BOLLWORM: RESPONSE TO METHYL PARATHION AND PERMETHRIN IN SOUTH TEXAS AND MEXICO

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Abstract

From 1969 to 1980s resistance to methyl parathion of the bollworm, *Helicoverpa zea* (Boddie), indicated by an LD_{50} value of >7.5 μg/larva, was found in populations from San Salvador, El Salvador and Tapachula, Chiapas and Tampico, Tamaulipas, Mexico. In 1992 a population from southern Tamaulipas was resistant to methyl parathion . This LD_{50} of >7.5 μg /larva was proposed as a resistance threshold for methyl parathion to populations of bollworm. Results were organized to show response of bollworm to 34 populations from methyl parathion in tropical areas of Mexico to subtropical areas of Mexico and south Texas. In 1990-1991, 1993 and 1995 resistance to methyl parathion was not found in populations of the bollworm collected from corn or cotton in south Texas or in northern Tamaulipas, Mexico. No resistance to permethrin was shown by 29 populations of bollworm collected from El Salvador, Mexico or from the United States in 1981-1983 and 1990-1991. None of the LD_{50} values for permethrin exceeded a proposed resistance threshold of >0.2 μg/larva. Many of the same collections were treated with both insecticides.

Introduction

Since the 1950s the extensive use of methyl parathion to control the boll weevil, *Anthonomus grandis* Boheman, in southwestern Chiapas, Mexico, and in all cotton growing areas of Central America may have caused the selection for resistance by the bollworm, *Helicoverpa zea* (Boddie). Methyl parathion was used to control both pests but the boll weevil is the major and consistent pest which could limit production of cotton year after year in these areas. This is the only Heliothine pest species found on cotton in southwestern Mexico and Central America. In 1969 resistance to methyl parathion was first shown for bollworms collected at Leon, Nicaragua, when an LD_{50} of 50 μg/larva was determined (Wolfenbarger et al 1971). This value was 45 fold greater than the LD_{50} determined for a reference strain of bollworm from Brownsville, Texas. In the late 1960s it was determined that this insecticide could be used with reliability against the bollworm in south Texas and northeastern Mexico (Wolfenbarger and McGarr 1970) and the early 1970s (Wolfenbarger et al 1973). In the early 1970s resistance was evident in most bollworm strains from Guatemala, Nicaragua and southwestern Mexico, but not in South America, El Salvador nor northern Tamaulipas (Wolfenbarger et al 1973). In 1980, bollworms from El Salvador were resistant to methyl parathion (Wolfenbarger et al. 1981).

Permethrin was the first 3-phenoxybenzyl pyrethroid tested in the laboratory and it was toxic to bollworm larvae (Davis et al 1975); a field test on cotton was established in 1974 but bollworms were not found in the plots. Resistance to permethrin had not been determined in strains of the bollworm from south Texas and Mexico prior to 1980 (Wolfenbarger et al 1981).

From 1981 to the mid-1990s LD_{50} values of both methyl parathion and permethrin were determined for bollworm strains collected from south Texas, Mexico and El Salvador; both insecticides were generally tested against each strain. If resistance by a strain from a new site was determined LD_{50}s from nearby sites in subsequent years could indicate the stability of resistance. Combining previously published LD_{50} values and LD_{50} values reported here, an LD_{50} value could be offered to establish a resistance thresholds for both these insecticides. If LD_{50} values exceeded the threshold for the insecticide in an area after being below the value for years resistance would be evident if it was present in subsequent years.

Materials and Methods

Insecticides

Technical grade methyl parathion and permethrin were obtained from Monsanto Company, St. Louis, MO, and FMC Corporation, Princeton, NJ, respectively.

Insect Collections

Eggs and larvae of the bollworm were collected at field-sites during the year. The town indicated was the closest to the field of corn, *Zea mays*, or cotton, *Gossypium hirsutum*. Immatures were collected from either host at sites in northern (Abasolo, Aldama, El Guayabo, Matamoros, San Fernando, San German and Rio Bravo) and southern Tamaulipas (Estacion Cuauhtemoc, Manuel and Tampico) , southwestern [Tapachula, Chiapas], western [Tepic, Nayarit] and eastern [Poza Rico and Veracruz, Veracruz and Merida, Yucatan] Mexico; San Salvador, El Salvador, Robstown and the Lower Rio Grande Valley (LRGV) of south Texas. Collections from each field-site were reared separately. The number of bollworm eggs and/or larvae per collection ranged from 10 to 100 in corn and 12 to 40 in cotton. Collection times ranged from one to four h for each collection site. Times varied depending of availability of populations.

