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MONITORING FOR PYRETHROID RESISTANCE IN BOLLWORM (HELICOVERPA ZEA) IN TEXAS-2005

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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, providing a summary of the context for pyrethroid use in cotton. Pyrethroid insecticides use is widespread in cotton and in other systems, such as corn and sorghum. This statewide monitoring program that evaluated resistance in male bollworm was conducted from April to September 2005, surveying nine 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. Bioassay results from a total of 5,041 moths from all areas were used for analysis. This number does not include additional moths that were tested but which bioassays were considered not valid for various reasons. Data from all areas in Texas was sent to Texas A&M University Toxicology Laboratory for analysis. Calculations included LC_{50}, LC_{90}, resistance ratios, 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 LC_{50} data, the most resistant populations were from Nueces, Uvalde, and Williamson Counties while the most susceptible populations were from Ellis, Fisher/Mitchell, Hockley, and Swisher Counties. Probit lines for Nueces County have not significantly changed from 2003 to 2005. Burleson County probit lines showed an improving situation, with a progressive return towards susceptibility from 2003 to 2005. However, the situation in Williamson County has deteriorated, with statistically significantly different probit lines indicating a progressive increase in pyrethroid resistance from 2003 to 2005.

Introduction

The purpose of this study was to access the susceptibility of bollworm to cypermethrin, a pyrethroid, in the main production regions of Texas, providing a summary of the context for pyrethroid use for the different counties. Our laboratory has monitored the evolution of resistance to pyrethroids in bollworm in Burleson and Nueces Counties since 1998 (Martin et al., 1999, 2000; Pietrantonio et al., 2000, Pietrantonio and Sronce, 2001). In the last three years we have more intensively monitored pyrethroid resistance in various counties (Pietrantonio *et* al., 2004, 2005) and we have detected the widespread presence of individuals with a resistant phenotype but at different frequencies in different locations. The resistance 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 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) similar to that described by Plapp et al. (1987, 1990) 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, 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 by Kanga et al. (1996) as discriminatory, possibly killing all susceptible bollworms. The probit analysis graph shown by these authors suggests 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 16:8 photoperiod on artificial diet. Bioassays were conducted on this colony in the spring of 2005. Results of these bioassays were: LC_{50} = 0.795 µg/vial and LC_{90} = 1.625 µg/vial, with 100 % observed mortality at 2.5 µg/vial. Notice that in bioassays from 2004 (Pietrantonio et al., 2005) we utilized both, the LC_{50} of a susceptible field population published by Kanga et al. (1996) (LC_{50} = 0.04 µg/vial), and the LC_{50} of a 2003 Texas susceptible field population as the denominators for calculation of the respective resistance ratios. In this paper, however, the resistance ratios were calculated with a less susceptible reference colony (LC_{50} = 0.795 µg/vial), so that in comparison to the resistance ratios of the 2004 populations, the resistance ratios of the Texas 2005 bollworm populations are underestimated.

Locations:

Nine Counties in Texas were included in the 2005 monitoring program, as follows: Hockley, and Swisher Counties in the High Plains production region; Fisher and Mitchell 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. Bioassays of 5,041 moths were utilized for analysis.

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. A baseline for susceptibility to cypermethrin in 2005 was established from a total of 432 moths tested from a susceptible laboratory colony obtained from the USDA-ARS laboratory in Stoneville, Mississippi. A LC_{50} value of 0.795 µg/vial and a LC_{90} value of 1.625 µg/vial were used to calculate resistance ratios. Confidence intervals for resistance ratios were calculated as described by Robertson and Preisler (1992). The lethal concentration resistant ratios of different populations were considered not significantly different if the 95% confidence intervals included 1 (Robertson and Preisler, 1992). 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.

Results and Discussion

Monitoring for bollworm resistance to cypermethrin was conducted from April to September of 2005, being more intensive in Nueces and Burleson Counties, due to their high pyrethroid resistance history in 2003, 2004 and previous years (Pietrantonio et al., 2004). Moths from **Nueces County** were collected from two traps located 1 mile apart. There were approximately 146,000 acres of cotton in Nueces County and 235,000 acres in the nearby region including the Nueces County figures. Transgenic *Bt* cotton accounted for 30% of the cotton acreage. Sorghum acreage in Nueces County was 186,000 acres with about 450,000 acres in the nearby region. Corn acreage in Nueces County was about 12,000 acres with about 50,000 acres in the nearby region. Cotton yields varied from 350 (low rainfall) to 1,700 (irrigated and high rainfall areas) pounds lint per acre. The average yield for Nueces County was about 650 lb/acre. *H. zea* and fall armyworm (headworm) infestations in sorghum were similar to those in 2004, that persisted for a long period with 80% of the sorghum acres being treated. No problems were experienced controlling *H. zea* in sorghum with medium to high rates of pyrethroids, nor at that time, were problems experienced in cotton. About 2-3 weeks following the sorghum treatments, problems controlling *H. zea* in cotton were experienced, or alternate materials were used in anticipation of the problem. Two treatments were applied to the cotton for *H. zea* control on about 60% of the acres. The 2005 cotton infestations were generally moderate to heavy where they occurred, prolonged egg lay was experienced, but the infestations did not last as long as in 2004. Plant coverage was good due to compact plant size, use of ground spray equipment, and less rainfall than that experienced in 2004. There were control problems with pyrethroids during the mid-season, with high label rates being used. It was also when peak numbers of larvae were present, thus, even leaving a small percentage alive would have resulted in unacceptable numbers and damage. Pyrethroid use was mainly represented by Mustang Max™ (zeta-cypermethrin), used at the mid-label rate of 0.0195 lb ai (active ingredient)/acre up to the high label rate of 0.0225 lb ai/acre; Karate™ (lambda cyhalothrin), used at the mid-label rate of 0.0325 up to the high label rate of 0.04 lb ai/acre and Decis™ (deltamethrin) used also at mid-label of 0.0245 lb ai/acre up to the high label rate of 0.03 lb ai/acre. The higher rates of pyrethroid were used on sorghum and from early- to just after mid-June on cotton. Denim™ (emamectin benzoate) was used at 0.01 lb ai/acre and Steward™ (indoxacarb) at 0.11 lb ai/acre. One of the mixtures used was the labeled rate of a pyrethroid (mid- to high-rate) plus Denim™ 0.16 EC at 0.007619 lb ai/acre which is one gallon per 21 acres of the Denim™. It is speculated that the early treatments with pyrethroid against the *H. zea* on sorghum relate to some extent to increased difficulty in controlling bollworm later in cotton. During the mid-season period of pyrethroid failure, control was achieved with Denim™ or Steward™. Several producers were concerned about difficulty controlling bollworm and the high cost of the alternatives and they requested to include 3 insecticide treatments in the cotton production budget for 2006. The evolution of pyrethroid resistance in Nueces Co. is shown in Table 1 for 2005, and in Fig. 1 for 2003-2005.

