MONITORING FOR PYRETHROID RESISTANCE IN BOLLWORM (*HELICOVERPA ZEA*) POPULATIONS IN TEXAS, TIFTON (OKLAHOMA) AND TAMAULIPAS (MEXICO) – 2009

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Abstract

A monitoring program evaluated the susceptibility of male bollworm, *Helicoverpa zea* (Boddie), populations to the pyrethroid insecticide cypermethrin using the adult vial test. Moths were trapped near cotton fields using a synthetic pheromone trap and glass vials coated with different concentrations of cypermethrin dissolved in acetone were used to assess moth mortality. Nine Texas counties were surveyed, along with Tifton in Oklahoma and Tamaulipas in Mexico, from April to September in 2009. Data were collected from all collaborators and sent to the Insect Toxicology Laboratory (AgriLife Research) for estimation of lethal concentrations (LC_{50} and LC_{90}), calculation of resistance ratios, and likelihood ratio tests of equality and parallelism. Uvalde and Nueces County populations exhibited the highest LC_{50} resistant ratios of 9.1 and 7.6, respectively. There were only two moth survivors at 60 µg/vial, the highest dosage tested, and none at 30 µg/vial, out of nearly 4,500 moths tested. Generally, populations monitored across other counties maintained susceptibility, and populations' resistance levels for the state of Texas were similar to those observed in 2008 and lower than those registered in the previous several seasons.

Introduction

The purpose of this study was to monitor the susceptibility of *H. zea* populations to the pyrethroid insecticide cypermethrin in the main cotton production areas of Texas in 2009. The Toxicology Laboratory (Texas AgriLife

Research) in collaboration with AgriLife Extension personnel has monitored bollworm populations for pyrethroid resistance in Burleson and Nueces Co. since 1998 (Martin *et al.*, 1999; 2000; Pietrantonio *et al.*, 2000; Pietrantonio and Sronce, 2001), and has more intensely monitored resistance in multiple Texas counties since 2003 (Pietrantonio *et al.*, 2004; 2005; 2006), with the addition of Rio Bravo, Tamaulipas, Mexico, in 2006 (Pietrantonio *et al.*, 2007; Junek *et al.* 2008) and Tipton, Oklahoma, in 2009.

These two neighboring locations were monitored in an attempt to evaluate the risk of migration of resistant moths into Texas. Pyrethroid insecticide use is widespread in cotton, grain sorghum, and corn production for control of *H. zea* and other insect pests. Continued pyrethroid susceptibility monitoring of *H. zea* populations is an important tool for resistance management in cotton and other cropping systems as well. A complete multi-season data set for pyrethroid resistance monitoring in Texas, publications and a tutorial to interpret probit lines is now available at http://insecticideresistance.tamu.edu.

Materials and Methods

The Adult Vial Test (AVT) was used to monitor the susceptibility of *H. zea* populations to cypermethrin as described in detail (Pietrantonio *et al.*, 2007). Concentrations evaluated were a control (acetone alone), 0.15, 0.3, 0.6, 1.0, 1.5, 2.5, 3.0, 5.0, 10.0, 30.0, and 60.0 µg cypermethrin/vial. The location-specific concentration range is dependent upon the resistance history of each location. In 2009 resistance was monitored in Rio Bravo, Tamaulipas, Mexico, and in Tipton, Oklahoma, as well as in the following nine Texas Counties in diverse production regions: Nueces County in the Coastal Bend; Uvalde County in the Winter Garden; Burleson County in the Brazos Valley; Williamson and Ellis Counties in the Blackland Prairies; Tom Green and Runnels Counties in the Southern Rolling Plains; and Parmer and Swisher Counties in the High Plains. Data were corrected for control mortality and analyzed using PoloPlus, Probit and Logit Analysis (LeOra Software; Robertson *et al.*, 2007), and dose-mortality regressions (probit lines) were plotted using SigmaPlot software. The probit analysis results of bioassays conducted with a field population collected in September 2005 from Burleson County was used as the baseline for susceptibility to cypermethrin, with corresponding LC_{50} and LC_{90} values of 0.33 µg/vial and 2.44 µg/vial, respectively. These values were used to calculate resistance ratios (RR) with 95% confidence intervals (CI) and likelihood ratio tests for equality and parallelism as calculated by the method of Robertson *et al.* (2007). The lethal concentration resistance ratios of different populations were not considered significantly different if the 95% confidence intervals included 1 (Robertson *et al.*, 2007). In the probit graphs, vertical lines at 3 and 10 µg/vial represent the discriminatory concentrations for susceptible insects (all susceptible moths should die at 3 µg/vial) and resistant insects (presumptively homozygotes), respectively.

Results and Discussion

Rio Bravo, Tamaulipas, Mexico

In Rio Bravo, a total of three traps were used to collect moths for bioassays in April. The 2009 LC_{50} and LC_{90} values were not significantly different from those of the 2005 susceptible Burleson Co. field population (Table 1). The April 2009 concentration-mortality probit line was parallel but significantly displaced towards the lower concentration range (Fig. 1), indicating a more susceptible population than those from July 2006 and May 2007 (Fig. 1).

Table 1. Rio Bravo, Tamaulipas, Mexico cypermethrin bioassay for male bollworms, *Helicoverpa zea*, collected from pheromone traps, 2009. Resistance ratios (RR) with 95% confidence intervals (CI) were calculated by the method of Robertson *et al.* (2007). RR marked with * indicate that LC are significantly different from the susceptible population ($p \leq 0.05$).

Date	n[a]	Slope ± SE	LC_{50}[b] (95% CI)	LC_{90}[b] (95% CI)	RR LC_{50} (95% CI)	RR LC_{90} (95% CI)	χ^2 (df)
Burleson[c]	217	1.48 ± 0.36	0.33 (0.08-0.60)	2.44 (1.52-5.77)	1	1	0.46 (3)
Apr 18-23	300	1.57 ± 0.34	0.44 (0.13-0.79)	2.88 (1.80-5.58)	1.33 (0.44-4.08)	1.18 (0.54-2.55)	1.72 (7)

[a]Number of insects tested.
[b]Lethal concentration expressed in micrograms of insecticide per vial with 95% confidence intervals.
[c] Bioassay of Burleson County September 2005 susceptible field population.

