EVALUATION OF THE SUSCEPTIBILITY OF THE BOLL WEEVIL (*Anthonomus grandis*, BOHEMAN) TO MALATHION IN THE LAGUNA REGION †

[EVALUACIÓN DE LA SUSCEPTIBILIDAD DEL PICUDO DEL ALGODONERO (*Anthonomus grandis,* BOHEMAN) AL MALATION EN LA REGIÓN LAGUNERA]

Christian Silva-Martínez[1], Pedro Cano-Ríos[1]*, Urbano Nava-Camberos[2], José Luis García-Hernández[3], José Luis Reyes-Carrillo[1], Francisco Javier Sánchez Ramos[1] and Saraí Shesareli Mendoza-Retana[3].

[1] Antonio Narro Agricultural Autonomous University - Laguna Campus (Universidad Autónoma Agraria Antonio Narro-Unidad Laguna) Periférico Raúl López Sánchez S/N, Colonia Valle Verde, CP. 27054.Torreón, Coahuila, México. E-mail: canorp49@hotmail.com
[2] School of Biological Sciences – Juarez University of the State of Durango (Facultad de Ciencias Biológicas-Universidad Juárez del Estado de Durango). Av. Universidad S/N, Fraccionamiento Filadelfia, CP. 35010. Gómez Palacio, Durango, México.
[3] School of Agriculture and Zootechnics - Juarez University of the State of Durango (Facultad de Agricultura y Zootecnia-Universidad Juárez del Estado de Durango). Carretera Gómez Palacio - Tlahualilo Km. 32. Ej. Venecia, Gómez Palacio, Durango. México.
**Corresponding author*

SUMMARY

Background. Resistance to an insecticide is what determines its effectiveness and the lack of information limits effectiveness in the control of the boll weevil in cotton crops. **Objective.** The objective of the study was to determine the susceptibility of the boll weevil (*Anthonomus grandis* Boheman) to malathion in the Laguna, Mexico. Currently, this pest is considered as the one with the highest economical impact on cotton cultivation in the region. **Methodology.** Three bioassays were carried out, one in the state of Coahuila in the 2017 agricultural season and in the 2018 and 2019 seasons in the states of Coahuila and Durango. Boll weevils reared in the laboratory, as well as those collected in the field were used to obtain DL50 and DL95. The mortality data was analyzed using the Probit analysis. **Results.** The mean lethal dose after 48 hours in the three agricultural cycles fluctuated between 0.19 and 0.39 µg/µl for adults raised in the laboratory and the data observed for adults collected in the field was between 0.37 and 0.44 µg/µl. **Implications.** Periodic bioassays are suggested to detect populations resistant to malathion. **Conclusion.** It was determined that the boll weevil populations in the Laguna Region were susceptible to malathion, since the mean lethal dosage for the 3 agricultural seasons was < 1 µg/µl.

Keywords: susceptibility; boll weevil; malathion; economic importance; bioassay; mean lethal dosage.

RESUMEN

Antecedentes. La resistencia a los insecticidas es considerada como la principal responsable de la efectividad de los mismos, la falta de información limita la efectividad en el control del picudo del algodonero. **Objetivo**. Evaluar la susceptibilidad del picudo del algodonero (*Anthonomus grandis* Boheman) al malation en la Comarca Lagunera, actualmente esta plaga se considera la de mayor importancia económica para el cultivo del algodón en la región. **Metodología.** Se realizaron tres bioensayos, en el ciclo agrícola 2017 se realizó únicamente en el estado de Coahuila y en los ciclos 2018 y 2019 en Coahuila y Durango, usando picudos emergidos en laboratorio y picudos colectados en campo, se obtuvo la DL50 y DL95, los datos de mortalidad se analizaron mediante análisis Probit. **Resultados.** La dosis letal media a las 48 horas en los tres ciclos agrícolas fluctuó de 0.19 a 0.39 µg/µl en adultos emergidos en laboratorio, los datos observados en adultos colectados en campo fueron de 0.37 a 0.44 µg/µl. **Implicaciones.** Se

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ORCIDS: C. Silva-Martínez - 0000-0002-2368-7218. P. Cano-Ríos - 0000-0003-4559-954X. J.L. Reyes-Carrillo - 0000-0001-6696-6981. F.J. Sánchez Ramos - 0000-0003-3372-3603. U. Nava-Camberos - 0000-0001-9192-6754. J.L. García-Hernández - 0000-0002-9190-8190. S.S. Mendoza-Retana - 0000-0002-9520-7332.

sugiere realizar bioensayos periódicamente para detectar poblaciones resistentes al malation. **Conclusión.** Se determinó que las poblaciones de picudo en la Región Lagunera son susceptibles al malation, ya que la dosis letal media de los 3 ciclos agrícolas es < 1 µg/µl.
Palabras clave: susceptibilidad; picudo del algodonero; malatión; importancia económica; bioensayo; dosis letal media.

