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MONITORING FOR PYRETHROID RESISTANCE IN BOLLWORM (*HELICOVERPA ZEA*) IN TEXAS - 2006

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Abstract

The purpose of this study was to assess the susceptibility of the cotton bollworm, *Helicoverpa zea* (Boddie) to the pyrethroid cypermethrin in the main production areas of Texas. Pyrethroid insecticides use is widespread in cotton and in other systems, such as corn and grain sorghum. This statewide monitoring program that evaluated resistance in male bollworm was conducted from April to September 2006 surveying 10 Texas Counties. Moths were trapped near cotton fields using pheromone, Hercon Luretape® with Zealure. Vials were prepared in the Toxicology Laboratory, Department of Entomology at Texas A&M University, College Station, Texas, and shipped as needed to Texas Cooperative Extension personnel. Data from all areas in Texas was sent to Texas A&M University Toxicology Laboratory for analysis. Calculations included lethal concentrations that killed half of the population (LC_{50}); 90% of the population (LC_{90}), resistance ratios (number of times that the amount of insecticide used to kill 50% of the susceptible population has to be multiplied by to kill half of the field populations), and the statistical significance test for the resistance ratios. A great variability in response to cypermethrin was detected in bollworms across the state. Based on the lethal concentration fifty (LC_{50}) data, the most resistant populations were from Nueces, Burleson, Williamson and Uvalde Counties. The most susceptible populations were from Tom Green, Hockley, Swisher, and Parmer Counties. The status of cypermethrin resistance in Nueces County populations has changed in that highly resistant populations were found earlier in the season. For the first time *H. zea* was tested in Tamaulipas, Mexico, with a resistance ratio of 4.34 for the LC_{50} while simultaneously, Nueces Co. populations, across the border, revealed a resistance ratio of 10. Burleson County populations showed an improving situation, with a progressive return towards susceptibility from 2003 to 2005 and stabilizing in 2006.

Introduction

The purpose of this study was to assess the susceptibility of bollworm to cypermethrin, a pyrethroid, in the majority of the cotton production regions of Texas. The entomology toxicology laboratory at Texas A&M University has monitored the evolution of resistance to pyrethroids in bollworm in Burleson and Nueces counties since 1998. In the last four years, pyrethroid resistance has been monitored in other production regions and widespread presence of individuals with a resistant phenotype but at different frequencies in different locations has been detected. The resistant ratios varied in different counties and in different years, exemplifying the local and varied nature of insecticide resistance in general and of cypermethrin resistance in particular. Additionally, immigration of pyrethroid resistant moths from Mexico into Texas and migration from neighboring Texas counties may add to the complexity of addressing pyrethroid resistance management in cotton bollworm in Texas.

Materials and Methods

Moth collection and vial assays:

