TEXAS RESISTANCE MONITORING PROGRAM REVEALS INCREASE IN CYPERMETHRIN SUSCEPTIBILITY IN *HELICOVERPA ZEA* (BODDIE) POPULATIONS IN 2010

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Abstract

The monitoring program in major cotton growing counties in Texas evaluated the susceptibility of males of bollworm, *Helicoverpa zea* (Boddie), populations to the pyrethroid insecticide cypermethrin. Moths were captured near cotton fields using traps with synthetic pheromone. Bioassays were conducted with the adult vial test for which glass vials are coated with different concentrations of cypermethrin dissolved in acetone. Seven Texas counties were surveyed from April to October in 2010. Data were collected from all collaborators for estimation of lethal concentrations (LC_{50} and LC_{90}), calculation of resistance ratios, and for likelihood ratio tests of equality and parallelism.

Nueces populations exhibited the highest LC_{50} (1.31 µg/vial) followed by Burleson (1.13 µg/vial) while maximum susceptibility to cypermethrin was exhibited by Hockley populations (LC_{50} 0.53µg/vial). The populations from Nueces and the Burleson Counties exhibited the highest LC_{50} resistance ratios of 3.9 and 3.4, respectively, indicating that in comparison to 2009 the Nueces population gained in susceptible individuals but yet remaining resistant, and the Burleson population maintained a similar resistance ratio to the 3.07 value for 2009. Uvalde populations with a LC_{50} of 0.79 µg/vial had a resistance ratio of 2.3 in 2010. The resistance ratios for Uvalde and Nueces showed the most drastic decline in comparison to 2009 when they exhibited the highest LC_{50} resistant ratios among counties surveyed, of 9.1 and 7.6, respectively.

Populations in the High Plains of Texas remained susceptible except in Parmer County. We found only one adult each in Hockley and Parmer counties which survived concentrations of 10- and 30-µg/vial, respectively. The populations' resistance levels for the state have shown some slight shifts towards susceptibility to pyrethroids except for Parmer Co. populations which for the first time showed a resistant population in July with a resistance ratio of 3. In summary, the Gaines, Hockley, Swisher, Nueces and Uvalde populations exhibited increased susceptibility with respect to 2009; however the Burleson populations maintained the same level of resistance compared to previous years. The overall slight decrease in resistance in 2010 may be attributed to several factors, such as the extensive

area subjected to drought in 2009 which caused a decrease in planting of summer crops in South Texas, especially sorghum and cotton, reducing source populations from sorghum and likely causing high mortality of pupae in the soil. The drought must have also reduced emergence of *H. zea* from non-crop hosts, because most collaborators also reported a decrease in overall densities, especially in Rio Bravo, Mexico and in South Texas. Reduced acreage dedicated to several crops also caused an overall reduced pesticide usage in 2009. Perhaps a larger area was planted with Bt corn in Texas in 2010, reducing source populations in early to mid-season. Finally, the impact of the area wide monitoring programs may not be neglected in creating awareness among growers regarding the resistance status of *H. zea* to pyrethroids (http://insecticideresistance.tamu.edu). Overall decreased population densities observed generally across the state may indicate areawide suppression by Bt crops in Texas.

Introduction

The purpose of this study was to monitor the susceptibility status of bollworm, *Helicoverpa Zea* (Boddie) populations to the pyrethroid insecticide cypermethrin in the main cotton production areas of Texas in 2010. The Toxicology Laboratory (Texas AgriLife Research) in collaboration with AgriLife Extension Service personnel has monitored bollworm populations for pyrethroid resistance in Burleson and Nueces Counties 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). Pyrethroid insecticide use is widespread in cotton, grain sorghum, and corn production for control of *H. zea* and other insect pests. Continued pyrethroid resistance monitoring of *H. zea* populations is an important tool for resistance management not only in cotton, but in other cropping systems as well.

