Monitoring Pyrethroid Resistance in Bollworm (Lepidoptera: Noctuidae) Moths in Missouri, 1988 to 1994[1]

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Abstract From 1988 to 1994, adult vial bioassays were conducted on bollworms, *Helicoverpa zea* (Boddie), collected from pheromone traps in Missouri to determine their susceptibility to pyrethroids. Although most moths were susceptible to cypermethrin, many assays contained individuals that survived concentrations of 5 and 10 µg per vial. The number of individuals that survived these concentrations increased each of the first 3 yrs, and then fluctuated from year to year. In some cases, moths with increased tolerance to cypermethrin occurred in locations where little or no pyrethroid insecticides were used for bollworm control. A likely explanation for tolerant bollworms in Missouri is immigration from more southerly locations, and evidence for long range dispersal of these insects is presented. Implications for regional resistance monitoring also are discussed.

Key Words *Helicoverpa zea,* cypermethrin, adult, resistance monitoring

The bollworm, *Helicoverpa zea* (Boddie), has become one of the primary pests of cotton in the United States. It is the most destructive agricultural insect in Missouri, with statewide cotton losses from this pest ranging from $1.5 to 5.5 million between 1988 and 1995 (Head 1989, 1990, 1991, 1992, 1993, Williams 1994, 1995, 1996). Despite attempts to utilize natural enemies, host plant resistance and other strategies, the use of conventional insecticides remains the most efficient and cost-effective means of bollworm control. The introduction of new insecticides has resulted in satisfactory control; however, prior to 1980, some populations of bollworm had developed resistance to the major classes of insecticides (chlorinated hydrocarbons, carbamates, and organophosphates) used for their control (Sparks 1981).

The widespread use of pyrethroid insecticides began in the early 1980's, and by 1984, pyrethroids constituted up to 90% of the insecticides applied for control of both the bollworm and the tobacco budworm, *Heliothis virescens* (F.), in some areas (Bacheler 1985). Pyrethroid insecticides comprised seven of the 12 products recommended by the University of Missouri Extension Service for control of bollworm (Sorenson and Nabors 1995). High tolerance to pyrethroids in tobacco budworm is a well-documented and particularly troublesome phenomenon (Luttrell et al. 1987,

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Leonard et al. 1988, Elzen et al. 1992); in 1996 and 1997, field failures of a pyrethroid in bollworm due to apparent resistance were found in South Carolina (T. Brown, pers. comm.). In Mississippi, some male and female adult bollworms reared from early-season wild hosts have survived a 10 µg per vial dose of cypermethrin (Stadelbacher et al. 1990).

Monitoring for resistance is essential to the organization and implementation of an insecticide resistance management program (Staetz 1985). Because resistant gene frequencies must be low for many resistance management strategies to be successful (Tabashnik and Croft 1982), it is crucial to implement a monitoring program prior to the development of resistance in the field. The objective of these studies was to determine the level of susceptibility of bollworm populations to cypermethrin for use in the development of programs to prevent or delay the onset of pyrethroid resistance in Missouri.

Materials and Methods

Wire cone traps (Hartstack et al. 1979) baited with sex pheromone lures (Hercon Environmental Corp., Emigsville, PA) were used to collect male bollworm moths from June through September from 1988 to 1994. Trapping protocols were similar to those described by Goodenough et al. (1987). Traps were checked two or three times per week. Field collections of moths were screened for susceptibility to the pyrethroid cypermethrin in four locations: southeast, central, south-central and southwest Missouri. The four areas were chosen for their diversity in agriculture and insecticide use. The southeast location is a region of intensive row crop agriculture and relatively heavy insecticide use. Areas within southeast Missouri where moths were collected for assay included Dunklin, Mississippi, New Madrid, Pemiscot, and Scott counties. The central location, Boone Co., is in a region of moderate levels of row crop agriculture and low insecticide use. The south central area, Wright Co., is in a region of very limited row crop production and little, if any, insecticide use. The southwest area, including Barton, Jasper and Vernon counties, is an area of moderately intense agriculture and low insecticide use.

