

**MONITORING FOR PYRETHROID RESISTANCE IN BOLLWORM  
(*HELICOVERPA ZEA*) IN TEXAS-2003**

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**Abstract**

A statewide monitoring program for males of bollworm (*Helicoverpa zea*) was conducted from May to October of 2003, surveying fifteen Texas Counties. Moths were trapped near cotton fields using pheromone, Hercon Luretape<sup>®</sup> with Zealure. Moths were collected early in the morning and assays were performed the same day. Vials were prepared in the Toxicology Laboratory, Department of Entomology at Texas A&M University, College Station, Texas and shipped as needed to Extension personnel. Vials were prepared using acetone only for controls, and cypermethrin (technical grade, 95.2% purity) at 0.3, 1, 3, 5, 10 and 30 µg/vial. One moth was placed in each vial and bioassays were evaluated after 24 h. Moths were classified as alive, dead, or "knocked-down". From Burleson County 1,814 moths were tested. Other counties in Texas participating in the monitoring program were: Castro, Hockley, Hale, Swisher and Martin Counties in the High Plains production region; Tom Green and Runnels Counties in the Southern Rolling Plains region; Ellis and Williamson Counties in the Blacklands region; Uvalde County in the Wintergarden region; Nueces and Wharton Counties in Coastal Bend region; and Hidalgo

County in the Lower Rio Grande Valley. A total of 4,134 moths were tested for all areas outside Burleson County. Data from all areas in Texas was sent to Texas A&M University Toxicology laboratory and analyzed using Probit-PC, Probit and Logit Analysis and graphed using SigmaPlot. Only data from valid bioassays were analyzed. A baseline for susceptibility to cypermethrin was established from two areas in Texas with low  $LC_{50}$  values, Hockley County and Wharton County, these values were pooled to obtain a baseline  $LC_{50}$  of 0.283  $\mu\text{g}/\text{vial}$  that was used to calculate relative resistance ratios for the rest of the counties. High levels of resistance were detected for Nueces, Burleson and Castro Counties; the  $LC_{50}$  resistance ratio as previously defined was 9 for the first two counties in July and between 4 and 10 for Castro Co., depending on the date. Bollworm populations were most susceptible to pyrethroids in 2003 in Parmer, Hale, Hockley, and Wharton Counties.

## **Introduction**

The occurrence of pesticide resistance stands as one of the most chronic problems in crop production. Texas was one of the first states to document resistance to the pyrethroids by tobacco budworms, *Heliothis virescens* (F.), in 1985. At that time, an extensive monitoring program was established that ran continuously from 1986 to 1992. Resistance concerns faded as new insecticides and new technologies were developed to assist producers in managing populations. However, as with any system that overly relies on insecticides, resistance has once again become a major issue in the state.

Initiation of the boll weevil eradication program in 1994 focused attention on the use of malathion for boll weevil control. The Texas Boll Weevil Eradication Foundation and Extension entomologists initiated many tests that showed that malathion was the most effective product for boll weevil control and the use of malathion would lead to the quickest route to reducing the boll weevil to a non-economic pest. The reliance on malathion lead researchers to implement a resistance monitoring program to ensure that malathion would continue to remain effective through the remainder of the boll weevil eradication effort (Pietrantonio and Juneck, 2002; Pietrantonio and Sronce, 2001; Pietrantonio et al., 2000).

We previously reported resistance to pyrethroids in bollworm (*Helicoverpa zea*) in Burleson Co., Texas (Pietrantonio et al., 2000; Pietrantonio and Sronce, 2001; Payne et al., 2001). Additional producer concerns in the High Plains and preliminary data from Lubbock in 2002 suggested that resistance to pyrethroids in bollworm might be present in that area as well. The widespread resistance to pyrethroids by the bollworm in cotton could lead to reduced yields for producers and higher production costs, including higher insecticide use. The development of resistance will also affect other crops such as green beans, watermelons and soybeans where the bollworm can be an economic pest and pyrethroids are the least expensive control tactic. Grain sorghum production in Texas would be also affected since the same species is present as part of the "headworm" complex. It was the purpose of this study to assess the susceptibility of bollworm to pyrethroids in the main producing areas of Texas.