Table 1. Nueces Co. Cypermethrin Bioassay for bollworm, *Helicoverpa zea*, 2005

Date	n	Slope ± SE	LC_{50} µg/vial	LC_{90} µg/vial	RR LC_{50} µg/vial	RR LC_{90} µg/vial	χ^2
Susceptible Colony	432	4.13 ± 0.52	0.79 (0.70-0.91)	1.62 (1.35-2.14)	--	--	1.47
04/07-12/05	400	1.91 ± 0.24	1.02 (1.75-1.29)	4.77 (3.61-7.10)	1.28 (0.95-1.73)	**2.93** (1.99-4.33)	4.62

05/23-25/05	280	1.96 ± 0.30	2.17 (1.63-2.85)	9.80 (6.52-19.75)	**2.73** (2.02-3.69)	**6.03** (3.43-10.62)	2.65
06/09-16/05	300	2.92 ± 0.59	3.35 (2.49-4.22)	9.21 (6.77-27.03)	**4.21** (3.18-5.58)	**5.66** (3.55-9.04)	2.55
06-20-05 to 07-03-05	400	1.46 ± 0.21	2.57 (1.70-3.61)	19.35 (12.16-40.70)	**3.23** (2.19-4.78)	**11.90** (6.42-22.05)	3.07
09-29-05 to 10-06-05	210	2.22 ± 0.57	1.91 (0.91-2.72)	7.26 (4.97-17.67)	**2.41** (1.51-3.85)	**4.46** (2.56-7.80)	3.57

n = number of insects tested. ( ) = 95% confidence intervals. RR = Resistance ratio based on susceptible colony LC = lethal concentrations. RR in red indicate the lethal concentrations of the susceptible colony and field insects are statistically significantly different.

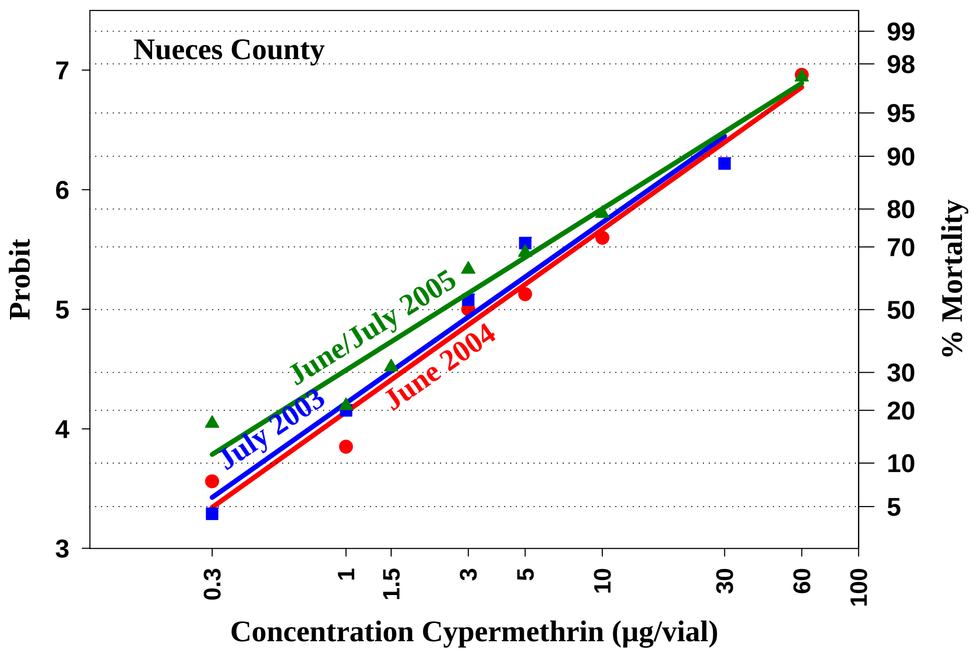


Figure 1. Concentration-mortality lines for bollworm moths collected in 2003, 2004, and 2005 in Nueces County and exposed to cypermethrin in the vial assay. Lines are statistically equal and parallel (*p* <0.05).

In **Williamson County** moths were collected from 3 traps, spaced 1/4 mile apart. There were approximately 32,000 acres planted in cotton, of which 80% was transgenic *Bt*; 72,000 acres of corn; and 30,000 acres of sorghum. The cotton yield was average at about 1 bale per acre. Bollworm pressure was lighter in 2005 as compared to the past 3 to 4 years. Overall populations of *H. zea* were light in cotton and moderate in corn and grain sorghum. Pyrethroids were avoided in cotton this season because of problems in previous years. No control problems or field failures were reported when Tracer^{TM} (spinosad) at a rate of approximately 2.2 - 2.5 oz/ac was used to achieve control. There were some pyrethroids used in grain sorghum for a combination of pests including sorghum midge, stink bugs and headworms. It is estimated that about 3,500 to 4,000 acres were treated. The products normally used on sorghum include Baythroid^{TM} (cyfluthrin) 1 gal./100 acres, Karate-Z^{TM} (lamba cyhalothrin) at 1.6 oz./acre, Asana^{TM} (esfenvalerate) at 6 oz./acre, and Mustang Max^{TM} (z-cypermethrin) at 1 gal to 45 acres. Generally, Baythroid^{TM} at 1.6 oz./acre was the product of choice. Some of the grain sorghum (about 40 acres) within 1 mile of where the traps were located had been sprayed with Baythroid^{TM} at 1.6 oz./A around mid-June. The majority of the late sorghum was sprayed around the 20th –28th of June. Results for Williamson County monitoring are shown in Table 2.

