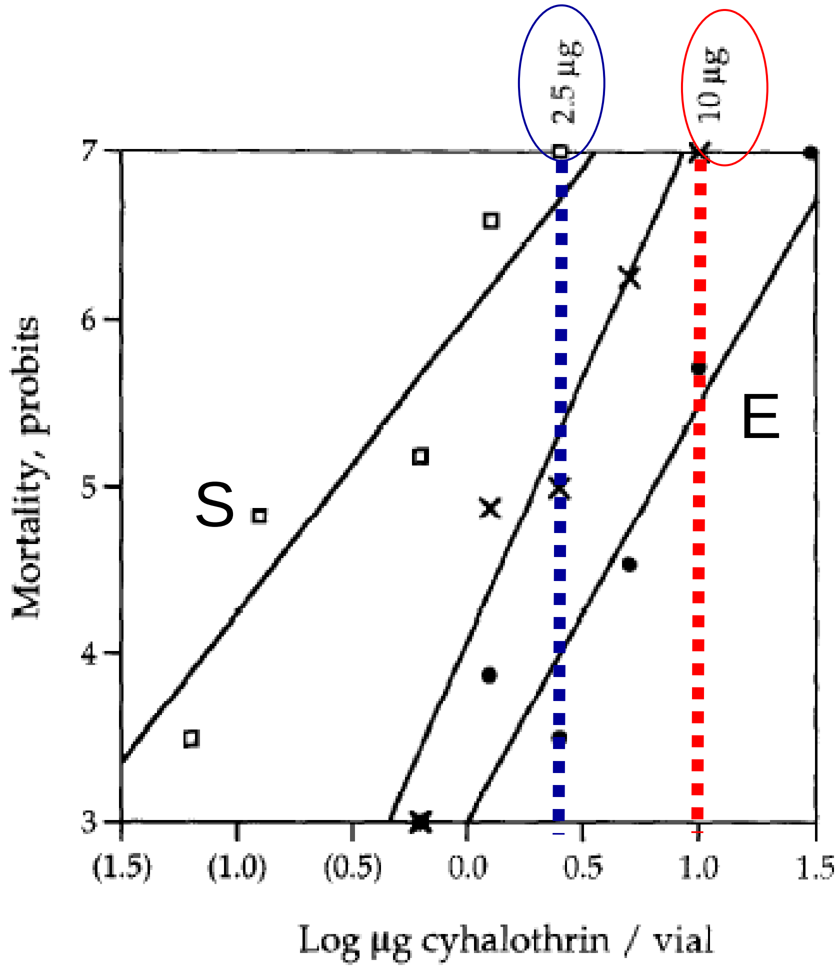


Figure 1. Susceptibility of adult *Helicoverpa zea* strains and hybrids exposed to cyhalothrin for 24 h. Open squares: *H. zea*-S; cross: Hybrid progeny of Estill96 and *H. zea*-S; filled circles: Estill96. Please refer to *Table 3* for statistical data.

- **Estill96 Resistant**

X Susc. x Estill96

First demonstration of cyhalothrin resistance in *H. zea*

First record of pyrethroid resistance in S. Carolina

T. Brown et al., 1998. Crop Protection 17: 441-445 Clemson Univ.

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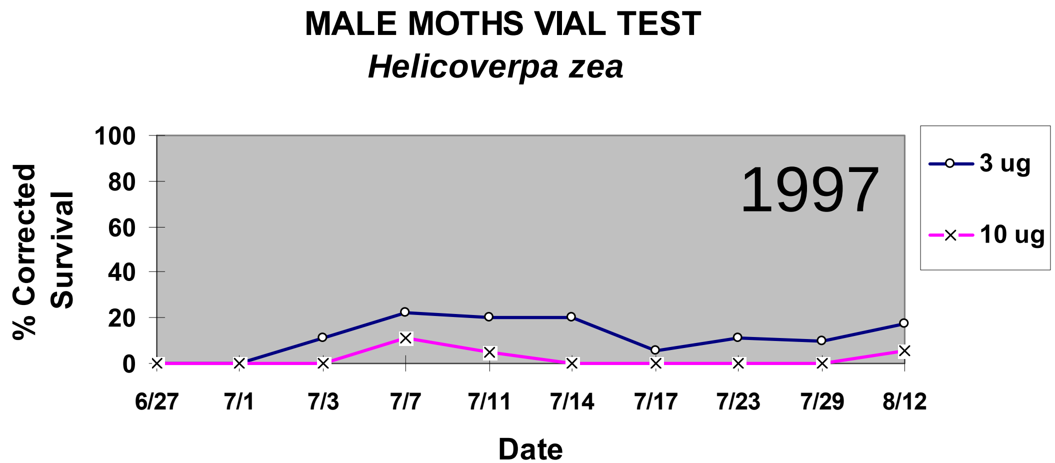
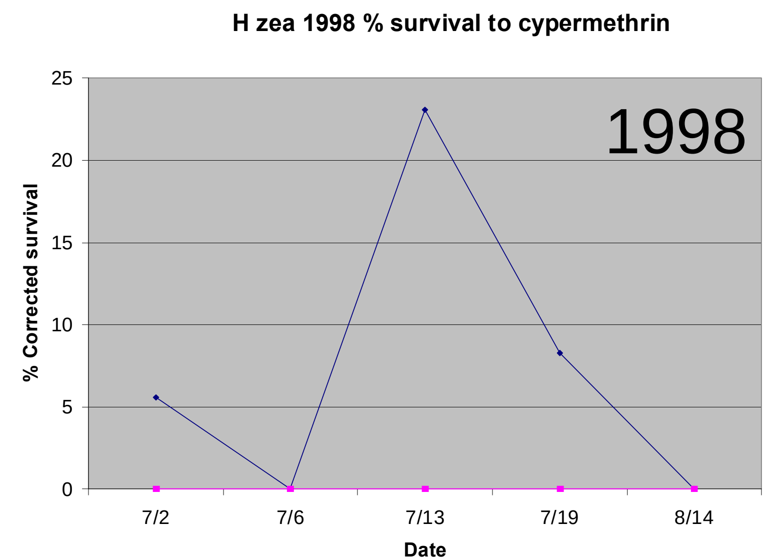
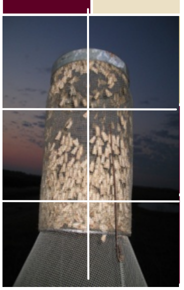
Bollworm (*Helicoverpa zea*) survival to 3 or 10 µg/vial cypermethrin, Burleson County, 1997 and 1998



INITIAL DATA ON SURVIVAL



Maximum of ~ 20% resistant males through July-August in both years



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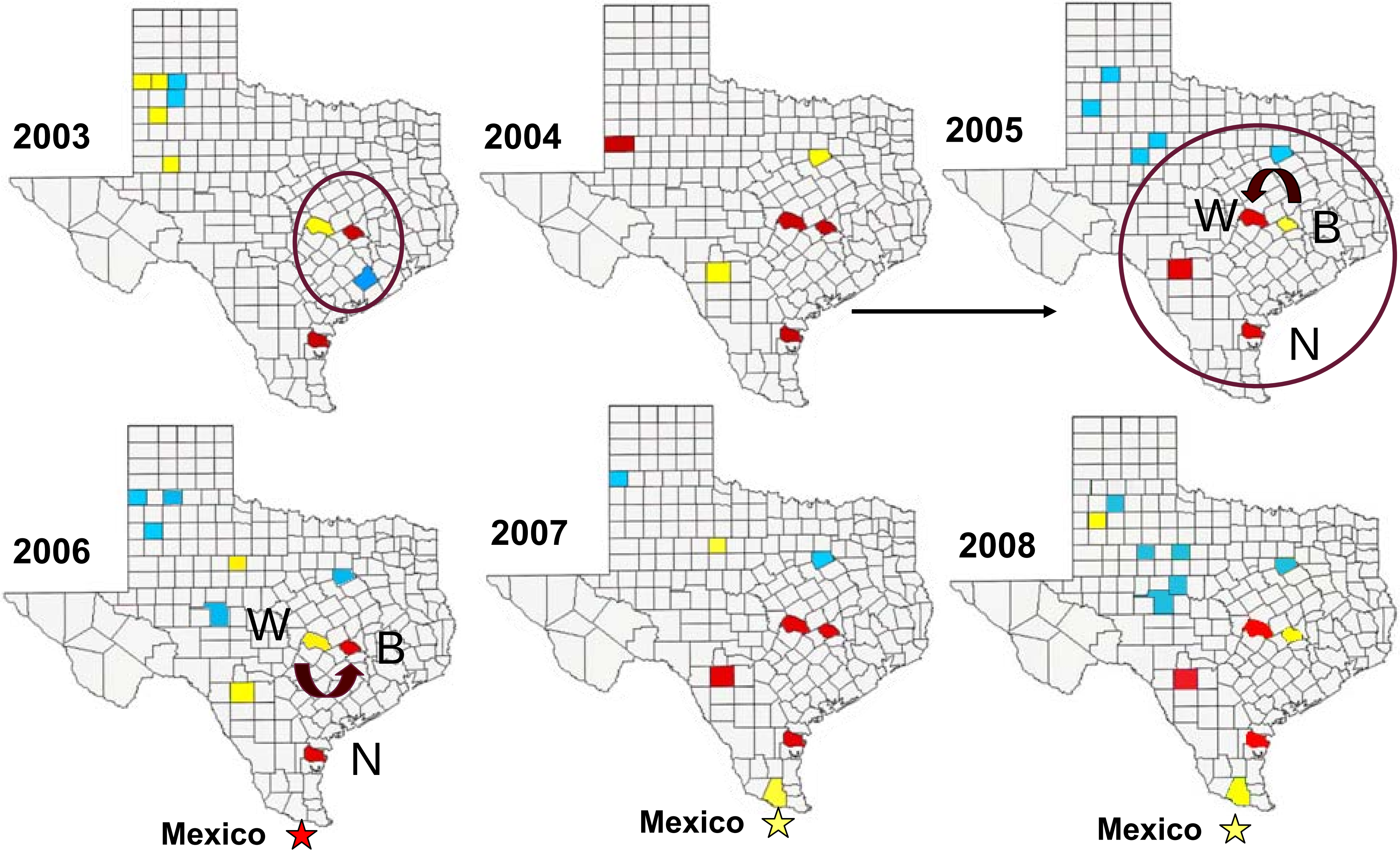
Temporal- Spatial Analysis: Highest LC_{50} Resistance Ratio



not significantly different from 1	significantly different from 1 (2X, 3X, 4X)	Resistant RR ≥ 5X

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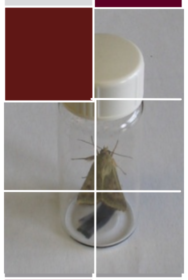
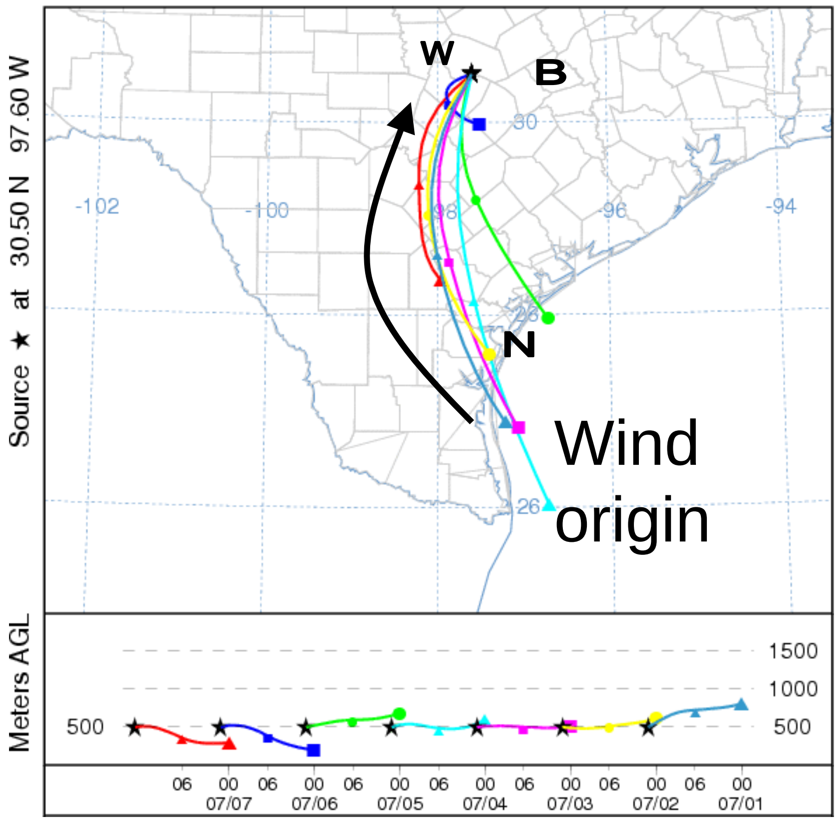
*Compared to susceptible population

