Response of Field-Collected Strains of Tobacco Budworm (Lepidoptera: Noctuidae) to Methyl Parathion in the Lower Rio Grande Valley, Texas, USA and Northeastern, North Central and Northwestern Mexico

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ABSTRACT

LD_{50}s of methyl parathion were determined for 40 populations of tobacco budworm, *Heliothis virescens* [F.], collected from cotton, *Gossypium hirsutum* L. and tomato, *Lycopersicum esculentum* Mill., in Mexico and the Lower Rio Grande Valley (LRGV), TX, United States of America [USA] from 1981 to 1983, 1989 to 1996 and 1998. LD_{50} values ranged from 0.11 to 32.97 µg/larva. Two other populations showed a non-significant regression for a total of 42 strains. A resistance threshold of LD_{50} >20.0 µg methyl parathion/larva was proposed. Reversion to susceptibility was determined from 1993 to 1998 in northwestern Mexico. Based on the threshold 95% of the populations were susceptible to methyl parathion.

RESUMEN

Se determinaron los valores de DL50 del paratión de metilo en 40 poblaciones del gusano de la yema del tabaco, *Heliothis virescens* [F.], colectado en algodón, *Gossypium hirsútum* L. y tomate, *Lycopersicum esculentum* Mill., en México y en el Bajo Valle del Río Grande, Texas, Estados Unidos [E.U.] de 1981 a 1983, 1989 a 1996 y 1998. Los valores de LD_{50} variaron de 0.11 a 32.97 µg/larva. Otras dos poblaciones mostraron una regresión no significativa para un total de 42 cepas. Se propuso un umbral de resistencia de DL_{50} >20.0 µg de paratión de metilo/larva. Se determinó la reversión de la susceptibilidad de 1993 a 1998 en el noroeste de Mexico. Con base en este umbral, el 95% de la población fue susceptible al paratión de metilo.

Key Words: Heliothine, insecticides, resistance

When an insecticide is applied over a period of time and no longer reduces the size of a target pest population it is suspected that resistance to that insecticide has developed. In the United States of America (USA) and Mexico methyl parathion has been used for control of the tobacco budworm, *Heliothis virescens* (F.), the bollworm, *Heliocoverpa zea* (Boddie) and the boll weevil, *Anthonomus grandis* Boheman on cotton, *Gossypium hirsutum* L. since the early 1950s.

Resistance to methyl parathion by the tobacco budworm on cotton was reported in the Mante-Tampico area of Tamaulipas, Mexico in the late 1960s and early 1970s [Adkisson 1969, Wolfenbarger and McGarr 1970, Wolfenbarger et al. 1973). From 1969 to 1972 *H. virescens* larvae from this area were deemed resistant because LD_{50} values ranging from 37.0 to 89.5 µg/larva were determined (Wolfenbarger et al. 1981). From 1969 and 1970 LD_{50} values of methyl parathion determined that strains of *H. virescens* collected from tobacco, *Nicotiana Tabacum* L., in Huejutla and San Andres Tuxtla, Veracruz, Mexico, were also resistant (Wolfenbarger et al.1981). In 1973 in southern Tamaulipas the highest LD_{50} value for H. virescens was 125.71 µg methyl parathion/larva (Wolfenbarger et al. 1984). All these populations were considered to be resistant.

LD_{50} values were determined from 1966 to 1980 in the LRGV of TX. In 1971, LD_{50} values ranged from 20 to 900 µg methyl parathion/larva for populations collected from cotton fields. Only 12% control of this pest was realized in cotton in 1971. Control increased from 1972 and then reverted to susceptibility from 1975 to 1980 (Wolfenbarger et al. 1984).

Resistance to methyl parathion was shown in Caborca and Hermosilla, Sonora, in northwestern Mexico, in 1987 and 1988 (Martinez-Carrillo et al. 1994). Populations from Valley del Yaqui, Sonora and Mexicali, Baja California were susceptible to methyl parathion from 1982 to 1992 (Martinez-Carrillo et al. 1994).

Methyl parathion has been used against the tobacco budworm in the LRGV of TX and cotton producing areas of Mexico for the past 40 years. It is used today and is effective against this pest in most fields at recommended use rates. Resistance and susceptibility to this insecticide have been determined in LRGV of TX and Mexico over time and space. With published information utilizing the topical application

Table 1. Toxicity of methyl parathion to larvae of the tobacco budworm collected from cotton in Mexico and the Lower Rio Grande Valley, Texas, 1981-1983, 1991-1996 and 1998.

