

# Response of Field-Collected Strains of Tobacco Budworm (Lepidoptera: Noctuidae) to Permethrin in the Lower Rio Grande Valley, TX, USA and Across Mexico

J. L. Martinez-Carrillo<sup>1</sup> and D. A. Wolfenbarger<sup>2</sup>

<sup>1</sup>CIRNO-INIFAP, Apartado Postal #515, Ote. Col. Campestre, Cd. Obregon, Sonora, Mexico 85760

<sup>2</sup>55 Calle Cenizo, Brownsville, TX 78520

## ABSTRACT

LD<sub>50</sub> values of permethrin for 32 strains of tobacco budworm, *Heliothis virescens* (F.) collected from cotton and tomatoes in Mexico, and the Lower Rio Grande Valley, TX, (LRGV) in the United States of America, (USA) from 1981 to 1982 and 1990 to 1996 ranged from 0.0088 to 0.9 µg/larva. A resistance threshold of >0.2 µg permethrin/larva was proposed. Most (72%) of the strains were susceptible to permethrin. The threshold resistance was exceeded from 1989 to 1991 in northwestern Mexico and in 1992 in north central Mexico. In Valle del Yaqui, Sonora, populations of strains exceeded the resistant threshold from 1989 to 1990 and then reverted to susceptibility from 1991 to 1996.

## RESUMEN

Los valores de DL50 de permetrina para 32 cepas del gusano de la yema del tabaco, *Heliothis virescens* ( F ), colectadas en algodón y en tomate en México y en el Bajo Valle del Río Grande (LRGV), Texas en Estados Unidos (E.U.) de 1981 a 1982 y de 1990 a 1996 variaron de 0.0088 a 0.9 µg/larva. Se propuso un umbral de resistencia de 0.2 µg de permetrina/larva. La mayoría de las cepas (72%) fueron susceptibles a la permetrina. El umbral de resistencia fue sobrepasado de 1989 a 1991 en el noroeste de México y en el 1992 en el área norte del centro de México. En el Valle del Yaqui, Sonora, las poblaciones de las cepas sobrepasaron el umbral de resistencia de 1989 a 1990 volviéndose de nuevo susceptibles de 1991 a 1996.

*Key Words: Insecticides, resistance*

When laboratory evaluations show that LD<sub>50</sub>s of an insecticide have increased after application in cotton fields over a period of time and continue to increase it is suspected that resistance to that insecticide has developed. Permethrin has been used on cotton by producers for control of the tobacco budworm, *Heliothis virescens* (F.) in the Lower Rio Grande Valley, Texas and Mexico since 1974 [Davis et al. 1975, Wolfenbarger et al. 1977 and 1984 and Wolfenbarger and Harding 1982]. No resistance to permethrin had been reported in northeastern Mexico or the LRGV, TX (Wolfenbarger et al. 1984). In 1987 the tobacco budworm showed resistance to this insecticide in northwestern Mexico [Martinez-Carrillo et al. 1991, Martinez-Carrillo 1991).

With published information on response of strains to permethrin and the results reported here we wanted to propose a resistance threshold for this pest on cotton in the LRGV of TX and northwestern, north central and northeastern Mexico. The resistance threshold is an arbitrarily selected LD<sub>50</sub> which separates resistant from susceptible populations. Using this threshold, resistance, susceptibility and reversion to susceptibility were determined for each strain. Reversion to

susceptibility to permethrin was elucidated from data shown here and the literature from four locations in Sonora and Baja California, Mexico.

## MATERIALS AND METHODS

Technical permethrin (93%) was obtained from FMC Corporation, Inc., Princeton, NJ.

**Insect collections:** Ten to 30 eggs and larvae of the test insects were collected from cotton in the LRGV of TX (USA) and northeastern, north central and northwestern Mexico from 1981 to 1998 [Table 1]. Insects were collected from a field nearest the indicated town in the north central and northeastern Mexico and the LRGV of TX and delivered to the laboratory at Brownsville or Weslaco, TX, USA. At each location in Caborca, Hermosilla, Guaymas and Valle del Yaqui, Sonora, and Mexicali, Baja California eggs or larvae were collected from three fields of cotton or tomato, *Lycopersicon esculentum* Mill. [Martinez-Carrillo 1991).