## **Materials and Methods**

### **Burleson Co.**

Adult male *Helicoverpa zea* moths were trapped using pheromone, Hercon Luretape<sup>®</sup> with Zealure from Great Lakes IPM (Vestaburg, MI). Six wire cone traps were placed in the Brazos River Bottom, Burleson Co., Texas near cotton and sorghum fields. In Burleson County most of the traps were located along County Road 265. Grain sorghum planted on that road was treated with Mustang Max<sup>™</sup> (Z-cypermethrin) three times during August against armyworms. The traps were near 100 acres of *Bt* cotton that were treated in June with Lorsban<sup>™</sup> (Chlorpyrifos). Moths were collected approximately every two weeks beginning May 14, 2003 and ending October 8, 2003. Moths were collected early in the morning and assays were performed the same day. Moths were supplied with a 10% sucrose solution until placed in vials. Only healthy, vigorous male moths with intact wing scales were used for the bioassays. An 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. The test vials were prepared by coating the inside of the vial with an acetone (EM Science, Gibbstown, NJ) solution of technical grade cypermethrin (95.2%). This was a generous gift of the FMC laboratory in Princeton, NJ, obtained through the collaboration of Chuck States. Acetone was dehydrated for at least 48 h on 4Å molecular sieves (EM Science) before use. The control vials were coated with acetone only. Insecticide dosages used in this study were 0.3, 1, 3, 5, 10, 30  $\mu\text{g}$  cypermethrin/vial. Vials were prepared by dispensing 0.5 ml of acetone or cypermethrin solutions and dried on a cold "hot-dog" roller under the hood for at least 15 min. One moth was placed in each vial and the vials were stored at 27°C. Mortality counts were taken after 24 h. 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. From Burleson County 1,814 moths were tested. Two discriminating cypermethrin dosages of 3  $\mu\text{g}/\text{vial}$  and 10  $\mu\text{g}/\text{vial}$  were used among the various tested. A 2.5  $\mu\text{g}/\text{vial}$  dosage was recommended by Kanga et al. (1996) as discriminatory possibly killing all susceptible bollworms. The probit analysis graph showed by these authors suggests the dosage of 5  $\mu\text{g}/\text{vial}$  as the ultimate discriminatory concentration for susceptibles. The IRAC procedure utilized the 5  $\mu\text{g}/\text{vial}$  for the same discrimination in previous monitoring efforts (Payne et al., 2001). The 10  $\mu\text{g}/\text{vial}$  dosage was chosen because previous experience with the budworm *Heliothis virescens* showed that this dosage should kill all heterozygote resistant as well as all susceptible insects (Plapp et al., 1987; Payne et al.,

2001). Therefore, bollworms surviving the 10 ug/vial are assumed to be homozygote resistant but we lack experimental verification of this in our laboratory.

### **Other Counties in Texas**

Other counties in Texas participating in cypermethrin monitoring were: Castro, Hale, Hockley, and Swisher Counties in the High Plains production region; Martin County in the Trans-Pecos production region; Tom Green and Runnels County in the Southern Rolling Plains region; Ellis and Williamson Counties in the Blacklands production region; Uvalde County in the Wintergarden production region; Nueces and Wharton Counties in Coastal Bend production region; and Hidalgo County in the Lower Rio Grande Valley production region. These counties represent every major production region of the state except for the Trans-Pecos.

Cypermethrin vials prepared at Texas A&M University, College Station, were shipped as needed to collaborators. Methods used for moth collection and evaluation were the same as those used for Burleson County, except that the numbers of wire cone traps used differed at each location (Table 1) and bioassays were performed at room temperature. A total of 4,134 moths were tested from other areas in Texas.

Data from all areas in Texas was sent to the Texas A&M University Toxicology laboratory to be analyzed using Probit-PC, Probit and Logit Analysis and graphed using SigmaPlot. A baseline for susceptibility to cypermethrin was established from two areas in Texas with low LC 50 values. Two bioassays from Hockley County and one from Wharton County were pooled to obtain a baseline LC 50 of 0.283 µg/vial. These values were used to calculate relative resistance ratios.

### **Results and Discussion**

A Texas-wide survey (Table 1, Fig. 1) showed high variation in the level of resistance to cypermethrin of males of *Helicoverpa zea* collected during the 2003 season (Table 2, Fig. 2). Although the exact reasons for the observed variability in susceptibility are unknown, the pyrethroid selection pressure and production characteristics of the diverse areas, adoption or not of *Bt* cotton, proximity of cotton fields (traps) to alternative hosts and prevalence of and treatment against other cotton pests may help explain these results. Migration of resistant moths from Mexico or neighboring States where resistance has previously achieved higher levels than in Texas is other possibly contributing factor but we lack quantitative information on it.