Table 2. Williamson Co. Cypermethrin Bioassay for bollworm, *Helicoverpa zea*, 2005

Date	n	Slope ± SE	LC_{50} µg/vial	LC_{90} µg/vial	RR LC_{50} µg/vial	RR LC_{90} µg/vial	χ^2
Susceptible Colony	432	4.13 ± 0.52	0.79 (0.70-0.91)	1.62 (1.35-2.14)	--	--	1.47
06/21/05	180	1.64 ± 0.32	1.63 (0.84-2.56)	9.89 (5.96-52.25)	**2.05** (1.20-3.50)	**6.08** (3.10-11.94)	3.14
07/07/05	144	1.83 ± 0.39	4.68 (2.84-7.49)	23.49 (13.05-79.45)	**5.89** (3.68-9.43)	**14.43** (6.37-32.71)	3.48

n = number of insects tested. ( ) = 95% confidence intervals. LC = lethal concentrations. RR = Resistance ratio based on susceptible colony. RR in red indicates that the lethal concentrations are significantly different.
The evolution of cypermethrin resistance is shown in Fig. 2. The probit lines from 2003-2005 are parallel but statistically significantly different, showing a rapid evolution towards high frequency of resistant individuals.

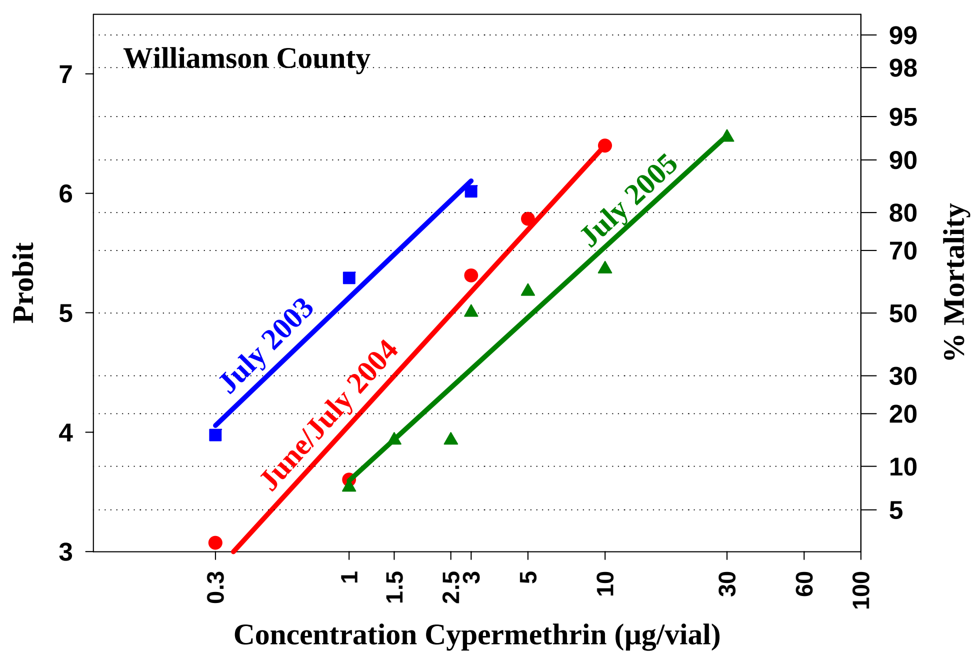


Figure 2. Concentration-mortality lines for bollworm moths collected in 2003, 2004, and 2005 in Williamson County and exposed 24h to cypermethrin in the vial assay. Lines are statistically parallel and all are significantly different (*p* <0.05) from each other.

In **Uvalde County** five traps were used, spaced 300 yards to 3/4 mile apart. In the vicinity of the trapping area, about 3,000 acres were planted in cotton. In the Winter Garden area (6 counties) there were 52,000 acres of cotton, 95% of which was transgenic *Bt*. In the vicinity of the trapping area, there were approximately 700 acres of sorghum and approximately 3000 acres of corn. Cotton yields across the area were average. Highest yields were better than in 2004, but the average yield was lower than in 2004; dry weather playing a major role in this. Bollworm pressure in 2005 as compared to the past 3-4 years was about average for cotton, but the pressure was lighter than the last two years. Bollworm populations were light to moderate throughout the region, including the trapping area and the six counties that make up the Winter Garden area. Trap catches also were light to moderate during the time the traps were being monitored. There were no known control problems or field failures. Bioassay results are shown in Table 3 for 2005 and Fig. 3 shows that the probit lines have not changed from 2004 to 2005.

Table 3. Uvalde Co. Cypermethrin Bioassay for bollworm, *Helicoverpa zea*, 2005

Date	n	Slope ± SE	LC_{50} µg/vial	LC_{90} µg/vial	RR LC_{50} µg/vial	RR LC_{90} µg/vial	χ^2
Susceptible Colony	432	4.13 ± 0.52	0.79 (0.70-0.91)	1.62 (1.35-2.14)	--	--	1.47
07/13/05	270	1.40 ± 0.26	2.36 (1.45-3.59)	19.38 (10.48-63.93)	**2.97** (1.90-4.66)	**11.91** (5.12-27.69)	1.47

n = number of insects tested. ( ) = 95% confidence intervals. LC = lethal concentrations. RR = Resistance ratio based on susceptible colony. RR in red indicates LC are significantly different.

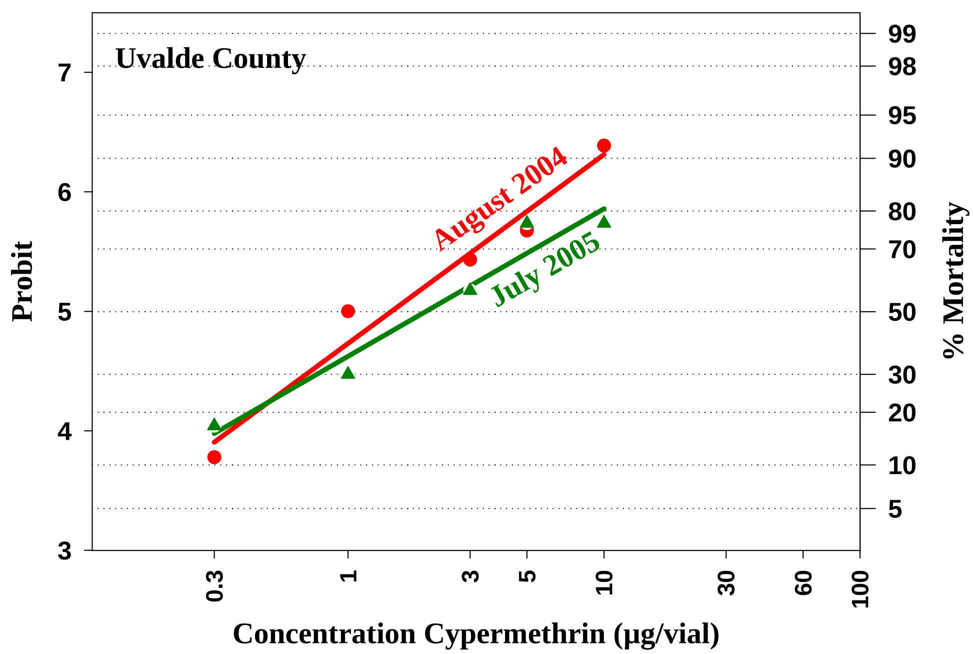


Figure 3. Concentration-mortality lines for bollworm moths collected in 2004 and 2005 in Uvalde County and exposed 24h to cypermethrin in the vial assay. Lines are statistically parallel and equal at *p* <0.05.