Materials and Methods

The Adult Vial Test (AVT) was used to monitor the susceptibility of *H. zea* populations to cypermethrin as described (Pietrantonio *et al.*, 2007). Cypermethrin concentrations evaluated were: 0.15, 0.3, 0.6, 1.0, 1.5, 2.5, 3.0, 5.0, 10.0, 30.0, and 60.0 µg/vial; the location-specific range depending on its resistance history. In 2010, locations monitored for resistance were seven Texas counties in diverse production regions : Nueces County in the Coastal Bend; Uvalde County in the Winter Garden; Burleson County in the Brazos Valley; Parmer, Swisher, Gaines and Hockley Counties in the High Plains (see maps Figs. 8, 9). 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. A field population collected in September 2005 from Burleson County was used as a 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 their 95% confidence intervals included 1 (Robertson *et al.*, 2007).

Results and Discussion

Nueces County

Monitoring was conducted from April to October of 2010 in Nueces County. Bollworm population densities were lower than in previous years. LC_{50} resistance ratios for bollworm populations in Nueces County indicated a resistant population but the resistance ratios were about 3 to 4 with a maximum value of 9 for the upper confidence limit of the resistance ratio (Table 1), and were lower than seen in all previous years (Pietrantonio *et al.* 2007; Junek *et al.*, 2008). The concentration-mortality probit line from October 2010 was parallel to those of all previous years except for 2008. In addition there was more use of pyrethroid insecticide on both sorghum and cotton in 2010 as a result of greatly reduced cost of these compounds as generics. Dr. R. Parker reported a gradual increase in survival throughout the season first with survivors observed at 5 µg/vial in April (Table 1) and July (not shown), and in October with about 10% survival at 5 µg/vial and a statistically significant resistance ratio for the LC_{90}. In previous years the percentage of survivors at 5 or more µg/vial decreased each year in late season compared with what had been recorded in mid-season. In 2010 survival at 5 µg/vial actually increased by the end of the season but populations had similar or lower resistance ratios in late season as in previous years. In 2010 individual moths only survived the 5 µg/vial while in 2008 and 2009 single moths survived the 10 µg/vial (Figure 1). The methodology for trapping was as in previous years except that in 2010 there was more corn planted near the moth collection site.

Table 1. Nueces County cypermethrin bioassay for male bollworms, *Helicoverpa zea*, collected from pheromone traps, 2010. 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 LC 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)
04/13-18/2010	132	1.75±0.36	1.25 (0.83-1.75)	6.78 (4.05-20.00)	**3.79* (1.57-9.16)**	**2.78* (1.12-6.86)**	4.41 (5)
6/22-26/2010	120	2.63 ± 0.48	1.06 (0.52-1.65)	3.26 (2.00-15.27)	**3.21* (1.37-7.55)**	1.34 (0.66-2.71)	3.24 (3)
10/3,4/2010	280	2.13 ± 0.36	1.31 (0.41-2.04)	5.22 (3.13-32.60)	**3.95* (1.67-9.32)**	**2.14* (1.07-4.25)**	7.75 (4)

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

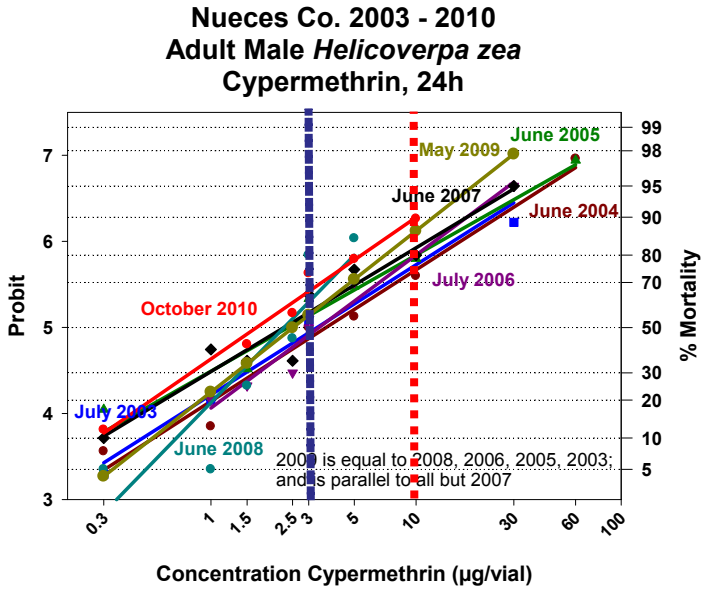

Figure 1. Concentration-mortality lines for the most resistant male bollworm populations collected from 2003 to 2010 in Nueces County and exposed 24 h to cypermethrin in the vial assay. Except for April, populations were less resistant than those surveyed in 2009, and the October 2010 probit line was parallel to those of all previous years except 2008.