The interiors of 20-ml glass scintillation vials were coated with a residual film of cypermethrin (Plapp et al. 1987). Vials were kept in the dark and below freezing until they were used. Six concentrations of cypermethrin were used in tests conducted 1988-1992: 0, 0.5, 1.0, 2.5, 5.0, and 10.0 µg per vial. In 1992, a decision was made to try to increase the number of assays in order to obtain more point assessments of population responses. Hence, during the 1992-1994 seasons, some or all assays were conducted with only three concentrations: 0, 2.5, and 5.0 µg per vial.

Only male moths that appeared to be healthy and in good condition were used in tests. Vials containing moths were kept on their sides at room temperature in the dark. After 24 h, moths were scored as "alive," "dead," or "down." Mortality was determined by removing the moths from the vials and tossing them into the air. Moths unable to fly were considered "down"; insects that were totally unresponsive to shaking, probing, or pressure were considered "dead." Acetone-treated vials were used to check for control mortality. All data were corrected for control mortality using Abbott's (1925) formula. Concentration-mortality regressions were estimated by probit analysis (SAS Institute 1985) for the six-concentration assays.

Ideally, a known susceptible control population would have been included in each assay; however, this was logistically impossible. Evaluations of this bioassay technique utilizing an inbred bollworm colony known to be susceptible to pyrethroids determined that the LC50 for this population was 0.72 μg/vial, with 95% confidence interval of 0.54-0.97 μg (Schreiber and Knowles 1991). Therefore, in the present study, populations with LC50 values greater than 0.72 μg/vial as indicated by non-overlap of 95% confidence intervals were classified as having elevated tolerance. Responses of this same inbred colony to 2.5 and 5 μg concentrations were 96% and 99.63% mortality, respectively.

The numbers and types of bioassays conducted each year are presented in Table 1.

Results

1988 Monitoring. Cypermethrin LC50s ranged from 0.21 to 1.82 μg per vial, which represented an 8.6-fold difference in tolerance. However, only one assay of 11 had a LC50 value exceeding that of the susceptible control population (Table 2). No moths survived 10 μg per vial, and only four of 125 moths (3.2%) survived 5 μg per vial.

1989 Monitoring. Cypermethrin LC50s ranged from 0.22 to 3.06 μg per vial, a 13.9-fold difference in tolerance. Three of the five assays from Wright Co. produced LC50's significantly higher than the susceptible population (Table 2); 13.3% of the moths tested survived a cypermethrin concentration of 5 μg per vial, and 5.3% survived a concentration of 10 μg per vial. The only other moths as tolerant occurred in a single Dunklin Co. assay. State-wide survival at the 5 μg and 10 μg per vial concentrations was 5.5 and 1.5%, respectively.

1990 Monitoring. Cypermethrin LC50's ranged from 0.16 to 3.13 μg per vial, a 20-fold difference in tolerance. In general, moths screened earlier in the season were less tolerant than those tested later in the season. Wright Co. bioassays conducted from 19 June through 20 July had LC50's less than 1.0 μg per vial; from 23 July through 11 September, seven of eight assays produced LC50s significantly higher than the susceptible population (Table 2). Ten percent of the moths from Wright Co. survived a 5 μg per vial concentration, and 5.8% survived a 10 μg per vial concentration. The only two other assays with high survival were from the southeast in September. State-wide survival at the 5 μg and 10 μg per vial concentrations was 5.6 and 3.2%, respectively. In addition to Wright Co., small numbers of moths collected in Dunklin and New Madrid counties survived a 10 μg per vial concentration.

1991 Monitoring. All assays in this year were conducted in Wright and Boone counties (Table 1). Cypermethrin LC50's ranged from 0.40 to 1.80 μg per vial representing a 4.5 fold range in tolerance. Three assays from Boone Co. produced LC50 values significantly greater than that of the susceptible population (Table 2). Survival in both counties at the 5 μg concentration was 4.2% (23 out 546) and at the 10 μg concentration, 0.7%.