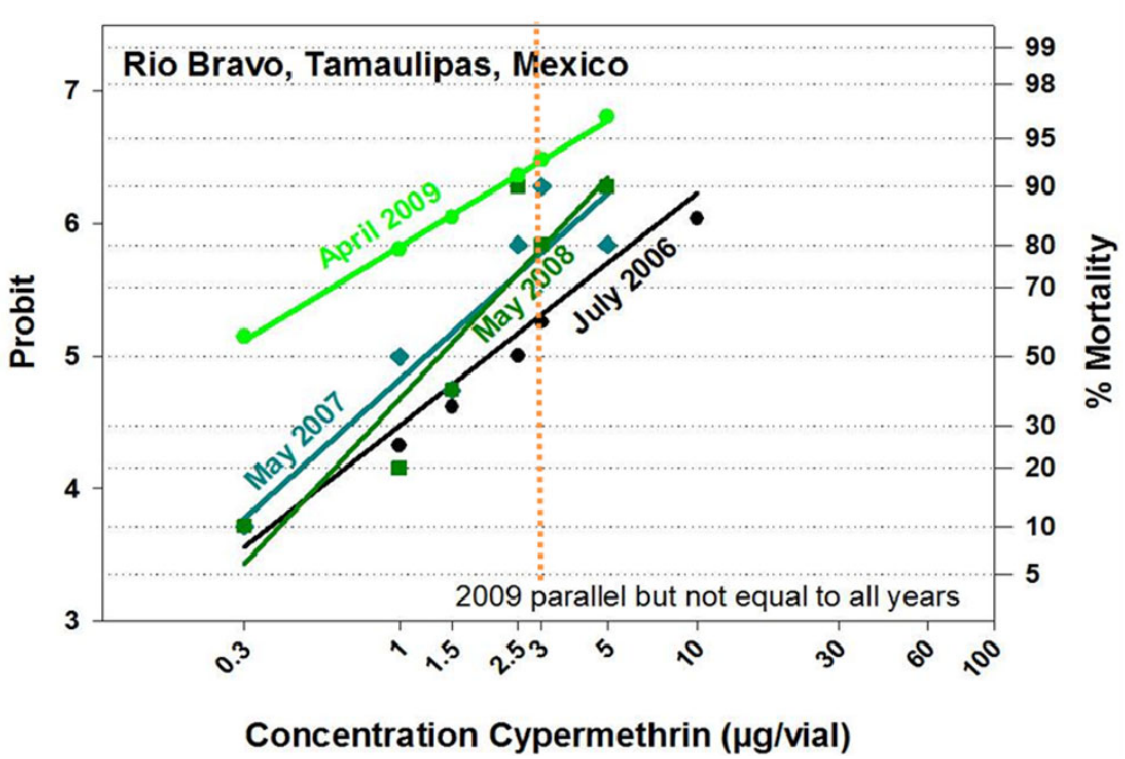

Figure 1. Concentration-mortality lines for the most resistant male bollworm populations collected from 2006, 2007, 2008, and 2009 in Rio Bravo, Tamaulipas, Mexico, and exposed 24 h to cypermethrin in the vial assay. The April 2009 population probit line was parallel but significantly less resistant than probit lines from all other years ($p \leq 0.05$).

Nueces County

Monitoring was conducted from April to September of 2009 in Nueces Co. Bollworm population densities were lower than in previous years. LC_{50} resistance ratios for bollworm populations in Nueces Co. remained high (3-7) throughout the season (Table 2), but were lower than seen in all previous years but 2008 (Pietrantonio *et al.* 2007; Junek *et al*., 2008). The concentration-mortality probit line from May 2009 was parallel to all years except 2008, and this population was significantly more resistant than the June 2008 one, but significantly less resistant than those from June/July 2004 (Fig. 2).

Table 2. Nueces Co. cypermethrin bioassay for male bollworms, *Helicoverpa zea*, collected from pheromone traps, 2009. Resistance ratios (RR) with 95% confidence intervals (CI) were calculated by the method of Robertson *et al.* (2007). RR marked with * indicate that LC are significantly different from susceptible population ($p \leq 0.05$).

Date	n[a]	Slope ± SE	LC_{50}[b] (95% CI)	LC_{90}[b] (95% CI)	RR LC_{50} (95% CI)	RR LC_{90} (95% CI)	χ^2 (df)
Burleson[c]	217	1.48 ± 0.36	0.33 (0.08-0.60)	2.44 (1.52-5.77)	1	1	0.46 (3)
Apr 15-20	300	1.82 ± 0.30	1.05 (0.62-1.47)	5.29 (3.76-8.95)	**3.17* (1.28-7.84)**	**2.17* (1.07-4.39)**	5.80 (7)
May 25-28	400	1.87 ± 0.18	2.51 (1.49-4.21)	12.16 (6.49-50.50)	**7.59* (3.29-17.51)**	**4.98* (2.52-9.85)**	26.32 (7)
Sept 2-3	180	1.58 ± 0.26	1.38 (0. 89-1.94)	8.92 (5.59-19.79)	**4.16* (1.70-10.17)**	**3.65* (1.60-8.32)**	5.95 (7)

[a]Number of insects tested.
[b]Lethal concentration expressed in micrograms of insecticide per vial with 95% confidence intervals.
[c]Bioassay of Burleson County September 2005 susceptible field population.