Laboratory Rearing of Insects

All eggs and larvae from each collection were reared to pupae on artificial diet of Shaver and Raulston (1971) under temperature conditions of 27±2˚C, 60-80% RH, and 12:12

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(L:D), according to methods of Raulston and Lingren (1972) and procedures recommended in Anonymous (1970).

Pupae of each collection for each generation were sexed, separated and placed in individual containers until moths emerged. In each generation 12 males were placed with an equal number of females in 3.78 liter-capacity containers, covered with gauze to provide oviposition sites, fed sugar water and allowed to mate for their lifetime. If additional bollworm moths were available they were placed in groups of 24 in additional containers until all available moths were paired. These were brother-sister matings. Gauze covers were changed daily and held in paper cups until the eggs hatched. The newly hatched larvae were placed individually on diet in 30 ml plastic cups and reared to size for testing.

Topical Treatments

Insecticides were diluted in acetone to provide doses as serial reductions of 50% as follows: 100.0-0.047 μg methyl parathion/larva and 0.062-0.000059 μg permethrin/larva. Bollworm larvae were treated with 5 to 10 doses upon obtaining weight of 25 ± 5 mg. All larvae were treated within one to three generations following each collection. Some strains died as larvae or failed to develop to adults in one of the generations or before adequate numbers of larvae were treated with the insecticides.

Following treatment larvae were held at the same temperature and conditions used for rearing. Treatments were replicated 2 to 4 times on different days, 4-100 larvae per replicate. Most of the strains were treated with both insecticides in the first generation. Additional larvae were needed for treatment when one of the insecticides, did not kill with the greatest dose tested or when all the larvae died when treated with the lowest dose This required rearing to the next generation. Two collections with 5 and 15 larvae were not treated in a generation to insure enough survivors for the next generation. Mortalities of larvae exposed to methyl parathion were determined after 48 h; those for permethrin were determined after 72 h because permethrin required longer to obtain maximum mortality. Larvae that did not move when gently probed with a blunt instrument were classified as dead.

Statistical Analysis

The LD_{50} values and the 95% confidence intervals (C.I.) are expressed as μg/toxicant/larva and the slope (regression coefficient) were estimated by SAS probit analysis (SAS, 1988). In the 1980s, the standard error (S.E.) of the slope was determined from the variance value of the covariance matrix by square root from SAS output for mainframe program. In the 1990s the S.E. of slope was a direct output of SAS analysis. If the *t* at $P \geq 0.05$ was $\leq$ 1.96 for ratio of slope/S.E. the slope value was not significantly different from zero. The LD_{50} values whose C.I.'s did not overlap were considered to be significantly different.

Results and Discussion

Corn and cotton are planted in different environments when grown in tropical and subtropical areas; in the tropics both corn and cotton are not irrigated and depend on rain to plant during the rainy season from June to November. In the tropics crops are harvested from December through February during the dry season. Southern Tamaulipas, southwestern Mexico and countries in Central America are tropical areas of corn and cotton production. Cotton and corn in subtropical south Texas and northern Tamaulipas are both irrigated and not irrigated; crops are planted in February and March and harvested in August and October. Crops in tropical southern Tamaulipas are harvested 90 days prior to planting the same crops in subtropical northern Tamaulipas and the LRGV. Crops in northern Tamaulipas and LRGV are harvested from June through August while corn and cotton are planted in southern Tamaulipas. Temperature differences are minimal in tropical and subtropical areas during the time of production.