2005 Wind trajectories from Nueces Co. ended more frequently in Williamson Co.





4 out of 6 years Williamson Co. populations have had resistance ratios above 5, similar to Nueces County

Star represents wind destination (Williamson)

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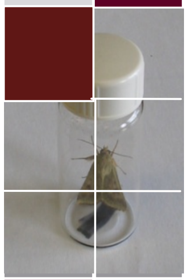
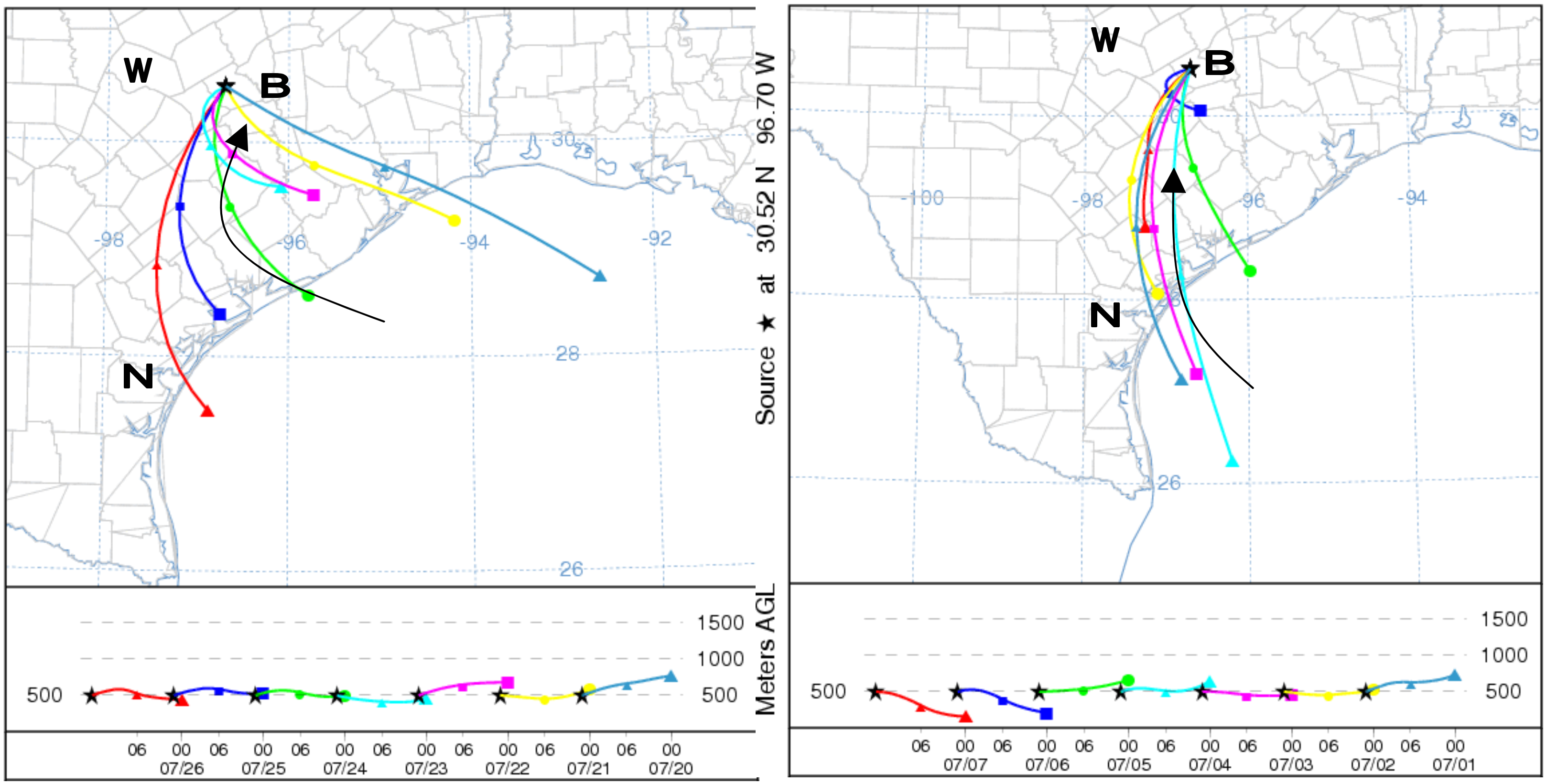
2005 Wind trajectories towards Burleson are less frequent from Nueces County, which has high resistance levels





Star represents wind destination (Burleson)

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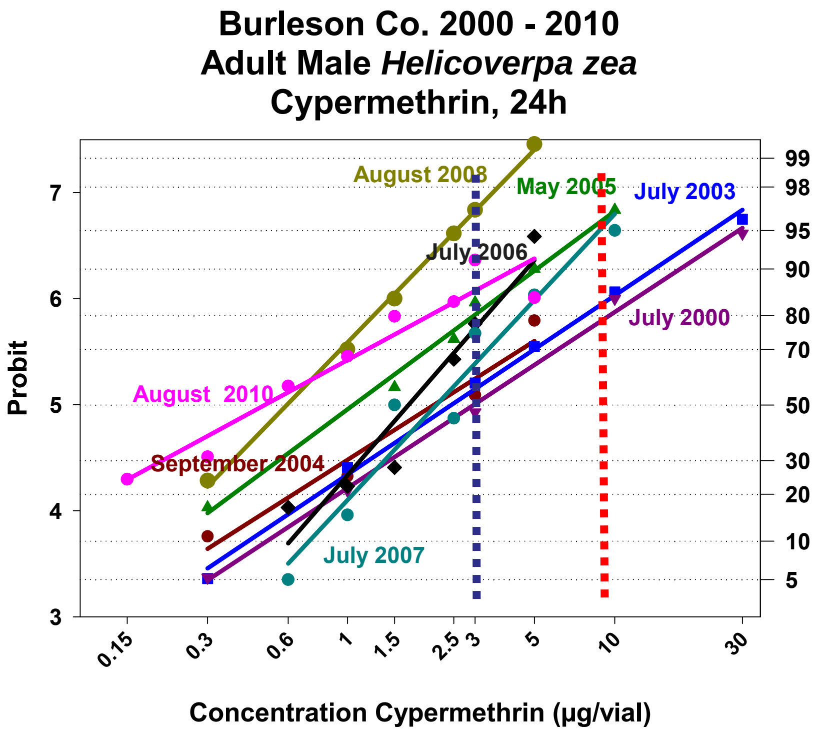
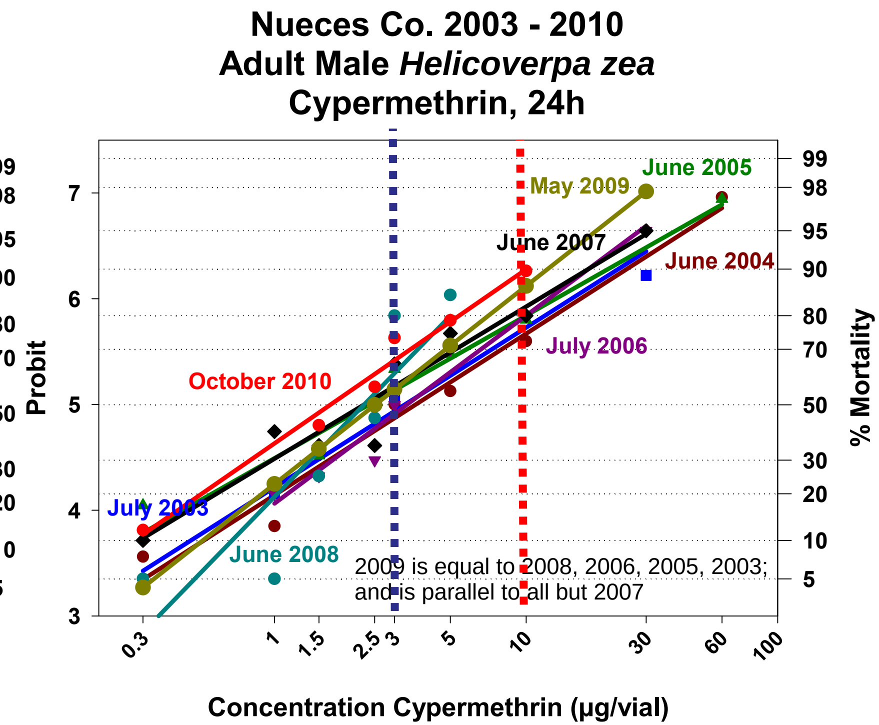
Burleson: Resistance is more variable perhaps depending on migration from known source



LC$_{50}$ 2010
1.13 µg/vial

Nueces: Constant source of resistant insects from system sorghum-cotton



LC$_{50}$ 2010
1.31 µg/vial

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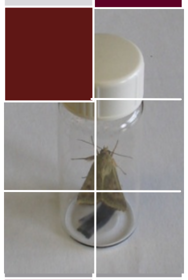
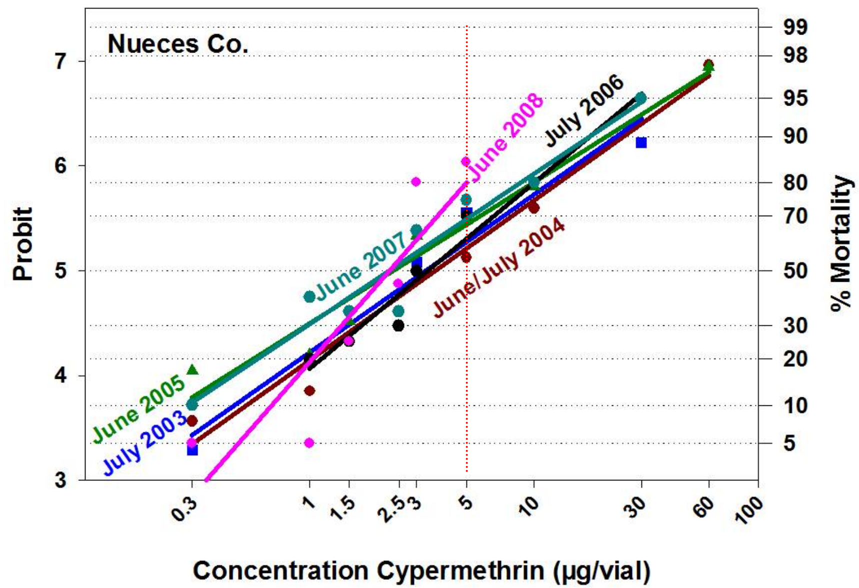
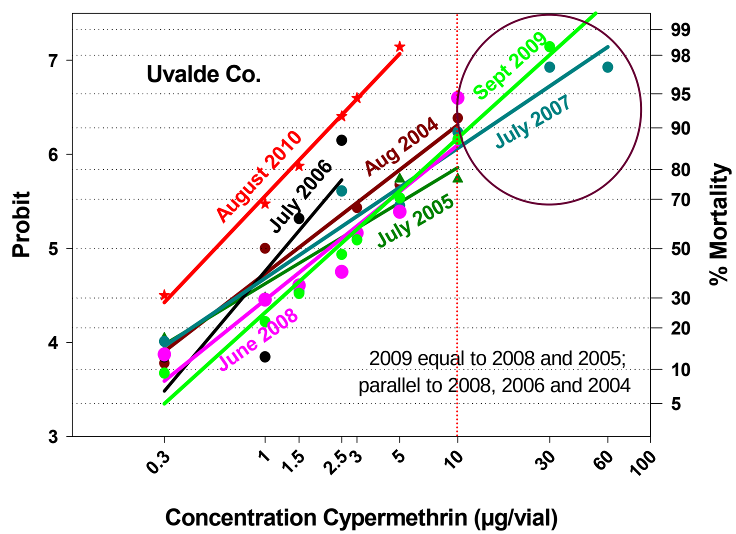
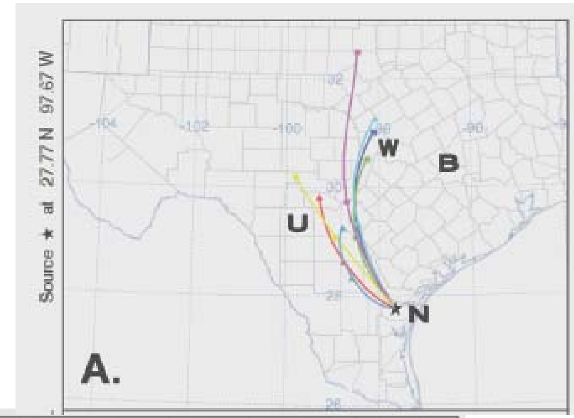
Predicted increased resistance level in Uvalde based on wind trajectories originating in Nueces and Rio Bravo in Mexico: did happen!