INTRODUCTION

Cotton (*Gossypium sp*.) is a vegetal fiber of considerable importance as raw material in the clothing industry. The cotton variety that is cultivated and commercialized in Mexico is *Gossypium hirsutum*, which is native to México and Central America (Hunter and Hinds, 1905). The boll weevil *Anthonomus grandis* (Boheman) is one of the most impacting pests for cotton cultivation in Mexico. This insect is considered native to Mexico and Central America (Pacheco, 1985). *Anthonomus grandis* has a widespread distribution on the American continent due to the cultivation and marketing of cotton and to the large amount of wild host plants that it uses for its development (Showler, 2009).

The boll weevil goes through a complete metamorphosis; it goes through the biological state of egg, larva, pupa and adult, all of which develop in squares and bolls. The female will oviposit individually on the flower buds of the cotton plant, perforating with its jaws into the squares and bolls, inserts the egg and covers the hole with a secreted substance from its ancillary gland. The female is capable of ovipositing between 100 and 300 eggs during her lifetime (Cross, 1973; SENASICA-DGSV, 2016). The damage that has economic consequences is produced by the larva as it feeds on the squares and small bolls. The attacked squares open up and fall to the ground where the larvae may be eliminated by high temperatures, predation, parasitism or infection by entomopathogens. The infected bolls will not open and are susceptible to rotting (Loera-Gallardo *et al*., 2008).

The population of these pests may increase considerably when the climatic conditions are favorable (average temperature of 30 °C and relative humidity greater than 70 %), as well as when there is enough and adequate food. Cotton cultivation requires a long cycle, which allows the boll weevil enough time to reproduce and grow (Martínez *et al*., 2002). The adult boll weevil hibernates or diapauses –does not eat– during the winter and returns to the cotton plant at the beginning of spring of the following year. The optimal temperature for the development of the immature states of *A. grandis* fluctuates between 15°C to 35° C. Well-fed insects that reach their hibernating place have enough fat content to allow them to survive well and in high densities (Greenberg *et al*., 2005). The critical period when the boll weevil attacks the plant is between 40 to 90 days of the emergence of the plants, which is the period of fixation and development of the productive structures (Manessi, 1997).

The Laguna Region which is located in the north-central part of Mexico, within the limits of the states of Coahuila and Durango and it is formed by 15 municipalities, 10 of which belong to Durango: Gómez Palacio, Lerdo, Tlahualilo, Mapimí, Rodeo, Nazas, Simón Bolívar, San Juan de Guadalupe, San Luis del Cordero and San Pedro del Gallo; and 5 municipalities belonging to Coahuila: Torreón, San Pedro, Matamoros, Francisco I. Madero and Viesca. In the 1970's and 1980's, about 60 to 70 thousand hectares of cotton were grown, whereas from 2004 to date, cotton is cultivated in close to 20 thousand hectares, with an average yield of 5 tons of seed cotton per hectare, in the Laguna, Mexico. From 2011 to 2014 the population of boll weevil increased considerably between September and October in the Laguna Region, as it is stated in the records of the Local Board for Plant Health of the Laguna Region in the states of Coahuila and Durango (Palomo *et al*., 2014).

The boll weevil has a high migration capacity and is able to travel within the fruit or product (Lanterini *et al*., 2003). This alerted other cotton growing zones close to the Laguna Region, and in 2013 the Binational Program for Suppression / Eradication of Pink Bollworm and Boll Weevil was implemented. The main action was aerial spraying of malathion ULV, complemented with trapping in order to detect the plague at an early stage and cultural actions like setting dates for weeding and clearing the ground, fallow, and sowing (SAGARPA, 2020). The detection of changes in susceptibility of *A. grandis* to malathion requires constant analysis, mortality curves are estimated based on response to the lethal dose (LD). Values can be calculated and compared using the Probit model (Finney, 1971).

Pacheco-Covarrubias (1993), considers the resistance to insecticides as the main gauge of their effectiveness. Therefore, it is necessary to constantly be evaluating the response of the *A. grandis* population to the malathion pesticide, as a fundamental action in effective combat of this pest. Since the susceptibility of *A. grandis* to malathion was unknown for the Laguna Region, the main objective of this study was to determine the susceptibility of the boll weevil, *A. grandis,* to malathion in the Laguna Region.