Three counties appear to have had the highest levels of resistance of bollworm to pyrethroids; these are Nueces, Burleson and Castro (Table 2, Fig. 2). In Burleson County approximately 10, 570 acres were planted in cotton in 2003, the majority being genetically modified. Approximately 6,300 acres were planted in sorghum and 7,500 in corn. For Burleson and Nueces Co. the highest pyrethroid resistance levels were recorded in July. The high resistance ratios obtained in the laboratory indicated the possibility of field control failures in these counties. In Burleson, with a resistance ratio of 9 for the lethal concentration 50 (populations were 9 times more resistant than susceptible field populations in Wharton and Hockley Co.), there were anecdotal reports that bollworms were not controlled on sorghum using pyrethroids in the Brazos River Bottom. The frequency of resistant individuals has increased in Burleson Co. in the last five years. While in 1998 there were no detected bollworms surviving the 10 ug/vial cypermethrin dosage (Pietrantonio, unpublished) there has been an increase in the percentage of survivorship at this dosage, being of 3.7 % in October 1999 (Pietrantonio and Sronce, 2000), of 2.6% in July of 2000 (Payne et al., 2001), 2.1% in July 2001 (Pietrantonio, unpublished) and up to 15.8% in July of 2003 (Table 3); there were no data collected in 2002. Similarly, adequate control of bollworm with pyrethroid insecticides was not achieved in many Texas Coastal Bend cotton fields during late June and early July of 2003, when the resistance ratio was 9.7 (Table 2). Treatment timing and application techniques were judged to be correct; additional reasons or combination of reasons for lack of control were examined. Problems identified which may have contributed to reduced control effectiveness included heavy leaf canopy, higher than normal number of bollworm larvae, extended egg lay and hatch, rainfall that reduced insecticide deposits on plants, and increased resistance of the species to pyrethroid insecticides. Although excellent control of the species had been achieved earlier in the season on sorghum, increased resistance observed in tested moths may have been the result of widespread use of pyrethroids on the sorghum crop since there were more acres of sorghum than cotton in the region. In agreement with these field observations, the percentage of moths surviving the 30 ug/vial dosage was 10 % and 11.2 % during early July (Table 4) and those surviving the 10 ug/vial dosage were up to 13.3 % in August (Table 4). In Nueces County, *Bt* cotton adoption has been, up to now, very low, about 16% of the planted acreage in 2003. In past years it has ranged from 12 - 15%. Pyrethroid use on sorghum, excellent *Helicoverpa zea* host, has been relatively high due to midge, "headworms", rice stink bugs, and even some early fall armyworms. Insecticide use on cotton in 2003 in the region averaged 4.3 treatments/acre for fleahoppers, thrips/aphids, bollworm, stink bugs, and a variety of other insect species. Of that number of treatments, it is estimated that 1.8 applications/acre was a pyrethroid while in previous years the use has been even less on cotton.

In Hidalgo Co. pyrethroid use was increased because growers were using it to control boll weevils, and while weevil populations were quite low in numbers this season, there was more pyrethroid use than what would have occurred three to four years or more ago.

In Castro County, in addition to cotton, there are high numbers of corn and sorghum acres. Sprays are regularly applied for bollworms on an annual basis, and sometimes more than one treatment is applied. Up to 10% of moths survived the 10 ug/vial dosage (Table 5) but no survivors were observed at 30 ug/vial. However, the results from Castro Co. should be interpreted cautiously because the trap used was the only one that collected enough moths for bioassays and was located northwest of an aerial applicator's headquarters. It is possible that these moths may not be representative of the overall situation in Castro County due to high pyrethroid selection pressure in that particular area. Since the resistance ratio was 10 in September (Table 2), resistance should be monitored next year early in the season.

In Uvalde County bollworms survived the 10 ug/vial dosage in July (4 moths/20 tested at 10 ug/vial = 20% survivorship) and the 30 ug/vial concentration in August (1/16 tested at 30 ug/vial= 6%), however data were insufficient and values had high heterogeneity as to allow calculate the  $LC_{50}$  and  $LC_{95}$ . In Uvalde County at least two pyrethroid treatments are applied to every acre of the winter greens grown in the Winter Garden area. Most of those are not good hosts of *H. zea*, but there are two to three crops in the winter each year that are relatively good hosts. Pyrethroids are also applied to some grain sorghum, but in most years that is a small percentage of the acreage. Resistance to pyrethroids should be carefully monitored in this area next season.