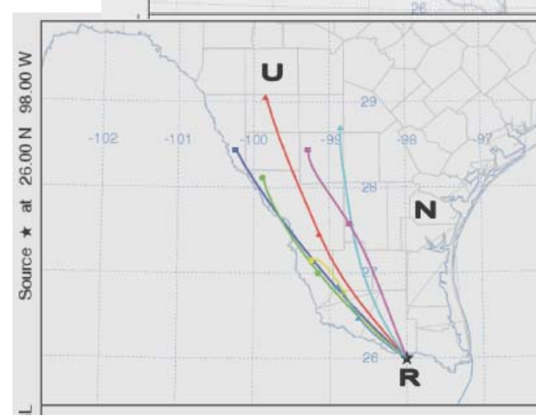






Pietrantonio et al. 2007. Environmental Entomology 36:1174-1188





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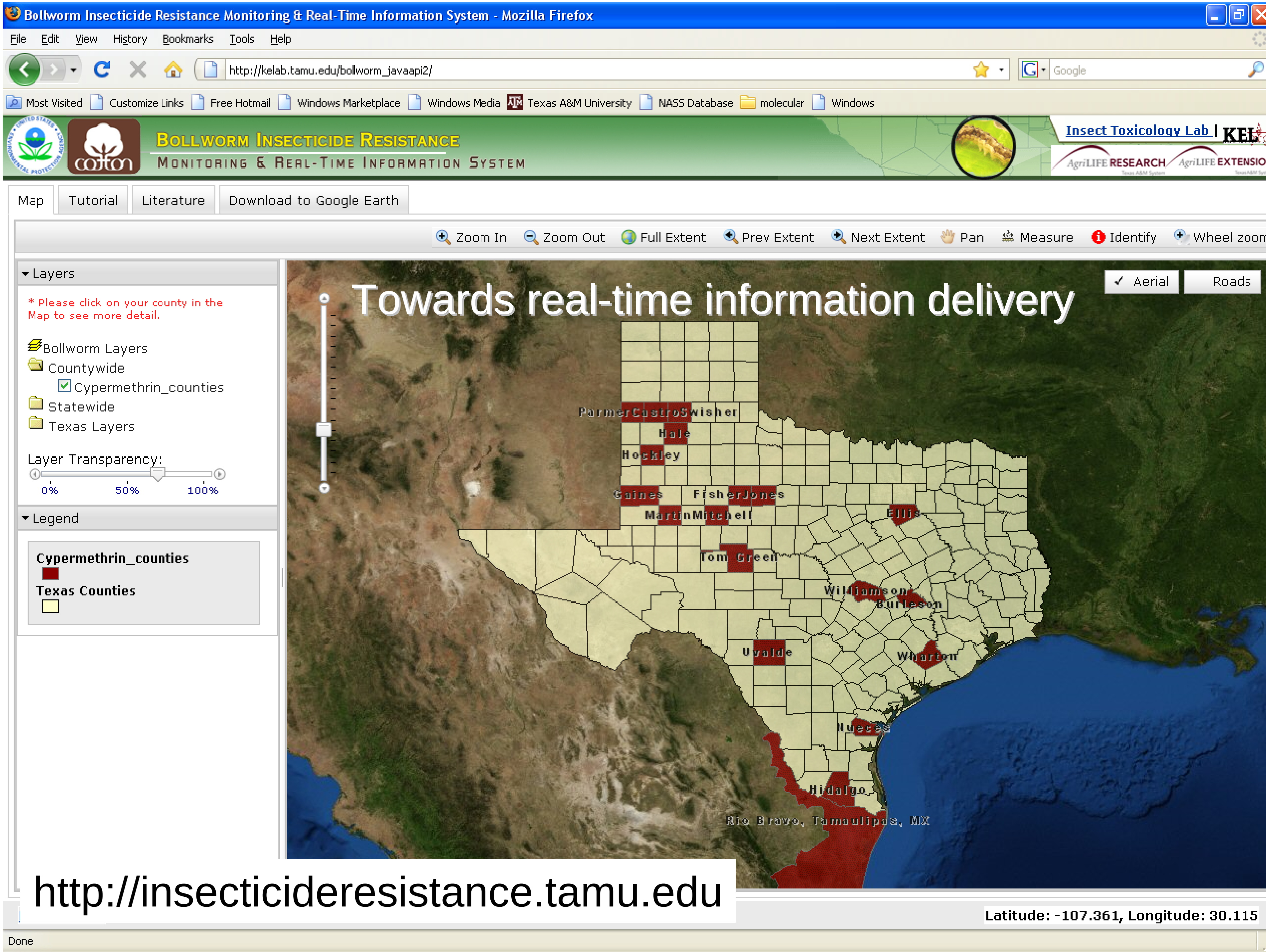
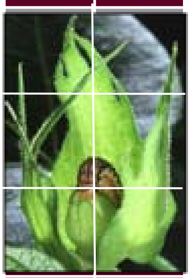
Towards real-time information delivery



http://insecticideresistance.tamu.edu

3 Pitfalls addressed



1. Pyrethroid applications are targeted to early larval stages but we used the AVT for resistance monitoring

2. Metabolism of pyrethroids can differ greatly depending upon insect growth stage and pyrethroid structure

Pitfall: Use one pyrethroid, cypermethrin, to conclude about the pyrethroid class

3. Use adults from pheromone traps of unknown geographic origin to estimate local resistance

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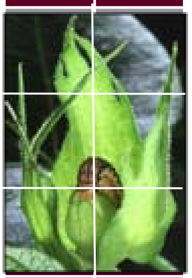
1. Validated AVT for *H. zea* males



- Determined the adult vial test is in fact diagnostic of larval resistance.
- Established baseline sensitivity of susceptible larvae and adults to structurally distinct pyrethroids.
- Used resistant field population from Uvalde, TX, to establish resistance ratios and compare the different pyrethroids.
- Failed to maintain a cypermethrin-resistance laboratory colony (fitness cost?)

Hopkins & Pietrantonio Pest Manag Sci 2010; **66**: 147–154

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ASSAY VALIDATED FOR 3 DIFFERENT PYRETHROIDS

Research Article

SCI

Received: 3 March 2009 Revised: 2 July 2009 Accepted: 3 July 2009 Published online in Wiley Interscience: 15 September 2009

(www.interscience.wiley.com) DOI 10.1002/ps.1847

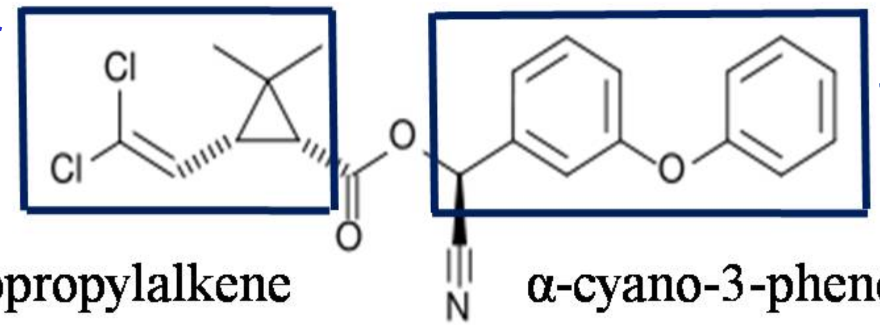
Differential efficacy of three commonly used pyrethroids against laboratory and field-collected larvae and adults of *Helicoverpa zea* (Lepidoptera: Noctuidae) and significance for pyrethroid resistance management†

Bradley W Hopkins and Patricia V Pietrantonio*

Pest Manag Sci 2010; **66**: 147–154

2. Not all Pyrethroids are Equal

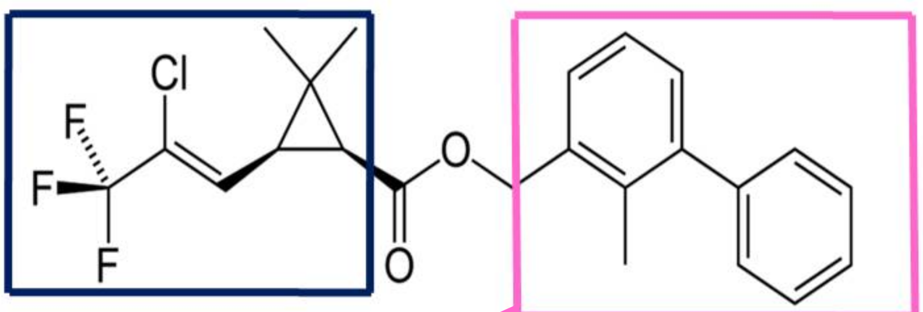
Cypermethrin



Aliphatic cyclopropylalkene acid moiety

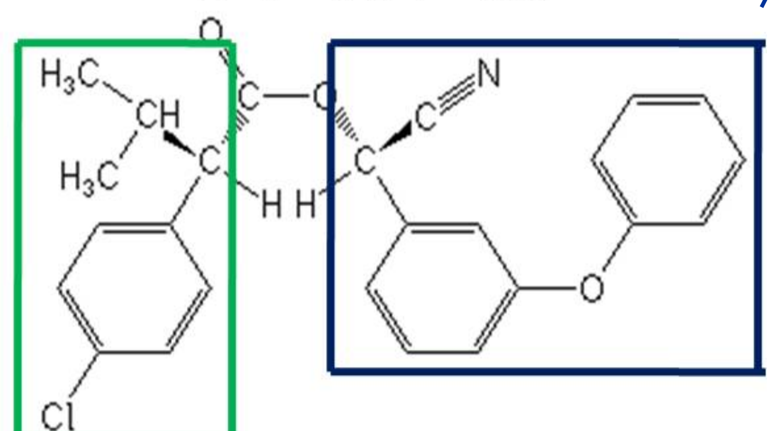
α-cyano-3-phenoxybenzyl alcohol moiety

Bifenthrin



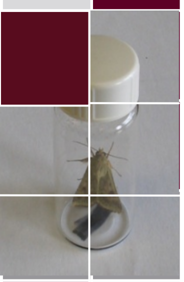
Non-cyano biphenyl alcohol moiety

Esfenvalerate

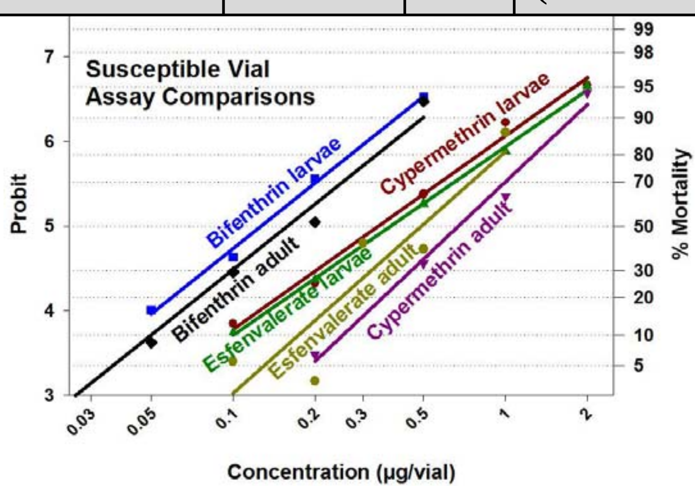


Aromatic benzene acid moiety

Susceptible larvae and adults respond similarly in vial tests

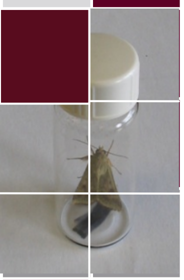


Insecticide	Growth stage	*n*	LC_{50} (95% CI)	LC_{90} (95% CI)	Slope (±SE)	$\chi2$ (*df*)
Cypermethrin	Third Instar	862	0.37 (0.37-0.44)	1.22 (0.97-1.67)	2.45 (±0.17)	25.03 (13)
	Adult	693	0.70 (0.60-0.81)	1.86 (1.50-2.54)	2.99 (±0.21)	17.84 (10)
Esfenvalerate	Third Instar	861	0.40 (0.29-0.54)	1.45 (1.02-2.55)	2.31 (±0.16)	55.28 (13)
	Adult	598	0.49 (0.39-0.65)	1.35 (0.95-2.47)	2.93 (±0.23)	29.58 (9)
Bifenthrin	Third Instar	854	0.13 (0.10-0.16)	0.38 (0.29-0.57)	2.80 (±0.20)	38.02 (13)
	Adult	798	0.17 (0.11-0.26)	0.51 (0.31-1.62)	2.62 (±0.18)	118.44 (13)