Adult male Helicoverpa zea moths were trapped using pheromone, Hercon Luretape® with Zealure^{TM} from Great Lakes IPM (Vestaburg, MI). Moths were collected early in the morning and bioassays were performed the same day in the laboratory. Moths were supplied with a 10% sucrose solution until placed in vials. Only healthy, vigorous male moths with intact wing scales were used for bioassays. The adult vial test (AVT) was used to monitor the susceptibility of bollworm to cypermethrin. Vials were prepared in the Department of Entomology, Toxicology Laboratory at Texas A&M University, College Station, Texas and shipped as needed to Texas Cooperative Extension collaborators throughout the state. Stock solutions were prepared by dissolving technical grade (95.2%) cypermethrin in dehydrated acetone. Acetone was dehydrated for at least 48 h on 4Å molecular sieves (EM Science) before use. Serial dilutions (2X) from each stock solution yielded the desired concentrations. Insecticide dosages used for this study were: 0.15, 0.3, 1, 1.5, 2.5, 3, 5, 10, 30 and 60 µg cypermethrin/vial. Test vials were prepared by coating the inside of the vial with an acetone solution of the respective insecticide concentration. The control vials were coated with dehydrated acetone only. Vials were prepared by dispensing 0.5 ml of acetone or cypermethrin solutions and dried on a cold "hot-dog" roller (heating element disconnected) under the hood for at least 15 min until the acetone had evaporated. One moth was placed in each vial and the vials were stored at 27 °C or room temperature. Mortality was counted after 24h. Moths were evaluated as alive, dead, or "knocked-down." Moths that were alive but could not fly in a normal manner were considered "knocked-down" and were included as dead for calculations of percentage of mortality. Two discriminating cypermethrin dosages of 3 µg/vial and 10 µg/vial were used among the various tested. A 2.5 µg/vial dosage was recommended as discriminatory, possibly killing all susceptible bollworms. Previous studies suggest the dosage of 5 µg/vial was the ultimate discriminatory concentration for susceptible moths. The IRAC (Insecticide Resistance Action Committee) procedure utilized the 5 µg/vial for the same discrimination in previous monitoring efforts (Payne et al., 2001). In 2005, the susceptible laboratory colony of H. zea used in this work was reared from pupae, received from the USDA/ARS at Stoneville, Mississippi. Insects from this colony were maintained at 27°C with a 6:8 photoperiod on artificial diet.

Locations:

Twelve counties in Texas were included in the 2006 monitoring program, as follows: Hale, Hockley, Parmer and Swisher Counties in the High Plains production region; Jones and Tom Green Counties in the Southern Rolling Plains region; Ellis and Williamson Counties in the Blacklands region; Burleson County in the Brazos River Bottom, Uvalde County in the Winter Garden region; and Nueces County in the Coastal Bend region, Hidalgo County in the Lower Rio Grande Valley and assays were also taken in Tamaulipas, Mexico.

Data analysis:
Data from all areas in Texas was sent to Texas A&M University Toxicology laboratory and analyzed using Polo PC, Probit and Logit Analysis program, and dose-mortality regressions were plotted using SigmaPlot software. Data were corrected for mortality using Abbott's (1925) formula. Confidence intervals for resistance ratios were calculated as described by Robertson and Preisler. The lethal concentration resistant ratios of different populations were considered not significantly different if the 95% confidence intervals included 1. Statistically significantly different resistance ratios higher than one are shown in red in the tables. Resistance ratios in blue in tables indicate that the field population is statistically more susceptible than the laboratory reference colony.

<u>Results and Discussion</u>

Cotton in the Burleson County area is planted to 95% Bt transgenic varieties. Most of Texas suffered from drought but this region had timely rains and irrigation that led to an above average crop. Bollworm pressure was light in this region much like the rest of the state. Overall, Burleson County resistance issues continue to improve with no resistance ratios above 5 at the LC_{90} values (Table 1).

Nueces County had 163,802 acres harvested in 2006 and 24% of that acreage was some type of Bt transgenic variety. Drought severely impacted the region. As in previous years, a significant amount (46%) of grain sorghum was treated for “headworm” problems. Bollworm was easier to control than in the past two years (2004-2005) since infestation levels were lower. Despite lower use in cotton, resistance issues continue to be a concern in the region. Resistance ratios were lower than in previous years (Table 2); however, resistance was evident throughout the season.

Tamaulipas, Mexico was surveyed for the first time in 2006 (Table 3). Some discussion has taken place that resistance may be a problem due to moths generated in Mexico but this data seem to contradict that statement. Resistance ratios were low for 2006. Moths were possibly treated in corn but bollworms were not a problem in the cotton.

As in previous years, resistance concerns remain low in the Southern Rolling Plains and High Plains cotton growing regions (Tables 4-8). Drought had a significant impact throughout the western growing regions and bollworm populations in fields were well below average. Bt cotton acreage continues to increase as producers choose varieties that offer the best opportunity to improve yields and fiber quality.

Table 1. Burleson Co. Cypermethrin Bioassay for bollworm, *Helicoverpa zea*, 2006. Area around TAMU.