Uvalde County

In Uvalde County monitoring was conducted in August, and populations and pressure in cotton remained low throughout the season; indeed Dr. Troxclair reported that 2010 was likely the year with the lowest ever pressure from bollworm in cotton and no treatment was applied to cotton in this area. The LC_{50} resistance ratios for August were on par with the baseline susceptible population (Table 2). The 2010 year showed a decline in the LC_{50} and resistance ratios values compared to the year 2009. The concentration-mortality probit line of August 2010 was almost parallel to all lines from previous years except those from July 2005 and 2007 (Figure 2). In spite of low resistance ratios this year one individual survived the threshold resistance concentration of 5µg/ vial.

Table 2. Uvalde County cypermethrin bioassay for male bollworms, *Helicoverpa zea*, collected from pheromone traps, 2010. 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 LC 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)
8/20,24/2010	418	2.56 ± 0.36	0.79 (0.33-1.17)	2.49 (1.75-4.36)	2.38 (0.99-5.66)	1.02 (0.55-1.90)	5.79 (4)

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

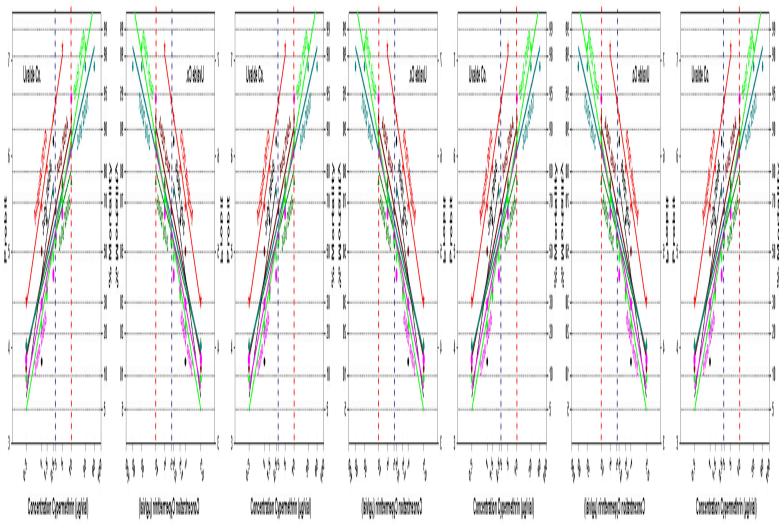

Figure 2. Concentration-mortality lines for the most resistant male bollworm populations collected from 2004 to 2009 in Uvalde County and exposed 24 h to cypermethrin in the vial assay. The August 2010 population line was parallel to all lines except those for July 2005 and 2007.

In Uvalde County, the pressure of *H. zea* was observed instead in corn as corn earworm larvae. For example, in non-Bt corn planted on August 4, there were about 3-4 larvae per ear. Treatments against *H. zea* larvae were made in other crops such as green beans and sweet corn; the latter was treated every day due to high *H. zea* density.

Burleson County

Monitoring in Burleson County was conducted from June to August 2010. Resistance was detected only in the populations collected in August (Table 3), but the susceptibility to cypermethrin was maintained in the region with no major shift observed in the resistance levels compared to the previous year. The August 2010 line was parallel to the July 2000, 2003 and 2004 lines (Figure 3).

Table 3. Burleson County cypermethrin bioassay for male bollworms, *Helicoverpa zea*, collected from pheromone traps, 2010. 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 LC 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)
6/25,30/2010	140	2.01 ± 0.46	0.77 (0.30-1.22)	3.31 (2.14-6.92)	2.31 (0.85-6.64)	1.36 (0.63-2.94)	3.80 (4)
8/6-30/2010	206	1.92 ± 0.47	1.13 (0.49-1.70)	5.26 (3.42-13.50)	**3.42* (1.30-8.97)**	2.15 (0.96-4.84)	1.83 (5)

Resistance ratios (RR) with 95% confidence intervals (CI) calculated by the method of Robertson et al. (2007). RR marked with * indicate that LC are significantly different from those of the susceptible population ($p \leq 0.05$).