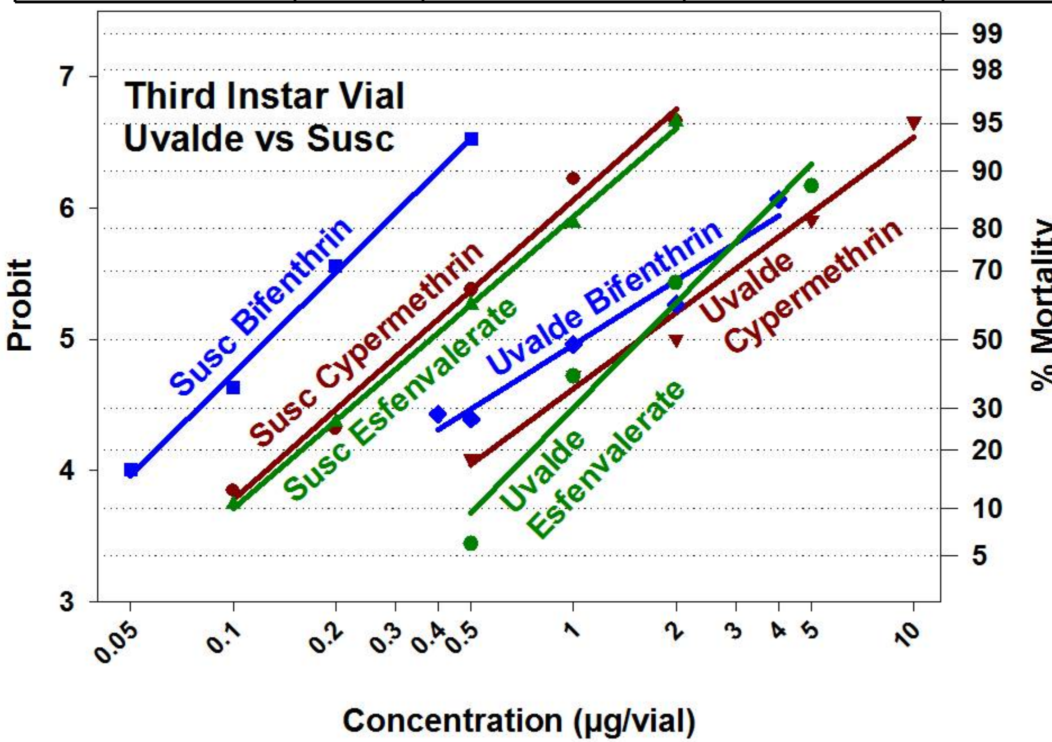


Individual insecticides have similar performance for larvae and adults, except Cypermethrin more effective on larvae.

Third Instar: Resistant (Uvalde) vs. Susceptible

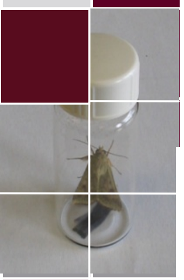


Insecticide	*n*	RR LC_{50} (95% CI)	RR LC_{90} (95% CI)	Equal	*χ2 (df), tail prob.*	Parallel	*χ2 (df), tail prob.*
Cypermethrin	1083	4.68 (3.37-6.48)	6.28 (3.89-10.14)	Reject	117. (2), 0.000	Do not reject	2.26 (1), 0.133
Esfenvalerate	1051	3.75 (2.89-4.87)	3.17 (2.05-4.92)	Reject	86.04 (2), 0.000	Do not reject	0.73 (1), 0.394
Bifenthrin	1019	9.41 (6.65-13.31)	15.22 (6.83-33.90)	Reject	215. (2), 0.000	Do not reject	3.82 (1), 0.051

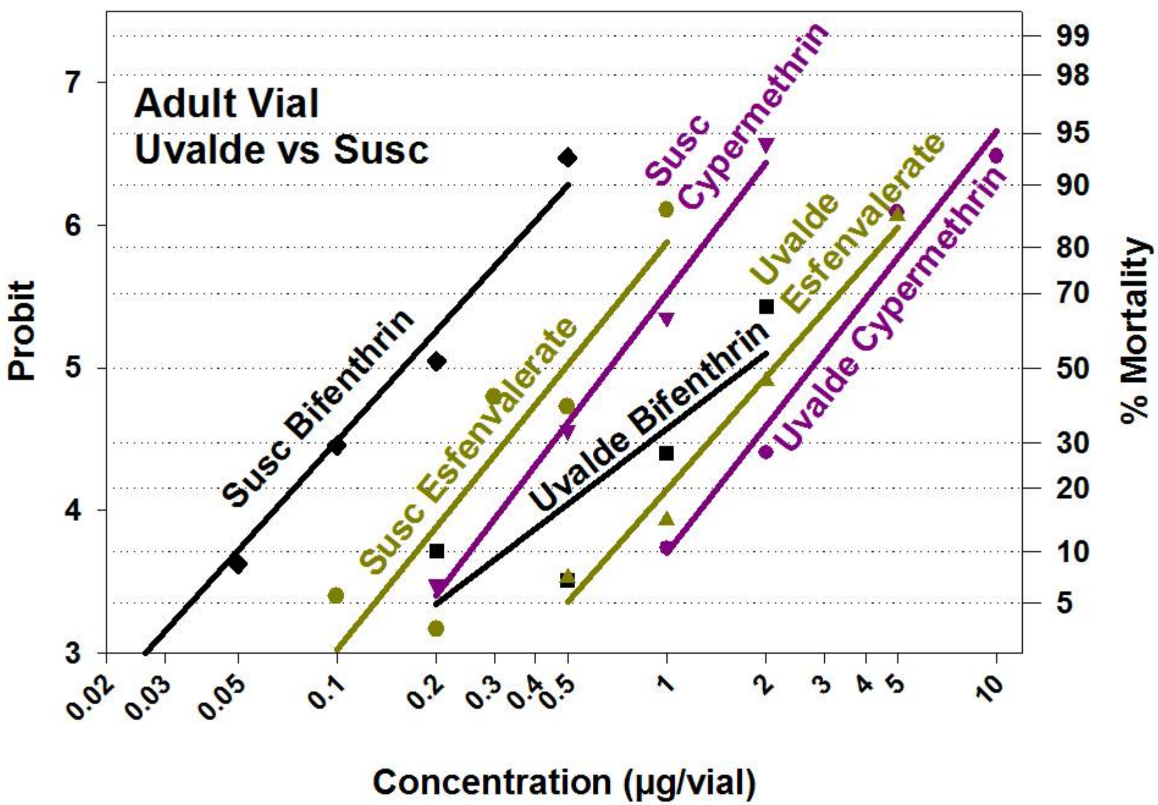


Resistance ratios of larvae are similar to resistance ratios of adults for individual insecticides = ~ 4 (Cyp and Esf) 9 for bifenthrin

Adult, Resistant (Uvalde) vs Susceptible



Insecticide	*n*	RR LC_{50} (95% CI)	RR LC_{90} (95% CI)	Equal	*χ2 (df), tail prob.*	Parallel	*χ2 (df), tail prob.*
Cypermethrin	848	4.16 (3.21-5.38)	3.82 (2.63-5.55)	Reject	104. (2), 0.000	Do not reject	0.28 (1), 0.597
Esfenvalerate	739	4.67 (3.48-6.27)	4.37 (2.63-7.26)	Reject	97.90 (2), 0.000	Do not reject	0.11 (1), 0.744
Bifenthrin	934	9.28 (7.32-11.76) ←	5.90 (4.02-8.64)	Reject	169. (2), 0.000	Reject	5.91 (1), 0.015



Resistance ratios of larvae are similar to resistance ratios of adults for individual insecticides = ~ 4 (Cyp and Esf) 9 for bifenthrin

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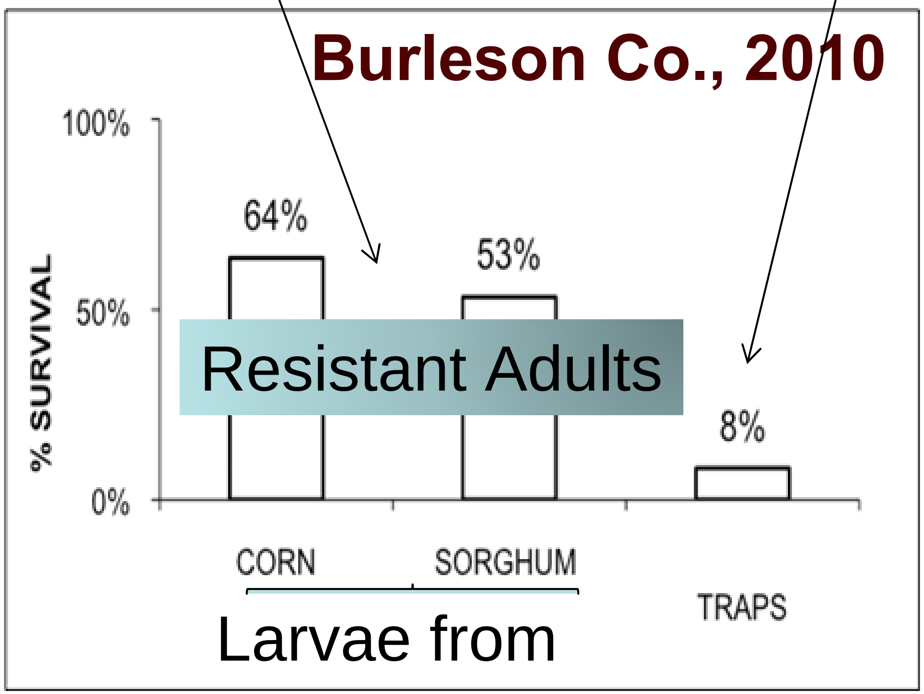
3. Crops vs. Traps. Percentage of <u>males</u> surviving the discriminatory dosage of 5 µg/vial varies among crops and traps: are we underestimating resistance?