The rest of the counties discussed below have resistance ratios for the  $LC_{50}$  lower than 5 when we use as a denominator for this calculation the  $LC_{50}$  of the most susceptible field populations for 2003 (see methods, Table 2, Fig. 2). However, if we utilized the  $LC_{50}$  of a laboratory susceptible population estimated by Kanga et al. (1996) all of the populations tested in 2003 with the exception of Wharton Co. would have had resistance ratios higher than 5 for the  $LC_{50}$ . This means that resistance is widespread in bollworm in Texas, with a few areas reaching critical resistance levels that could result field control failures.

In Hockley County this season there was light worm pressure and few acres were treated. Three traps were placed in three different cotton fields; only two of them received one pyrethroid application against bollworm. One field was treated with Ammo (cypermethrin) 3.2 oz and the other with Baythroid (cyfluthrin) 2.1 oz. Two traps were in close proximity to forage sorghum and another near a few rows of sweet corn. *Bt* cotton has not been widely adopted in Hockley and Cochran Counties. Up to this point *Bt* cotton has not precluded the use of pyrethroids in those acute situations mid- to particularly late-season. There has been pyrethroid use against *Lygus* in early-season in some situations.

In Parmer County pyrethroid applications were infrequent in 2003 in corn, and were rare in sorghum. Pyrethroid use was greater in 2002 in corn. Many more fields were treated against mites (bifenthrin) and corn borers (various pyrethroids) in 2002 than in 2003. Therefore, bollworm pyrethroid exposure in corn was far greater in 2002 than in 2003. A total of 9,000 acres of green bean fields in 2003 were treated an average of 3.5 times each with pyrethroids, mostly with zeta-cypermethrin. Corn earworm (cotton bollworm) spends 2 generations in corn before moving to cotton in large numbers after the corn begins to decline. Exposure to pyrethroids will occur if fields are sprayed for spider mites or southwestern corn borer. Also, sorghum is a host for bollworm, where it is known as a member of the headworm complex. Headworms are usually not treated, but when they are, pyrethroids are often used. Approximate acreage figures for 2002 were 80,500 in cotton, 41,700 in corn and 57,300 in sorghum.

For Hale and Swisher Counties, about 30 to 40% of the acreage was treated for cotton bollworm in 2003. Products used were Ammo® (cypermethrin), Karate® (cyhalothrin), Asana® (esfenvalerate) and Fury® (Z-cypermethrin). Ammo® was the predominant product used because of its lower cost (\$3 to \$3.85/acre). In 2002, one hundred percent of the cotton acreage was treated for cotton bollworm, with about 1.2 applications per acre. Ammo® was sold out first in 2002, then other products were used.

In Williamson County approximately 20,000 acres of cotton were planted in 2003, about 80% of the cotton was *Bt* and probably less than 1 % of that was sprayed with a pyrethroid. Much of the non-*Bt* cotton required on average 1-2 foliar sprays for worm control. This was generally done with either a pyrethroid only, or a tank-mix with products such as Larvin® (thiodicarb), Curacron® (profenofos) or Denim® (emamectin benzoate). The pyrethroids used in our area were mainly Mustang Max®, and Baythroid®. Karate® (lambda-cyhalothrin) and Asana® (esfenvalerate) were used to the lesser extent. Some growers reported control problems with pyrethroids alone. These could have been caused by uneven coverage and sub-optimal application time, when caterpillars are too large to be controlled. Tank mixtures of pyrethroids with the above mentioned products provided satisfactory field control. However, the resistance ratio for the  $LC_{50}$  of this population is 16.6 when calculated using the  $LC_{50}$  of a susceptible bollworms calculated in 1988 by Kanga et al. (1996) of 0.05 ug/vial, and the resistance ratio calculated with this season field population baseline is of 2.9.

In Wharton County there were no reported field failures to control bollworm, in agreement with resulting the most susceptible population tested in the laboratory in 2003 (Table 2). Pyrethroid use was probably above average in other crops, with stink bugs present in grain sorghum and soybeans.