[a]Number of insects tested.

[b]Lethal concentration expressed in micrograms of insecticide per vial with 95% confidence intervals (CI).

[c]Bioassay of Burleson County September 2005 susceptible field population.

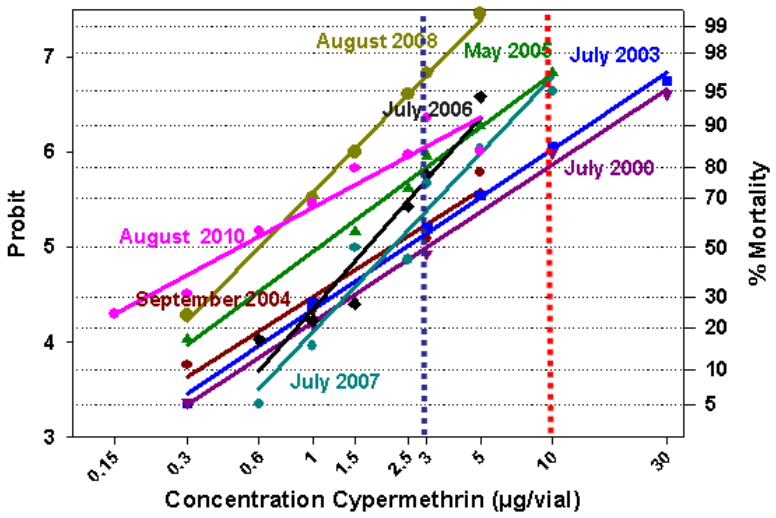

Figure 3. Concentration-mortality lines for the most resistant male bollworm populations collected from 2000 and 2003 to 2010 in Burleson County and exposed 24 h to cypermethrin in the vial assay. The July 2009 population line was parallel with the July 2000, 2003 and, 2004.

Parmer County

Monitoring in Parmer County was conducted from July to August in 2010. The LC_{50} calculated from the July population was significantly different from those of the 2005 susceptible Burleson Co. field population (Table 4, Figure 4). A slight decrease in susceptibility has been observed in the Parmer 2010 population compared to populations surveyed in previous years, which had remained on par with the baseline susceptible Burleson populations of 2005. The resistance ratio of 3.09 of the population collected in July was statistically significant indicating a resistant population. The LC_{50} could not be calculated for the bioassay performed in August because

only 72 moths were captured, but the highest concentration survived by a single moth was 2.5 µg/vial, indicating that both the level and frequency of resistance were low. The 2010 general bollworm pressure in cotton for the Parmer County area would be considered moderate for the most part and somewhat sporadic especially when compared to the extreme acute infestations observed in the past. Bt technology has been readily adopted in the area's corn and cotton production and may have played a role in this shift in population dynamics in cotton.

Table 4. Parmer County cypermethrin bioassay for male bollworms, *Helicoverpa zea*, collected from pheromonetraps, 2010. 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 LC 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)
7/23/2010	90	2.86 ± 0.80	1.02 (0.57-1.47)	2.87 (1.90-8.44)	**3.09* (1.25-7.61)**	1.17 (0.52-2.63)	2.65 (3)

[a]Number of insects tested.
[b]Lethal concentration expressed in micrograms of insecticide per vial with 95% confidence intervals (CI).