Adults from larval collections were from pesticide <u>untreated</u> fields vs. <u>adults from traps</u>



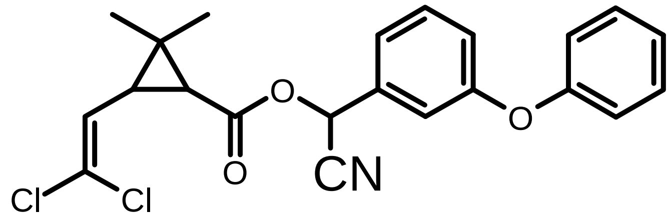
1. **Corn and sorghum could act as a source of resistant adults.**

2. **There may be differences in resistance among populations associated with different host-plant species.**

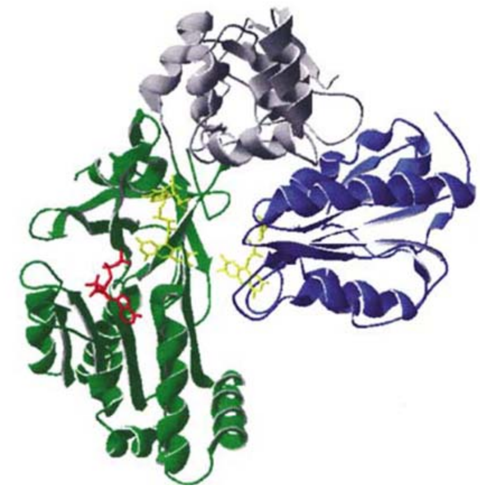
($X2$= 13.85; d.f.= 2; $P < 0.01$)*(Collaborator Raul Medina)*

Resistance mechanisms

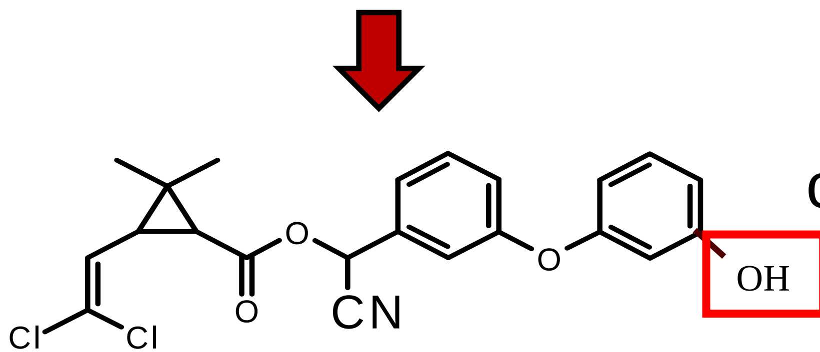
Metabolism: Cytochrome P450 (CYP)



cypermethrin

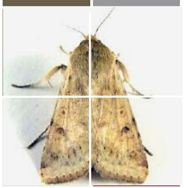


cytochrome P450



inactive metabolite; hydroxylated phenoxybenzyl alcohol

- ***Less active ingredient reaches sodium channel***



Most Resistant Field Insects for Analysis

Specimen	Date	Location	[C] survived
R1	6/21/2005	Nueces	60 µg/vial
R2	6/18/2006	Nueces	30 µg/vial
R3	7/05/2006	Nueces	30 µg/vial
R4	7/01/2003	Burleson	30 µg/vial
R5	7/10/2003	Burleson	30 µg/vial
R6	7/14/2004	Burleson	30 µg/vial
R7	8/19/2004	Burleson	30 µg/vial
R8	6/22/2004	Nueces	60 µg/vial
R9	7/13/2007	Uvalde	30 µg/vial
R10	7/08/2008	Williamson	60 µg/vial
R11	7/07/2006	Nueces	30 µg/vial
R27	6/30/2009	Uvalde	60 µg/vial

Insects surviving 3 µg/vial are considered resistant

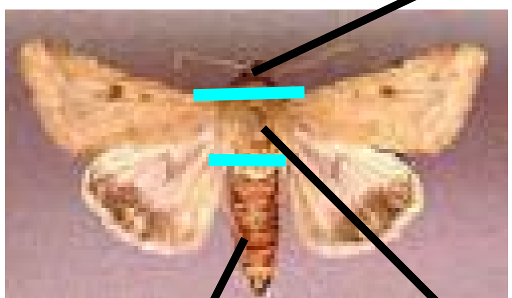
Synthesis of templates for analyses of mechanism of resistance from single moths

Head – mRNA, cDNA synthesis, ***Sodium Channel*** cloning

multiple alleles?

alternative splicing?

RNA editing?



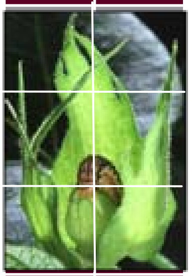
confirmation

Abdomen – total RNA, cDNA, RT-PCR, qPCR for ***Cytochrome P450s***

Thorax – genomic DNA

Brad Hopkins dissertation researc

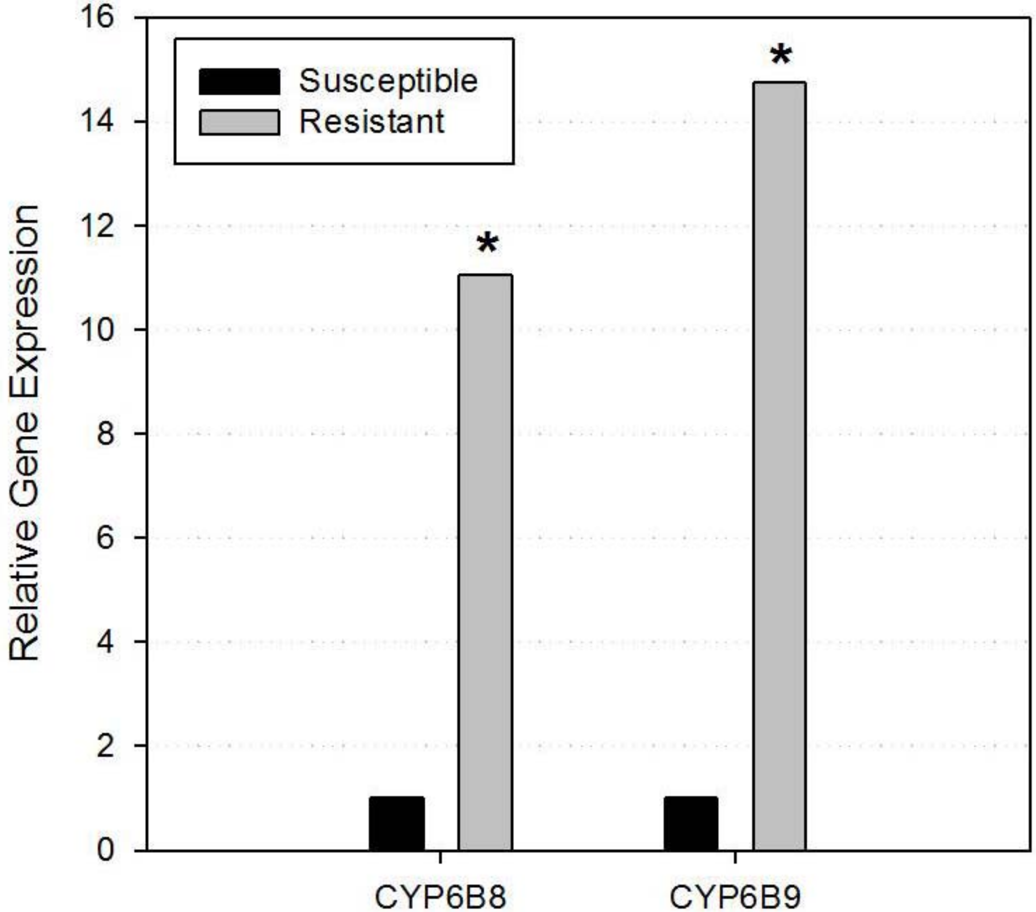
Metabolism: Background



Known *CYP* sequences from *H. zea*

Gene	Reference	Genebank Accession #
CYP321A1	Sasabe et al. 2004	AY113689
CYP4M6	Sasabe et al. 2004	AY113687
CYP4M7	Sasabe et al. 2004	AY113688
CYP6B8 ☆	Li et al. 2000	AF102263
CYP6B27	Li et al. 2002	AF285829
CYP6B28	Li et al. 2002	AF285186
CYP6B9 ★	Pimprale and Brown 1999	AF140278
CYP9A12	Chen and Li 2007	DQ788839
CYP9A14	Chen and Li 2007	DQ788840

Increased CYP450 Metabolism in *H. zea* survivors
Relative Transcript Expression



Survivors (resistant males) have higher transcript levels (by a factor of ~11-15) than susceptible males

One-way ANOVA: *CYP6B8* – P = 0.008; *CYP6B9* – P= 0.004

(Hopkins et al. In press; Pest Manag. Sci.)

Metabolism Results and Significance

- Pooled resistant group had significantly higher transcriptional expression of *CYP6B8* and *CYP6B9* than susceptible
- Individual expression varied for both cDNAs
  - ***CYP6B8* from 3.7 to 33.3**
  - ***CYP6B9* from 5.6 to 39.6**
- Resistance was completely abolished with PBO addition in Uvalde 2009 field experiments
- Likely due to mutation in common transcriptional regulatory mechanism

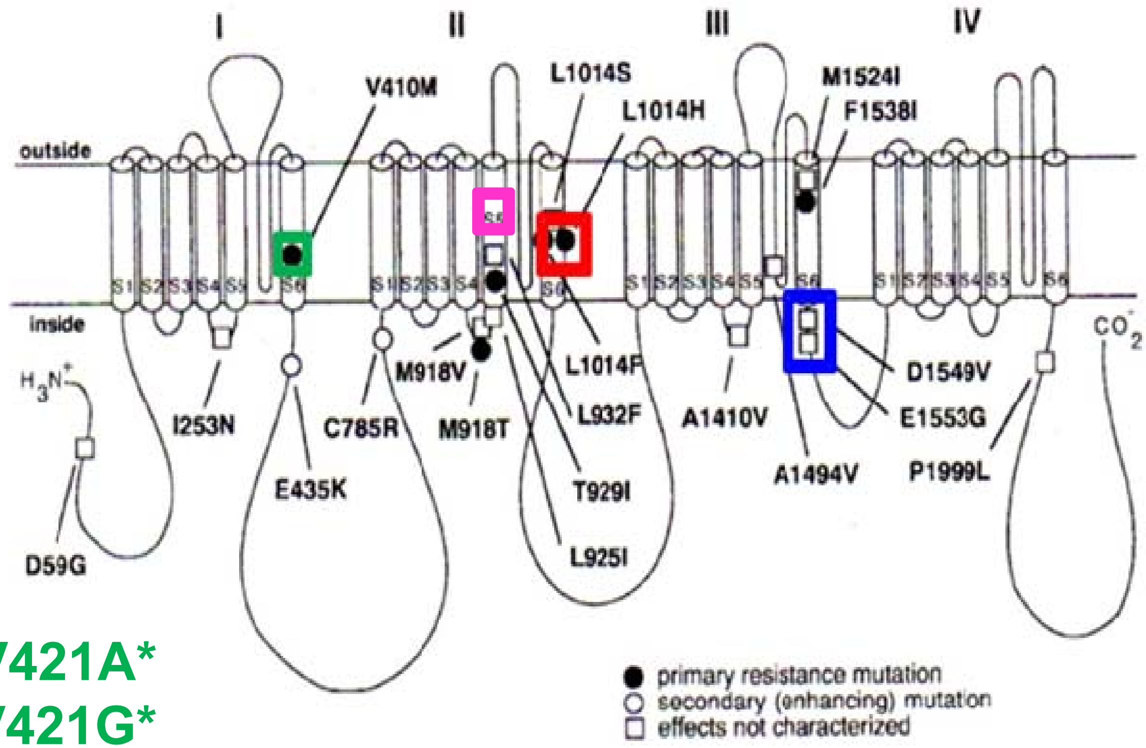
Voltage-gated sodium channel Target Site Mutations

H. virescens

V410M
L1014H
D1549V
E1553G

H. zea

V421M   V421A*
L1029H   V421G*
I951V*



H. armigera

D1549V
E1553G

-Modified from Soderlund and Knipple, 2003.

Sodium channel mutations in resistant H. zea males may also reveal geographic differences

Survivors were heterozygotes even at high dosages. Mutations associated with location in red and blue.