Date	n[a]	Slope ± SE	LC_{50}[b] (95% CL)	LC_{90}[b] (95% CL)	RR[c] LC_{50} (95% CI)	RR[c] LC_{90} (95% CI)	χ^2 (df)
Burleson[d]	400	1.47 ± 0.35	0.33 (0.08-0.59)	2.44 (1.51-5.76)	1	1	0.45 (3)
04-21-06	187	2.37 ± 0.78	1.14 (0.22-1.81)	3.97 (2.65-11.62)	**3.47** (1.21-9.90)	1.63 (0.75-3.50)	0.82 (4)
05-11-06	189	2.57 ± 0.57	1.32 (0.77-1.82)	4.17 (2.94-8.13)	**4.01** (1.64-9.82)	1.70 (0.82-3.52)	2.27 (4)
06-09-06	299	2.05 ± 0.33	1.11 (0.74-1.47)	4.66 (3.35-8.00)	**3.35** (1.40-8.03)	1.91 (0.94-3.86)	1.53 (5)
06-23-06	198	1.53 ± 0.32	0.99 (0.48-1.51)	6.80 (4.09-19.03)	**2.99** (1.14-7.82)	**2.78** (1.15-6.73)	2.36 (5)
07-21-06	200	3.39 ± 1.04	1.88 (0.98-2.43)	4.49 (3.38-10.73)	**5.80** (2.37-13.70)	1.84 (0.91-3.72)	2.22 (5)
08-17-06	150	1.53 ± 0.45	0.56 (0.14-1.00)	3.88 (2.03-25.40)	1.71 (0.57-5.09)	1.58 (0.54-4.65)	3.30 (4)
09-07-06	250	2.68 ± 0.84	0.93 (0.29-1.34)	2.81 (2.60-14.42)	**2.83** (1.08-7.43)	1.15 (0.56-2.35)	2.90 (4)
09-22-06	200	2.80 ± 0.78	0.71 (0.43-0.97)	2.04 (1.37-6.35)	2.15 (0.89-5.16)	0.83 (0.37-1.87)	1.32 (2)

[a]Number of insects tested.
[b]Lethal concentration expressed in micrograms of insecticide per vial with 95% confidence limits.
[c]Resistance ratio, RR, with 95% confidence intervals as calculated by the method of Robertson and Preisler (1992) using the susceptible Burleson County September 2005 field population as the ratio divisor.
[d] Bioassay of Burleson County September 2005 susceptible field population.

Table 2. Nueces Co. Cypermethrin Bioassay for bollworm, *Helicoverpa zea*, 2006

Date	n[a]	Slope ± SE	LC_{50}[b] (95% CL)	LC_{90}[b] (95% CL)	RR[c] LC_{50} (95% CI)	RR[c] LC_{90} (95% CI)	χ^2 (df)
Burleson[d]	400	1.47 ± 0.35	0.33 (0.08-0.59)	2.44 (1.51-5.76)	1	1	0.45 (3)
05/11-15/2006	370	3.16 ± 0.37	3.47 (2.95-4.12)	8.83 (6.95-12.61)	**10.51** (4.60-24.04)	**3.62** (1.90-6.88)	2.15 (3)
06/06-18/2006	600	2.09 ± 0.25	2.92 (2.31-3.55)	11.94 (9.08-17.88)	**8.85** (3.83-20.43)	**4.89** (2.52-9.47)	5.05 (6)
07/01-06/2006	200	1.82 ± 0.35	3.32 (2.41-4.86)	16.68 (9.46-54.98)	**10.05** (4.19-24.09)	**6.83** (2.60-17.91)	1.74 (4)
07/18-20/2006	280	2.30 ± 0.31	2.58 (2.09-3.20)	9.29 (6.66-15.83)	**7.81** (3.38-18.04)	**3.80** (1.87-7.72)	2.75 (5)
09/11-20/2006	300	1.78 ± 0.26	2.26 (1.64-3.08)	11.85 (7.61-24.50)	**6.85** (2.88-16.29)	**4.85** (2.18-10.78)	2.23 (3)
10/02-04/2006	140	1.10 ± 0.30	1.00 (0.34-1.73)	14.32 (6.09-189.06)	**3.02** (1.06-8.57)	**5.85** (1.45-23.59)	4.91 (5)