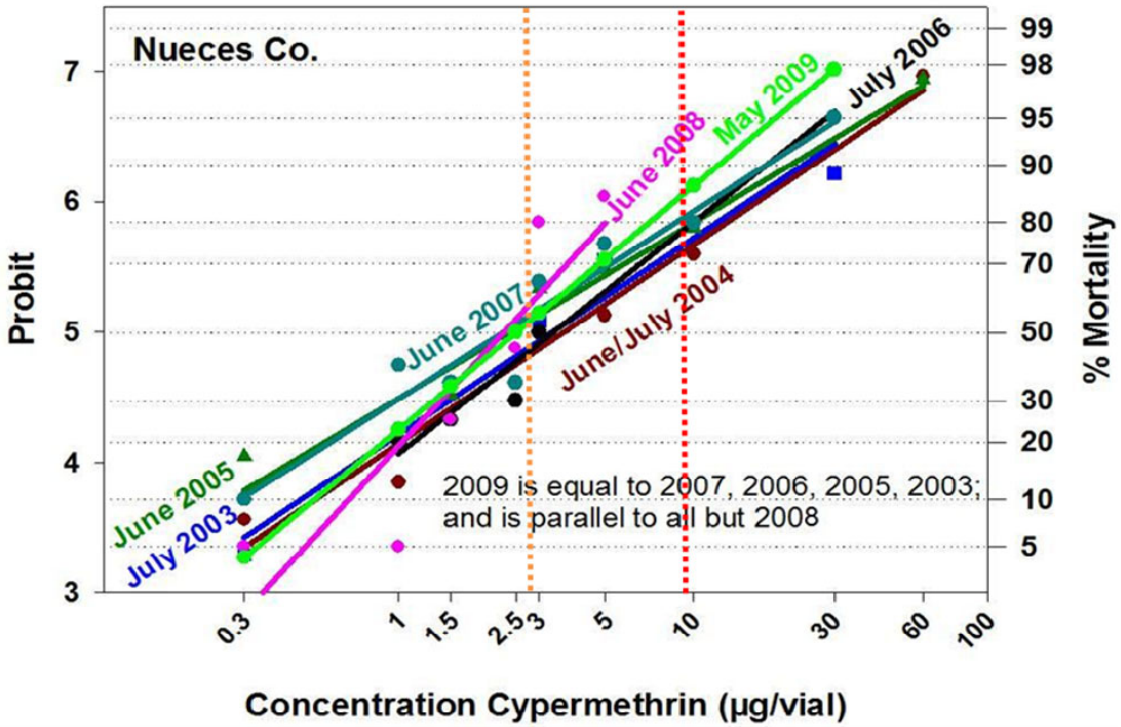

Figure 2. Concentration-mortality lines for the most resistant male bollworm populations collected from 2003 to 2009 in Nueces Co. and exposed 24 h to cypermethrin in the vial assay. The population from May 2009 was significantly more resistant than that of June 2008, significantly less resistant that June/July 2004, and the 2009 probit line was parallel to those of all years except 2008 ($p \leq 0.05$).

Uvalde County

In Uvalde Co. monitoring was conducted from May to September. The LC_{50} resistance ratios for May, June, and Sept were significantly higher than one, indicating a resistant population and the September resistance ratio was the highest seen for all counties in 2009 (Table 3). The concentration-mortality probit line from Sept 2009 was parallel to all lines except July 2005 and 2007 and not significantly different from the June 2008 and July 2005 probit lines (Fig. 3).

Table 3. Uvalde Co. cypermethrin bioassay for male bollworms, *Helicoverpa zea*, collected from pheromone traps, 2009. Resistance ratios (RR) with 95% confidence intervals (CI) were calculated by the method of Robertson *et al.* (2007). RR marked with * indicate that LC are significantly different from those of the susceptible population ($p \leq 0.05$).

Date	n[a]	Slope ± SE	LC_{50}[b] (95% CI)	LC_{90}[b] (95% CI)	RR LC_{50} (95% CI)	RR LC_{90} (95% CI)	χ^2 (df)
Burleson[c]	217	1.48 ± 0.36	0.33 (0.08-0.60)	2.44 (1.52-5.77)	1	1	0.46 (3)
May 13	400	2.18 ± 0.33	0.99 (0.64-1.31)	3.82 (2.91-5.59)	**2.98*** **(1.24-7.19)**	1.56 (0.81-3.01)	1.86 (7)
June 30	400	1.66 ± 0.28	2.56 (0.97-4.20)	15.17 (9.08-43.85)	**7.75*** **(3.08-19.51)**	**6.21*** **(2.96-13.05)**	9.56 (7)
Sept 15-16	400	2.13 ± 0.30	3.01 (1.37-4.81)	12.05 (7.07-48.89)	**9.12*** **(3.89-21.39)**	**4.93*** **(2.48-9.81)**	18.42 (7)

[a]Number of insects tested.
[b]Lethal concentration expressed in micrograms of insecticide per vial with 95% confidence intervals.
[c]Bioassay of Burleson County September 2005 susceptible field population.