Moth ID	County	Survived (μg/vial)	Sodium channel mutation	Genotype
R3	**Nueces**	**30**	**V421A**	**SR**
R8	**Nueces**	**60**	**L1029H (*kdr*)**	**SR**
R19	**Nueces**	**10**	**V421M**	**SR**
R26	**Nueces**	**10**	**V421M**	**SR**
R22	**Nueces**	**10**	**V421G**	**SR**
R6	**Burleson**	**30**	**I951V**	**SR**
R37	**Burleson**	**10**	**V421A**	**SR**
R39	**Burleson**	**10**	**V421M**	**SR**

Either
mutation
Na channel
Or
Increased
Cyp450
Never both

2.5μg/vial killed all susceptible individuals.

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Target Site Findings Significance

- Allows further research on lepidopteran sodium channels through modeling and expression
- Only heterozygotes found: fitness cost for RR?
- First description of target site mutations in *H. zea*
  - **L1029H and V421M**
- Novel mutations at the V421 (to Ala and Gly)
- First description of I951V mutation in field-collected resistant insect
  - Within mutually exclusive exon region
  - Likely due to RNA-editing

Summary

- Adult vial tests predicted larval resistance, validating method for current monitoring programs over the range of geographic distribution of *H. zea*
- Resistance ratios with one pyrethroid may not be predictive of resistance ratios for others (not all pyrethroids are equally effective)
- Cypermethrin is a good, practical choice for resistance monitoring (does not underestimate larval resistance; adults are harder to kill)
- Understanding molecular mechanisms responsible for resistance may aid in finding other environmental or temporal associations such as crop-host or uncultivated host, location, month of the year, wind patterns, barriers, etc.

Summary (cont.)

- In susceptible populations, both larvae and adults were most sensitive to bifenthrin and adults more sensitive to esfenvalerate than cypermethrin
- Both growth stages gave similar resistance ratios for each of the three pyrethroids
- In both growth stages, LC_{50} resistance ratios were double for bifenthrin compared to esfenvalerate or cypermethrin

Summary - Significance

- Both target site and metabolic resistance have been found in Texas *H. zea*
  - Never found in same individual
  - Sometimes found at same date and location: different hosts?
- Temporal analysis: Most recent years metabolic resistance most frequent
- Current resistance monitoring strategy is effective, but future will allow for more specific, high-throughput molecular-based assays
  - Melting temperature shift qPCR
  - ELISA-based *CYP* assays

Thank you!

Brad Hopkins, Ph.D., currently with Dow AgroSciences

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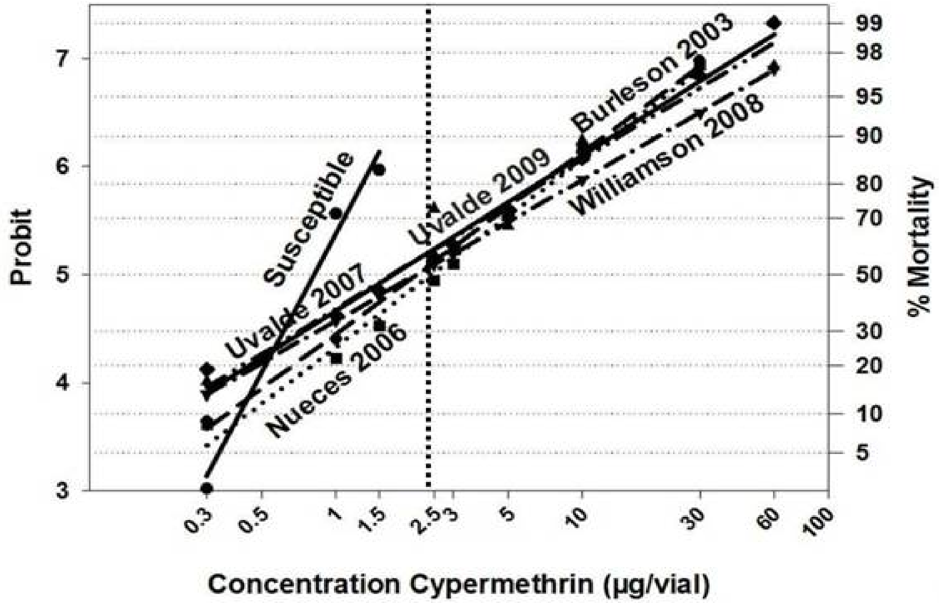
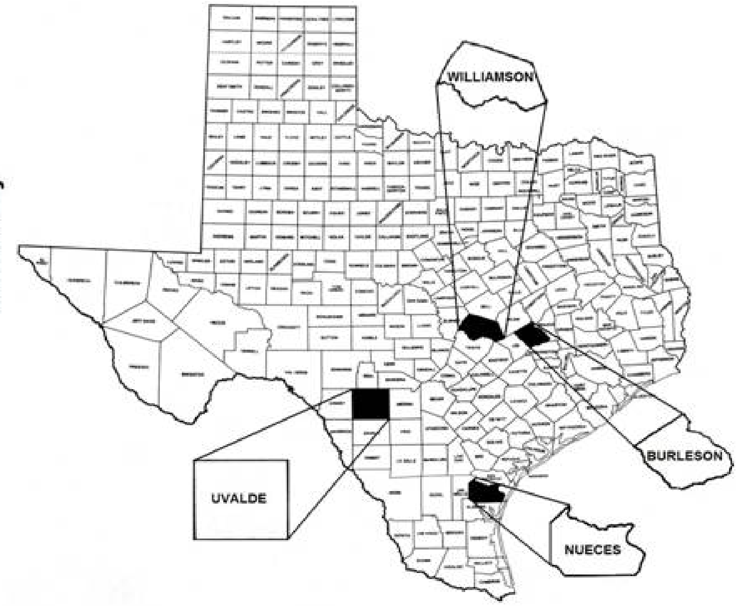
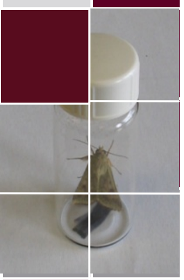
Metabolism Results



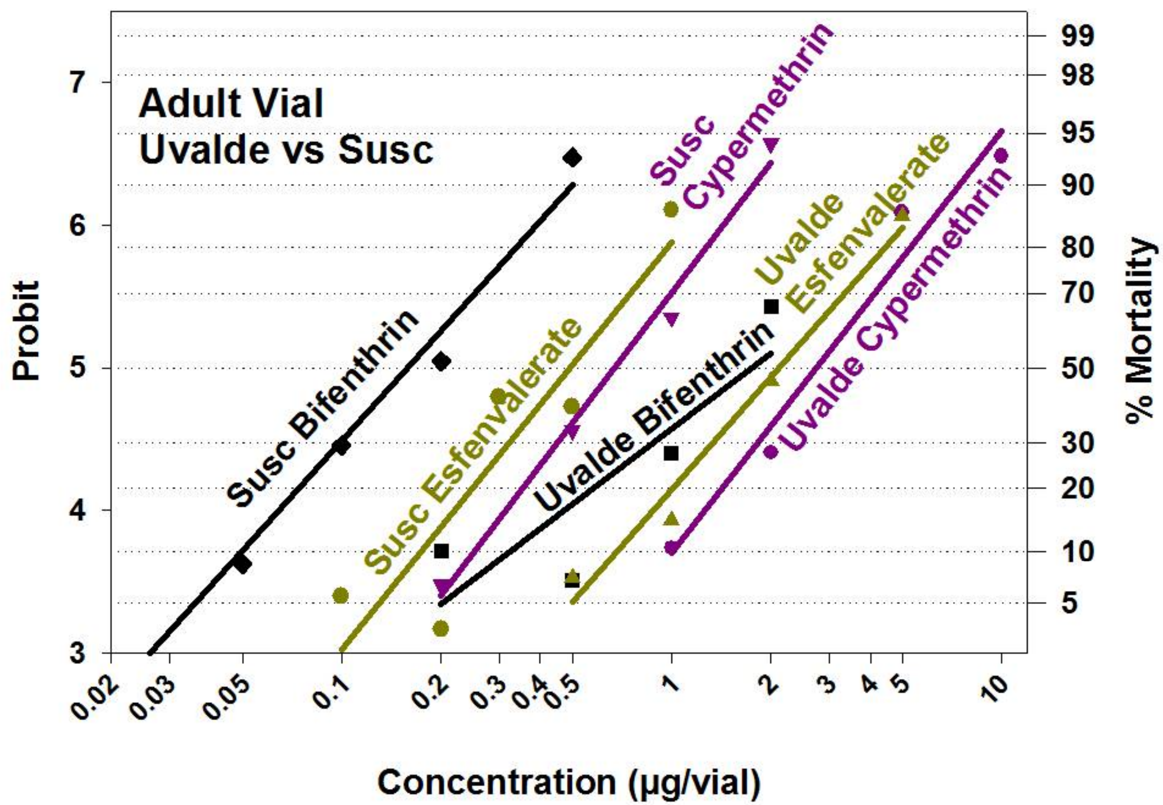


Individual Specimen	County	Concentration Survived (µg/vial)	Date Collected	CYP6B8 Relative Expression	CYP6B9 Relative Expression
Susceptible	—	—	—	1.00	1.00
R2	Nueces	30	June 18, 2006	3.72	5.56
R4	Burleson	30	July 1, 2003	33.33	39.65
R5	Burleson	30	July 10, 2003	5.80	6.38
R9	Uvalde	30	July 13, 2007	12.38	23.64
R10	Williamson	60	July 8, 2008	6.68	7.78
R27	Uvalde	60	June 30, 2009	34.92	37.43

Results: Adult, Uvalde vs Susc



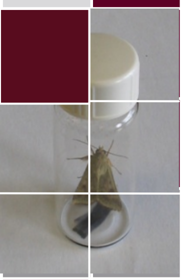
Insecticide	*n*	RR LC_{50} (95% CI)	RR LC_{90} (95% CI)	Equal	*χ2 (df), tail prob.*	Parallel	*χ2 (df), tail prob.*
Cypermethrin	848	4.16 (3.21-5.38)	3.82 (2.63-5.55)	Reject	104. (2), 0.000	Do not reject	0.28 (1), 0.597
Esfenvalerate	739	4.67 (3.48-6.27)	4.37 (2.63-7.26)	Reject	97.90 (2), 0.000	Do not reject	0.11 (1), 0.744
Bifenthrin	934	9.28 (7.32-11.76)	5.90 (4.02-8.64)	Reject	169. (2), 0.000	Reject	5.91 (1), 0.015