Insecticide usage in other crops prior to bollworm moth movement into cotton was very light to non-existent; grain sorghum acreage was down and insect pressure that would generate insecticide usage was virtually non-existent in grain sorghum; drier weather than the past two growing seasons may have been a factor. Producers did not communicate concerns, and growers and consultants indicated that there was no pyrethroid use in the area this year in cotton, corn, and sorghum. Together, the lack of pyrethroid use and the bioassay results showing a significant resistant ratio of 2.97 (Table 3) suggest that pyrethroid resistance in Uvalde may be maintained by resistant immigrant moths from south Texas (Westbrook et al., 1998).

In **Burleson County** 7-8 traps were used, spaced over 4 miles along Co. Road 265 and Hwy 60. There were 11,000 acres planted in cotton, most of which was transgenic *Bt*. There were 6,100 acres planted in sorghum and 7,900 acres of corn. Cotton yield for the county was considered good. In the trapping area, *H. zea* pressure was similar to the past 3-4 years. There was more pressure in cotton from fall armyworms and stink bugs than from bollworms in

2005. There was one major flight of *H. zea* in July during which the cotton field in the trapping area was treated with full rate (1 gal/32 acres) of Mustang Max™ (z-cypermethrin). Sorghum in the area was treated in early June for headworms with full rate of Mustang Max™ and in later June with Lannate™ (methomyl), to alternate chemistries. Lorsban™ (chlorpyrifos) was used to control midge. The LC_{50} of the bollworm population was similar to that of the susceptible population except in July when the resistance ratio was significantly different from 1 (Table 4). The resistance ratio of 0.41 in blue (Table 4) was statistically significantly lower than 1 indicating that the field population was more susceptible in September than the reference susceptible laboratory colony.

Table 4. Burleson Co. Cypermethrin Bioassay for bollworm, *Helicoverpa zea*, 2005

Date	n	Slope ± SE	LC_{50} µg/vial	LC_{90} µg/vial	RR LC_{50} µg/vial	RR LC_{90} µg/vial	χ^2
Susceptible Colony	432	4.13 ± 0.52	0.79 (0.70-0.91)	1.62 (1.35-2.14)	--	--	1.47
05/05/05	200	2.36 ± 0.44	0.64 (0.41-0.87)	2.24 (2.15-6.70)	0.80 (0.56-1.18)	1.34 (0.86-2.20)	0.93
05/26/05	300	1.96 ± 0.32	1.16 (0.72-1.59)	5.23 (5.42-15.66)	1.46 (0.98-2.18)	**3.22** (2.05-5.05)	0.47
06/23/05	180	1.89 ± 0.42	1.04 (0.46-1.60)	4.98 (3.29-10.78	1.31 (0.75-2.29)	**3.06** (1.74-5.40)	3.51
07/2005 pool	400	2.15 ± 0.35	1.12 (0.83-1.44)	4.43 (3.16-7.92)	**1.41** (1.05-1.91)	**2.72** (1.68-4.42)	2.51
09/2005 pool	400	1.48 ± 0.36	0.33 (0.08-0.60)	2.44 (1.52-5.77)	**0.41** (0.18-0.95)	1.50 (0.81-2.78)	0.46

n = number of insects tested. ( ) = 95% confidence intervals. LC = lethal concentrations. RR = Resistance ratio based on susceptible colony. RR in red and blue indicate LC are significantly different.

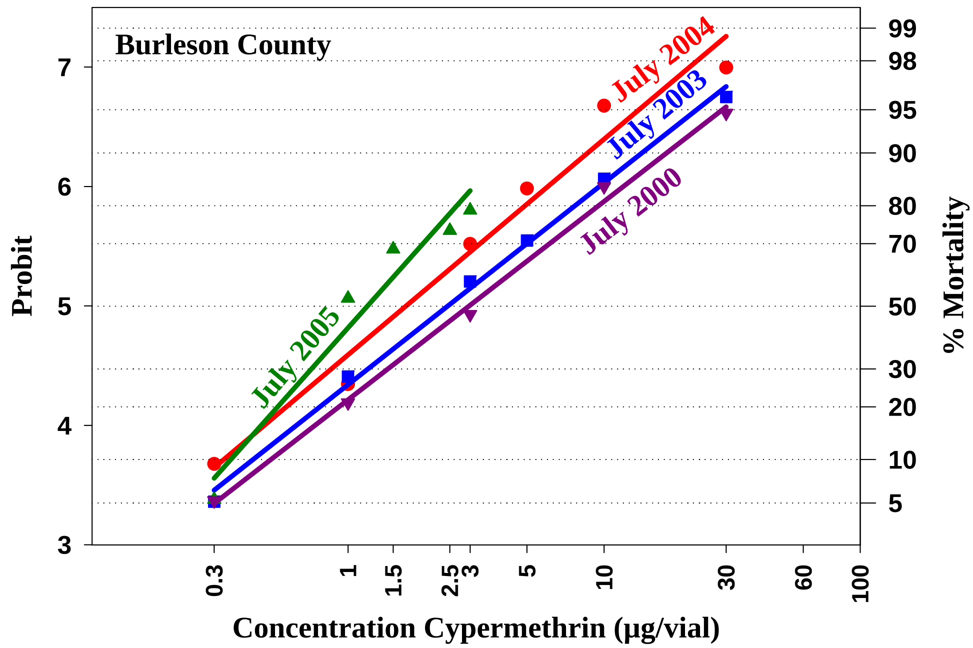


Figure 4. Concentration-mortality lines for bollworm moths collected in 2000, 2003, 2004, and 2005 in Burleson County and exposed 24h to cypermethrin in the vial assay. Lines are statistically parallel but not equal: The probit line for 2005 is significantly different from those of 2004, 2003, and 2000 (*p* <0.05). The LC_{90} for 2004 is significantly different from that in 2003, but the LC_{50}s were similar.