MATERIALS AND METHODS

Location and management of the sampling sites

The collections of biological material for the bioassays performed in 2017 were collected only in the state of Coahuila in the municipalities of Francisco I. Madero and San Pedro and the collections carried out in the years 2018 and 2019 were collected from both states, in Coahuila from the municipalities of Francisco I. Madero and San Pedro and in Durango from the municipalities of Gómez Palacio and Tlahualilo.

Methodology and Evaluation of Susceptibility

Squares were collected from the previously selected cotton fields. The squares were then assorted at the laboratory, casting aside those that had more than one oviposit and those that were damaged by feeding, leaving only the squares with one oviposit and the clean squares for feeding. Boll weevils adults (field boll weevils) were also removed for later topical application immediately after its collection in the field. The squares that had only one oviposit were placed in the environmental chamber to be dried at an average temperature of 29°C and a 13:11 h (L:D) photoperiod for the duration of the study. After a period of 5 to 7 days, the dried out squares were inspected and pupae were collected into petri dishes with previously moistened vermiculite where they remained until becoming adults. The emerging adults (laboratory boll weevils) were individually placed in petri dishes and given a clean square for food and a moist cotton daily for three consecutive days. Afterwards, the topical application was carried out using the standardized method only on those individuals whose weight was between 10.0 and 16.0 mg (Entomological Society of America, 1968; Armstrong *et al*., 2006). Technical grade (99.5 0%, Chem Serve, West Chester, PA) Malathion was used diluted in acetone HPLC grade (99.9% HPLC grade, Acros, Fairlawn, NJ) applied on the backside using a Burkard micro applicator (Burkard Scientific, Uxbridge, Reino Unido) and a 29 caliber needle. The doses used in 2017 were 0, 0.125, 0.250, 0.500 and 1 µg of malathion / µl of acetone and in 2018 and 2019 were 0, 0.125, 0.250, 0.500, 1 and 2 µg of malathion / µl of acetone, with a total of 40 adults for each dose. Mortalities were registered at 24, 48 and 72 h after application of the treatment. After the first 24 hours, if the treated specimen was still alive, it was given a square for food and a wet cotton. The bioassays performed in 2017 were from collections taken in the months of September and October and only in the state of Coahuila. In 2018, collections were carried out in the months of September and October, and in 2019, the collections were done in September. The collections for both years were from the states of Durango y Coahuila.

Statistical Analysis

The effects of malathion on the mortality percentage of *A. grandis*, were analyzed using Probit regression (Finney, 1971) using the PROBIT procedure (SAS, 2002), to obtain values for DL50 and DL95 and its fiducial limits (95 %), for field and laboratory boll weevils.

RESULTS AND DISCUSSION

Terán-Vargas and Wolfenbarger (2001), they established that susceptibility is indicated when DL50 is less than 1 µg/µl. A range between 1-10 µg/µl, indicates an intermediate level of resistance and a mean lethal dose that exceeds 10 µg/µl indicates resistance. Seven localities studied did not exceed the 1 µg/µl range and were therefore considered as susceptible populations. However, in 2017 the mean lethal dose reached after 24 hours was 1.67 µg/µl for boll weevils collected on the field in the state of Coahuila (Table 1). Reiser *et al.* (1953) mention that as the growing season advances, the fat percent of the *A. grandis* constantly increases, consequently, it is able to generate greater resistance to pesticides. It was also observed that the surviving insects were considerably larger than those raised in the laboratory, Lagunes and Vazquez (1994) consider that the quantity and quality of the food could affect the size and survival capacity of the insect, considering that there is a difference in tolerance between newly fed individuals and those that have been without food for a certain time.

The data obtained for the mean lethal dose from the localities being studied for boll weevil emerged in a laboratory, after 48 h., varied from 0.199 up to 0.392 µg/µl. An increase in resistance of the *A. grandis* can clearly be observed for the specimens collected in Coahuila compared to those collected in Durango (Table 2). According to data obtained from the Local Board for Plant Health of the Laguna Region for Coahuila and Durango in 2017, the average applications were 4.5 and 5.3. In 2018, 4.5 and 8.5 were performed and in 2019 the average was 3.3 and 7.9 applications of Malathion ULV. It can be observed that although on average there was a higher number of applications in the state of Durango, the *A. grandis* populations are more susceptible in comparison to the ones in the state of Coahuila which are considered to have been less exposed to malathion. A similar case to the one shown by Bottrell *et al.* (1973) and Pruitt *et al.* (1978), where after having been treated with 10 to 17 applications through several years, *A. grandis* was not able to develop resistance to malathion.

