# RESPONSE OF BOLL WEEVIL (COLEOPTERA: CURCULIONIDAE) TO MALATHION AND METHYL PARATHION IN SOUTHERN TAMAULIPAS

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## ABSTRACT

From 1994 to 1996 and in 1999, $LD_{50}$ values of technical malathion and methyl parathion were determined for boll weevil, *Anthonomus grandis* Boheman, populations collected near Estacion Cuauhtemoc, in southern Tamaulipas, Mexico. In 1998 an $LD_{50}$ for malathion was determined from populations of boll weevils collected near Ebano, San Luis Potosi, Mexico. Both locations are in a tropical cotton production area. In 1995, the $LD_{50}$ for malathion was 10.69 $\mu$g/adult. This is the greatest value ever reported for malathion and indicates that resistance was present that year. In 1996 and 1998, $LD_{50}$'s for weevils were <0.95 $\mu$g/adult which suggested that the populations had reverted to susceptibility. In 1999 an $LD_{50}$ of 1.44 $\mu$g/adult was determined. Aqueous foliar sprays of an emulsifiable concentrate formulation of malathion were not toxic (<30% mortality) to boll weevils on plants in cages from 1993 to 1996 and 1999. Cross resistance of insecticides was not consistently determined by either bioassay. In 1998, the $LD_{50}$ of malathion following a single dose of butifos, a synergist, was 17-fold less than the $LD_{50}$ of malathion alone. The $LD_{50}$'s of methyl parathion indicated susceptibility to boll weevil populations from 1994 to 1996 and 1999; $LD_{50}$'s were <0.067 $\mu$g/weevil. Foliar sprays of methyl parathion were toxic to boll weevils in cages (61% to 100% mortality) in 1993, 1994, 1996 and 1999. In 1995 mortalities of both insecticides were <13%. Resistance to both insecticides was indicated in 1995 when foliar sprays were applied.

## INTRODUCTION

Malathion and methyl parathion are registered for use on cotton in Mexico. Methyl parathion is the most widely used insecticide against the boll weevil in the tropical area of southern Tamaulipas, Mexico, near the Gulf of Mexico. Malathion is not used against the boll weevil in Mexico. Resistance has not been reported for malathion against this insect from any area in the United States of America (USA). ULV malathion [>95%] is the insecticide of choice in the eradication program in the USA. There is concern that resistance could appear in populations of boll weevil in the USA from populations which might be resistant and disperse from Mexico. If a resistant population was found it is unknown why this would be in Mexico without any selection pressure from malathion.

$LD_{50}$'s of malathion to boll weevils were determined from 1991 to 1993 in Estacion Cuauhtemoc in southern Tamaulipas (Teran-V. and Vargas-C. 1995). $LD_{50}$'s of methyl parathion indicated susceptibility of boll weevils collected in southern Tamaulipas from 1978 to 1983 by Wolfenbarger et al. (1986) and again from 1991-1993 by Teran-V. and Vargas-C. (1995).

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From 1994 to 1996 and in 1999, toxicity of malathion and methyl parathion was determined for the same boll weevil populations from Estacion Cuauhtemoc. Toxicity of malathion was determined in 1998 against boll weevil populations from Ebano, San Luis Potosi, Mexico. From 1993 to 1996 and in 1999, mortalities of malathion and methyl parathion to field-collected boll weevils in field-cage tests were determined at Estacion Cuauhtemoc. In 1998 toxicity of malathion with two synergists was also evaluated to determine if one might prevent resistance factors.

## MATERIALS AND METHODS

Technical forms of both insecticides were obtained from Cheminova, Inc., Lemvig, Denmark. Technical piperonyl butoxide (PBO) was obtained from DOW-Elanco, Inc., Indianapolis, IN and technical butifos from Bayer, Inc., Kansas City, KS. Both insecticides and synergists were diluted in acetone and delivered to the dorsum of the thorax in 1 $\mu$l using a ISCO, Inc., Lincoln, NE. micro-applicator. Doses of malathion tested were 10, 1, 0.32, 0.1, 0.046, 0.021 and 0.01 $\mu$g/adult. Doses of methyl parathion tested were 1, 0.5, 0.25, 0.125, 0.0625, 0.031 and 0.0155 $\mu$g/weevil.

To test synergism, 1 $\mu$g of butifos or PBO/weevil was applied to 160 weevils. After 15 minutes 10, 1, 0.1, or 0.01 $\mu$g malathion/weevil was applied to 10 to 15 weevils. The test consisted of three replicates and an untreated check. When synergist and malathion were applied, $LD_{50}$'s were determined for malathion doses only.

From 1994 to 1996 and 1998-1999 1 ha of untreated cotton /year was sampled for bolls infested by the boll weevil at Estacion Cuauhtemoc and Ebano. Estacion Cuauhtemoc is about 60 km northeast of Ebano. In Estacion Cuauhtemoc fields were within 3 km of each other. Cotton is planted in this tropical area in July and harvested from December to February. In October or November 1000 to 2000 small or medium green bolls infested with immatures of boll weevils were collected. Weevils which emerged were fed non-infested green bolls until treated 3 to 5 days post-emergence.