[a]Number of insects tested.
[b]Lethal concentration expressed in micrograms of insecticide per vial with 95% confidence limits.
[c]Resistance ratio, RR, with 95% confidence intervals as calculated by the method of Robertson and Preisler (1992) using the susceptible Burleson County September 2005 field population as the ratio divisor.
[d] Bioassay of Burleson County September 2005 susceptible field population.

Table 3. Rio Bravo, Tamaulipas, Mexico Cypermethrin Bioassay for bollworm, *Helicoverpa zea*, 2006

Date	n[a]	Slope ± SE	LC_{50}[b] (95% CL)	LC_{90}[b] (95% CL)	RR[c] LC_{50} (95% CI)	RR[c] LC_{90} (95% CI)	χ^2 (df)
Burleson[d]	400	1.47 ± 0.35	0.33 (0.08-0.59)	2.44 (1.51-5.76)	1	1	0.45 (3)
05-11-06	100	3.03 ± 1.14	1.43 *(0.73-2.02)	3.79 *(2.56-13.56)	**4.34** (1.71-10.99)	1.55 (0.64-3.76)	2.04 (3)
06-04-06	100	1.13 ± 0.47	0.39 *(0.02-0.80)	5.31 *(2.36-182.65)	1.19 (0.27-5.22)	2.17 (0.44-10.65)	0.75 (3)

[a]Number of insects tested.
[b]Lethal concentration expressed in micrograms of insecticide per vial with *90% confidence limits
[c]Resistance ratio, RR, with 95% confidence intervals as calculated by the method of Robertson and Preisler (1992) using the susceptible Burleson County September 2005 field population as the ratio divisor.
[d] Bioassay of Burleson County September 2005 susceptible field population. All confidence limits on LC50 and LC90 are 90%.

Table 4. Swisher Co. Cypermethrin Bioassay for bollworm, *Helicoverpa zea*, 2006

Date	n[a]	Slope ± SE	LC_{50}[b] (95% CL)	LC_{90}[b] (95% CL)	RR[c] LC_{50} (95% CI)	RR[c] LC_{90} (95% CI)	χ^2 (df)
Burleson[d]	400	1.47 ± 0.35	0.33 (0.08-0.59)	2.44 (1.51-5.76)	1	1	0.45 (3)
08/02/2006	80	0.69 ± 0.82	0.05*	3.87*	0.16 (0.001-23.42)	1.58 (0.003-774.36)	1.72 (1)

[a]Number of insects tested.
[b]Lethal concentration expressed in micrograms of insecticide per vial with *< 90% confidence limits
[c]Resistance ratio, RR, with 95% confidence intervals as calculated by the method of Robertson and Preisler (1992) using the susceptible Burleson County September 2005 field population as the ratio divisor.
[d] Bioassay of Burleson County September 2005 susceptible field population.