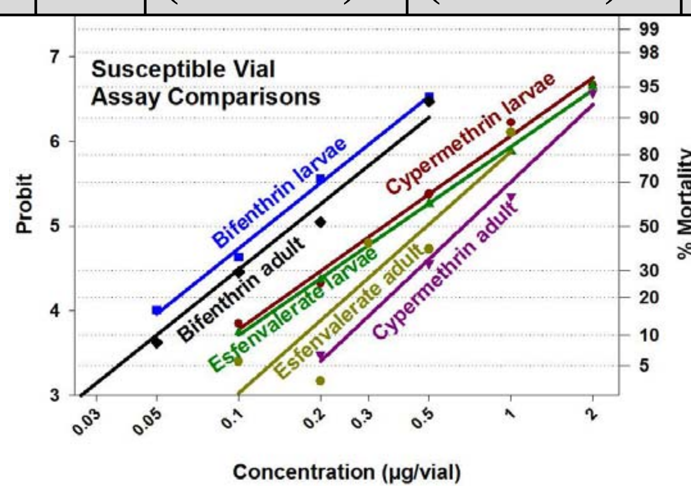
Literature Cited

- Chen, S. and X. Li. 2007. Transposable elements are enriched within or in close proximity to xenobiotic-metabolizing cytochrome P450 genes. *BMC Evol. Biol*. 7: 46-59.
- Fitt, G.P. 1989. The ecology of *Heliothis* species in relation to agroecosystems. Ann. Rev. Entomol. 34: 17-52.
- Li, X., M.R. Berenbaum, and M.A. Schuler. 2000. Molecular cloning and expression of *CYP6B8*: a xanthotoxin-inducible cytochrome P450 cDNA from *Helicoverpa zea*. *Insect Biochem. Molec. Biol.* 30: 75-84.
- Park, Y., Taylor, M.F.G., and Feyereisen, R. 1999. Voltage-gated sodium channel genes *hscp* and *hDSC1* of *Heliothis virescens* F. genomic organization. *Insect Molec. Biol.* 8: 161-170.
- Pietrantonio, P.V., T.A. Junek, R. Parker, D. Mott, K. Siders, N. Troxclair, J. Vargas-Camplis, J.K. Westbrook, and V.A. Vassilou. 2007. Detection and evolution of resistance to the pyrethroid cypermethrin in *Helicoverpa zea* (Lepidoptera: Noctuidae) populations in Texas. *Environ Entomol* **36**: 1174-1188.
- Pimprale and Brown. 1999. Genebank Accession AF140278.
- Plapp, F.W., Jr. (1987) Managing resistance to synthetic pyrethroids in the tobacco budworm. In: Richter, D. (ed.) *Proceedings Beltwide Cotton Conferences*. National Cotton Council of America, Memphis, TN, pp. 224-226.
- Sasabe, M., Z. Wen, M.R. Berenbaum, and M.A. Schuler. 2004. Molecular analysis of CYP321A1, a novel cytochrome P450 involved in metabolism of plant allelochemicals (furanocoumarins) and insecticides (cypermethrin) in *Helicoverpa zea*. *Gene* 338: 163-175.
- Sato, C., Y. Ueno, K. Asai, K. Takahashi, M. Sato, A. Engel, and Y. Fujiyoshi. (2001) The voltage-sensitive sodium channel is a bell-shaped molecule with several cavities. *Nature* **409**: 1047-1051.
- Soderlund, D.M. 2005. Sodium channels. In: Gilbert, L.I., Iatrou, K., and Gill, S.S. (eds.) *Comprehensive Molecular Insect Science*, 2005.
- Soderlund, D.M. and D.C. Knipple. 2003. The molecular biology of knockdown resistance to pyrethroid insecticides. *Insect Biochem. Molec. Biol.* 33: 563-577.
- Williams, M.R. 2005. Cotton insect losses – 2004., pp. 1828-1843. *In* D. Richter (ed.) Proc. 2005 Beltwide Cotton Conferences. National Cotton Council of America, Memphis, TN.
- Williams, M.R. 2006. Cotton insect loss estimates – 2005., pp. 1135-1150. *In* D. Richter (ed.) Proc. 2006 Beltwide Cotton Conferences. National Cotton Council of America, Memphis, TN.
- Williams, M.R. 2007. Cotton insect loss estimates – 2006., pp. 1027-1042. *In* D. Richter (ed.) Proc. 2007 Beltwide Cotton Conferences. National Cotton Council of America, Memphis, TN.
- Williams, M.R. 2008. Cotton insect loss estimates – 2007., pp. 980-995. *In* D. Richter (ed.) Proc. 2008 Beltwide Cotton Conferences. National Cotton Council of America, Memphis, TN.

Results: Susceptible Vial Assays



Insecticide	Growth stage	*n*	LC_{50} (95% CI)	LC_{90} (95% CI)	Slope (±SE)	*χ2 (df)*
Cypermethrin	Third Instar	862	0.37 (0.37-0.44)	1.22 (0.97-1.67)	2.45 (±0.17)	25.03 (13)
	Adult	693	0.70 (0.60-0.81)	1.86 (1.50-2.54)	2.99 (±0.21)	17.84 (10)
Esfenvalerate	Third Instar	861	0.40 (0.29-0.54)	1.45 (1.02-2.55)	2.31 (±0.16)	55.28 (13)
	Adult	598	0.49 (0.39-0.65)	1.35 (0.95-2.47)	2.93 (±0.23)	29.58 (9)
Bifenthrin	Third Instar	854	0.13 (0.10-0.16)	0.38 (0.29-0.57)	2.80 (±0.20)	38.02 (13)
	Adult	798	0.17 (0.11-0.26)	0.51 (0.31-1.62)	2.62 (±0.18)	118.44 (13)



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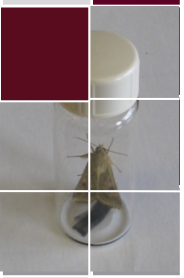
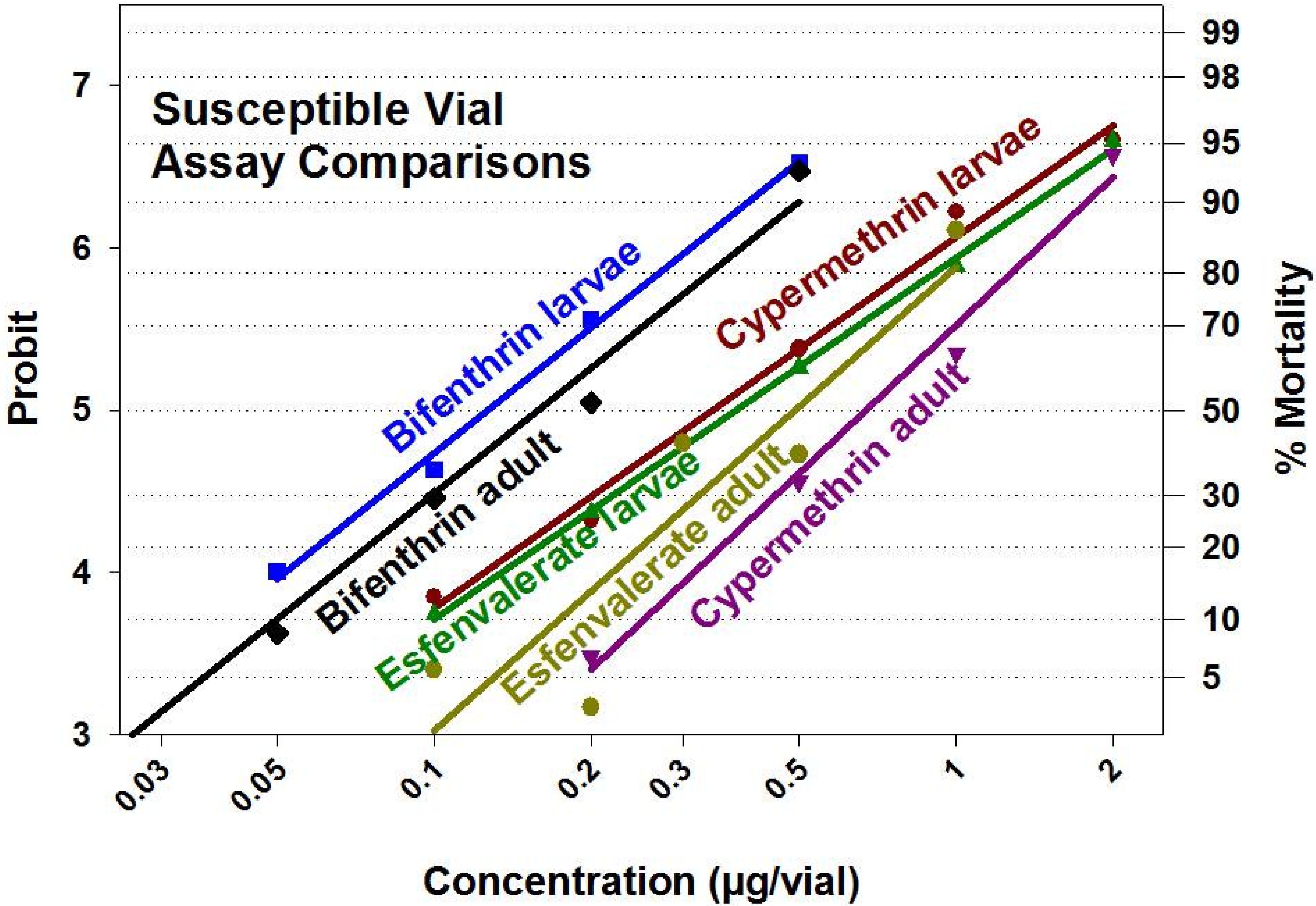
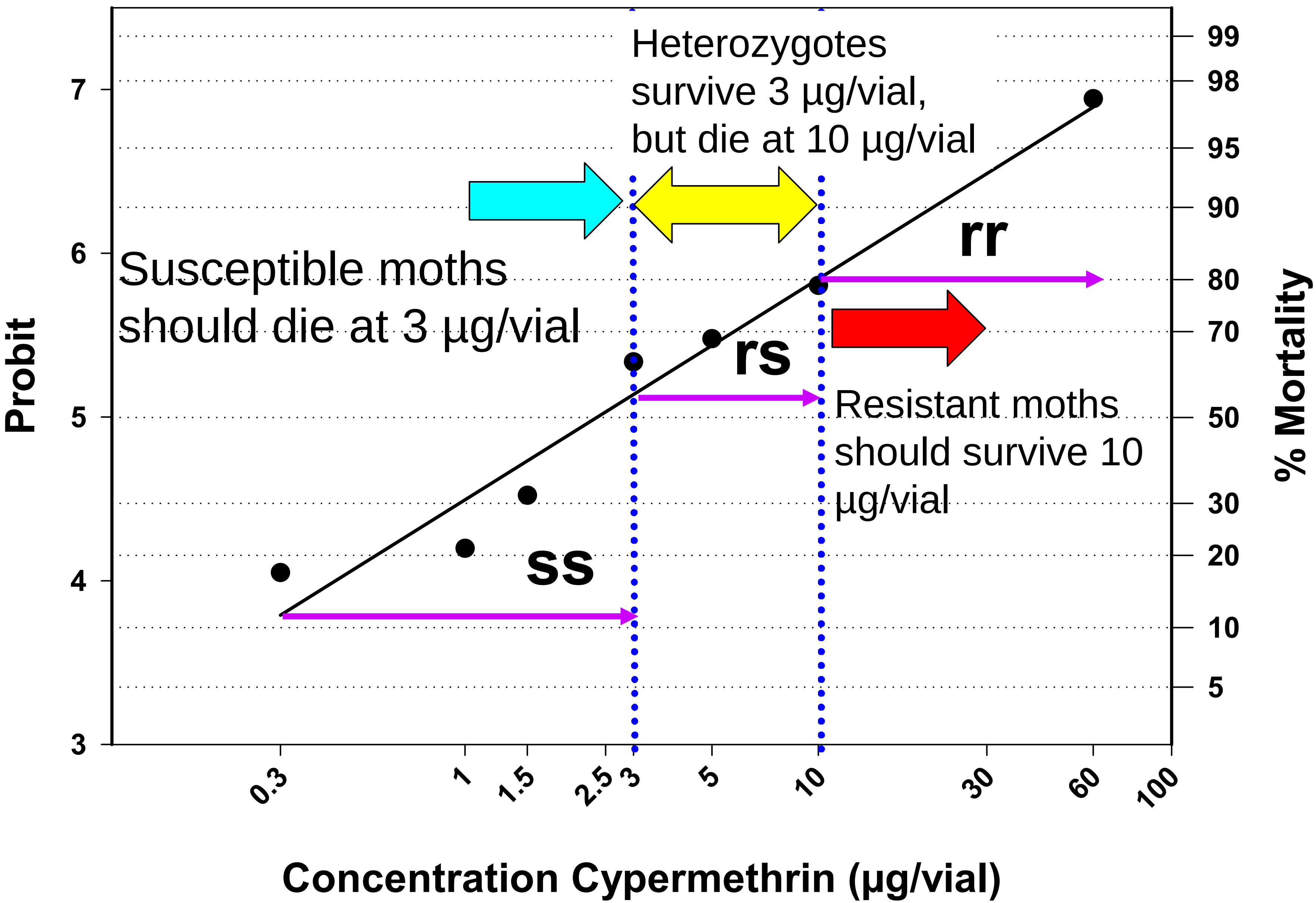
Results: Susceptible Vial Assays





Probit Lines: used for *H. virescens* and confirmed for *H. zea* data from South Carolina 1998 (