Methyl Parathion

Thirty four field collected strains of bollworm from south Texas and northern and southern Tamaulipas, eastern, western and southwestern Mexico and El Salvador were treated and LD_{50} values determined from 1981 to 1983, 1991 to 1993 and 1995. Thirty-three showed significant regression (Table 1). In 1991 one strain collected from cotton near Rio Bravo showed a non-significant regression; 170 insects were tested and slope±S.E. were 0.47±0.3l (Vargas-Camplis and Wolfenbarger 1992). At a high dose of 1.56, doses of 0.39 and 0.095 and the lowest rate of 0.0015 μg/larva, mortalities of 100% to 60% were determined and the strain was highly susceptible. Results describe a response for an adequate number of insects treated and because a comparison of toxicity was shown with permethrin which exhibited an LD_{50}. Strain was susceptible but did not survive the second generation so further treatments were not possible.

LD_{50} values were ranked from greatest to lowest for each year. LD_{50} values ranged from 0.1 to 346.7 μgs/larva, which represented a 3,467 fold difference. Sixty-three percent of LD_{50} values ranged from 0.21 to 0.6 μg/larva and 84% showed LD_{50} values <2μg/larva indicating that the vast majority of populations of bollworm in south Texas and Tamaulipas, Mexico were susceptible. Six percent showed LD_{50} values >100 μg/larva.

Slope values of 34 strains (which included significant and non-significant regressions) showed that 29% were flat (<1), 62% were intermediate (1 to 2) and 9% were steep (>2). The slope of the non-significant regression was equal to the slope of a significant regression for the resistant strain from Tapachula, Chiapas, Mexico in 1982.

For each strain the number of larvae treated with methyl parathion ranged from 88 to 753; for number of larvae treated and occurrences in parentheses <100 (2), 101 to 200 (12), 201 to 300 (9), 301 to 400 (2) and 401 to 753 (9). Results clearly show that the number of larvae of each strain varied and the results followed a normal distribution. The non-significant regression showed the same number of larvae (170) as shown for the range of 101 to 200.

In 1981 resistance was not indicated in populations from Mexico and south Texas (Table 1). LD_{50} values were <2.0 μg/larva which is less than the resistance threshold value.

In 1982 LD_{50s} of 31.21 and 9.07 μg/larva were determined from collections made in Tapachula and Tampico, Mexico, respectively. Both populations were classified as resistant and the LD_{50} value from Tampico was determined in the northernmost area of the tropics. The tropics are about 500 km south of LRGV. However, during the same year, LD_{50} values of a collection from Manuel, 75 km west of Tampico, as well as from four sites in south Texas, ranged from 0.24 to 0.58 μg/larva. All these populations were classified as susceptible. Resistance did not spread in 1983 or the early 1990s to any site in northern Tamaulipas or south Texas.

Bollworms were collected from fields in subtropical and tropical environments. Northern Tamaulipas is subtropical while southern Tamaulipas is tropical. The Tropic of Cancer divides the state of Tamaulipas. Collection sites in Poza Rica, Tampico, Manuel, Estacion Cuauhtemoc, Aldama, Veracruz and Merida are tropical and on or near the east coast of Mexico. Tepic and Tapachula are tropical and on or near the west and southwest coast of Mexico, respectively. San Salvador is in El Salvador, Central America.

In 1971, 1977, 1978 and 1979 LD_{50} values of 0.78, 45.0, 30.5, and 40.5 μg methyl parathion/larva, respectively, were determined from collections of eggs and larvae from cotton near Tapachula, Chiapas, Mexico (Wolfenbarger et al 1981). The collection in 1971 was susceptible, but those from 1977 to 1979 were resistant. In 1982 and 1983, LD_{50} values >7.5 μg/larva for methyl parathion were obtained for collections from Tapachula and from a field near San Salvador, El Salvador, Central America. These results confirm reports on stability (Wolfenbarger et al 1973, 1981) of resistance in populations from cotton in southwestern Mexico and Central America during the early 1970s and 1980.