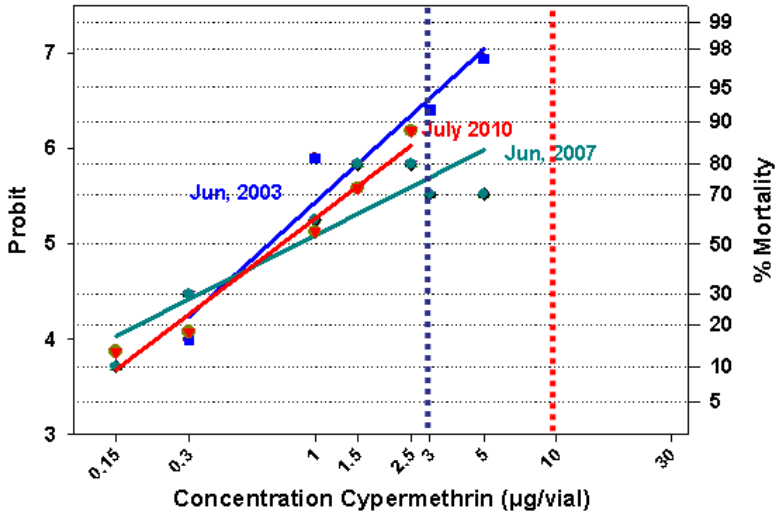

Figure 4. Concentration-mortality lines for the most resistant male bollworm populations collected in 2003, 2007, and 2010 in Parmer County and exposed 24 h to cypermethrin in the vial assay. The July 2010 population line was neither parallel nor equal to probit lines from June 2003 and June 2007 populations.

Swisher County

Monitoring in Swisher County was conducted in August in 2010 (Figure 5). The populations of this region maintained susceptibility with no significant difference in the LC_{50} of the current year population and the baseline susceptible 2005 Burleson population (Table 5).

Table 5. Swisher County cypermethrin bioassay for male bollworms, *Helicoverpa zea*, collected from pheromone traps, 2010. 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 LC 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)
8/23/2010	113	2.31 ± 0.80	0.80 (0.12-1.36)	2.89 (1.77-11.03)	2.43 (0.81-7.29)	1.18 (0.51-2.73)	1.27 (3)

Resistance ratios (RR) with 95% confidence intervals (CI) calculated by the method of Robertson et al. (2007). RR marked with * indicate that LC are significantly different from those of the susceptible population ($p \leq 0.05$).

[a]Number of insects tested.

[b]Lethal concentration expressed in micrograms of insecticide per vial with 95% confidence intervals (CI).

[c]Bioassay of Burleson County September 2005 susceptible field population.

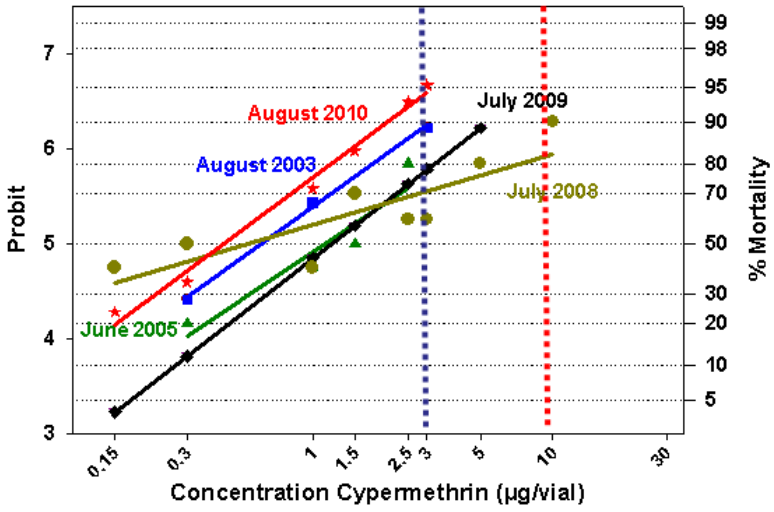

Figure 5. Concentration-mortality lines for the most resistant male bollworm populations collected from 2003 to 2010 in Swisher County and exposed 24 h to cypermethrin in the vial assay. The August 2010 population line was parallel to those of August 2003, June 2005 and July 2009.

Hockley County

Monitoring in Hockley County was conducted from July to September in 2010 (Figure 6). The populations of this region also maintained susceptibility with no significant difference in the LC_{50} of the current year population and the baseline susceptible 2005 Burleson population (Table 6). There was an overall slight increase in the susceptibility compared to the 2008 populations. Due to the low number of insects (51) captured in July the results could not be analyzed by probit analysis but these insects were, overall, also susceptible. In the July bioassay, only one moth was resistant and survived 10 µg/vial dosage.