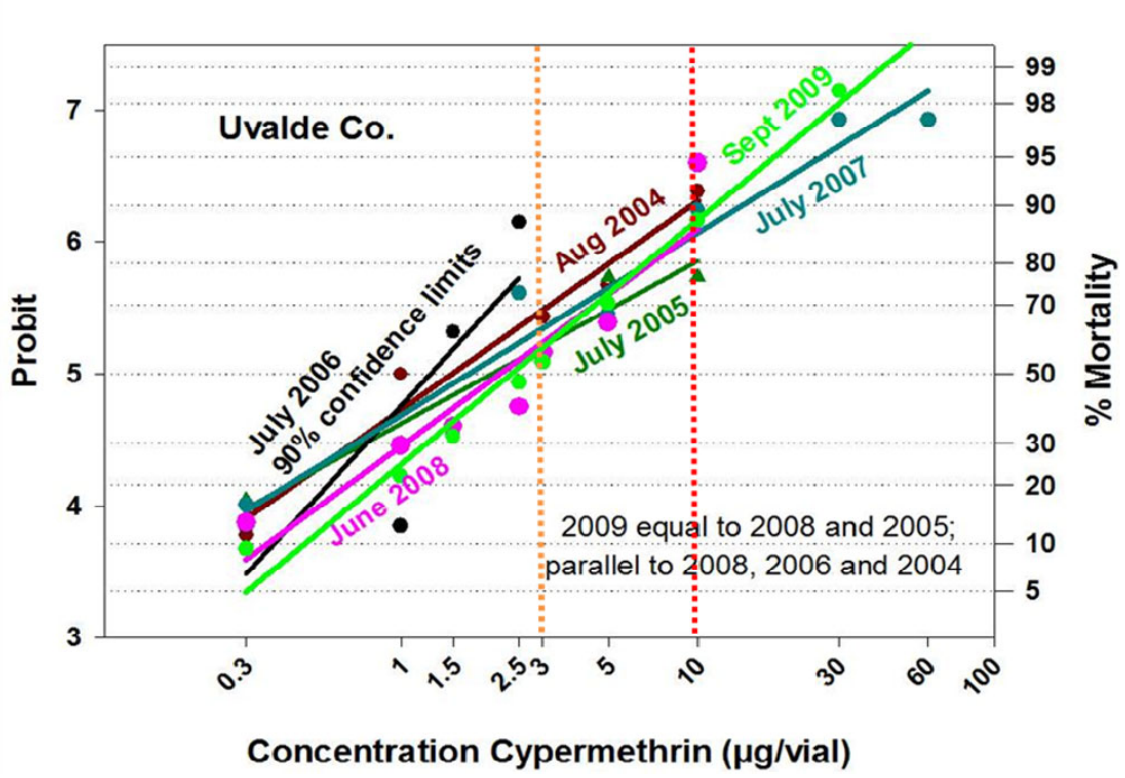

Figure 3. Concentration-mortality lines for the most resistant male bollworm populations collected from 2004 to 2009 in Uvalde Co. and exposed 24 h to cypermethrin in the vial assay. The Sept 2009 population line was parallel to all lines except those for July 2005 and 2007 and not significantly different from the June 2008 and July 2005 probit lines ($p \leq 0.05$).

Burleson County

Monitoring in Burleson Co. was conducted from May to September in 2009. The only sampling date in which resistance was detected was June 10 (Table 4), but resistance levels were low for Burleson Co. in 2009. The July 2009 line was parallel to the July 2000, 2003, 2004, and 2005 lines and the 2009 population is significantly less resistant than populations analyzed in other years except for the July 2005 (Fig. 4).

Table 4. Burleson Co. cypermethrin bioassay for male bollworms, *Helicoverpa zea*, collected from pheromone traps, 2009. Resistance ratios (RR) with 95% confidence intervals (CI) were calculated by the method of Robertson *et al.* (2007). RR marked with * indicate that LC are significantly different from susceptible population ($p \leq 0.05$).

Date	n[a]	Slope ± SE	LC_{50}[b] (95% CI)	LC_{90}[b] (95% CI)	RR LC_{50} (95% CI)	RR LC_{90} (95% CI)	χ^2 (df)
Burleson[c]	217	1.48 ± 0.36	0.33 (0.08-0.60)	2.44 (1.52-5.77)	1	1	0.46 (3)
May 6	400	2.82 ± 0.65	0.50 (0.20-0.78)	1.43 (0.96-2.24)	1.52 (0.56-4.10)	0.59 (0.29-1.17)	2.91 (7)
June 10	200	7.39 ± 2.54	1.02 (0.66-1.19)	1.52 (1.29-2.63)	**3.07*** **(1.34-7.08)**	0.62 (0.33-1.15)	4.14 (7)
July 24	88	1.47 ± 0.44	0.58 (0.08-1.25)	4.26 (2.04-21.14)	1.74 (0.47-6.50)	1.74 (0.60-5.05)	4.35 (4)

[a]Number of insects tested.
[b]Lethal concentration expressed in micrograms of insecticide per vial with 95% confidence intervals.
[c]Bioassay of Burleson County September 2005 susceptible field population.

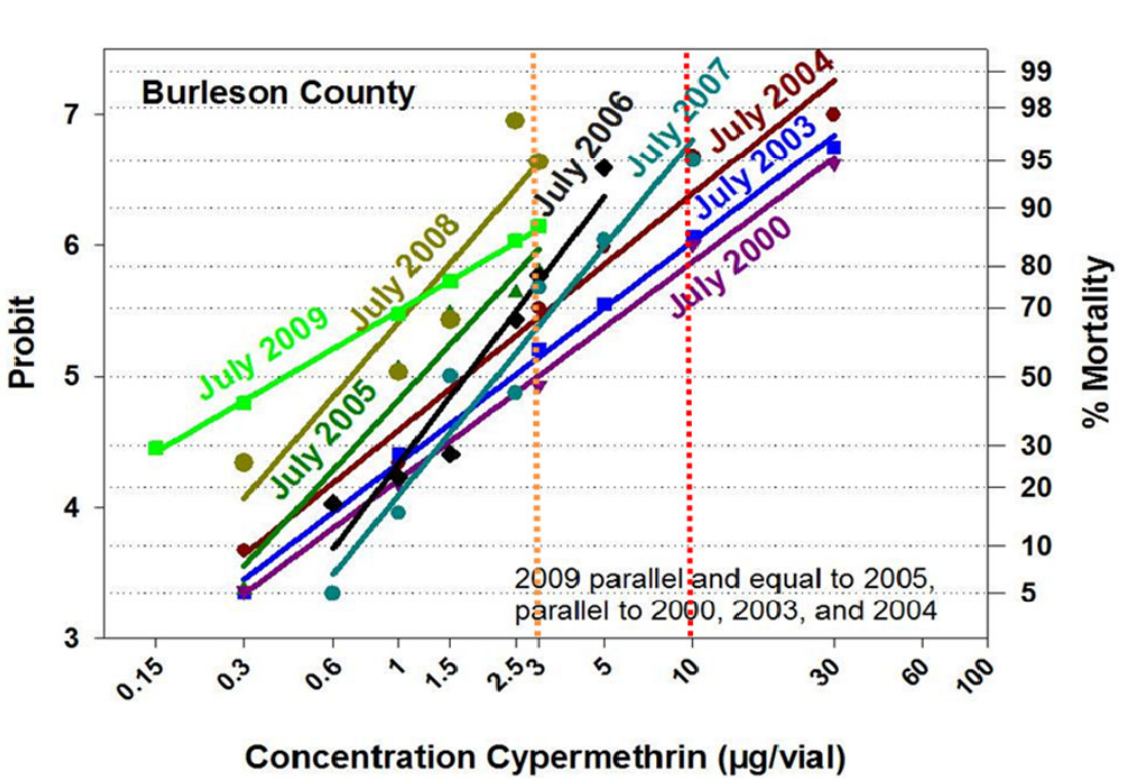

Figure 4. Concentration-mortality lines for the most resistant male bollworm populations collected from 2000 and 2003 to 2009 in Burleson Co. and exposed 24 h to cypermethrin in the vial assay. The July 2009 population line was parallel with the July 2000, 2003, 2004, and 2005 lines and is significantly less resistant than all probit lines except for July 2005 ($p \leq 0.05$).

