Stability of Resistance to Permethrin and Malathion in Mexican Populations of *Aedes aegypti* (Diptera: Culicidae) with Recommendations for Rotational Use[1]

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Abstract *Aedes aegypti* (L.) (Diptera: Culicidae) management in Mexico primarily involves destruction of breeding sites and application of insecticides. There are many cases of field resistance and few studies on the instability of this phenomenon, despite its usefulness in management of this pest. The goal of this research was to estimate, under laboratory conditions, the stability of resistance to permethrin and malathion in 3 field-collected Mexican populations of *Ae. aegypti*. Early fourth-instar larvae were used for the bioassays according to the methodology of the World Health Organization (WHO). Initially, we estimated the level of insecticide resistance in F_1 larvae. Then, each population was individually reared without selection pressure through 7 generations, and the insecticide response was monitored and compared with the susceptible New Orleans strain. The resistance response (RR_{95}) to permethrin in the F_1 generation varied from 53.8 to 267.9X at the LC_{95} level. In malathion, the RR_{95} of the F_1 generation ranged from 5.6 to 10.5X. In most cases, the highest rate of resistance decline occurred from F_1 to F_2. The response to permethrin and malathion after 7 generations without selection pressure was similar to that observed in the susceptible reference strain.

Key Words pyrethroid, organophosphate, dengue, disease

Mosquitoes can transmit harmful viruses, bacteria, protozoa, and nematodes that cause severe human diseases (Becker et al. 2010, World Health Organization [WHO] 2016). The yellow fever mosquito, *Aedes aegypti* (L.) (Diptera: Culicidae), is one of the most dangerous animal species because it transmits different arboviruses that cause diseases to humans, such as dengue, dengue hemorrhagic fever, yellow fever, zika, chikungunya, and Mayaro virus (Gloria-Soria et al. 2016, Lindsay et al. 2017). About 100–400 million dengue infections occur worldwide each year, and 3.9 billion people are at risk of being affected by this disease (Louise et al. 2015, WHO 2023). In Mexico, dengue is the most important vector-borne disease, threatening people residing in 30 of the 32 Mexican states, with

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13,025 confirmed cases of dengue in 2023 until epidemiological week 34 (Secretaría de Salud 2015, 2023). The fight against this vector focuses on larval habitat destruction and the use of insecticides (WHO 2012). However, the overuse and misuse of insecticides have led to many cases of resistance. The continual use of temephos for more than 30 yr and permethrin for more than 10 yr has resulted in numerous reported cases of resistance to these insecticides throughout Mexico (Centro Nacional de Programas Preventivos y Control de Enfermedades [CENAPRECE] 2018, Flores et al. 2013). The Arthropod Pesticide Resistance Database (Michigan State University 2023) reports 53 cases of *Ae. aegypti* resistance to pyrethroid and organophosphorus insecticides in Mexico, jeopardizing their use as the main mosquito vector control tool.

Schechtman and Souza (2015) documented that, in the absence of insecticide selection pressure, the level of population resistance usually decreases. As a result, it would be possible to reintroduce insecticides that were previously discontinued due to resistance issues (Abbas et al. 2015, Carrière and Tabashnik 2001, Contreras et al. 2008, Ferguson 2004, Kristensen et al. 2000, Rehan et al. 2011). When a field population is partially resistant to insecticides, it may reverse toward susceptibility in a few generations when the population exists in insecticide-free conditions. However, if homozygosity is reached, the insect population will remain in this stage unless exposed to the immigration of susceptible individuals (Abedi and Brown 1960). Therefore, the instability of insecticide resistance is of paramount importance to implement effective rotational programs (Afzal et al. 2014, Contreras et al. 2008, Ullah and Shad 2017). The aim of this research was to estimate, under laboratory conditions, the stability of resistance to permethrin and malathion in 3 Mexican field-collected populations of *Ae. aegypti* from the states of Baja California Sur, Sinaloa, and Jalisco.

Materials and Methods

Mosquitoes. This research was conducted from June 2017 to December 2018 at the Colegio de Postgraduados, Campus Montecillo, Texcoco, State of Mexico, Mexico. Three populations of *Ae. aegypti* were field-collected using ovitraps and the methodology of the Centro Nacional de Programas Preventivos y Control de Enfermedades (CENAPRECE 2015) (Table 1). We used the New Orleans strain of this species as a reference of susceptibility. This strain was provided by Universidad Autónoma de Nuevo Leon, Mexico.