Table 5. Hockley Co. Cypermethrin Bioassay for bollworm, *Helicoverpa zea*, 2006

Date	n[a]	Slope ± SE	LC_{50}[b] (95% CL)	LC_{90}[b] (95% CL)	RR[c] LC_{50} (95% CI)	RR[c] LC_{90} (95% CI)	χ^2 (df)
Burleson[d]	400	1.47 ± 0.35	0.33 (0.08-0.59)	2.44 (1.51-5.76)	1	1	0.45 (3)
06/27,28/ 2006	170	0.90 ± 0.26	0.15 (0.007-0.42)	4.013 (1.83-23.50)	0.46 (.008-2.55)	1.64 0.52-5.13)	4.55 (5)
07/11,16/ 2006	199	1.07 ± 0.23	0.17 (0.04-0.34)	2.81 (1.52-8.92)	0.54 (0.16-1.75)	1.15 (0.43-3.01)	0.35 (5)
08/02, 07/ 2006	300	0.91 ± 0.19	0.30 (0.10-0.55)	7.63 (3.64-35.92)	0.92 (0.30-2.77)	3.12 (0.98-9.85)	3.02 (5)
08/14, 16/ 2006	100	0.79 ± 0.41	*0.16	*6.78	0.50 (0.007-3.51)	2.77 (0.17-44.84	1.00 (3)

[a]Number of insects tested.
[b]Lethal concentration expressed in micrograms of insecticide per vial with 95% confidence limits, *<90% confidence limits.
[c]Resistance ratio, RR, with 95% confidence intervals as calculated by the method of Robertson and Preisler (1992) using the susceptible Burleson County September 2005 field population as the ratio divisor.
[d] Bioassay of Burleson County September 2005 susceptible field population.

Table 6. Parmer Co. Cypermethrin Bioassay for bollworm, *Helicoverpa zea*, 2006

Date	n[a]	Slope ± SE	LC_{50}[b] (95% CL)	LC_{90}[b] (95% CL)	RR[c] LC_{50} (95% CI)	RR[c] LC_{90} (95% CI)	χ^2 (df)
Burleson[d]	400	1.47 ± 0.35	0.33 (0.08-0.59)	2.44 (1.51-5.76)	1	1	0.45 (3)
07/06/2006	100	1.98 ± 0.96	0.61*	2.70*	1.85 (0.55-6.20)	1.10 (0.29-4.22)	0.0007 (1)
09/29/2006	115	1.80 ± 0.67	0.39** (0.13-0.72)	2.00** (0.99-18.13)	1.18 (0.38-3.65)	0.82 (0.21-3.07)	1.68 (2)

[a]Number of insects tested.
[b]Lethal concentration expressed in micrograms of insecticide per vial with 95% confidence limits. *<90% confidence limits, ** 90% confidence limits.
[c]Resistance ratio, RR, with 95% confidence intervals as calculated by the method of Robertson and Preisler (1992) using the susceptible Burleson County September 2005 field population as the ratio divisor.
[d]Bioassay of Burleson County September 2005 susceptible field population.

Table 7. Jones Co. Cypermethrin Bioassay for bollworm, *Helicoverpa zea*, 2006

Date	n[a]	Slope ± SE	LC_{50}[b] (95% CL)	LC_{90}[b] (95% CL)	RR[c] LC_{50} (95% CI)	RR[c] LC_{90} (95% CI)	χ^2 (df)
Burleson[d]	400	1.47 ± 0.35	0.33 (0.08-0.59)	2.44 (1.51-5.76)	1	1	0.45 (3)
09/07/2006	100	2.08 ± 0.56	1.57*	6.48*	**4.75** (1.89-11.95)	2.65 (0.91-7.66)	4.33 (3)

[a]Number of insects tested.
[b]Lethal concentration expressed in micrograms of insecticide per vial with 95% confidence limits. *< 90% confidence limits
[c]Resistance ratio, RR, with 95% confidence intervals as calculated by the method of Robertson and Preisler (1992) using the susceptible Burleson County September 2005 field population as the ratio divisor.
[d]Bioassay of Burleson County September 2005 susceptible field population.