Williamson County

Monitoring in Williamson Co. was conducted from May to July in 2009. This population was susceptible and indeed the LC_{90} resistance ratio was significantly low, indicating that this population was more susceptible than the susceptible reference population at the 90th percentile (Table 5). The concentration-mortality probit line from June 2009 was parallel but significantly less resistant than probit lines from all previous years (Fig. 5).

Table 5. Williamson Co. cypermethrin bioassay for male bollworms, *Helicoverpa zea*, collected from pheromone traps, 2009. Resistance ratios (RR) with 95% confidence intervals (CI) were calculated by the method of Robertson *et al.* (2007). RR marked with * indicate that LC are significantly different from susceptible population ($p \leq 0.05$).

Date	n[a]	Slope ± SE	LC_{50}[b] (95% CI)	LC_{90}[b] (95% CI)	RR LC_{50} (95% CI)	RR LC_{90} (95% CI)	χ^2 (df)
Burleson[c]	217	1.48 ± 0.36	0.33 (0.08-0.60)	2.44 (1.52-5.77)	1	1	0.46 (3)
June 15	190	1.76 ± 0.57	0.13 (0.01-0.28)	0.69 (0.33-1.39)	0.34 (0.09-1.70)	**0.28* (0.13-0.63)**	5.66 (7)

[a]Number of insects tested.
[b]Lethal concentration expressed in micrograms of insecticide per vial with 95% confidence intervals.
[c] Bioassay of Burleson County September 2005 susceptible field population.

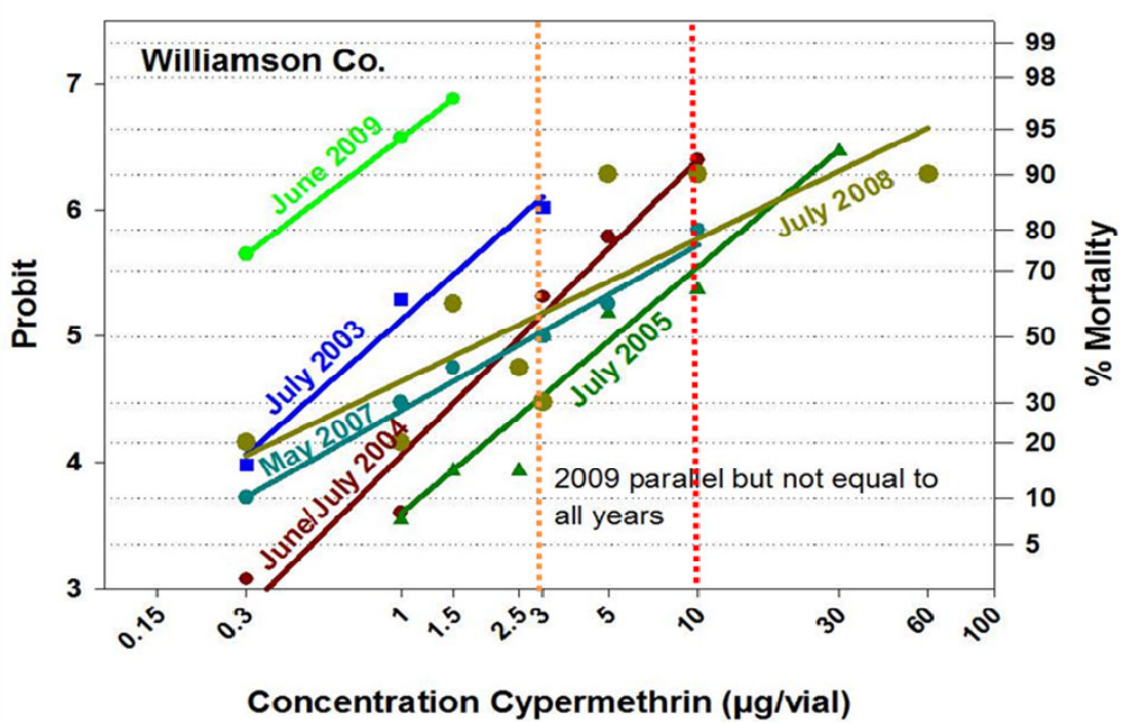

Figure 5. Concentration-mortality lines for the most resistant male bollworm populations collected in 2003 to 2005 and 2007 to 2009 in Williamson Co. and exposed 24 h to cypermethrin in the vial assay. The June 2009 population line was parallel but significantly less resistant than probit lines from all previous years ($p \leq 0.05$).

The results for 2009 should be interpreted with caution because they are from only one date and are very different from those obtained in 2008 It appears that among the locations in this study Williamson Co. has the highest variability in susceptibility to pyrethroids across different seasons.

Ellis County

Monitoring in Ellis Co. was conducted from May to July in 2009. The LC_{50} and LC_{90} resistance ratios indicate this population was susceptible, not significantly different than the 2005 susceptible Burleson Co. field population (Table 6). The concentration-mortality probit line from June 2009 was parallel to probit lines from all previous years, but significantly different from the July 2004 probit line, indicating a reversion towards susceptibility in the last few years (Fig. 6).

Table 6. Ellis Co. cypermethrin bioassay for male bollworms, *Helicoverpa zea*, collected from pheromone traps, 2009. Resistance ratios (RR) with 95% confidence intervals (CI) were calculated by the method of Robertson *et al.* (2007). RR marked with * indicate that LC are significantly different from susceptible population ($p \leq 0.05$).