Table 1. Susceptibility of adult boll weevils, *Anthonomus grandis*, collected directly from the field in different agricultural seasons in the Laguna Region, to the Malathion pesticide.

Agricultural seasons	n	Time exposed (h)	Slope ± SE	DL_{50} (FL 95%)	DL_{95} (FL 95%)	$\chi 2$	Pr > $\chi 2$
2017	200	24	1.58 ± 0.40	1.67 (1.00-6.38)	18.51 (5.30-717.29)	15.52	<0.0001
		48	2.25 ± 0.35	0.45 (0.36-0.58)	2.42 (1.50-5.74)	40.70	<0.0001
		72	2.65 ± 0.38	0.38 (0.31-0.46)	1.57 (1.09-2.92)	49.45	<0.0001
2018	240	24	1.74 ± 0.25	0.75 (0.58-1.01)	6.59 (3.67-18.09)	47.84	<0.0001
		48	2.31 ± 0.29	0.37 (0.30-0.46)	1.91 (1.35-3.29)	64.77	<0.0001
		72	2.15 ± 0.29	0.29 (0.23-0.37)	1.70 (1.18-3.06)	56.58	<0.0001
2019	240	24	1.85 ± 0.25	0.62 (0.49-0.81)	4.84 (2.90-11.33)	53.35	<0.0001
		48	1.97 ± 0.26	0.39 (0.30-0.64)	2.64 (1.72-5.30)	55.49	<0.0001
		72	2.21 ± 0.29	0.31 (0.24-0.38)	1.72 (1.19-3.08)	58.30	<0.0001

n = number of insects treated. Slope of the regression line ± the standard error (SE). DL50 = Mean Lethal Dose expressed in µg adult^{-1} and fiducial limits at 95 %. DL95 = Lethal Dose 95 expressed in µg adult^{-1} and fiducial limits at 95 %.

Table 2. Susceptibility of adult boll weevils, *Anthonomus grandis*, emerged in the laboratory from pupae extracted from infested squares collected from different agricultural seasons in the Laguna Region, to the Malathion pesticide.

Agricultural seasons	State	n	Time exposed (h)	Slope ± SE	DL_{50} (FL 95%)	DL_{95} (FL 95%)	$\chi 2$	Pr > $\chi 2$
2017	Coah.	200	24	2.00 ± 0.34	0.46 (0.36-0.62)	3.04 (1.73-9.00)	34.49	<0.0001
			48	2.63 ± 0.38	0.29 (0.23-0.35)	1.22 (0.86-2.17)	48.25	<0.0001
			72	2.75 ± 0.43	0.20 (0.16-0.24)	0.80 (0.46-1.38)	41.63	<0.0001
2018	Coah.	240	24	1.92 ± 0.26	0.70 (0.55-0.91)	5.02 (3.03-11.54)	55.00	<0.0001
			48	2.57 ± 0.30	0.39 (0.32-0.48)	1.71 (1.25-2.75)	71.23	<0.0001
			72	2.94 ± 0.35	0.32 (0.27-0.39)	1.18 (0.90-1.78)	70.97	<0.0001
	Dgo.	240	24	2.88 ± 0.37	0.27 (0.22-0.32)	0.99 (0.74-1.52)	62.04	<0.0001
			48	3.70 ± 0.52	0.21 (0.18-0.25)	0.60 (0.47-0.89)	50.84	<0.0001
			72	4.09 ± 0.61	0.20 (0.16-0.23)	0.49 (0.39-0.72)	44.30	<0.0001
2019	Coah.	240	24	2.12 ± 0.27	0.54 (0.44-0.68)	3.23 (2.13-6.23)	62.85	<0.0001
			48	2.68 ± 0.32	0.33 (0.27-0.40)	1.37 (1.01-2.15)	69.71	<0.0001
			72	3.04 ± 0.37	0.30 (0.25-0.36)	1.04 (0.80-1.57)	68.76	<0.0001
	Dgo.	240	24	2.64 ± 0.35	0.25 (0.20-0.30)	1.03 (0.76-1.66)	56.83	<0.0001
			48	3.65 ± 0.54	0.20 (0.17-0.24)	0.56 (0.44-0.85)	46.27	<0.0001
			72	3.61 ± 0.58	0.171 (0.14-0.20)	0.49 (0.38-0.76)	38.37	<0.0001

n = number of insects treated. Slope of the regression line ± the standard error (SE). DL50 = Mean Lethal Dose expressed in µg adult^{-1} and fiducial limits at 95 %. DL95 = Lethal Dose 95 expressed in µg adult^{-1} and fiducial limits at 95 %.