Table 6. Hockley County cypermethrin bioassay for male bollworms, *Helicoverpa zea*, collected from pheromone traps, 2010. 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 LC 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)
9/6,8,12/2010	132	1.62 ± 0.31	0.53 (0.34-0.77)	3.31 (1.93-9.48)	1.61 (0.66-3.96)	1.36 (0.54-3.39)	1.36 (3)

Resistance ratios (RR) with 95% confidence intervals (CI) calculated by the method of Robertson et al. (2007).
RR marked with * indicate that LC are significantly different from those of the susceptible population ($p \leq 0.05$).

[a]Number of insects tested.

[b]Lethal concentration expressed in micrograms of insecticide per vial with 95% confidence intervals (CI).

[c]Bioassay of Burleson County September 2005 susceptible field population.

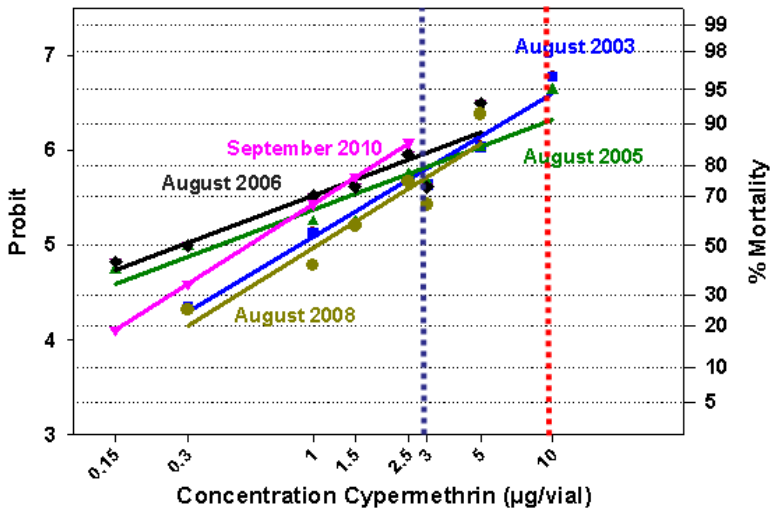

Figure 6. Concentration-mortality lines for the most resistant male bollworm populations collected from 2003 to 2010 in Hockley County and exposed 24 h to cypermethrin in the vial assay. The September 2010 population line was parallel to those of August 2003 and August 2008.

Gaines County

The populations of Gaines County also maintained susceptibility with no significant difference between the LCs of August 2010 and the September 2005 susceptible Burleson population (Table 7). There was an overall increase in susceptibility compared to the August and September 2004 populations which had LC_{50} values of 1.76 and 2.36µg/vial, respectively (Pietrantonio et al., 2005). Despite the high susceptibility shown by this year's population, one resistant adult was found surviving the concentration of 5 µg/vial (Figure 7).

Table 7. Gaines County cypermethrin bioassay for male bollworms, *Helicoverpa zea*, collected from pheromone traps, 2010. 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 LC 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)
8/11-17/2010	164	1.25 ± 0.29	0.45 (0.05-1.03)	4.77 (2.04-56.54)	1.37 (0.45-4.18)	1.95 (0.71-5.36)	5.66 (5)

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

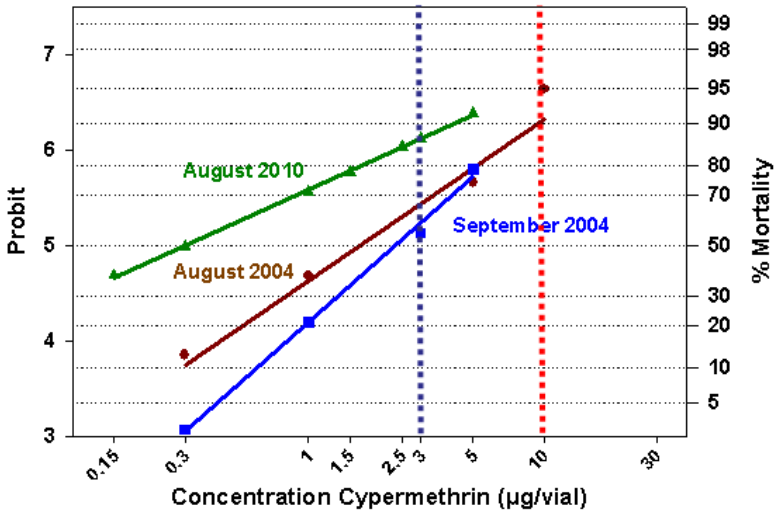

Figure 7. Concentration-mortality lines for the most resistant male bollworm populations collected from 2004 and 2010 in Gaines County and exposed 24 h to cypermethrin in the vial assay.