Date	n[a]	Slope ± SE	LC_{50}[b] (95% CI)	LC_{90}[b] (95% CI)	RR LC_{50} (95% CI)	RR LC_{90} (95% CI)	χ^2 (df)
Burleson[c]	217	1.48 ± 0.36	0.33 (0.08-0.60)	2.44 (1.52-5.77)	1	1	0.46 (3)
June 15	166	1.23 ± 0.23	0.28 (0.12-0.48)	3.14 (1.81-8.17)	0.86 (0.31-2.41)	1.28 (0.53-3.14)	2.62 (7)

[a]Number of insects tested.
[b]Lethal concentration expressed in micrograms of insecticide per vial with 95% confidence intervals.
[c] Bioassay of Burleson County September 2005 susceptible field population.

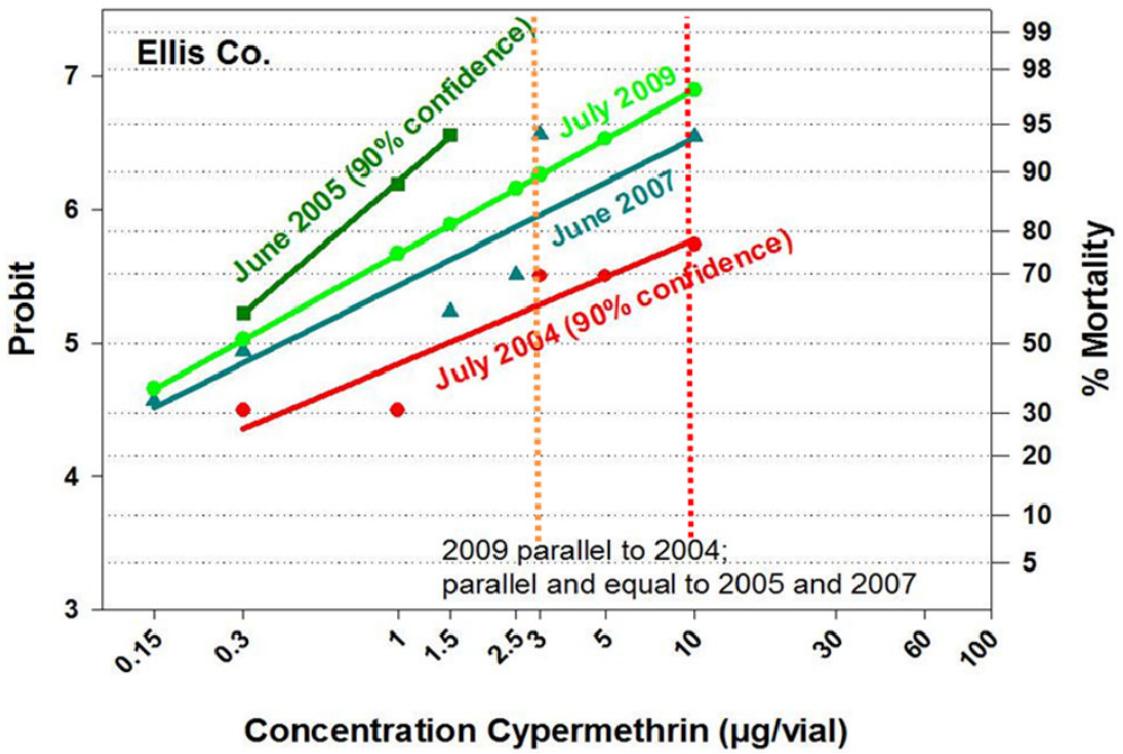

Figure 6. Concentration-mortality lines for the most resistant male bollworm populations collected in 2004, 2005, 2007, and 2009 in Ellis Co. and exposed 24 h to cypermethrin in the vial assay. The June 2009 population line was parallel to probit lines from all previous years, but this population was significantly less resistant than the July 2004 population ($p \leq 0.05$).

Tom Green and Runnels Counties

Monitoring in Tom Green and Runnels Co. was conducted from June to September in 2009. Probit analysis of bioassay data could not be done due to a high level of control mortality in those bioassays. Despite this, the highest concentration of cypermethrin survived by any moths was a single survivor collected at 10 µg/vial (Fig. 9), and overall resistance levels appeared to be low.

Parmer County

Monitoring in Parmer Co. was conducted from June to August in 2009. The LC_{50} and LC_{90} values were not significantly different than those from the 2005 susceptible Burleson Co. field population (Table 7). The concentration-mortality probit line from July 2009 was parallel and equal to probit lines from July 2007 and Sept 2006 indicating a stable susceptible population (Fig. 7).

Table 7. Parmer Co. cypermethrin bioassay for male bollworms, *Helicoverpa zea*, collected from pheromone traps, 2009. Resistance ratios (RR) with 95% confidence intervals (CI) were calculated by the method of Robertson *et al.* (2007). RR marked with * indicate that LC are significantly different from susceptible population ($p \leq 0.05$).

Date	n[a]	Slope ± SE	LC_{50}[b] (95% CI)	LC_{90}[b] (95% CI)	RR LC_{50} (95% CI)	RR LC_{90} (95% CI)	χ^2 (df)
Burleson[c]	217	1.48 ± 0.36	0.33 (0.08-0.60)	2.44 (1.52-5.77)	1	1	0.46 (3)
July 18	200	1.12 ± 0.21	0.30 (0.10-0.55)	4.20 (2.36-11.08)	0.90 (0.30-2.75)	1.72 (0.69-4.28)	5.29 (7)

[a]Number of insects tested.
[b]Lethal concentration expressed in micrograms of insecticide per vial with 95% confidence intervals.
[c] Bioassay of Burleson County September 2005 susceptible field population.