Eggs and larvae were reared to pupation on artificial diet [Shaver & Raulston 1971) at 27± 2° C, 60 to 80% rh and 12:12

h of light : dark [Raulston and Lingren 1972). As moths emerged, 5 to 15 pairs were placed in a 3.78 L cardboard container and fed 5% sugar-water. Each additional 15 pairs were placed in another container and handled similarly. Cloth covers that provided oviposition sites were changed daily and held in sealed 336 g paper cups until eggs hatched. Upon eclosion neonate larvae were placed singly on artificial diet in 30 ml cups with cardboard caps and held for testing.

When possible all strains were treated with permethrin within one generation. We either treated enough larvae in

generation one for an LD<sub>50</sub> value and the strain was discarded or enough larvae were treated in generation two to complete the LD<sub>50</sub> from both generations. Totals of larvae for each dose from both generations were used to determine the LD<sub>50</sub>.

**Topical treatments.** Permethrin was diluted and maintained in technical grade acetone. Three to 10 doses, as µg/larva, of 0.000059, 0.00048, 0.00096, 0.0076, 0.015, 0.031, 0.62, 0.12, 0.24, 0.48 0.96 and 1.92 permethrin were used to treat all available third stage larvae of each strain each day with procedures for the topical application technique (Anonymous

**Table 1.** Toxicity of permethrin to larvae of the tobacco budworm collected from cotton in Mexico and the Lower Rio Grande Valley, TX, 1981-1982, and 1989-1996.

Site of Collection	Number of Larvae Tested	Slope ± SE	LD <sub>50</sub> (µg/larva)	(95% C.I.)
<u>1981</u>				
Felipe Carrillo Puerto, Michoacan	100	1.61 ± 0.12	0.12	(0.98-0.13)
Torreon, Coahuila	207	1.72 ± 0.11	0.059	(0.015-0.068)
Brownsville, TX	245	0.9 ± 0.31	0.043	(0.026-0.068)
Pharr, TX	175	1.75 ± 0.17	0.038	(0.026-0.062)
Raymondville, TX	346	1.41 ± 0.2	0.037	(0.026-0.055)
Caborca, Sonora	545	1.27 ± 0.17	0.031	(0.019-0.049)
Guaymas, Sonora	191	0.93 ± 0.17	0.029	(0.016-0.055)
Mexicali, Baja California	102	1.54 ± 0.83	0.024	(0.021-0.027)
Hermosillo, Sonora	349	0.94 ± 0.17	0.017	(0.008-0.026)
Valle del Yaqui, Sonora (tomato)	668	1.22 ± 0.24	0.011	(0.007-0.015)
Valle del Yaqui, Sonora	342	1.77 ± 0.19	0.0088	(0.00039-0.016)
<u>1982</u>				
Estacion Cuauhtemoc, Tamaulipas	193	1.71 ± 0.4	0.13	(0.093-0.19)
<u>1989</u>				
Mexicali, Baja California	250	1.59 ± 0.23	0.47	(0.38-0.58)
Weslaco, TX	172	1.65 ± 0.8	0.18	(0.13-0.28)
<u>1990</u>				
Valle del Yaqui, Sonora (late season)	250	1.85 ± 0.19	0.56	(0.45-0.69)
Valle del Yaqui, Sonora (early season)	250	1.86 ± 0.22	0.48	(0.38-0.59)
<u>1991</u>				
Valle del Yaqui, Sonora (end season)	250	1.71 ± 0.22	0.44	(0.35-0.56)
Valle del Yaqui, Sonora (begin season)	250	2.36 ± 0.22	0.32	(0.27-0.38)
Rio Bravo, Tamaulipas	96	1.31 ± 0.69	0.011	(∞-∞)
<u>1992</u>				
Torreon, Coahuila	171	1.16 ± 0.16	0.9	(0.054-1.41)
La Blanca, TX (field 4)	117	1.27 ± 0.24	0.64	(0.38-1.45)
Valle del Yaqui, Sonora	250	1.95 ± 0.23	0.47	(0.38-0.5)
La Blanca, TX (field 2)	229	0.87 ± 0.13	0.14	(0.084-0.31)
La Blanca, TX (field 1)	320	0.99 ± 0.17	0.1	(0.064-0.21)
San Perlita, TX	98	1.22 ± 0.34	0.053	(0.011-0.11)
Rio Bravo, Tamaulipas	251	1.17 ± 0.13	0.039	(0.027-0.057)
Valle Hermosa, Tamaulipas	256	0.51 ± 0.18	0.03	(0.0021-0.38)
La Blanca, TX (field 3)	141	0.82 ± 0.16	0.013	(0.00049-0.026)
<u>1993</u>				
Valle del Yaqui, Sonora	250	1.62 ± 0.17	0.2	(0.16-0.26)
<u>1994</u>				
Valle del Yaqui, Sonora	250	1.65 ± 0.17	0.18	(0.14-0.22)
<u>1995</u>				
Valle del Yaqui, Sonora	250	1.62 ± 0.17	0.19	(0.15-0.24)
<u>1996</u>				
Valle del Yaqui, Sonora	250	1.59 ± 0.16	0.18	(0.14-0.23)