1992 Monitoring. None of the seven, six-concentration assays conducted in Boone, Barton, and Vernon produced LC50 values significantly different from that of the susceptible population. In these assays, using a total of 840 moths, LC50's ranged from 0.30 to 0.96 μg per vial. This low range of LC50 values (compared to other years in the study) is due at least in part to the limited number and geographic range of the assays. Survival at the 2.5 μg concentration in the three dose assays was as high as 25% in one assay but survival at 5 μg did not exceed 6% in any instance (Table 3). No assays were conducted in the southeastern counties.

1993 Monitoring. State-wide survival at the 2.5 μg concentration was 14.0% and at the 5 μg concentration survival was 4.2% (Table 4). The elevated state-wide survival rates in 1993 were due to increased survival in Barton Co. and high relative

Table 1. Number and type of bollworm adult-vial cypermethrin bioassays in Missouri, 1988-1994.

Year	County	No. six-concentration assays	No. three-concentration assays
1988	Boone	5	—
	Dunklin	2	—
	Pemiscot	4	—
1989	Boone	6	—
	Dunklin	3	—
	Pemiscot	1	—
	Wright	5	—
1990	Boone	3	—
	Dunklin	7	—
	New Madrid	5	—
	Scott-Mississippi	3	—
	Wright	13	—
1991	Boone	10	—
	Wright	18	—
1992	Barton	1	3
	Boone	5	6
	Vernon	1	5
	Wright	0	11
1993	Barton	0	10
	Boone	0	7
	Dunklin	0	17
	New Madrid	0	12
	Pemiscot	0	14
	Wright	0	16
1994	Barton	0	15
	Boone	0	3
	New Madrid	0	17
	Pemiscot	0	8
	Wright	0	17

Table 2. Six-concentration bollworm cypermethrin response assays in Missouri in which LC50 values were significantly higher than that for a known susceptible population, 1988-1992.

Date	County	No. Moths	Slope (±SE)	LC50*	95% Confidence Limits
8/15/88	Boone	60	2.42 (0.38)	1.82	1.13-3.25
7/25/89	Dunklin	150	2.73 (0.37)	1.75	1.34-2.36
8/24/89	Wright	60	1.69 (0.46)	3.06	1.56-6.67
8/29/89	Wright	60	2.03 (0.65)	2.03	1.17-3.47
8/30/89	Wright	120	3.75 (0.71)	1.73	1.23-2.47
9/13/90	Dunklin	120	1.74 (0.33)	1.71	1.11-2.48
9/14/90	Scott-Miss.	84	2.06 (0.42)	1.63	1.03-2.43
7/23/90	Wright	120	0.75 (0.28)	3.13	1.28-14.8
7/25/90	Wright	96	1.45 (0.34)	1.92	1.08-3.17
7/26/90	Wright	120	1.32 (0.30)	2.49	1.51-4.23
8/16/90	Wright	102	5.10 (1.03)	1.58	1.25-2.01
8/17/90	Wright	90	3.32 (0.62)	1.94	1.43-2.60
9/09/90	Wright	120	2.72 (0.43)	1.94	1.46-2.57
9/11/90	Wright	60	2.37 (0.53)	2.15	1.33-3.36
6/5/91	Boone	120	2.66 (0.43)	1.80	1.34-2.39
6/7/91	Boone	120	2.48 (0.41)	1.74	1.27-2.33
8/6/91	Boone	120	2.21 (0.39)	1.45	1.01-1.99

* µg/vial

survival in some assays from the southeastern counties. Survival greater than 10% at the 5 µg concentration was observed a total of 12 times (15.7% of bioassays); nine of these instances occurred in the southeast; the other three instances occurred in Barton Co.