Site of Collection	Number Larvae Tested	Slope ± SE	LD_{50} (µg/larva)	(95% C.I.)
1981				
Caborca, Sonora	659	0.5 ± 0.097	32.97	(10.97-366.17)
Raymondville, TX	1426	0.68 ± 0.059	20.70	(14.72-32.70)
San Perlita, TX	260	0.65 ± 0.13	19.73	(8.77-93.96)
Felipe Carrillo Puerto, Michoacan	260	1.04 ± 0.17	14.17	(8.39 - 31.05)
Weslaco, TX	756	0.96 ± 0.15	9.80	(5.92-22.10)
Torreon, Coahuila	200	1.51 ± 0.09	5.48	(4.80-6.32)
Valle del Yaqui, Sonora (tomato)	727	0.95 ± 0.15	4.94	(2.73-8.61)
Valle del Yaqui, Sonora	327	0.95 ± 0.15	4.86	(3.01-9.70)
Hermosillo, Sonora	446	0.94 ± 0.23	4.81	(2.15-30.20)
Pharr, TX	1564	1.10 ± 0.19	4.56	(2.56-10.61)
Guaymas, Sonora	648	1.10 ± 0.19	3.42	(2.12-7.50)
Brownsville, TX	474	0.98 ± 0.21	1.51	(0.73-2.93)
Mexicali, Baja California	547	1.71 ± 0.12	1.29	(1.10-1.15)
1982				
Monte Alto, TX	258	0.92 ± 0.19	3.41	(2.28-6.21)
Estacion Cauahtemoc, Tamaulipas	193	0.75 ± 0.12	1.90	(1.30-3.00)
1983				
La Feria, TX	566	0.99 ± 0.11	6.31	(4.72-8.13)
Mercedes, TX	139	1.33 ± 0.12	1.81	(1.40-2.40)
1989				
Weslaco, TX	314	0.59 ± 0.29	5.4	(∞-∞)
1991				
Rio Bravo, Tamaulipas	94	1.95 ± 0.84	0.11	(∞-∞)
1992				
La Blanca, TX (field 2)	237	0.7 ± 0.12	5.45	(3.08-12.38)
Torreon, Coahuila	151	0.84 ± 0.20	3.10	(1.61-15.89)
Rio Bravo, Tamaulipas (2nd collection)	220	1.03 ± 0.15	2.55	(1.69-4.21)
La Blanca, TX (field 1)	166	0.5 ± 0.22	2.26	(0.23-8.73)
Valle Hermoso, Tamaulipas	286	1.82 ± 0.47	2.02	(1.24-5.52)
Brownsville, TX	156	0.96 ± 0.21	1.71	(0.68-5.33)
Rio Bravo, Tamaulipas (1st collection)	234	0.83 ± 0.18	1.07	(0.29-3.17)
Rio Bravo, Tamaulipas (3rd collection)	214	2.34 ± 0.28	0.92	(0.74-1.18)
La Blanca, TX (field 3)	195	0.5 ± 0.17	0.80	(0.02-1.68)
Estacion Cuauhtemoc, Tamaulipas	270	1.51 ± 0.37	0.64	(0.21-1.68)
La Blanca, TX (field 4)	84	0.95 ± 0.24	0.36	(0.095-0.74)
1993				
Matamoros, Tamaulipas	458	0.58 ± 0.81	12.1	(6.53-21.20)
Valle del Yaqui, Sonora	250	1.53 ± 0.16	3.26	(2.54-4.17)
Rio Bravo, Tamaulipas	104	1.31 ± 0.29	1.26	(0.49-4.15)
1994				
Valle del Yaqui, Sonora	250	1.45 ± 0.16	3.49	(2.7-4.48)
1995				
Matamoros, Tamaulipas	372	0.94 ± 0.11	5.73	(3.97-9.12)
Valle del Yaqui, Sonora	250	1.52 ± 0.17	4.44	(3.48-5.80)
Rio Bravo, Tamaulipas	303	1.46 ± 0.15	2.17	(1.63-2.95)
1996				
Valle del Yaqui, Sonora	250	1.51 ± 0.16	4.10	(3.19-5.28)
1998 Valle del Yaqui, Sonora (late season)	250	2.19 ± 0.23	3.00	(2.50-3.60)
Valle del Yaqui, Sonora (early season)	250	1.65 ± 0.18	1.16	(0.88-1.47)

bioassay and the results reported here a resistance threshold is proposed for this insecticide against strains of this pest. Response was evaluated against this pest in north central Mexico and western Mexico for the first time. By using a resistance threshold, resistance and susceptibility and reversion to susceptibility for each strain was determined. Reversion to susceptibility was elucidated so producers would understand that methyl parathion could be considered for use in their cotton insect control program.

MATERIALS AND METHODS

Technical methyl parathion (98% AI) was obtained from Monsanto, Inc., St. Louis, MO. The insecticide was diluted and maintained in technical grade acetone.