Most of the pyrethroid insecticide applications in Ellis and Navarro Counties during the 2003 season targeted mid- and late season boll weevil outbreaks. Bollworm/tobacco budworm numbers were very light and did not require treatment, except in some of the fields being treated for boll weevil. Approximately 9,000 acres of Ellis county cotton was treated with a pyrethroid insecticide for in-season boll weevil. Depending on weevil pressure, fields received from 1 to 5 applications against boll weevil. The average would be about 3 applications at intervals of 5 days. In Navarro County, we were only aware of 3 fields totaling 175 acres that were treated with a pyrethroid insecticide against bollworm. The pyrethroid insecticides used were; cyhalothrin (Karate®), cyfluthrin (Baythroid®) and deltamethrin (Decis®). Actual lbs. applied/acre ranged from 0.036 to 0.18 with an average of about 0.108. In essence, bollworm/tobacco budworm pressure has been light the last several seasons with only a few fields requiring treatment, and in most cases they were treated with a non-pyrethroid and low impact control agent such as spinosad (Tracer®). Only this season there was an increase in pyrethroid insecticide use principally for boll weevil. It is noteworthy that in the last four Counties mentioned, Hale, Swisher, Ellis and Navarro there were no bollworms surviving the 3 ug/vial dosage indicating that bollworm populations were susceptible to pyrethroids (Fig. 1).

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Table 1. Area-wide monitoring for bollworm resistance to cypermethrin: production regions and counties surveyed.

<b>Region</b>	<b>County</b>	<b>Collaborator</b>	<b># Traps</b>
High Plains	Castro	Emilio Nino	2
High Plains	Hale & Swisher	Greg Cronholm	5
High Plains	Hockley	Kerry Siders	3
High Plains	Parmer	Pat Porter	8
High Plains	Martin	Russell Baker	2
Southern Rolling Plains	Runnels & Tom Green	Chris Sansone & Rick Minzemayer	4
Blacklands	Ellis	Glen Moore	2
Blacklands	Williamson	Dale Mott	6
Brazos River Bottom	Burleson	Terry Junek	6
Wintergarden	Uvalde	Noel Troxclair	3
Coastal Bend	Nueces	Roy Parker	4
Coastal Bend	Wharton	Dan Fromme	2
Lower Rio Grande Valley	Hidalgo	John Norman	1