Burleson County probit lines showed an improving situation, with a progressive return towards susceptibility from 2003 to 2005. No survivors were observed beyond the 5 µg/vial concentration in July of 2005 (Fig. 4). The resistance ratio of the LC_{50} was statistically significantly higher than one only in July (Table 4).

In **Hockley County** 2 traps were used, spaced 880 yards apart. Crop acreage for the area was: 230,000 acres of cotton (15% transgenic *Bt*), 12,000 acres of sorghum, and no corn. Cotton yield for the area was for dryland 425 lb lint/acre and for irrigated 725 lb lint/acre. The past two years experienced unusually light bollworm pressure, with 2005 having generally low activity in most crops. There were no reported control problems and no pyrethroids were applied. This was perhaps due to the fact that there were few or no applications targeting boll weevils and beneficial insects were present in good numbers. The bioassays support these field observations in that the resistance ratios for the LC_{50}s were lower than 1 (0.28 and 0.51), indicating these populations were more susceptible than the laboratory reference colony (Table 5).

Table 5. Hockley Co. Cypermethrin Bioassay for bollworm, *Helicoverpa zea*, 2005

Date	n	Slope ± SE	LC_{50} µg/vial	LC_{90} µg/vial	RR LC_{50} µg/vial	RR LC_{90} µg/vial	χ^2
Susceptible Colony	432	4.13 ± 0.52	0.79 (0.70-0.91)	1.62 (1.35-2.14)	--	--	1.47
07/11-13/05, 07/26/05, and 08/01/05 pool	324	1.51 ± 0.21	0.22 (0.13-0.32)	1.57 (1.12-2.53)	**0.28** (0.18-0.44)	0.96 (0.61-1.52)	4.19
08/22/05, 08/24/05 and 08/29/05 pool	360	0.90 ± 0.16	0.40 (0.18-0.66)	10.95 (5.70-37.88)	**0.51** (0.28-0.94)	**6.72** (2.76-16.35)	4.14

n = number of insects tested. ( ) = 95% confidence intervals. LC = lethal concentrations. RR = Resistance ratio based on susceptible colony. RR in red and blue indicate LC are significantly different.

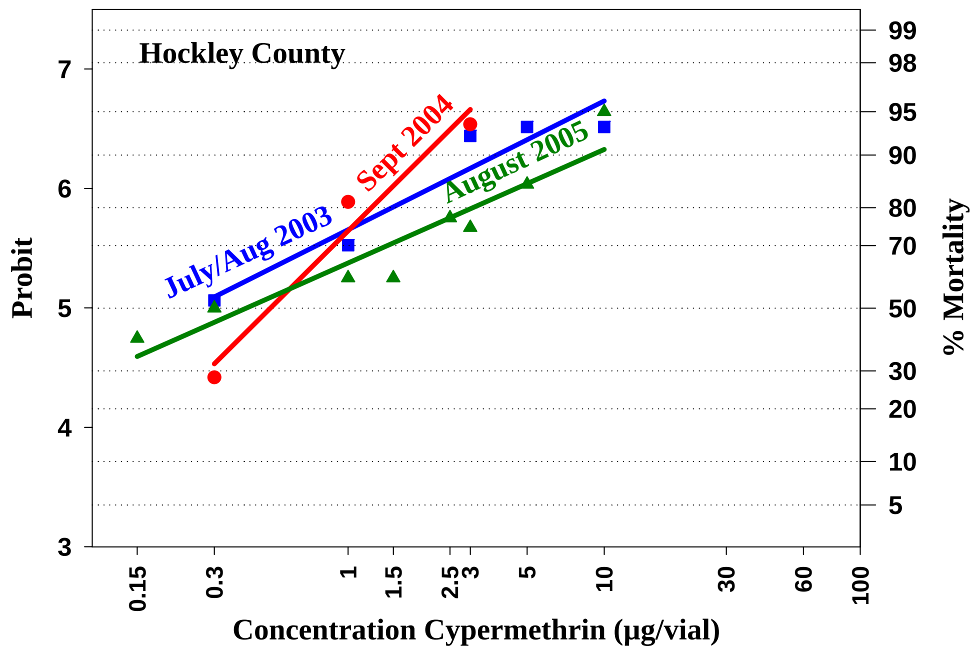


Figure 5. Concentration-mortality lines for bollworm moths collected in 2003, 2004, and 2005 in Hockley County and exposed 24h to cypermethrin in the vial assay. Probit lines for 2005 and 2003 are statistically parallel but are significantly different, and the LC_{90} resistance ratio was also statistically different. Probit lines for 2005 and 2004 are neither equal nor parallel, and the LC_{90} resistance ratio was also statistically different. Probit lines for 2004 and 2003 are equal but not parallel (p<0.05).

For Hockley County, pyrethroid resistance appears to be in higher frequency in 2005 that in any of the two previous years (Fig. 5). This is confirmed by the significantly higher resistance ratio for the LC_{90} of 6.7 for August (Table 5) and the fact that the probit line of 2005 is parallel but lower than the one of 2003, indicating an overall reduction in the effectiveness of pyrethroids in these populations (Fig. 5).

In **Swisher County** 3 traps were used, spaced l.25 miles apart. Crop acreage for the Hale and Swisher County area was: 350,000 acres of cotton (20-25% transgenic *Bt*), 150,000 acres of sorghum, and 75,000 acres of corn. Bollworm pressure was more widespread and generally higher than in the past 3-4 years and populations were widespread on cotton. There were anecdotal reports of possible field failures with Baythroid™ (cyfluthrin) that could not be confirmed as due to resistance; these failures were probably due to improper application and insects were controlled with reapplication of another pyrethroid. Limited applications were made to sorghum and corn. About 90% of non-*Bt*-cotton and several Bollgard I™ fields were treated for bollworms. Application rates were: Cypermethrin (Generic) 0.08 to 0.1 lb ai/acre; Baythroid™ (Cyfluthrin) 0.03 to .04 lb ai/acre; Karate™ (Cyhalothrin) 0.03 to .04 lb ai/acre. Cypermethrin was the most commonly used pyrethroid since the 0.08 lb ai rate could be applied for $2.56 per acre plus application cost, which ranged from $3.50 to $4.50 per acre aerial application. There were no observed significant control failures. The bioassay results also confirmed the field observations (Table 6). There were, however, resistant individuals in the population as detected by the July bioassay showing a significant LC_{90} resistance ratio of 2.37 (Table 6). In Fig. 6 the probit line for August suggests an increase in the frequency of susceptible individuals in the population in comparison to the June probit line, suggesting that the allele(s) conferring pyrethroid resistance, more frequent in June, might also inflict a fitness cost in the absence of, or decline in selection pressure by pyrethroids in August, as the season progresses.