Insect collections. Ten to 30 eggs and larvae of the test insects, identified as populations, were collected once from a cotton field at each location (the nearest town) in the LRGV, TX, and western, north central and northeastern Mexico from 1981 to 1998. Insects were delivered to the laboratory in Brownsville or Weslaco, TX. Most (72%) of the 42 collections tested here were separated into halves and treated with permethrin (Martinez-Carrillo and Wolfenbarger 2004).

In northeastern Mexico eggs or larvae were collected by the senior author from fields of cotton or tomato, *Lycopersicum esculentum* Mill., near Valle del Yaqui and from cotton in Caborca, Hermosilla, Guaymas, Sonora and Mexicali, Baja California, Mexico (Martinez-Carrillo, et al. 1994). They were delivered to the laboratory in Valle de Yaqui, near Cd. Obregon, Sonora.

All larvae from the Brownsville, Weslaco and Valle de Yaqui laboratories were reared to pupation on artificial diet [Shaver & Raulston 1971) at 27 ± 2º C, 60 to 80% rh and 12:12 h of light : dark (Raulston and Lingren 1972) with procedures recommended in Anonymous (1970). As moths emerged, a maximum of 15 pairs were placed in a 3.78 L cardboard container to and fed 5% sugar in 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.

Topical treatments. Three to 10 doses of methyl parathion were used to treat all available larvae of each strain at Brownsville, Weslaco and Valle de Yaqui, on diet each d to obtain these LD_{50}s. Doses were 0.012, 0.0975, 195, 0.39, 0.78, 1.56, 3.125, 6.25, 12.5, 25, 50 and 100 µg methyl parathion in one microliter/larva. Applications, using a micro-applicator (ISCO, Inc., Lincoln, NE), when larvae were 4 to 7 d old and weighed 22 ± 6 (16 to 28) mg. All available larvae of each collection were treated in the third stage each generation on different days. Each day of treating was considered to be a replicate and, depending on availability, 4 to 100 larvae/dose/replicate were treated. Larvae of different strains grew at different rates. For two strains 4 larvae/dose were treated in one replicate because the rate of growth by that strain was slow. Shown is the total number of larvae treated in all replicates and generations. Mortalities were taken after 48 h. Larvae were considered dead when they did not respond when probed with a blunt rod. LD_{50} values and the 95% confidence interval (CI) as µg/larva and slope ± standard error (SE) were determined (SAS 1988). Total number of larvae treated and total number killed by each dose were used in the statistical analysis. LD_{50} 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. Non-significant regressions were considered to be a response to methyl parathion. Only three doses were tested against the strains which showed the non-significant regressions because few larvae were available to treat. LD_{50} values were ranked from highest to lowest each y.

The standard for maximum mortalities of treated controls is 10%. Mortalities of larvae, treated with 0.012 µg/larva, were determined in generation one for certain collections from locations in northeastern, northwestern, north central or western Mexico and the LRGV, TX. Following treatment and mortality determinations in generation one survivors were pooled (including larvae treated with 0.012 µg/larva) and reared to pupation for generation two. If a significant dose-mortality curve was determined in generation one the strain was discarded. All strains were discarded after two generations. The regression was determined from dead and total larvae after one or two generations.

RESULTS AND DISCUSSION

The Tropic of Cancer divides the subtropical and tropical cotton producing areas of Mexico. Subtropical Tamaulipas includes cotton grown along and within 25 km of the Rio Grande River, the LRGV of Mexico. The sub-tropical areas are planted in February-March; the tropical areas are planted in June-July. In the 1990s >95% of the cotton in Mexico was planted in subtropical areas.

All locations. Forty LD_{50} values with significant regressions ranged from 0.11 to 32.97, a 291 fold difference (Table 1). Two regressions were not significant, so the regression did not differ from 0. In 1992 a non-significant regression was shown by a strain from San Perlita, TX, in the LRGV. The strain was treated with 6.25, 1.56 and 0.39 µg/larva. Mortalities ranged from 25%-37%. The number of larvae and slope ± SE were 77 and 0.58 ± 0.3, respectively. In 1993 a strain from San Fernando, northeastern Tamaulipas, was treated with the same three doses. Mortalities ranged from 13% - 23%. Number of larvae and slope ± SE were 72 and 0.63 ± 0.57, respectively. The San Perlita strain may have been susceptible while the San Fernando strain may have been resistant. Both strains were discarded following generation one.

A frequency distribution of 40 LD_{50} values ranged from 0.11 to 1.0 (13%), 1.1 to 2 (23%), 2.1 to 4 (28%), 4.1 to 5 (10%), 5.1 to 10.0 (15%), 10.1 to 20 (8%) and >20.0 (5%) µg/larva. Distribution approximated a normal curve. Results reflect the response pattern of a series of LD_{50} values over years and locations which justify the number of strains sampled.

In 1971 LD_{50} values of 20 to 900 µg methyl parathion/larva showed poor control in field plots (Wolfenbarger et al. 1984). No other similar relationship has been shown for this insect.