In 1983, LD_{50} values of >2 and <4 μg/larva were determined from collections from corn at Santa Rosa and San Benito (17 km apart), respectively in the LRGV of Texas. In 1995, a strain collected from cotton near San Fernando, Tamaulipas, about 110 km south of Brownsville in LRGV and Matamoros in Tamaulipas, Mexico showed an LD_{50} within the above range. Brownsville and Matamoros are sister cities which are in different countries separated by the Rio Grande river. These results may show initiation of factor(s) of resistance to methyl parathion in south Texas but only additional LD_{50} values are needed to confirm or reject this hypothesis. In addition, the slope values for the two collections from the LRGV were equal to those from Tapachula and San Salvador, indicating that the response patterns of strains from these sites were the same.

Until resistance is shown to be widespread and stable in a given area methyl parathion can be used for control of bollworm populations. If 80% of strains showed LD_{50}s greater than the resistance threshold in an area, i.e. south Texas, then this population of insects is resistant to that insecticide. Since corn is the major host of this pest and most corn is not treated resistance will not be prevalent in south Texas or northern Tamaulipas. This is because selection pressure has not been applied to its favorite host. Strain from El Salvador and southwestern, Mexico were found in cotton while strain from southern Tamaulipas were collected from corn.

Methyl parathion is applied to both these hosts for control of this pest in these subtropical and tropical areas. More insecticide is applied to cotton because of the boll weevil than corn in these areas therefore the selection pressure is stronger.

A bollworm strain classed as resistant was collected from cotton in 1971 in Nicaragua and showed an LD_{50} of 54 μg methyl parathion/larva in the first larval generation, (Wolfenbarger et al 1973). These results are presented because they describe a response by a population that was resistant and then reverted to susceptibility even though a selection regime was conducted. Each larva of each single pair was treated for even greater levels of resistance for four generations, but the reverse response occurred (Wolfenbarger 1996). The strain was lost after the fourth generation because it reverted to susceptibility. In generation four 100 μg/larva killed 96% of the larvae while 88% were killed in generation 1. This result was determined for only this strain.

LD_{50} values of methyl parathion to larvae of the same laboratory reared reference strain were determined and all were <2 μg/larva. They were 0.25 and 1 μg/larva in 1966-1968 (Wolfenbarger and McGarr 1970), 0.35 μg/larva in 1970 (Wolfenbarger 1972), and 0.52 μg/larva in 1980 (Wolfenbarger et al 1981). From pooled treatings in 1989-1990 and 1993 it was 0.12 μg/larva (Wolfenbarger et al 1998).

Permethrin

LD_{50} values were estimated for 29 field collected strains of bollworm from 1981 to 1983 and 1990 and 1991; 28 showed significant regressions (Table 1). One regression for a collection from corn near Manuel, Tamaulipas, Mexico in

1982 was not significant. Regression was determined for 77 insects and the slope±S.E. was 1.27±1.15. A dose of 0.031 μg permethrin/larva (the greatest dose tested) killed 100% of the larvae and all other doses (0.00775 to 0.0019 μg/larvae) killed >50% with great variation. Strain was susceptible to permethrin. Strain was also treated with methyl parathion and LD_{50} value indicated susceptibility.

LD_{50} values ranged from 0.00034 to 0.043 μg/larva, a difference of 127 fold.

Seventy eight percent of the LD_{50} values ranged from 0.0011 to 0.02 μg/larva. LD_{50} values only indicate susceptibility.

Slope values of 29 strains show that 13% were flat (<1), 74% were intermediate (1 to 2) and 13% were steep (>2). Slope value of non-significant regression was classed as intermediate indicating a similarity to slopes of significant regression.

For each strain the number of larvae treated with permethrin ranged from 77 to 690. In number of larvae treated and occurrence in parenthesis were <100 (1), 101 to 200 (12), 201 to 300 (5), 301 to 400 (3) and 401 to 690 (7). Number of larvae from the various strains follow a normal distribution. A similar distribution was also shown for methyl parathion but this was confirmed and expected because insects were from the same collection.

The number of progeny produced from groups of reproducing moths caused the variation. As a guideline this author strives for numbers to treat which ranged from 100 to 200 individuals. However, significant regressions of mortalities at doses tested is the most important criteria for evaluating the variability of a study and this can sometimes be achieved with smaller sample sizes.

In 1982 and 1983 permethrin was as toxic to the strains from El Salvador and Tapachula, Mexico as it was to the strains from other locations in Mexico and south Texas. In 1990 and 1991 two strains of bollworm from south Texas and northern Tamaulipas were shown to be susceptible to permethrin.