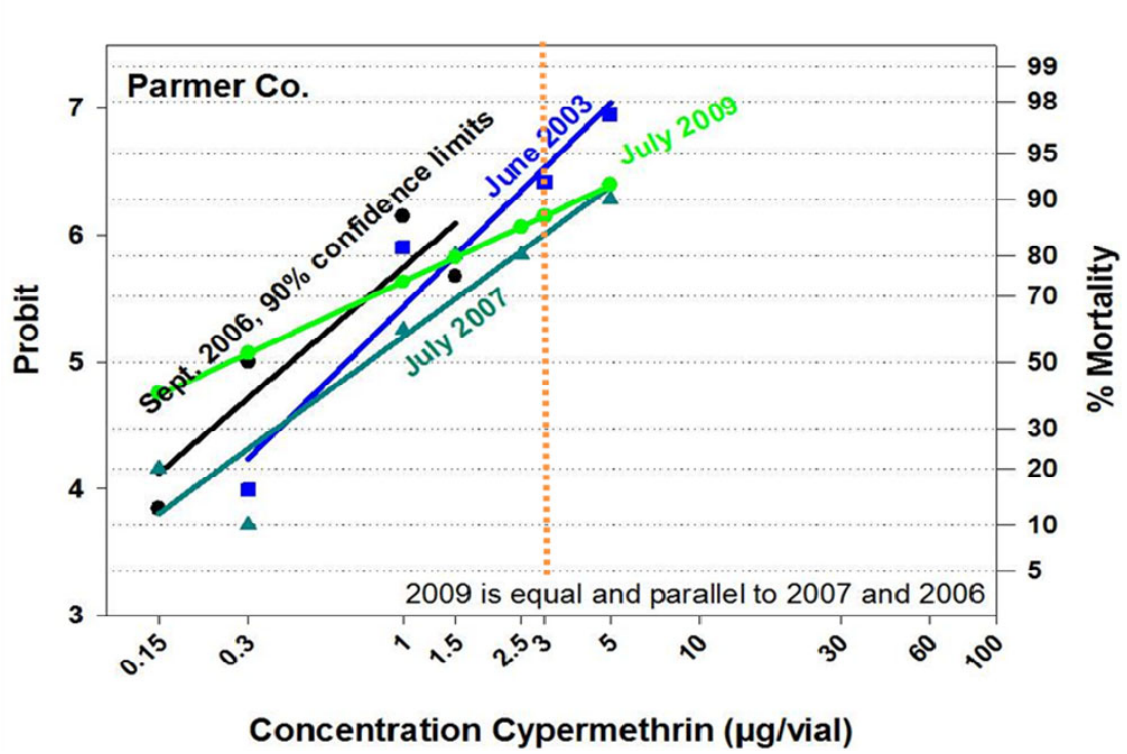

Figure 7. Concentration-mortality lines for the most resistant male bollworm populations collected in 2003, 2006, 2007, and 2009 in Parmer Co. and exposed 24 h to cypermethrin in the vial assay. The July 2009 population line was parallel and equal to probit lines from July 2007 and Sept 2006 populations ($p \leq 0.05$).

Swisher County

Monitoring in Swisher Co. was conducted from June to September in 2009. The only sampling date with a significant LC_{50} resistance ratio was July 22 (Table 8), but the resistance level was low.

Table 8. Swisher Co. cypermethrin bioassay for male bollworms, *Helicoverpa zea*, collected from pheromone traps, 2009. Resistance ratios (RR) with 95% confidence intervals (CI) were calculated by the method of Robertson *et al.* (2007). RR marked with * indicate that LC are significantly different from susceptible population ($p \leq 0.05$).

Date	n[a]	Slope ± SE	LC_{50}[b] (95% CI)	LC_{90}[b] (95% CI)	RR LC_{50} (95% CI)	RR LC_{90} (95% CI)	χ^2 (df)
Burleson[c]	217	1.48 ± 0.36	0.33 (0.08-0.60)	2.44 (1.52-5.77)	1	1	0.46 (3)
July 22	60	1.97 ± 0.46	1.20 (0.42-2.52)	5.36 (2.54-53.15)	**3.62*** **(1.38-9.54)**	2.19 (0.83-5.80)	8.63 (7)
Aug 20	200	1.88 ± 0.44	0.71 (0.24-1.19)	3.41 (2.21-6.65)	2.15 (0.75-6.20)	1.39 (0.66-2.97)	2.92 (7)

[a]Number of insects tested.
[b]Lethal concentration expressed in micrograms of insecticide per vial with 95% confidence intervals.
[c] Bioassay of Burleson County September 2005 susceptible field population.

Tipton, Oklahoma

Monitoring in Tipton, Oklahoma, was conducted from June to August in 2009. The LC_{50} and LC_{90} resistance ratios were statistically significant (Table 9), but were below 5, indicating low to moderate levels of resistance.

Table 9. Tipton, Oklahoma, cypermethrin bioassay for male bollworms, *Helicoverpa zea*, collected from pheromone traps, 2009. Resistance ratios (RR) with 95% confidence intervals (CI) were calculated by the method of Robertson *et al.* (2007). RR marked with * indicate that LC are significantly different from susceptible population ($p \leq 0.05$).

Date	n[a]	Slope ± SE	LC_{50}[b] (95% CI)	LC_{90}[b] (95% CI)	RR LC_{50} (95% CI)	RR LC_{90} (95% CI)	χ^2 (df)
Burleson[c]	217	1.48 ± 0.36	0.33 (0.08-0.60)	2.44 (1.52-5.77)	1	1	0.46 (3)
July 22	50	1.36 ± 0.39	1.14 (0.39-2.44)	10.02 (4.09-138.52)	**3.45* (1.14-10.46)**	**4.10* (1.00-16.87)**	3.96 (7)

[a]Number of insects tested.
[b]Lethal concentration expressed in micrograms of insecticide per vial with 95% confidence intervals.
[c] Bioassay of Burleson County September 2005 susceptible field population.

Conclusions

Analysis of the 2009 monitoring season revealed similar or lower levels in bollworm resistance to pyrethroid insecticides than seen in 2008. Population densities of *H. zea* were relatively low throughout most growing areas compared with previous years, likely due to excessive drought and a large reduction in overall cropping acreages. Populations exhibiting the highest LC_{50} resistance ratios were from Uvalde and Nueces Counties, with ratios of 9.1 and 7.6, respectively. These were the only two counties that had LC_{50} resistance ratios of greater than 5 (Fig. 8); however, these values are still lower than has typically been observed in these counties in previous seasons (Pietrantonio *et al.*, 2007; Junek *et al.*, 2008). Only two moths survived 60 µg/vial (both from Uvalde Co.), and there were no survivors at 30 µg/vial, with a total of almost 4,500 moths tested, indicating a very low percentage of moths surviving the most extreme rates of cypermethrin (Fig. 9). Populations from Burleson and Swisher Counties, Texas, and Tipton, Oklahoma, were significantly more resistant than the 2005 susceptible Burleson Co. field population based on likelihood ratio tests for equality, but the extent of resistance was still relatively low based upon resistance ratios. All other locations tested maintained general susceptibility to pyrethroids for 2009.