Conclusions

The 2010 monitoring program revealed similar or lower levels of bollworm cypermethrin resistance than seen in 2009 except for Parmer County. Population densities of *H. zea* were relatively low throughout most growing areas compared with previous years, perhaps a consequence of the excessive drought and a large reduction in overall cropping acreages during 2009. Populations exhibiting the highest LC_{50} resistance ratios were from Nueces and Burleson Counties, with resistance ratios of 3.95 and 3.42, respectively. All the counties had LC_{50} resistance ratios of less than 5 (Figure 8) and all of these values are lower than those observed in previous seasons (Pietrantonio *et al.*, 2007; Junek *et al.*, 2008) except for Parmer Co. A few moths from each county (Burleson, Uvalde, Gaines and Nueces) survived 5 µg/vial (Figure 9). Populations from Nueces, Burleson and Parmer Counties were significantly more resistant than the 2005 susceptible Burleson County field population but the level of this difference was relatively low based on resistance ratios values. Populations were also different based on likelihood ratio tests for equality. This is the first year in which the Nueces County populations did not reach a resistance ratio of 5 or higher for the LC_{50} since we began monitoring in 2003 when the resistance ratio was 9 (Pietrantonio et al., 2004). Resistant individuals are, however, still present in these populations and increased pyrethroid pressure may increase the frequency of resistance in 2011. All other locations tested maintained general susceptibility to pyrethroids for 2010.

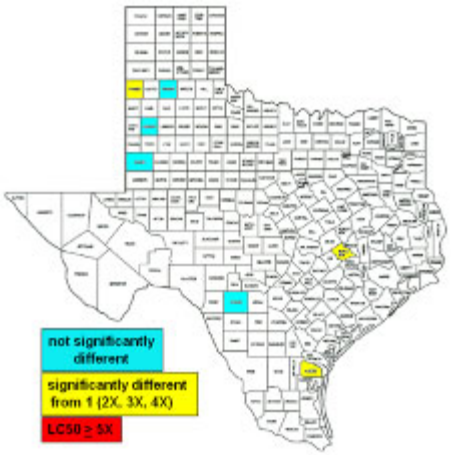

Figure 8. Highest LC_{50} resistance ratios for populations in counties in 2010: The LC_{50} obtained for the different counties are respectively divided by the LC_{50} value of the 2005 susceptible population from Burleson County. Values in blue represent a resistance ratio not significantly different 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 with possibility of field control failures. None of the populations were highly resistant in 2010.

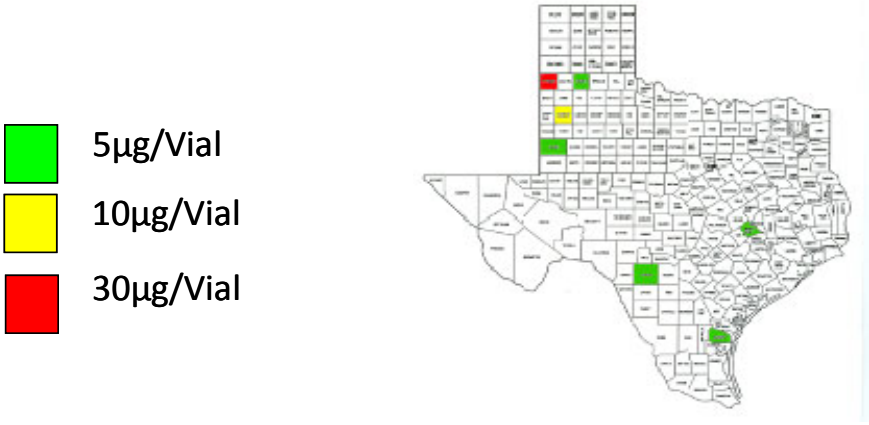

Figure 9. Highest concentration of cypermethrin in µg/vial at which individual moth survivorship was observed in 2010.

Acknowledgements

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