The same reference strain of bollworm used in the methyl parathion test was used for the permethrin studies. LD_{50} values of permethrin for this strain were equal or less than LD_{50} values of permethrin from the field collected strains. In 1974 an LD_{50} of 0.028 μg permethrin/larva was determined (Davis et al 1975); in 1976 an LD_{50} of 0.021 μg/larva was determined (Davis et al 1977); in 1980 an LD_{50} of 0.0002 μg/larva was determined (Wolfenbarger et al 1981). From pooled bioassays in 1989, 1990 and 1993 an LD_{50} of 0.0058 μg/larva was determined (Wolfenbarger et al 1998).

These results and previous information on permethrin from the LRGV of Texas and Mexico as well as other areas of Mexico (Davis et al 1975), El Salvador (Wolfenbarger et al 1981) and [Vargas-Camplis and Wolfenbarger 1992) gave us an opportunity to propose a resistance threshold LD_{50} of >0.2 μg permethrin/larva. This threshold should be able to separate resistant from susceptible strains. It is an arbitrary value but I think it is a needed guideline.

In summary, no resistance by any strain of bollworm from south Texas nor northern Tamaulipas treated with methyl parathion to 1995 and permethrin to 1991 was shown. The single LD_{50} indicating resistance in 1982 to methyl parathion in southern Tamaulipas was not confirmed after other strains were treated from the area. Resistance has to be stable and this was not shown with these results.

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Table 1. Toxicity of two insecticides to larvae of bollworms collected in Central America, Mexico and South TX. 1981-1983, 1990-1991, 1993 and 1995.

Host	Number larvae tested	Slope ± SE	LD_{50} µg/larva	95% C.I.
Methyl parathion				
1981 Poza Rica, Veracruz				
corn	145	2.02±0.27	1.82	[0.74-2.91]
Tepic, Nayarit				
corn	175	1.27±0.094	0.58	[0.32-0.79]
Raymondville, TX				
corn	680	1.60±0.17	0.22	[0.17-0.29]
1982 Tapachula, Chiapas				
cotton	88	0.47±0.14	31.21	[21.56-48.68]
Tampico, Tamaulipas				
corn	135	1.23±0.62	9.07	[2.61-∞∞-∞∞]
Mercedes, TX				
corn	234	1.28±0.15	0.58	[0.4-0.75]
Brownsville, TX				
corn	221	1.53±0.14	0.54	[0.47-0.63]
Manuel, Tamaulipas				
corn	245	1.15±0.36	0.47	[0.19-0.63]
San Sebastian, TX				
corn	178	1.29±0.19	0.44	[0.35-0.54]
Monte Alto, TX				
corn	179	2.74±0.47	0.41	[0.34-0.51]
Brownsville, TX				
corn	450	1.44±0.16	0.38	[0.25-0.52]
Estacion Cuauhtemoc, Tamaulipas				
corn	175	1.93±0.23	0.26	[0.2-0.34]
1983 Tapachula, Chiapas				
cotton	286	0.6±0.16	346.7	[104.6-3,264.5]
San Salvador, El Salvador				
cotton	121	0.66±0.19	136.43	[32.71-10,649.16]
Santa Rosa, TX				
corn	173	0.58±0.18	3.65	[1.6-30.97]
San Benito, TX				
corn	222	0.64±0.17	2.32	[1.13-12.96]
Santa Maria, TX				
corn	615	1.26±0.11	0.93	[0.76-1.97]
Aldama, Tamaulipas				
corn	288	1.27±0.17	0.74	[0.56-1.0]
Matamoros, Tamaulipas				
corn	102	0.88±0.15	0.72	[0.41-1.54]
Vera Cruz, Vera Cruz				
corn	168	1.47±0.26	0.62	[0.45-0.83]
Brownsville, TX				
corn	753	1.57±0.14	0.52	[0.44-0.62]
Abasolo, Tamaulipas				
corn	321	0.69±0.17	0.46	[0.2-1.2]
San Sebastion, TX				
corn	178	1.29±0.19	0.44	[0.35-0.54]
El Guayabo, Tamaulipas				
corn	210	1.64±0.28	0.43	[0.26-0.75]
San German, Tamaulipas				
corn	412	1.36±0.14	0.42	[0.34-0.53]
Merida, Yucatan				
corn	179	1.68±0.41	0.41	[0.025-0.83]
Rio Bravo, Tamaulipas				
corn	500	1.84±0.36	0.4	[0.19-0.69]
Mercedes, TX				
corn	450	1.44±0.16	0.38	[0.26-0.53]
Brownsville, TX				
corn	453	1.56±0.4	0.25	[0.092-0.48]
Abasolo, Tamaulipas				
corn	492	0.97±0.17	0.24	[0.12-0.42]
Weslaco, TX				
corn	324	2.69±0.08	0.1	[0.057-0.23]
Estacion Cuauhtemoc, Tamaulipas				
corn	246	0.93±0.3	0.089	[4.43^{10-9}.-0.21]
San Fernando, Tamaulipas				
cotton	218	1.36±0.15	3.52	[2.28-5.14]
Permethrin				
1981 Tepic, Nayarit				
corn	569	1.26±0.34	0.0075	[0.0024-0.046]