1970). When larvae on the diet were 3 to 7 d old and weighed  $22 \pm 6$  mg (from 16 to 28 mg) they were treated using a micro-applicator (ISCO, Inc., Lincoln, NE). First d of fourth stage weigh 30 to 32 mg. Each day of treating was considered to be a replicate and, depending on availability, 4 to 100 larvae/dose were treated in each replicate. Larvae of different strains grew at different rates so different numbers of larvae were treated in each replicate. Larvae were weighed daily within the range of weights indicated. Larvae that weighed  $<16$  mg were discarded after eight d. Mortalities were taken after 48 h. Larvae were considered dead when they did not respond when probed with a blunt rod.

LD<sub>50</sub> values, the 95% confidence interval (CI) as  $\mu\text{g}/\text{larva}$  and slope  $\pm$  standard error (SE) were determined by [SAS 1988]. Total number of larvae treated and total number killed by each dose in both generations were used in the statistical analysis. LD<sub>50</sub> values with overlapping CI values were not significantly different. If the "t" at  $P < 0.05$  for the ratio of slope/SE was  $<1.96$  the regression was not significant and did not differ from 0. Results were summarized by insecticide. LD<sub>50</sub> values were ranked from highest to lowest each year regardless of location.

The combination of the three strains from northwestern Mexico allowed a large supply of larvae for treating in the first generation. Control larvae to determine natural mortality were not needed nor used in these combined collections. Larvae of this species from field collections rarely die from natural causes. Control larvae were used for three of the single field collections in generation two from northeastern Mexico and the LRGV, USA because a few neonate to second stage larvae died from natural causes in generation one. When control larvae were not used  $0.0000059 \mu\text{g}/\text{larva}$  was used because it does not kill  $>1\%$ . The standard for maximum mortalities of untreated control larvae is 10%. Following treatment and mortality determinations, survivors were pooled and reared to pupation for generation two.

## RESULTS

LD<sub>50</sub> values (32) of permethrin with significant regressions ranged from 0.0088 to 0.9, a 102 fold difference (Table 1). The frequency distribution of LD<sub>50</sub> values of 0.0088 to 0.03, 0.031 to 0.099, 0.1 to 0.19 and  $>0.20 \mu\text{g}/\text{larva}$  were 25%, 22%, 25% and 28%, respectively. LD<sub>50</sub> values were equally distributed over the 100 fold range. Factors for response were equally distributed among the strains in both time and space.

Field control data was shown by Wolfenbarger and Harding (1982) using permethrin in field plots against the tobacco budworm from the LRGV. At  $0.11 \text{ kg(AI)}/\text{ha}$ , in 1977 and 1981, percentage control was 39% and 48% while in 1978 and 1979 percentage control was 70% and 87%, respectively. No trend for control or the failure to control populations was shown in the four years the field tests were conducted. LD<sub>50</sub>s were not determined from any collection from any of these plots. A review of all LD<sub>50</sub> values found in the literature for Mexico and the LRGV, TX and the results shown here suggests a proposed resistance threshold of  $>0.2 \mu\text{g}/\text{larva}$  for this insect.

Using this threshold 72% of the strains were susceptible.

The range of number of larvae tested and percentage of strains within the range were  $<100=9\%$ ,  $101-200=25\%$ ,  $201-300=47\%$ ,  $301-400=13\%$  and  $401-700 = 6\%$ . Percentages of larvae used for each strain treated followed a normal distribution.

Slopes of regressions showed 22%,  $<1$ , 75%  $>1.1$  to 2 and 3%,  $>2$ . The flatter slopes are considered to have more factors for resistance..

**Northwestern and North Central Mexico.** In 1981, LD<sub>50</sub> values of 11 collections ranged from 0.0088 (Valle de Yaqui, Sonora,) to  $0.12 \mu\text{g}/\text{larva}$  (Felipe Carrillo Puerto, Michoacan], a 15 fold difference. Five LD<sub>50</sub> values were determined for strains collected from Torreon, Caborca, Guaymas, and Mexicali, Mexico: all strains were susceptible.