Table 8.Tom Green Co. Cypermethrin Bioassay for bollworm, *Helicoverpa zea*, 2006

Date	n[a]	Slope ± SE	LC_{50}[b] (95% CL)	LC_{90}[b] (95% CL)	RR[c] LC_{50} (95% CI)	RR[c] LC_{90} (95% CI)	χ^2 (df)
Burleson[d]	400	1.47 ± 0.35	0.33 (0.08-0.59)	2.44 (1.51-5.76)	1	1	0.45 (3)
07/10/2006	100	0.65 ± 0.50	0.04*	3.92*	0.13 (.001-12.51)	1.60 (0.05-46.34)	2.26 (3)

[a]Number of insects tested.
[b]Lethal concentration expressed in micrograms of insecticide per vial with 95% confidence limits. *< 90% confidence limits
[c]Resistance ratio, RR, with 95% confidence intervals as calculated by the method of Robertson and Preisler (1992) using the susceptible Burleson County September 2005 field population as the ratio divisor.
[d]Bioassay of Burleson County September 2005 susceptible field population.

Williamson and Uvalde Counties had average conditions with irrigation saving the Uvalde crop and timely rains helping the Williamson County crop. Resistance problems were not evident and resistance ratios were fairly low (Tables 9-10).

Table 9 . Uvalde Co. Cypermethrin Bioassay for bollworm, *Helicoverpa zea*, 2006

Date	n[a]	Slope ± SE	LC_{50}[b] (95% CL)	LC_{90}[b] (95% CL)	RR[c] LC_{50} (95% CI)	RR[c] LC_{90} (95% CI)	χ^2 (df)
Burleson[d]	400	1.47 ± 0.35	0.33 (0.08-0.59)	2.44 (1.51-5.76)	1	1	0.45 (3)
08/09/2006	100	5.63 ± 2.38	1.48* (0.90-1.97)	2.51* (1.91-7.14	**4.49** (1.87-10.77)	1.03 (0.49-2.15)	0.77 (2)

[a]Number of insects tested.
[b]Lethal concentration expressed in micrograms of insecticide per vial with * 90% confidence limits
[c]Resistance ratio, RR, with 95% confidence intervals as calculated by the method of Robertson and Preisler (1992) using the susceptible Burleson County September 2005 field population as the ratio divisor.
[d]Bioassay of Burleson County September 2005 susceptible field population.

Table 10. Williamson Co. Cypermethrin Bioassay for bollworm, *Helicoverpa zea*, 2006

Date	n[a]	Slope ± SE	LC_{50}[b] (95% CL)	LC_{90}[b] (95% CL)	RR[c] LC_{50} (95% CI)	RR[c] LC_{90} (95% CI)	χ^2 (df)
Burleson[d]	400	1.47 ± 0.35	0.33 (0.08-0.59)	2.44 (1.51-5.76)	1	1	0.45 (3)
05/26/2006	100	2.65 ± 1.22	1.35*	4.12*	**4.10** (1.58-10.63)	1.68 (0.52-5.47)	0.67 (2)

[a]Number of insects tested.
[b]Lethal concentration expressed in micrograms of insecticide per vial with *< 90% confidence limits
[c]Resistance ratio, RR, with 95% confidence intervals as calculated by the method of Robertson and Preisler (1992) using the susceptible Burleson County September 2005 field population as the ratio divisor.
[d]Bioassay of Burleson County September 2005 susceptible field population.

Conclusions

Overall, the resistance concerns seem to have stabilized in 2006. There were no major control problems reported in the majority of the state. Isolated areas had a few control problems, including Williamson County in the Southern Blacklands. Corpus Christi continues to be an area of concern with relatively high resistance values compared to the rest of the state; especially the higher values seen early in the cotton season. Migration of moths according to predominant wind patterns must be also overlaid in the analysis of resistance evolution, and for the development of resistance management strategies since immigration of resistant moths from Mexico or counties with higher frequencies of resistant individuals can result in lower pyrethroid effectiveness even in populations without previous local pyrethroid use or exposure. This can be particularly useful for north Texas where the first moths captured in the season are believed to be migrant individuals. It is critical to preserve pyrethroid susceptibility in north Texas since there is evidence of fall reverse migration of bollworms towards south Texas. This may aid in diluting the resistance pool and contribute to pyrethroid use sustainability.