Table 6. Swisher Co. Cypermethrin Bioassay for bollworm, *Helicoverpa zea*, 2005

Date	n	Slope ±	LC_{50}	LC_{90}	RR	RR	χ^2

		SE	μg/vial	μg/vial	LC_{50} μg/vial	LC_{90} μg/vial	
Susceptible Colony	432	4.13 ± 0.515	0.79 (0.70-0.91)	1.62 (1.35-2.14)	--	--	1.47
*06/15/2005	50	1.68 ± 0.66	*1.157	*6.67	1.45 (0.81-2.63)	4.10 (0.87-19.29)	0.84
07/19/2005 & 07/27/2005	180	1.90 ± 0.47	0.81 (0.35-1.26)	3.86 (2.40-11.08)	1.02 (0.59-1.79)	2.37 (1.22-4.65)	1.20
08/24/2005	90	2.35 ± 0.76	0.65 (0.29-1.14)	2.29 (1.27-16.40)	0.82 (0.47-1.43)	1.40 (0.58-3.40)	0.08

n = number of insects tested. ( ) = 95% confidence intervals. LC = lethal concentrations. RR = Resistance ratio based on susceptible colony. RR in red indicates LC are significantly different. *no 95% confidence intervals, LC_{50} and LC_{90} intervals have a confidence level of 90%.

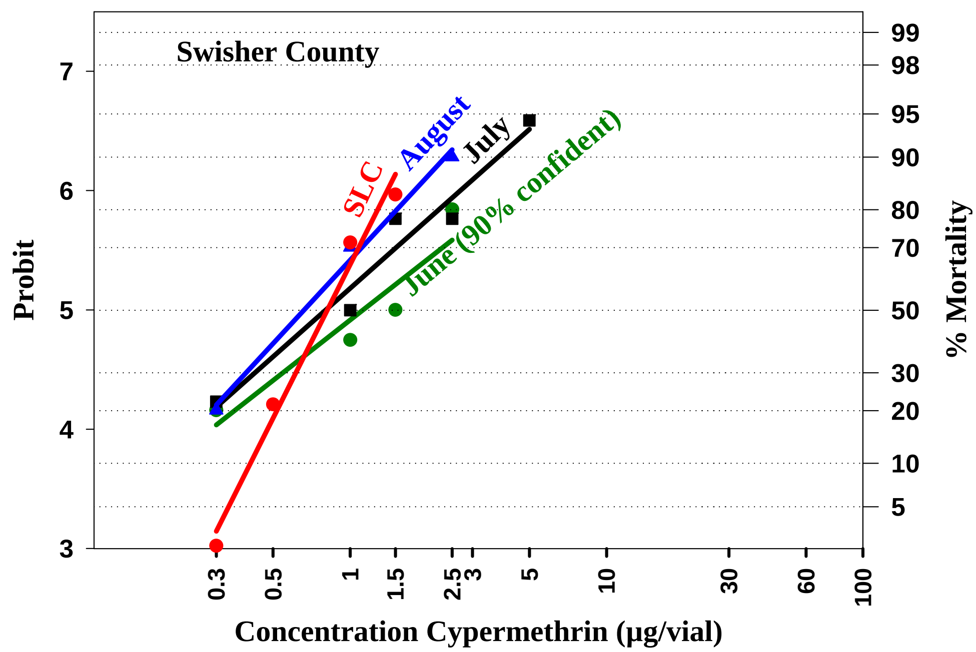


Figure 6. Concentration-mortality lines for bollworm moths collected in 2005 in Swisher County and exposed 24h to cypermethrin in the vial assay.

In the **Fisher/Mitchell County** region 6 traps, spaced 0.5 miles apart were used. There were 1.1 million acres planted in cotton (35% transgenic *Bt*), 300,000 in sorghum, and 8,000 in corn. Cotton yield for 2005 was above average. Bollworm pressure was moderate in 2005 but higher than in past 3-4 years. Fifty percent of conventional fields were treated twice and 10% of Bollgard™ cotton were treated once. Control problems were observed with fall armyworms, which originated faulty reports of bollworm control failures due to misidentification of larvae. Bollworm control was achieved with pyrethroid Karate™ (lambda cyhalothrin), 0.04 lb/acre, applied the 3rd week in July. The only bioassay available in late season showed no resistance to pyrethroids (Table 7 and Fig. 7).

Table 7. Fisher/Mitchell Co. Cypermethrin Bioassay for bollworm, *Helicoverpa zea*, 2005

Date	n	Slope ± SE	LC_{50} µg/vial	LC_{90} µg/vial	RR LC_{50} µg/vial	RR LC_{90} µg/vial	χ^2
Susceptible Colony	432	4.13 ± 0.52	0.79 (0.70-0.91)	1.62 (1.35-2.14)	--	--	1.47
09/03/05 & 09/07/05	180	1.83 ± 0.52	0.51 (0.20-0.86)	2.60 (1.44-13.56)	0.65 (0.36-1.18)	1.59 (0.67-3.79)	0.72

n = number of insects tested. ( ) = 95% confidence intervals. LC = lethal concentrations. RR = Resistance ratio based on susceptible colony.