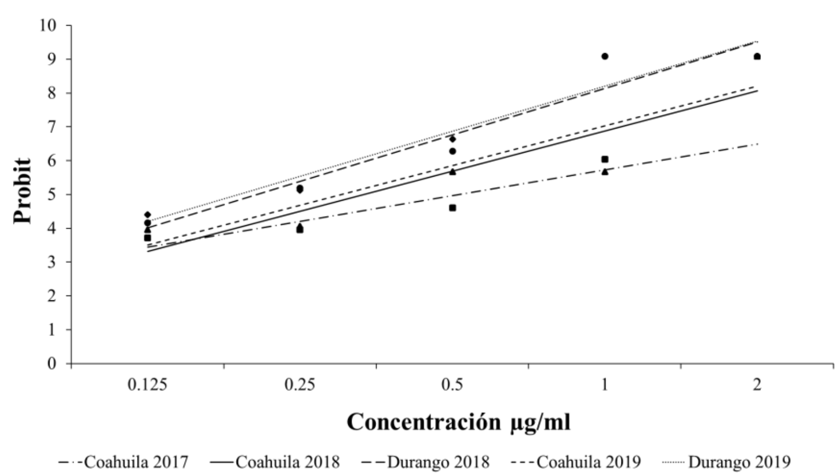

Figure 1. Average ratio of the *Anthonomus grandis* populations between the Probit mortality after 48 hours and the logarithm of the concentration of Malathion for adults emerged in the laboratory, obtained from the bioassays performed for the 2017, 2018 and 2019 agricultural seasons in the Laguna México.

The values determined for the slope of the regression varied from 1.5757 to 2.6469 for adults collected on the field and values from 1.9199 to 4.0888 were observed for adults emerged in the laboratory. Figure 1 shows that the slopes corresponding to the populations of the state of Coahuila present less inclination compared to the populations of the state of Durango, which indicates that the populations in Coahuila require a higher concentration of the active ingredient to obtain the same response in comparison to mortality with Durango.

In similar studies, Pacheco-Covarrubias (1994), in research done in the state of Sonora, reported mean lethal doses after 48 hours in a range from 0.0143 up to 0.6382 for the years from 1985 to 1993. Terán-Vargas and Wolfenbarger (2001), in the states of Tamaulipas and San Luis Potosí, in the years from 1994 to 1998 reported mean lethal doses ranging from 0.93 up to 10.69 µg/µl. This latter one was reported in 1995 when populations manifested resistance to malathion. Scott Armstrong *et al.* (2006) reported results of 2.01 in a research study done in the Texas Valley. In other studies they reported doses of 2.0 and 2.6 for Delicias, Chihuahua in 2008, for Cameron County doses of 5.4, for Medina County doses of 7.8 and for Hidalgo County doses of 10.2. All these counties are in the state of Texas and correspond to 2009 (Armstrong, 2010). The latter one considered as resistant to malathion in comparison to the Laguna Region where the populations are considered as susceptible since they do not exceed the 1 µg/µl established by Terán-Vargas and Wolfenbarger, 2001.

CONCLUSIONS

Based on the results obtained in this research study it may be concluded that the *A. grandis* populations in the Laguna region are susceptible to malathion, and greater susceptibility to malathion was observed for the *A.grandis* populations in the state of Durango than for those in the state of Coahuila.

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Conflict of interest. The authors declare that there is no conflict of interest. The investigation was conducted in the absence of any commercial or financial relationship that could be interpreted as a possible conflict of interest.

Compliance with ethical standards. The research did not include measurements with humans or animals. The study site is not considered a protected area nor is the species under study protected or in danger of extinction, therefore, its use has negligible effects on the wider functioning of the ecosystem.

Data availability. The authors confirm that all data underlying the findings are fully available without restriction, upon reasonable request to the corresponding author, Ph. D. Pedro Cano-Ríos, by e-mail canorp49@hotmail.com All relevant data needed to replicate this study are described in the document.

Author contribution statement (CRedit). Ch. Silva-Martínez - Data curation, Formal Analysis, Investigation, Visualization, Writing – original draft., **P. Cano-Ríos** - Funding acquisition Project administration, Resources, Writing – review & editing., **U. Nava-Camberos** – Conceptualization, Methodology, Formal Analysis, Writing – review & editing., **J.L. García-Hernández** – Supervision, Validation, Writing – review & editing., **J.L. Reyes-Carrillo** - Supervision, Validation., **F.J. Sánchez Ramos** - Supervision, Validation., **S.S. Mendoza-Retana** - Supervision, Validation.

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