For each of 3 to 10 replicates, topical applications of 10 to 40 weevils were used to treat with each dose. Each day of treating was a replicate. All available boll weevils were used in the test. Mortalities for both insecticides were taken 48 h post-treatment. Each year the same number of untreated boll weevils used for a dose of either insecticide were used to correct for natural mortality. Weevils that did not move upon probing were considered to be dead.

Data were analyzed by the probit regression of Polo PC (1987) according to Teran-V. and Vargas-C. (1995). $LD_{50}$'s and 95% confidence interval were shown as $\mu$g/adult. Slope and standard error (S.E.) of slope were also determined. $LD_{50}$'s were equal when confidence intervals overlapped. If the "t" at the 5% level of probability for the ratio of slope/SE was $<1.96$, the slope was not significantly different from 0.

From 1993 to 1996 and in 1999, field-cage tests were conducted. For each insecticide cages were placed over plots 6 or 8 rows wide x 10 m long in either September or October. Three replicates of 1 cage/replicate were used in 1993, four replicates were used in 1994, 1995 and 1999 and five were used in 1996. In 1993, 8 or 10 weevils/replicate were tested for each insecticide; in 1994, 18 to 20 weevils/replicate were tested; in 1995, 1996 and in 1999, 25 weevils/replicate were tested. Boll weevils used for the cage test bioassay were from untreated cotton and allowed to age 3 to 5 d prior to the bioassay.

The boll weevils were placed inside a cylindrical (20 cm diameter x 40 cm tall) wire cage encircled with 2 mm mesh plastic cloth. Cages with an open top were put on the top of the plant and immediately sprayed with a $CO^2$ powered sprayer at 155 kg/cm$^2$

through a single nozzle/row. One hundred and fifty L/ha aqueous solutions of emulsifiable concentrate formulations of either malathion (1000 g/L) at 2 kg (AI) /ha or methyl parathion (720 g/L) at 0.72 kg (AI)/ha were applied. Immediately following the spray the open top of each cage was covered with 2 mm mesh plastic cloth and sealed. Untreated check cages with untreated boll weevils were established in the untreated cotton area at the same time the boll weevils were sprayed.

Mortalities were taken 72 h posttreatment. The same number of untreated weevils used for the spray treatments were used to determine natural mortalities. Mortalities were corrected using Abbott's formula [Abbott 1925]. Weevils that did not move legs or proboscis when probed with a blunt rod were considered dead. Analysis of variance was determined for percentage mortality of insecticides each year. Mean mortality of insecticides was separated by least significant difference [$P \leq 0.05$ (SAS Institute 1988)].

## RESULTS AND DISCUSSION

The $LD_{50}$ of 10.69 $\mu$g malathion/adult in 1995 indicated resistance by the boll weevil in southern Tamaulipas (Table 1). This is the first report of resistance by the boll weevil to malathion. Resistance was evident without selection pressure by malathion. From 1991 to 1993, $LD_{50}$'s of 1.75 to 4.38 $\mu$g malathion/adult were determined (Teran-V. and Vargas-C. 1995). $LD_{50}$'s determined in 1991 and 1992 (Teran-V. and Vargas-C. 1995) were equal to those determined in 1994, 1996 and 1999 (Table 1). All these values for malathion were determined in southern Tamaulipas, Mexico, and were significantly greater than any determined from northwestern Mexico (Pacheco-C 1994) where $LD_{50}$'s for malathion ranged from 0.036 to 0.18 $\mu$g/adult from 1991 to 1993.

Slope values of regression for malathion determined in 1994 to 1996 and 1998 to 1999 ranged from 1.5 to 2.42. Values indicate intermediate (1.0 to 2.0) to steep (>2.1) slopes. SE values showed no trends. Number of insects tested was adequate. Natural mortalities for methyl parathion and malathion in topical application tests were 0 in 1993 for both insecticides, 2% and 5% in 1994, 1% for both insecticides in 1995, 0 and 7% in 1996, 2% in 1998 and 0 and 1% in 1999, respectively.

Based on results from Pacheco-C (1994) and the results here, we propose that $LD_{50}$'s of <1 $\mu$g malathion/adult indicate susceptibility, $LD_{50}$'s of >1 to 10 $\mu$g/adult

TABLE 1. Toxicity of Malathion and Methyl Parathion to Boll Weevil from Estacion Cuauhtemoc, Tamaulipas, and Ebano, San Luis Potosi, Mexico. 1994 - 1998.