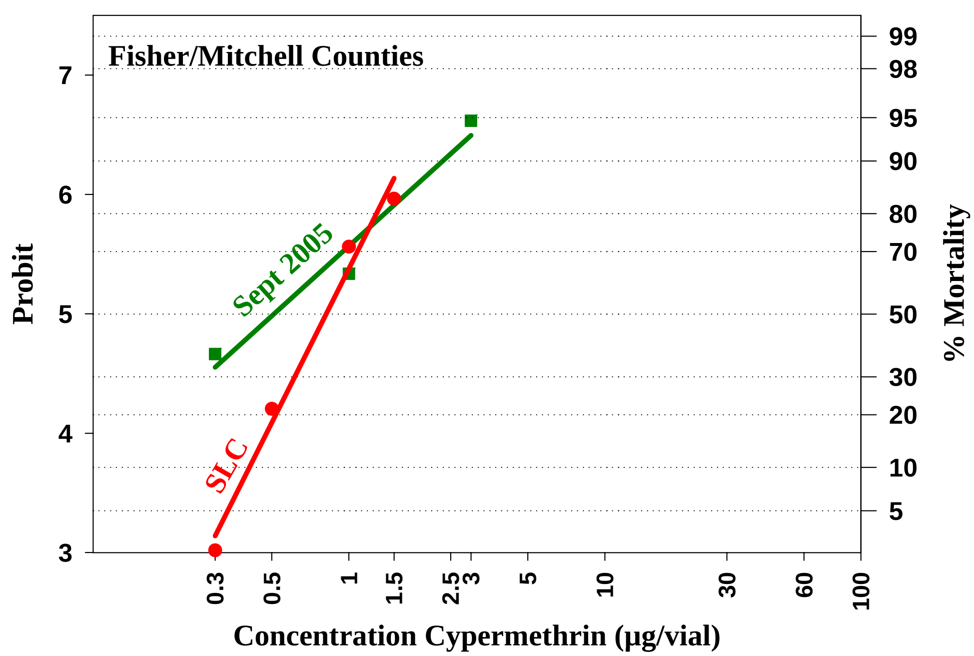


Figure 7. Concentration-mortality lines for bollworm moths collected in 2005 in Fisher/Mitchell Counties and exposed 24h to cypermethrin in the vial assay.

In **Ellis County,** 2 traps were used and spaced 1 mile apart. Crop acreage for this county was 30,000 acres of cotton (80% transgenic *Bt*), 48,000 acres of corn, and 10,000 acres of sorghum. Cotton yield was above average. *H. zea* pressure in cotton and other crops was much lighter than in 2004 and as compared to past 3-4 years; a hot and dry spring and early summer, and the absence of wild host plants for *H. zea* could have been contributing factors. Bollworm numbers were so low this season that insecticidal control was not necessary. There were no reports of control failures or problems. The only pyrethroid treatments were applications made against boll weevil during midseason, 3000 acres were treated with Karate[TM] (lambda cyhalothrin) at 0.04 lb/acre. Bioassays confirmed that the population was susceptible even during June, in fact, more susceptible than the reference colony with a resistance ratio of 0.28 (Table 8).

Table 8. Ellis Co. Cypermethrin Bioassay for bollworm, *Helicoverpa zea*, 2005

Date	n	Slope ± SE	LC_{50} µg/vial	LC_{90} µg/vial	RR LC_{50} µg/vial	RR LC_{90} µg/vial	χ^2
Susceptible Colony	432	4.13 ± 0.52	0.79 (0.70-0.91)	1.62 (1.35-2.14)	--	--	1.47
*06/20-23/05	193	1.89 ± 0.75	*0.23	*1.09	**0.28** (0.11-0.79)	0.67 (0.32-1.43)	0.01

n = number of insects tested. ( ) = 95% confidence intervals. LC =lethal concentrations. RR = Resistance ratio based on susceptible colony. RR in blue indicate LC are significantly different. *no 95% confidence intervals could be estimated, LC_{50} and LC_{90} have a confidence level of 90%.

In 2005 the population appears to have been more susceptible than in 2004 as shown in Fig. 8, the probit lines for June 2005 and July 2004 are statistically significantly different but parallel. Notice that these are results for only one month and may not accurately represent the entire season.

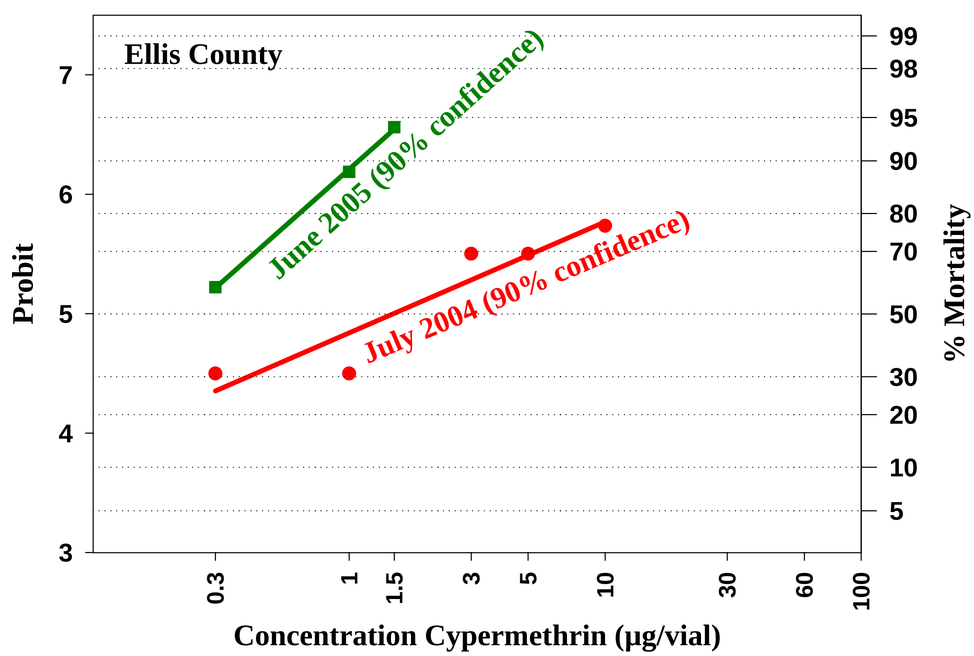
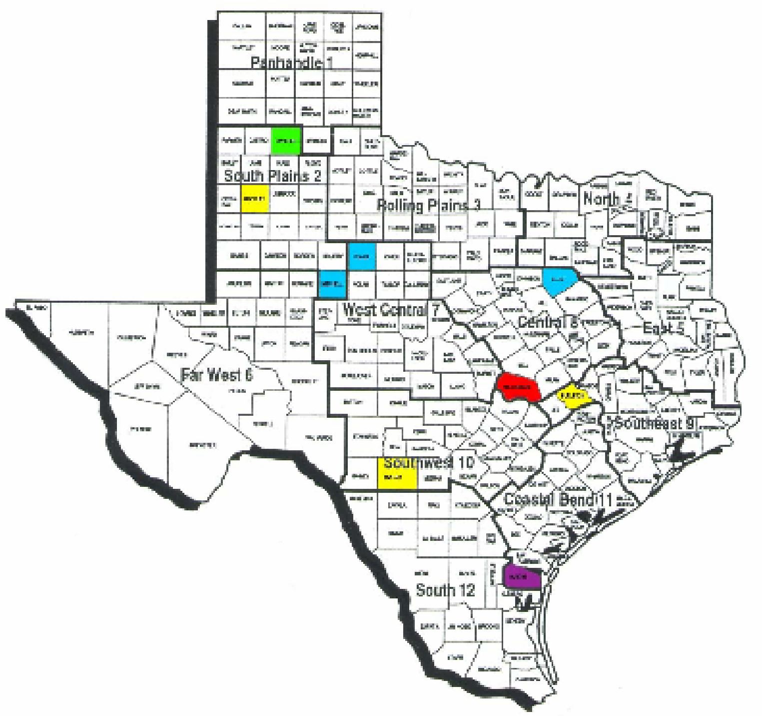
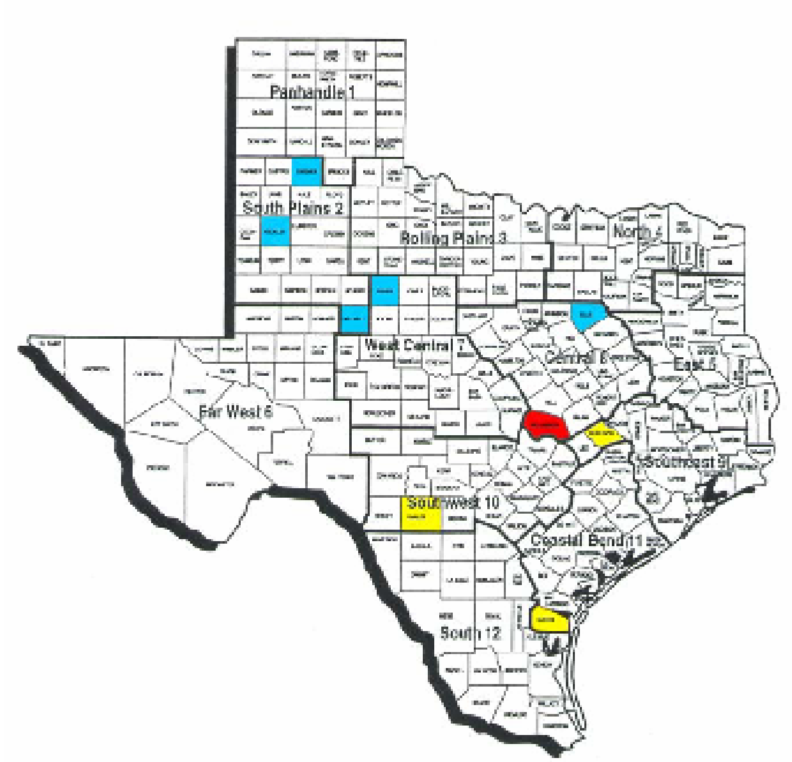