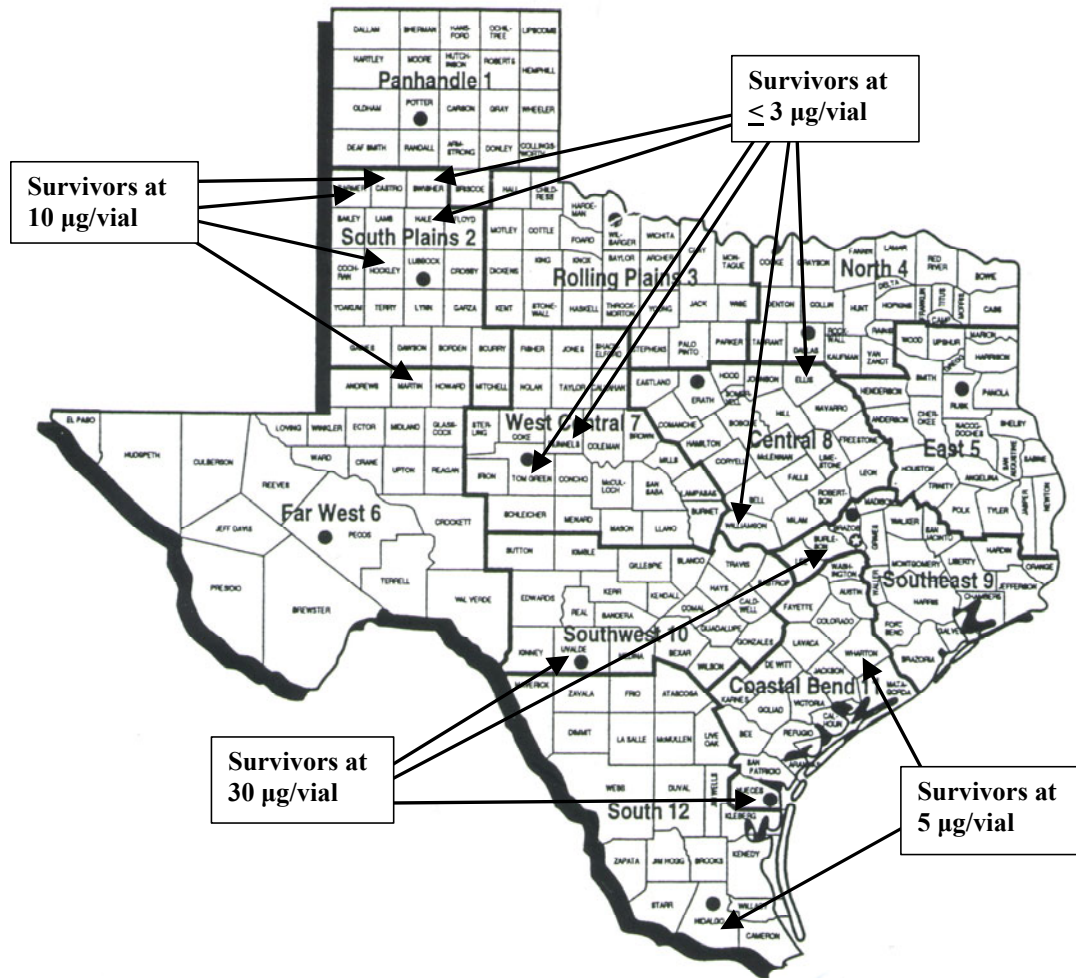


Figure 1. Texas map with cypermethrin survivorship by county.

Table 2. Cypermethrin Bioassay for bollworm, *Helicoverpa zea*, 2003.

County	Date	LC 50, µg/vial (C.L.)	RR LC 50	LC 95, µg/vial (C.L.)	$\chi^2$	df	# Moths Tested
Castro Co.	8/26	1.207 (.718-1.797)	4.265	11.570 (6.409-35.050)	2.476	3	140
Castro Co.	9/02	2.833 (.882-4.238)	10.011	12.791 (7.668-97.611)	.396	3	140
Hale Co.	8/19	.609 (.210-1.138)	2.152	4.740 (2.020-215.744)	.0258	1	130
Hockley Co. (pooled)	7/23,29, 8/18	.283 (0.84-.510)	1	6.990 (3.415-35.137)	1.400	3	308
Hockley Co.	8/25-27	.949 (.510-1.456)	3.353	12.022 (6.566-37.047)	.623	3	189
Parmer Co.	6/12	.669	2.364	3.0575	5.358	2	280
Martin Co (pooled)	7/3, 7/30	.977 (.605-1.409)	3.452	10.738 (6.413-25.022)	1.599	3	280
Williamson Co. (pooled)	7/18, 7/25	.833 (.487-1.255)	2.943	5.432 (2.970-21.872)	.5137	1	196
Burleson Co. May	5/14/03	.467 (.111-.857)	1.650	6.035 (2.595-111.550)	.075	1	210
Burleson Co. June (pooled)	6/04, 6/18	.939 (.584-1.292)	3.318	4.005 (2.728-8.100)	.2539	2	420
Burleson Co. July (pooled)	7/1, 7/10, 7/22	2.524 (1.987-3.129)	8.919	21.613 (14.992-36.327)	1.295	4	554
Burleson Co. August (pooled)	8/7, 8/20	2.009 (.591-3.085)	7.099	7.603 (4.641-52.664)	3.865	3	280
Burleson Co. September (pooled)	9/9, 9/24	.711 (.377-1.085)	2.512	10.073 (5.769-26.881)	.140	3	270
Nueces Co. July (pooled)	7/4 7/15-19	2.750 (1.700-4.089)	9.717	37.671 (20.267-108.840)	2.687	3	340
Nueces Co. August	8/15-8/22	1.587 (.764-2.588)	5.608	13.905 (7.188-59.929)	1.595	3	112
Wharton Co.	7/7-11	.209	.739	7.213	.001	1	124

C.L. = Confidence Limits

RR = Resistance Ratio

Resistance ratio calculated from baseline LC 50 (.283 µg/vial) derived from Hockley and Wharton County bioassays

Total moths used for analysis: 3,973

Table 3. Burleson County % corrected survival at different concentrations of cypermethrin.

Date	0.3 µg	1 µg	3 µg	5 µg	10 µg	30 µg
5/14/03	62.5	29.1	12.5	0	0	0
6/04/03	72.4	31	6.9	0	0	0
6/18/03	95.9	54.1	0	4.1	0	0
7/01/03	93.1	75.9	37.9	24.1	13.8	3.3
7/10/03	100	78.9	42.1	21.1	15.8	5.3
7/22/03	96.7	66.7	46.7	40	0	0
8/07/03	100	100	22.2	11.1	0	0
8/20/03	86.7	76.7	20	16.7	3.3	0
9/09/03	94.1	47.1	23.5	17.6	5.9	0
9/24/03	57.7	34.6	15.3	7.7	0	0

Table 4. Nueces County % corrected survival at different concentrations of cypermethrin.