| Year | Number tested | Slope ± SE | $LD_{50}$ [$\mu$g/adult] | 95% Confidence Interval |
|---|---|---|---|---|
| Malathion | | | | |
| 1994 | 360 | 2.05 ± 0.44 | 1.45 | 0.87 - 2.03 |
| 1995 | 320 | 1.62 ± 0.19 | 10.69 | 6.76 - 14.95 |
| 1996 | 360 | 1.50 ± 0.14 | 0.94 | 0.73 - 1.24 |
| 1998 | 240 | 2.3 ± 0.63 | 0.93 | ∞-∞ |
| 1999 | 200 | 2.42 ± 0.51 | 1.44 | 0.48-2.27 |
| Methyl Parathion | | | | |
| 1994 | 280 | 2.00 ± 0.23 | 0.035 | 0.014 - 0.064 |
| 1995 | 360 | 1.67 ± 0.15 | 0.067 | 0.052 - 0.084 |
| 1996 | 240 | 2.70 ± 0.55 | 0.060 | 0.04-0.079 |
| 1999 | 320 | 1.86 ± 0.19 | 0.0060 | ∞-∞ |

indicate intermediate level of resistance and $LD_{50}$'s >10 $\mu$g/adult indicate resistance. With the exception of 1995, susceptibility and intermediate levels of resistance are evident in cotton at Estacion Cuauhtemoc from 1991 to 1993 (Teran-V. and Vargas-C 1995) and 1994 and 1999 (Table 1). Populations collected in 1996 and 1998 were susceptible to malathion. No explanation is offered for the reversion to susceptibility to malathion; but a similar case of boll weevil resistance to azinphosmethyl, followed by susceptibility, was reported by Wolfenbarger et al. 1998.

Response to malathion has not previously been determined in the Lower Rio Grande Valley in northern Tamaulipas, Mexico. In 1995, for 139 weevils, a slope ± SE of 1.8 ± 0.37, an $LD_{50}$ of 1.69 and an 95% CI as $\mu$g/adult of 1.01-2.59 was determined. Resistance was classified as intermediate. This $LD_{50}$ was equal to those determined for Estacion Cuauhtemoc in 1994, 1996 and 1999 and Ebano in 1998.

Only susceptibility was shown for methyl parathion from 1991 to 1993 (Teran-V. and Vargas-C. 1995) and from 1994, 1996 and 1999 (Table 1). $LD_{50}$'s were no greater than any shown by Wolfenbarger et al. (1986) from southern Tamaulipas a decade earlier nor any shown by Pacheco-C. et al. (1995) in northwestern Mexico. Slope values of regressions for methyl parathion ranged from 1.67 to 2.7 and were in the same range shown for malathion. Number of insects tested was adequate.

Aqueous sprays of malathion caused <30% mortality to field-collected boll weevils in cages from 1993 to 1996 and 1999 (Table 2). Mortality of weevils was only 5% in 1995.

In 1995, aqueous sprays of methyl parathion caused 12% mortality of boll weevils but there was a significant difference in mortality of the field collected boll weevil by malathion and methyl parathion (Table 2). Mortalities ranged from 61% to 100% the other three years of the bioassay. No explanation is offered for this year to year variation. Natural mortality of field collected boll weevils in untreated check in the cage tests from 1993 through 1996 and in 1999 were 12%, 11%, 13% and 23% and 10% for 58, 80, 100, 100 and 100 boll weevils, respectively.

TABLE 2. Percentage Mortality of Boll Weevil in Cages on Plants Sprayed with Malathion and Methyl Parathion from Estacion Cuauhtemoc, Tamaulipas, Mexico.[a]

| 1993 | 1994 | 1995 | 1996 | 1999 |
|---|---|---|---|---|
| | | Malathion | | |
| 9b | 30b | 5a | 26b | 26b |
| | | Methyl Parathion | | |
| 100a | 90a | 12a | 53a | 61a |

[a] Means followed by the same letter each year were not significantly different at 5% probability

In 1995 there was no cross resistance of malathion and methyl parathion by boll weevil when topically applied. Results suggest that resistance to malathion will not result in resistance to methyl parathion by this major pest in this tropical environment.

The synergist butifos and malathion had an $LD_{50}$ 17 fold less than that for malathion alone in 1998 (Table 3). This population was considered to be susceptible to malathion in 1998 (Table 1), but it was even more susceptible when treated with the synergist.

TABLE 3. Toxicity of Malathion with synergist Butifos to Boll Weevil from Ebano, San Luis Potosi, Mexico. 1998.

| Synergist | Number tested | Slope ± SE | $LD_{50}$ [$\mu$g/adult] | 95% Confidence Interval |
|---|---|---|---|---|
| Butifos | 160 | 0.44 ± 0.16 | 0.056 | 0.001-0.54 |

The synergist PBO and malathion had a non-significant regression of $16.63 \pm 2x10^6$ for 160 insects indicating that mortalities did not differ from 0. PBO and butifos alone did not cause >4% toxicity to boll weevils. The slope for butifos and malathion was flat while the slope for malathion was steep. Authors suggest that flatness of the slope was caused by interaction of butifos with esterase enzymes. Inhibition of esterases by butifos could partially or totally prevent resistance to malathion shown in 1995. If resistance to malathion becomes a concern, perhaps a mixture of malathion and butifos could be sprayed on cotton at the end of the season for both defoliation and enhanced toxicity of boll weevil.

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