LD<sub>50</sub> values exceeded the resistance threshold from 1987-1993 in Valle del Yaqui, 1987-1988 in Hermosilla, 1985-1988 in Caborca, and 1986-1989 in Mexicali (Martinez-Carrillo 1991). Six to nine years after resistance was determined, there was reversion to susceptibility to permethrin in Valle del Yaqui from 1994 to 1996 (Table 1) as there was in 1984-1985, 1985-1986 and 1985 in populations in Mexicali, Hermosillo and Caborca, respectively (Martinez-Carrillo 1991).

In Valle del Yaqui in 1990 and 1991 LD<sub>50</sub> values of permethrin determined at the beginning of the season (Table 1) and at the end of the season were not significantly different. All four populations were resistant. Populations were also resistant to permethrin in 1992 and 1993. Then there was a reversion to susceptibility by the populations sampled from 1994 through 1996.

In 1992 the LD<sub>50</sub> value of  $0.9 \mu\text{g}$  permethrin/larva from a collection from Torreon, Coahuila, was the greatest of the 32 values determined (Table 1). After a decade of use in 1991 and 1992 resistance was determined.

**Northeastern Mexico and LRGV, TX, USA.** In 1981 three strains from the LRGV, TX were susceptible to permethrin (Table 1). In 1982 a collection from Estacion Cuauhtemoc, Tamaulipas, was susceptible.

In 1991-1992 collections from LRGV in Rio Bravo, Tamaulipas, Mexico, were susceptible (Table 1). The collections from Estacion Cuauhtemoc in 1991 and 1994 in the tropical area of northeastern Mexico exhibited resistance to permethrin (Teran- Vargas 1994). In 1992 a strain from field 4, La Blanca, TX, LRGV, was resistant to permethrin. That same year three strains collected from three other fields near La Blanca were susceptible. There were more resistant strains to permethrin in the early 1990s than in the early 1980s in the LRGV of both TX and Mexico.

In 1987 and 1993 LD<sub>50</sub> values of 0.89 and  $3.83 \mu\text{g}$  permethrin/larva, respectively, were shown in Uvalde, TX (USA), in the Winter Garden area 400 km north of the LRGV [Sparks et al. 1988 and Wolfenbarger and Vargas-Camplis 1997]. Both strains were resistant to permethrin. These LD<sub>50</sub> values are in contrast to our results. If all our LD<sub>50</sub> values were similar to those in the Winter Garden area all of the strains would be resistant to permethrin.

Resistance to permethrin was more prevalent by the strains of this insect in the Winter Garden area, northwestern Mexico,

northeastern Mexico and the LRGV. An LD<sub>50</sub> of permethrin in north central Mexico exhibited resistance in 1992, but the LD<sub>50</sub> only showed susceptibility in 1981.

Natural mortalities of all larvae of the tobacco budworms in all collections prior to treatment are minimal for neonate through second stage larvae. None of the collections exceeded 10% in any of the collections. Control larvae were not used in the first generation of the single larvae collection from Rio Bravo, Tamaulipas in 1981 and San Perlita and La Blanca, LRGV in 1982 when five to 11 larvae in each collection did not grow to 16 mg. In generation two 15 to 28 larvae from each of the strains were used as controls. One to three percent of the larvae of each collection died. The low dose of 0.0000059 µg/larva killed 0 to 3% in each replicate for each strain. Untreated controls were not needed to estimate natural mortalities for these 32 field collections.

### DISCUSSION

The Tropic of Cancer divides the tropical cotton producing areas in southern Tamaulipas and western Mexico from the subtropical and temperate cotton producing areas in northern Mexico. Northern Tamaulipas includes cotton grown along and within 25 km of the Rio Grande River, the LRGV of Mexico. The subtropical producing areas are planted in February-March. The tropical areas are planted in June-July. Insect collections were made in all these areas. LD<sub>50</sub> values were not determined each year for each strain because they were not available.

In the 1990s >95% of the cotton in Mexico was planted in subtropical and temperate areas. In the 1990s no cotton was planted in western Mexico. Heterogeneity for response to permethrin is shown with these regressions. It was a natural occurrence in collections for all the locations identified here. Numerous mechanisms for resistance factors can be offered for this insect, but the variation in these factors from insect to insect has not been determined.

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