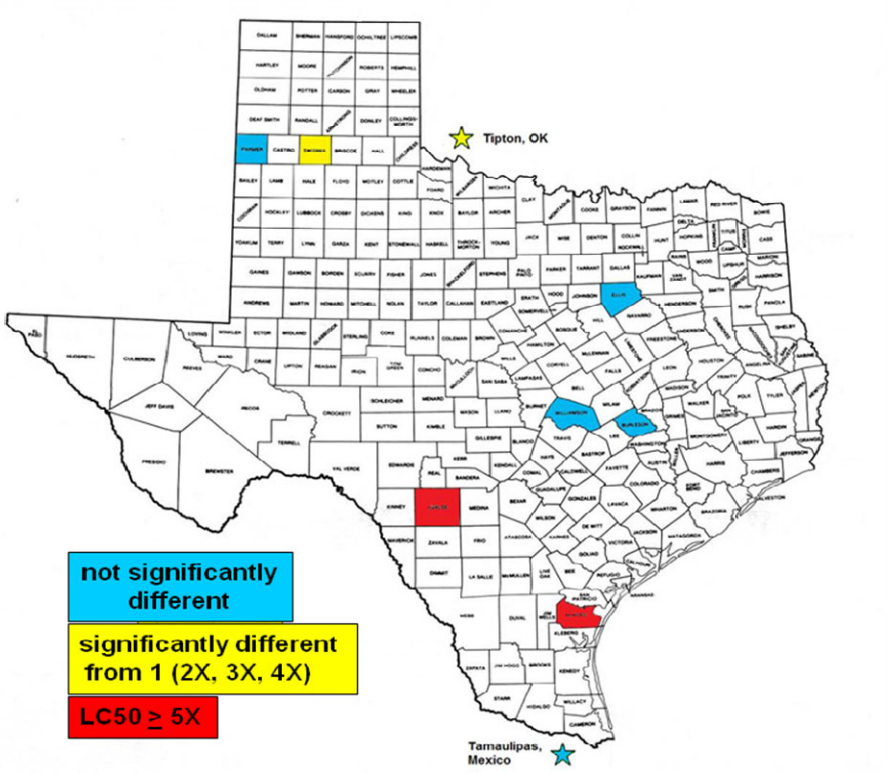

Figure 8. Highest LC_{50} resistance ratios in counties in 2009: The LC_{50} obtained for the different counties are respectively divided by the LC_{50} value of the 2005 susceptible population from Burleson Co. Values in blue represent a resistance ratio not significantly different from 1 (no resistance in the population). Values in yellow represent a resistance ratio significantly different than 1, but less than 5 (e.g., 2, 3, 4) (resistance is present but likely does not result in field control failures). Values in red represent a resistance ratio greater than 5, indicating a highly resistant population.

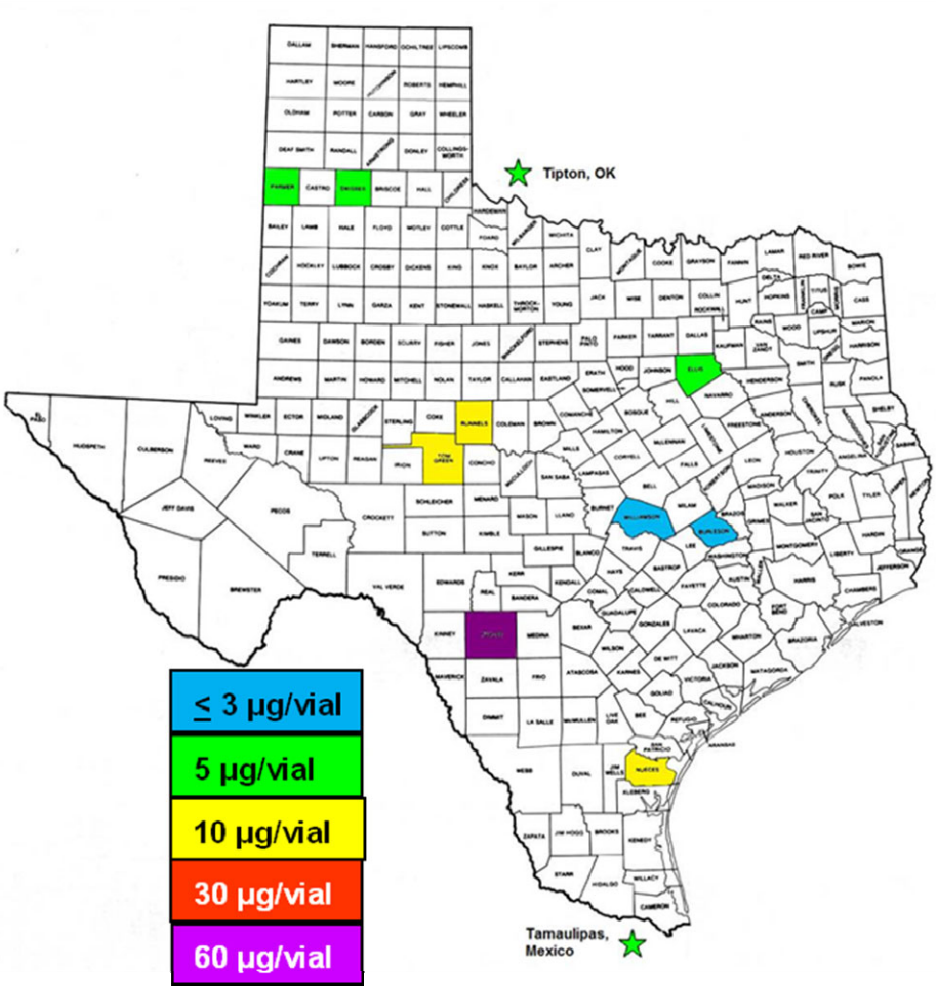

Figure 9. Highest concentration of cypermethrin in µg/vial at which individual moth survivorship was observed in 2009.

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