1994 Monitoring. State-wide survival at the 2.5 µg concentration was 12.13% and at the 5 µg concentration 2.55% (Table 5). Survival at the 5 µg concentration exceeded 10% in four instances (6.6% of bioassays), one in Barton Co., twice in Wright Co., and once in New Madrid Co.

Over the course of the study, survival at the 5 µg appeared to increase between 1988 and 1990 and then fluctuated at lower levels the remaining four years (Table 6).

Discussion

The fact that some moths survived 5 and 10 µg per vial concentrations of cypermethrin suggests the presence of pyrethroid tolerant bollworm in Missouri. However, because the higher LC50's observed in these studies were not directly associated

Table 3. Susceptibility of male bollworm moths to cypermethrin in Missouri during 1992, three-concentration assays*.

Month (No. assays)	Mean % Survival	
	2.5 µg/vial (range)	5.0 µg/vial (range)
	Barton Co.	
June (1)	16	5
August (1)	25	0
September (1)	0	0
	Boone Co.	
June (1)	11	0
July (1)	16	0
August (2)	17 (6-22)	0
September (2)	8.5 (6-11)	0
	Vernon Co.	
June (1)	6	0
July (2)	0	0
August (2)	14 (10-18)	3 (0-6)
	Wright Co.	
June (1)	6	6
July (3)	12 (5-20)	0
August (5)	7.4 (5-15)	1 (0-5)
September (2)	10.5 (5-16)	2.5 (0-5)

* n = 20 moths/concentration

with field control failures, these moths cannot be considered resistant using the criteria of Ball (1981). Plapp et al. (1987) reported that tobacco budworm moths surviving a 10 µg per vial concentration of cypermethrin were homozygous for resistance, and that this should be considered a discriminating concentration for resistance monitoring programs for this species. The 10 µg per vial concentration has been used to document pyrethroid resistance for tobacco budworm in several cotton producing states (Luttrell et al. 1988, Campanhola and Plapp 1989, Mullins et al. 1991). No discriminating concentration has been reported for the closely related bollworm. However, the occurrence of bollworm moths from multiple locations in Missouri surviving a 10 µg per vial concentration of cypermethrin in all years between 1989 and 1992 suggests that some proportion of bollworms in this state are highly tolerant and capable of developing resistance to pyrethroid insecticides.

The gradual increase over the first 3 years in numbers of moths that survived the 5 and 10 µg per vial concentrations of cypermethrin suggests an increase in pyrethroid tolerance over time. The increased survival by bollworm populations at the 10

Table 4. Susceptibility of male bollworm moths to cypermethrin in Missouri during 1993, three-concentration assays*.

Month (No. Assays)	Mean % Survival	
	2.5 µg/vial (range)	5.0 µg/vial (range)
	Barton Co.	
July (6)	19.5 (0-65)	9.5 (0-25)
August (4)	18 (5-39)	4 (0-11)
	Boone Co.	
June (1)	12	0
July (4)	16 (6-24)	6 (0-12)
August (1)	13	6
September (1)	17	0
	Dunklin Co.	
June (2)	3 (0-6)	3 (0-6)
July (6)	12 (0-31)	4.2 (0-13)
August (8)	21.5 (11-39)	5.2 (0-15)
September (1)	6	0
	New Madrid Co.	
July (2)	26 (22-30)	16 (15-17)
August (9)	14.9 (0-39)	4.4 (0-11)
September (1)	0	0
	Pemiscot Co.	
July (5)	11 (0-25)	1.2 (0-6)
August (7)	25.7 (0-50)	6.4 (0-30)
September (2)	11	6.5 (6-7)
	Wright Co.	
June (4)	8 (0-15)	0.7 (0-3)
July (3)	11 (5-20)	4 (3-6)
August (6)	11.7 (3-21)	3.7 (0-10)
September (3)	7 (0-18)	5.3 (3-8)

* n = 20 moths/concentration

µg per vial concentration from 0% in 1988 to 2.3% in 1990 strongly parallels bollworm tolerance patterns in Mississippi, where male bollworm moth survival at the 10 µg per vial concentration increased from 0% in 1988 (Luttrell et al. 1988) to 2.9% in 1990 (Stadelbacher et al. 1990).