Date	0.3 $\mu\text{g}$	1 $\mu\text{g}$	3 $\mu\text{g}$	5 $\mu\text{g}$	10 $\mu\text{g}$	30 $\mu\text{g}$
7/4/03	90	63.3	46.7	30	3.3	10
7/15,18,19/03	88.9	94.4	38.9	22.2	5.6	11.2
8/15-22/03	93.3	66.7	20	20	13.3	0

Table 5. Castro County % corrected survival at different concentrations of cypermethrin.

Date	0.3 $\mu\text{g}$	1 $\mu\text{g}$	3 $\mu\text{g}$	5 $\mu\text{g}$	10 $\mu\text{g}$	30 $\mu\text{g}$
8/26/03	90	45	30	10	10	0
9/02/03	94.1	82.4	47.1	29.4	5.9	0

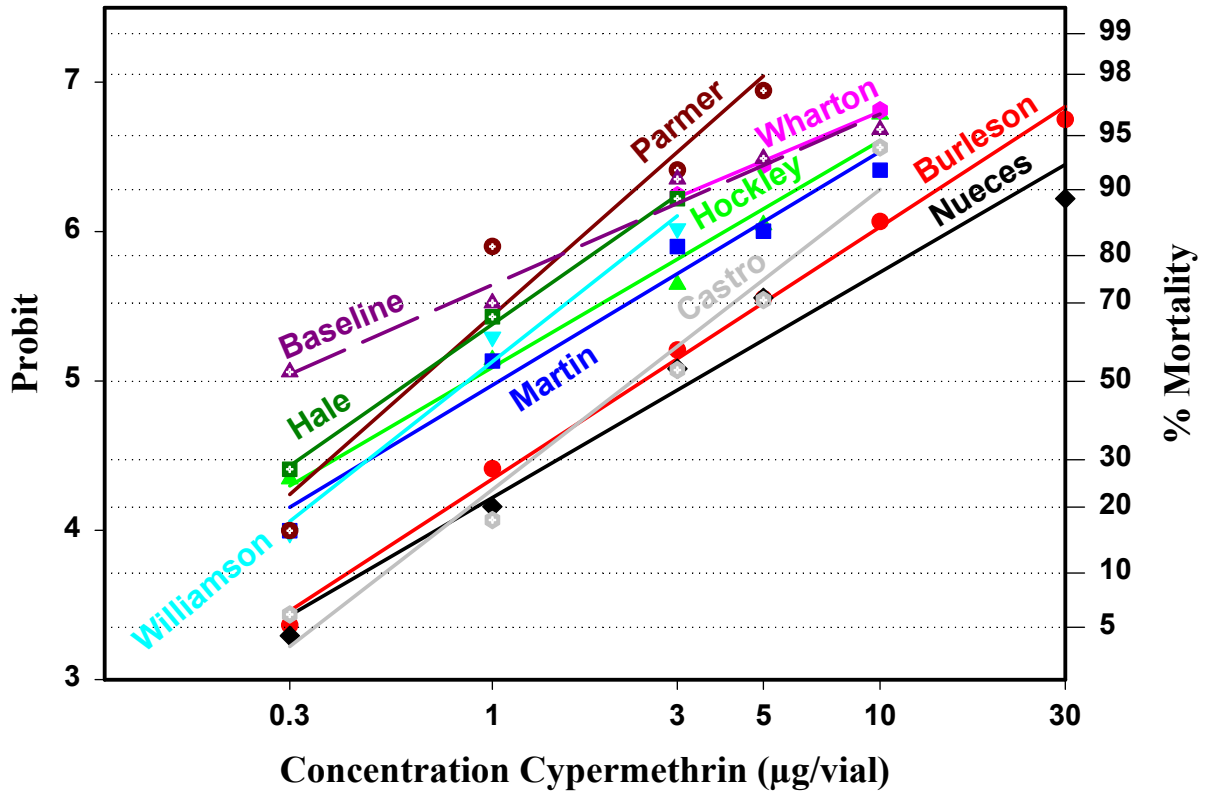


Figure 2. Cypermethrin mortality by Texas county, linear regression.