Figure 8. Concentration-mortality lines (90% confidence) for bollworm moths collected in 2004 and 2005 in Ellis County and exposed 24h to cypermethrin in the vial assay. Lines are statistically parallel but not equal (*p* <0.05).



Blue **≤ 3 µg/vial**
Green **5 µg/vial**
Yellow **10 µg/vial**
Red **30 µg/vial**
Purple **60 µg/vial**

Figure 9. Highest concentration at which moth survivorship was observed in each county.
As shown in the insert in Fig. 9, the colors represent the different vial concentrations at which surviving moths were observed. In Fig. 9 counties in blue are Fisher/ Mitchell and Ellis. Counties in yellow are Hockley, Burleson and Uvalde. In green is Swisher County; in red, Williamson County, and in purple, Nueces County. This map does not refer to the frequency of the resistant individuals present, but only to the highest level of resistance observed. Notice that for example, Swisher County is represented in green, with some moths surviving the 5µg/vial concentration (Fig. 9), but Fig. 10 shows that Swisher Co. has a LC_{50} similar to that of the laboratory reference colony. Contrary to the Swisher County example, Nueces Co. has individuals that survived the highest concentration of 60 µg/vial (purple in Fig. 9) however, the LC_{50} is lower than that of Williamson County (red in Fig. 10) that had some moths that survived only up to the 30 µg/vial concentration. This means that the LC_{50}s shown in Fig. 10 are representative of the average susceptibility status of a population while the information in Fig. 9 represents the extreme level of pyrethroid that individual moths have survived.



Blue **not different**
Yellow **resistance ratio < 5**
Red **resistance ratio $\geq$ 5**

Figure 10. Highest LC_{50} Resistance Ratio (RR) detected by county in 2005. Blue counties indicate LC_{50} RR not significantly different from the susceptible laboratory colony or LC_{50} of field population lower than that of the laboratory colony. Yellow indicates LC_{50} RR significantly different from the susceptible laboratory colony but lower than 5. Red indicates a statistically significant resistant population with LC_{50} RR of at least 5.

Conclusions

The analysis of the 2005 monitoring season together with those in 2003 and 2004 reflect a fragile situation for pyrethroid use in Texas. South Texas (Nueces County) resistant populations have stabilized in the last three years (Fig. 1) and continue to have survivorship at the highest concentration of 60 µg/vial. The situation in Williamson County (Fig. 2) has deteriorated this year, with a rapid loss of pyrethroid efficacy on those populations occurring in the last three years. The improvement of the situation in Burleson County was surprising, but shows that alternating chemistries with different modes of action and using higher rates of pyrethroids when resistance is in high frequency can aid in eliminating pyrethroid resistant heterozygote individuals in the population. The high adoption of *Bt* cotton in the area may have contributed to this improvement. The high rate of pyrethroid is not recommended routinely for counties with low LC_{50}s such as those in northern Texas. Hockley County should be more closely monitored next season considering that individuals surviving the 10µg/vial dosage were detected (Fig. 5). Similarly, although statistically the probit lines for Uvalde County do not differ between 2004 and 2005, the situation must continued to be monitored closely since there are individuals surviving the 10 µg/vial dosage. This could be due to immigrating resistant moths from south Texas.

In both, Nueces and Burleson Counties, the LC_{50} of the populations decreased toward the end of the season after the high insecticide pressure subsided; this may reflect a fitness cost of the resistance allele(s) in the absence of pyrethroid selection and in competition with susceptible individuals in the field. This may also explain why the LC_{50} at the beginning of the season for both counties is low. 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 (Rummel et al., 1986). It is critical to preserve pyrethroid susceptibility in north Texas since there is evidence of fall reverse migration of bollworms towards south Texas (Pair et al., 1987). This may aid in diluting the resistance pool and contribute to pyrethroid use sustainability.

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