		1982 Estacion Cuauhtemoc, Tamaulipas		
corn	176	1.54±0.95	0.043	[0.038-0.05]
		Monte Alto, TX		
corn	187	2.07±0.04	0.017	[0.014-0.022]
		San Sebastion, TX		
corn	570	1.49±0.69	0.016	[∞∞-∞∞]
		Tapachula, Chiapas		
cotton	251	1.11±0.32	0.011	[0.0093-0.014]
		Brownsville, TX		
corn	363	1.38±0.35	0.01	[0.0012-0.014]
		Mercedes, TX		
corn	450	1.23±0.44	0.0089	[0.0045-0.014]
		Robstown, TX		
corn	155	1.23±0.44	0.004	[0-0.013]
		1983 Tapachula, Chiapas		
cotton	634	1.43±0.20	0.015	[0.0087-0.025]
		Santa Rosa, TX		
corn	143	1.73±0.22	0.014	[0.011-0.019]
		San Salvador, El Salvador		
cotton	102	1.56±0.42	0.014	[0.0089-0.034]
		San Benito, TX		
corn	123	1.96±0.26	0.013	[0.011-0.018]
		Brownsville, TX		
corn	195	1.49±0.35	0.012	[0.0089-0.034]
		Veracruz, Veracruz		
corn	239	1.42±0.52	0.012	[∞∞-∞∞]
		Brownsville, TX		
corn	571	1.12±0.14	0.012	[0.0093-0.015]
		Santa Maria, TX		
corn	236	1.34±0.21	0.0089	[0.0047-0.017]
		Merida, Yucatan		
corn	259	1.48±0.41	0.0079	[0.0016-0.019]
		Rio Bravo, Tamaulipas		
corn	108	1.35±0.21	0.0073	[0.0041-0.013]
		Brownsville, TX		
corn	178	2.32±0.29	0.0059	[0.0046-0.0075]
		Aldama, Tamaulipas		
corn	347	2.72±0.25	0.0058	[0.0051-0.0067]
		San German, Tamaulipas		
corn	678	1.77±0.33	0.0036	[0.0019-0.0072]
		El Guayabo, Tamaulipas		
corn	300	2.51±0.71	0.0028	[0.0011-0.0075]
		Mercedes, TX		
corn	451	0.47±0.20	0.0023	[0.000046-1.29]
		Matamoros, Tamaulipas		
corn	690	0.92±0.20	0.0013	[0.00037-0.0029]
		Abasolo, Tamaulipas		
corn	185	0.57±0.20	0.0011	[0.000001-∞∞-∞∞]
		Abasolo, Tamaulipas		
corn	122	0.77±0.15	0.00034	[0.000084-0.00075]
		1990 - Weslaco, TX		
corn	305	1.19±0.07	0.0022	[0.00013-0.01]
		1991 - Rio Bravo, Tamaulipas		
cotton	177	1.72±0.62	0.012	[0.0099-0.047]