These results led us to accept the LD_{50} value of 20 µg/larva as the resistance threshold because it demonstrated failure to control this insect. Two LD_{50} values were >20 µg/larva (Table 1). Using the threshold, 95% of the strains were susceptible.

For the 42 strains the number of larvae treated with methyl parathion and percentage in each range was <100 (10%,), 101-200 (19%), 201-300 (36%), 301-400 (10%), 401-500 (7%) and 501-600, 601-700, 701-800 and >800 were 5% each. There was an >8 fold difference in number of larvae collected over years and locations.

Slopes of 42 significant and non-significant regressions were <1 (55%), 1.1 to 2 (40%) and >2 (5%). Greater than 50% of the curves were flat (<1). Several factors for response to methyl parathion were present in these strains. Each of the factors was considered to cause different responses which reflect on the flat regressions.

Northwestern, Western and North-Central Mexico. In 1981 six LD_{50} values of methyl parathion against strains collected from cotton in northwestern Mexico were shown (Table 1). The strain from Caborca, Sonora, was resistant. Strains from Valle del Yaqui, Hermosillo, and Guaymas, Sonora and Mexicali, Baja California and Felipe Carrillo Puerto, Michoacan, Mexico were susceptible. Felipe Carrillo Puerto is the southernmost area in Mexico for this pest species on cotton. The strain from Torreon was susceptible to methyl parathion. This is the first information on response to this insecticide in Felipe Carrillo Puerto and Torreon.

In northwestern Mexico, resistance to methyl parathion was first shown in Caborca in 1981 and again in 1988 (Martinez-Carrillo et al. 1994). In Hermosilla and Valle del Yaqui resistance was shown in 1986 and 1988 (Martinez-Carrillo 1994).

Susceptibility to methyl parathion was shown by strains from Hermosillo in 1981 (Table 1), 1984 to 1986 and Caborca from 1985 to 1987 (Martinez-Carrillo 1994). In 1992 the strain from Torreon was susceptible. Susceptibility was shown in Valle del Yaqui in 1982 and 1984-1992 (Martinez-Carrillo 1994) and again in 1993-1998; LD_{50} values ranged from 1.16 to 19.94 µg/larva. In 1998 the LD_{50} value determined at the end of the season in Valle del Yaqui was significantly greater than the one at the beginning of the season, but both were susceptible.

LRGV, TX, USA, and Northeastern Mexico. In 1981 an LD_{50} of >20 µg methyl parathion/larva was shown by a strain collected near Raymondville, TX, LRGV, signifying resistance (Table 1). LD_{50} values of four other strains from the LRGV were susceptible to the insecticide. LD_{50} values of 20 strains determined in 1982-1983, 1991-1996 and 1998 ranged from 1.16 to 12.1 µg methyl parathion/larva only indicate variation in susceptibility.

In 1991 and 1994 there was no significant difference in LD_{50} values from northern Tamaulipas [Rio Bravo] (Table 1) and LD_{50} values in Estacion Cuauhtemoc, southern Tamaulipas (Teran-Vargas 1996). All strains were susceptible. Collections from these subtropical and tropical areas were 500 km apart.

In 7% of the 45 strains tested multiple resistance to methyl parathion and permethrin was evident among the strains at Mexicali, Baja California and Caborca, Sonora in 1988 and Hermosillo in 1987-1988 (Martinez-Carrillo 1991, Martinez-Carrillo et al. 1991 and Martinez-Carrillo 1994). Multiple resistance is exhibited when there is resistance to insecticides representing two or more classes (i.e. pyrethroid and a organophosphorus insecticide). In time (from 1981 to the 1990s) and space (northwestern, north central and northeastern Mexico and LRGV, TX, USA) 93% of the strains treated with methyl parathion and permethrin were susceptible.

It was clear that strains of the tobacco budworm reverted to susceptibility to methyl parathion in northwestern Mexico and northeastern Mexico in the mid to late 1990s. Populations were resistant to methyl parathion in the 1970s in southern Tamaulipas, but none were resistant in the 1980s and 1990s (Wolfenbarger et al. 1984). The literature showed there was resistance for 8 y in northwestern Mexico. Resistance to methyl parathion was more prevalent in the strains from northwestern Mexico than from northeastern Mexico and the LRGV.

Mortalities of controls for strains were determined for both methyl parathion and permethrin. The dose of 0.012 µg methyl parathion/larva killed 0% to 6% of larvae from San Perlita, Pharr and Brownsville in 1981, La Feria and Mercedes in 1982, Rio Bravo, Brownsville and Matamoros (1993) and Matamoros (1995).

In conclusion, most of the strains were susceptible. The choice of 20 µg /larva as a resistance threshold for methyl parathion was easy to elucidate. The 42 strains provided data for establishing this resistance threshold.

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