Table 5. Susceptibility to cypermethrin of male bollworm moths collected in pheromone traps in 1994, three-concentration assays*.

Month (No. Assays)	Mean % Survival	
	2.5 µg/vial (range)	5.0 µg/vial (range)
	Barton	
June (9)	13.11 (5-30)	4.2 (0-15)
July (1)	6	0
August (4)	17.4 (5-37)	5 (0-10)
October (1)	0	0
	Boone	
July (1)	0	0
August (1)	0	0
September (1)	15	5
	New Madrid	
July (4)	23.3 (17-33)	4 (0-11)
August (9)	17.4 (0-71)	0.7 (0-7)
September (4)	9 (0-25)	0
	Pemiscot	
August (7)	7.1 (0-20)	2.1 (0-10)
September (1)	0	0
	Wright	
June (4)	22 (15-37)	2.8 (0-10)
July (2)	5.5 (0-11)	8 (0-16)
August (3)	6.7 (5-10)	0
September (6)	11 (0-20)	3.3 (0-10)
October (2)	0	2.5 (0-2.5)

* n = 20 moths/concentration

Geographic distribution of populations with apparently elevated tolerance was variable. In 1988 and 1991, moths sampled in Boone Co. had the greatest tolerance. In 1989, moths sampled in Wright Co. had the greatest tolerance. Although all locations had moths with elevated tolerance at the end of the season in 1990, seven samples in Wright Co. and two from the southeast exhibited elevated tolerance.

Examination of the response to 5 µg over the 7 years supports the assessment that tolerance increased for the first 3 years (Table 6). However, state-wide survival rates at this concentration the last 4 years fluctuated at levels generally lower than the maximum observed in 1990. While not all areas were sampled in all years, each

Table 6. Survival of male bollworm moths exposed to cypermethrin at a concentration of 5 µg per 20 ml vial, 1988-1994.

Year	Area	Mean Percent Survival at 5 µg
1988	Statewide	3.2
1989	Statewide	5.5
	Wright	13.3
1990	Statewide	5.6
	Wright	10.0
1991	Wright and Boone	4.2
1992	Boone, Barton, Vernon	1.2
1993	Statewide	4.2
1994	Statewide	2.5

year's data included assays from areas where elevated tolerance had been observed in the first 3 years. This fluctuation may be due to differences in immigrant source populations or perhaps may reflect changes in corn (*Zea mays* L.) hectareages in source areas. Greater corn hectareages in source areas could generate large numbers of relatively susceptible moths because field corn typically receives very little, if any, pyrethroid insecticide.

While average survivals at 5 µg were generally lower the last 4 years than the peak year (1990, Table 6), instances of apparent high tolerance were still recorded. Survival at the 5 µg concentration exceeded 10% in 16 out 136 assays (approximately 12% of total assays) in 1993 and 1994. Again, while no control failures were observed in any of the test areas, instances of high survival at the 5 µg concentration suggest that these populations have experienced selection pressure.