Mutually Exclusive Exons c and d - 54 Amino Acids in Length

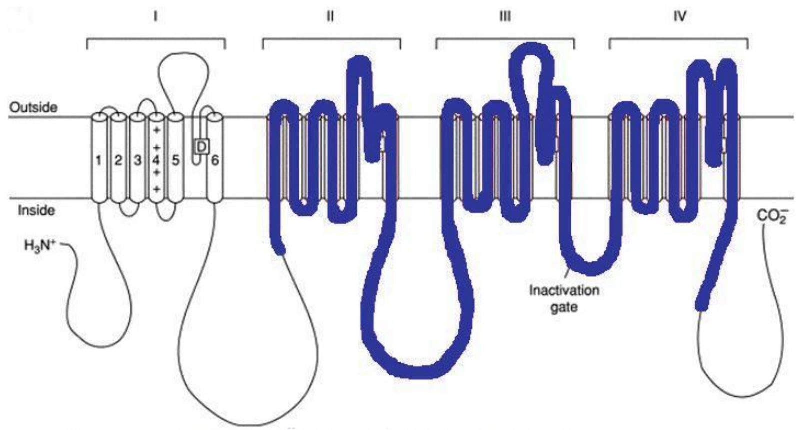
```
                                                     *
   Helze c  LRVFKLAKSWPALNLIISIMGRTVGALGNLTFVLCIIIFIFAVMGMQLFGKNYT
   Helze d  ...........T...L......M..............................V
Helze R6 c  ......................................................
Helze R6 d  ...........T...L......M..............V...............V
   Helvi c  ......................................................
   Helvi d  ...........T...L......M..............................V
   BmNav c  ......................................................
   BmNav d  ...........T...L......M..............................V
    para c  ...........T...L......................................
    para d  ...........T...L......M..............................H
```

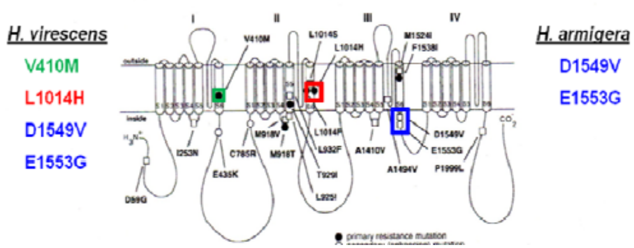
Alignment of mutually exclusive exons c and d from *Helicoverpa zea* (*Helze*) with *Heliothis virescens* (*hscp*), *Bombyx mori* (*BmNav*) and *Drosophila melanogaster* (*para*) (GenBank accession nos. **GU574730**, **AF072493**, **EU822499** and **M32078**, respectively) (Park et al. 1999; Shao et al. 2009; Loughney et al. 1989). *Helze* R6 represents a cDNA identified from a resistant specimen that contained a mutation homologous to I951V (*) of *para*, potentially an RNA-editing event associated with resistance to pyrethroid insecticides.

Helicoverpa zea novel sodium channel cDNA and P450 enzymes probes

Target site insensitivity   Confidential

3183 nt; 1061 amino acids sequenced; ~ 62.5% of sequence



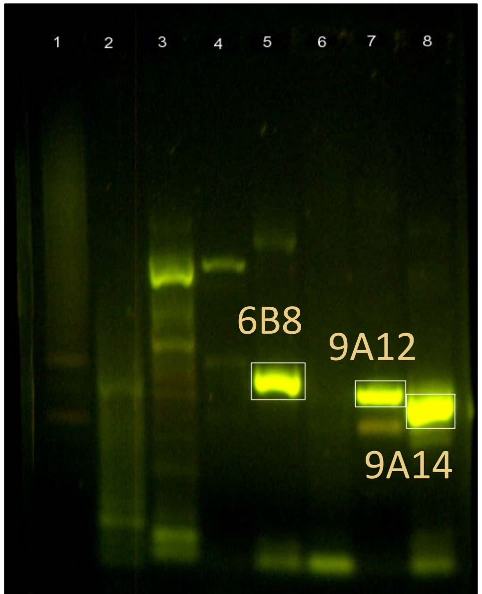


96.70% nucleotide and 98.96% Aa similarity with budworm (*Heliothis virescens*) channel

B. Hopkins et al. UNPLUBLISHED

P450s cloned, sequenced

CYP6B8
CYP9A12
CYP9A14



Helze cDNA with specific CYP primers. (1) Marker; (2) CYP321A1; (3) CYP4M6; (4) CYP4M7; (5) CYP6B8; (6) CYP6B9; (7) CYP9A12; (8) CYP9A14. Bands were cut from Lanes 5, 7, and 8. March 6, 2008.

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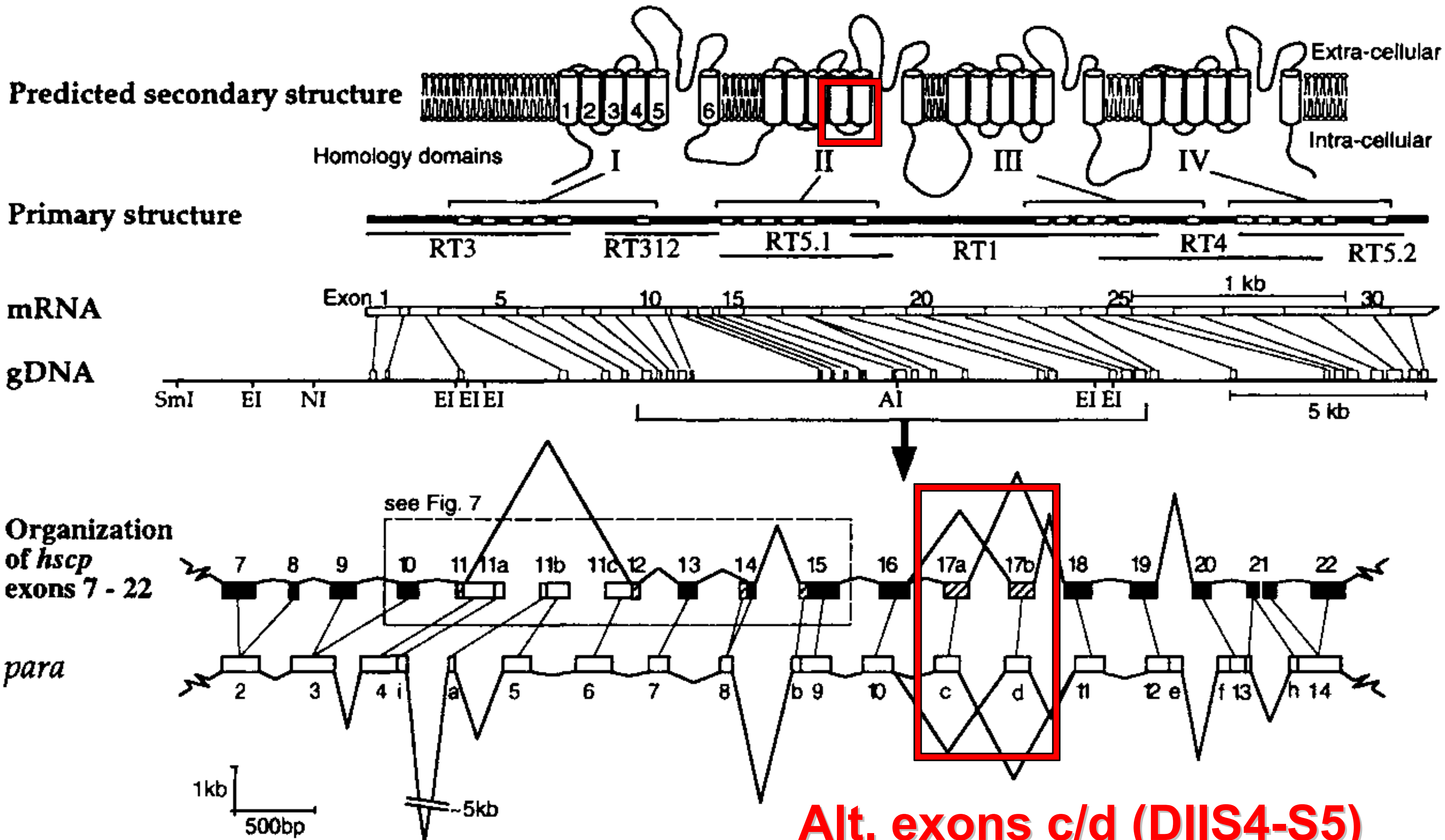
H. virescens Sodium Channel Gene Organization

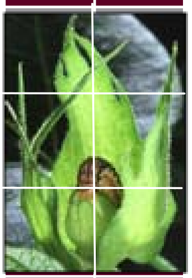


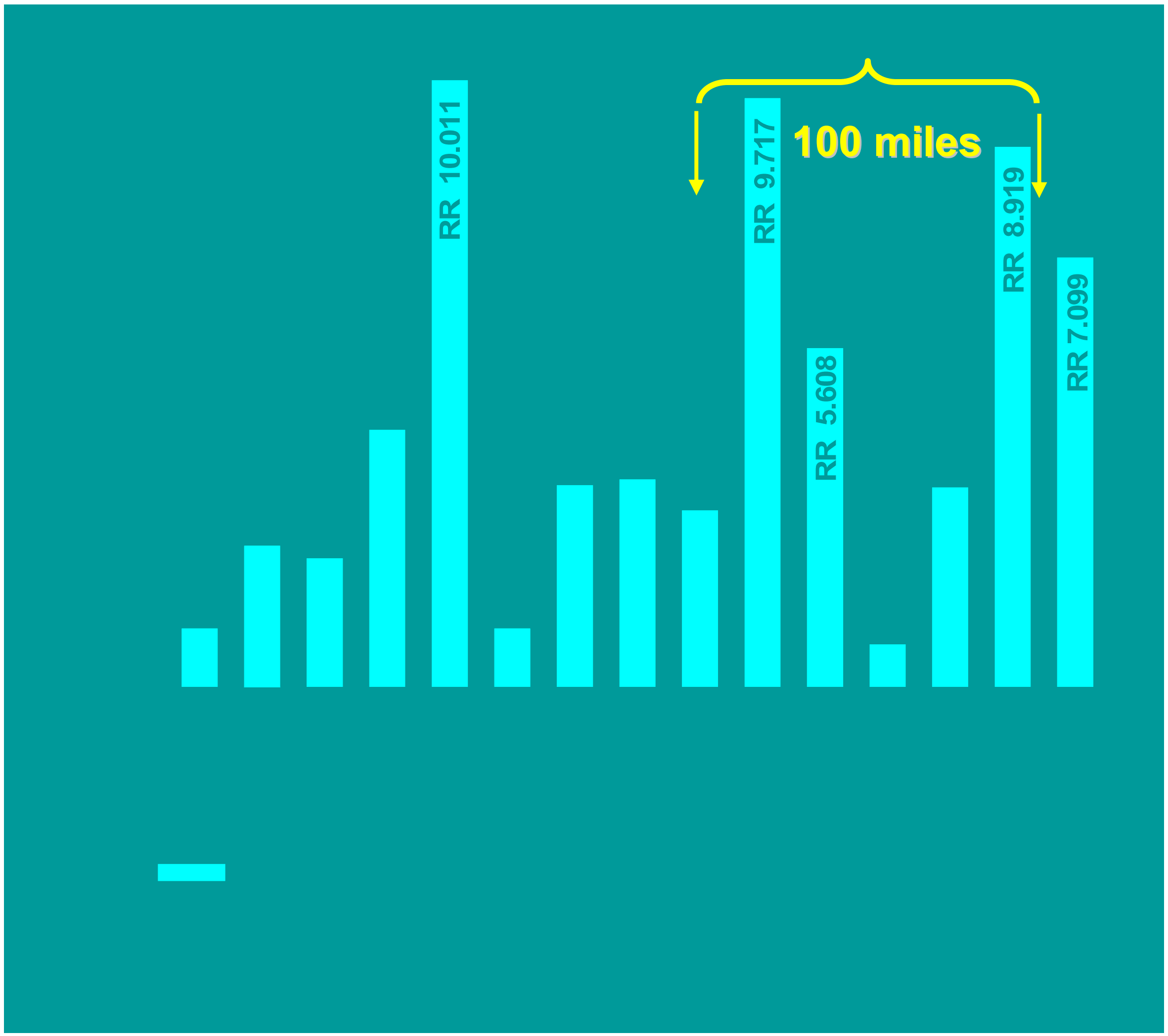
Alt. exons c/d (DIIS4-S5)

Park et al., 1999

Spatial diversity: Evolution of resistance has a strong local component

2003 Texas Counties LC50 (µg/vial) with resistance ratios





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