The evolution of insecticide tolerance is determined by genetic and biological factors, such as intensity of insecticide application, immigration, host refugia, and resistance gene frequency (Georghiou and Taylor 1977). Because the development of pyrethroid tolerance should occur in areas with the greatest pyrethroid use, and hence the strongest selection pressure for increased tolerance (Crowe 1957), the difference in insecticide use between sampling areas should be reflected in relative LC50's. A 1990 Pesticide Impact Assessment Survey of insecticide use on all row crops in Missouri revealed that pyrethroid use was greatest in the southeast and immeasurably low in the south-central and central regions (G. S. Smith, pers. comm.). The most tolerant moths tested in 1989 and 1990 were from an area with almost no row crop production (row crop acreage in 1988 was less than 400 ha, primarily corn and soybeans, Walsh and Schlegal 1989). Pyrethroid insecticide use against bollworm in this area is rare. The most tolerant populations in 1988 were in Boone Co., an area with a corn-soybean rotation system of agriculture that rarely requires insecticide treatment for bollworm control. The southeastern region is an area of intense agricultural production that includes 122,000 to 162,000 ha of cotton, of which bollworm is a primary pest. During 1988, 81,000 ha received on average 1.5 applications of insecticide for bollworm control (Head 1989), and over 90% of insecticides used for bollworm control in Missouri are pyrethroids (A. S., unpub. data). However, the pyrethroid use rates in southeast Missouri are much lower than those in Mississippi, southern Arkansas, and Louisiana (Head 1989, 1990, 1991, 1992, 1993, Williams 1994, 1995, 1996), and it is reasonable to expect selection pressure would be lower in Missouri compared to these more southerly areas.

The occurrence of elevated pyrethroid tolerance in bollworm in central and south-central Missouri, locations where pyrethroids are used rarely, if at all, suggests that the selection for tolerance could not have occurred locally. The most likely explanation for insect tolerance to pyrethroids in areas of low pyrethroid insecticide use is that these individuals emigrated from areas of more intense pyrethroid use. The movement of adult Lepidoptera influencing resistance gene frequency, sometimes over long distances, has been reported for several species. Daly and Fitt (1990) described immigration of susceptible *H. armigera* (Hübner) as the most likely cause for the annual decline in resistance frequencies in New South Wales, Australia. Young (1979) reported that migration of resistant populations of fall armyworm, *Spodoptera frugiperda* (J. E. Smith), from Florida or Mexico was responsible for the occurrence of carbaryl-resistant fall armyworm in the eastern and southeastern United States. Long-range movement of tobacco budworm and bollworm from Mexico into Texas and the mid-South has been reported to explain the rapid development and spread of methyl parathion resistance throughout the United States cotton belt (Lukefahr 1970, Wolfenbarger et al. 1973). The sudden occurrence of pyrethroid resistant tobacco budworm throughout western Texas in 1985 was thought to have been due to atmospheric transport of adults from southern Texas or northern Mexico (Westbrook et al. 1990).

Bollworm is not known to over-winter in Missouri, except in the extreme southeastern portion of the state (Keaster 1961); even in that part of the state, immigrants may comprise a large proportion of the population. Bollworm moths are known to disperse over long distances. By using pollen markers to pinpoint adult feeding sites, Hendrix et al. (1987) determined that moths collected from pheromone traps in northeastern Arkansas originated from southern Texas or northern Mexico. Harstack et al. (1986) described the long-range movement of bollworm from Texas and northern Mexico across the Great Plains and northeastward up the Mississippi River Valley. Raulston et al. (1986) reported the collection of dispersing bollworm moths at varying heights up to 1767 m. Westbrook et al. (1990) provided data suggesting pyrethroid resistant tobacco budworm moths moved from southern Texas or Mexico into western Texas during a single 12-h period at an altitude of approximately 500 m. Texas has widespread tobacco budworm and bollworm infestations that have elevated tolerance or resistance to pyrethroid insecticides (Plapp et al. 1989). While it is likely that southeastern Missouri populations are experiencing some slight selection pressure through pyrethroid applications to cotton crops, we feel that long-range emigration of moths from more southerly locations is the most likely explanation for cypermethrin-tolerant bollworms in Missouri.

Because of the development of pyrethroid resistance in tobacco budworm over much of the cotton belt and the increase in pyrethroid tolerance in bollworm, a species capable of long-range movement, the need to develop a coordinated regional resistance monitoring and management program for both species is apparent. The development of these strategies should not be limited to states that are experiencing pyrethroid control problems. States that are currently on the periphery of areas with resistance problems, and within the dispersal range of populations that have resistance, should actively promote strategies that will allow early detection and management of resistance. These states should integrate such strategies into a flexible regional program that accommodates the unique crop production and protection tactics of the individual states.

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