Once in the laboratory, the egg papers were placed in containers with 8 L of tap water for egg hatching which were maintained in bioclimatic chambers (Thermo Scientific Model TFFU2065FWA, Waltham, MA) under controlled conditions of 27 ± 2°C and photoperiod of 12: 12 h (light: darkness). Larvae were fed every third day with rodent food for (Rodent Lab Chow®5001, Nestlé Purina, St. Louis, MO). After the adult mosquitoes emerged, they were introduced into entomological cages (40 × 40 × 40 cm) covered with organza fabric and kept at 27 ± 2°C, 75 ± 5% relative humidity (RH), and on a photoperiod of 12: 12 h (light: darkness). The adult mosquitoes were provided with a 10% sugar solution, and females were fed with pig, *Sus scrof* (L.), blood treated with 7 mL of heparin (Inhepar® 1000 IU, Pisa, Mexico) per liter of blood, as an anticoagulant. The treated pig blood was refrigerated at 4°C and, before dispensing, it was heated in a water bath at 40°C until reaching room temperature. Then, blood (5 mL) was poured

Table 1. Field-collected Mexican populations of *Ae. aegypti* evaluated to estimate the stability of resistance to insecticides in the absence of selection pressure.

Population	Origin	Number of Egg-Papers	Total Number of Eggs	Date of Field-Collection
La Paz	La Paz, Baja California Sur	66	14,903	May, 2017
Culiacan	Culiacan, Sinaloa	227	40,779	June, 2017
Guadalajara	Guadalajara, Jalisco	119	23,786	June, 2017

into plastic cups and covered with Parafilm-M®, Bemis Co., Neenah, WI). One cap was placed upside down on the upper part of each entomological cage thus allowing females to feed through the parafilm membrane. We introduced one ovitrap per cage to collect the eggs. The ovitrap consisted of a container covered with kraft paper and 200 mL of tap water which was replaced every 3 d to collect the eggs. The rearing was managed in order to obtain discrete generations. The F_1 adults were identified with the morphological taxonomic key of Darsie and Ward (1981) to discard other species of mosquitoes.

Insecticides. Commercial formulations of insecticides were used: Aqua Reslin Super® (permethrin, aqueous solution, 108.7 g of the active ingredient per L, Bayer de México S. A. de C. V., Mexico) and Verthion® (malathion, concentrated solution, 410 g of the active ingredient per L, Agricultura Nacional, Mexico). We prepared the required insecticide concentrations using distilled water as a diluent.

Bioassays. The bioassays were conducted according to the standardized procedure of the WHO (2005) with some adjustments. One mL of the required insecticide concentration was added to a 127-mL disposable cup containing 100 mL of tap water and 20 early fourth-instar larvae. Five replications were established on different days, and each replication included an untreated control. After 24 h of exposure to the insecticide, mortality was recorded as the percentage of larvae unable to exhibit diving after water disturbance. The maximum mortality accepted for the untreated control was 10%, and it was adjusted using Abbott's (1925) formula.

Initially, we determined the biological response window, which refers to the range of concentrations in which 0 and 100% mortality occur. Then, we included 7 to 10 equidistant concentrations within this established range. The bioassays were conducted on the early fourth-instar larvae of each generation (F_1 to F_7) reared free of insecticide exposure.

Statistical analysis. The median lethal concentration (LC_{50}), the LC_{95}, and their respective 95% confidence limits for each population and generation were estimated using probit analysis (Proc Probit) of the Statistical Analysis System (SAS 9.4; SAS Institute, Inc. 2013). The relative response (RR) was calculated by dividing the respective $LC_{50(95)}$ of the field population by the $LC_{50(95)}$ of the New Orleans strain. The average rate of reduction of resistance (R_{95}) at 95% mortality was calculated as follows: $R_{95} = [\log (LC_{95}\ \text{final}) - \log (\text{initial}\ LC_{95})]/n$, where n is the number of generations without selection pressure (Basit et al. 2013, Rehan et al. 2011, Saddiq et al. 2016, Ullah and Shad 2017, Wirth et al. 2010). The relative variation in resistance on a per generation basis was calculated as follows: Resistance variation (RV) = $[(RR_{50(95)Fn} - RR_{50(95)Fn\text{-}1})/RR_{50(95)F1}]\times 100$. To compare the $LC_{50(95)}$ among generations or between a field-collected population and the reference strain, we used the criterion of Robertson et al. (2017) who considered that, at the same level of mortality, two responses are statistically different if their 95% confidential limits do not overlap.

Results and Discussion

The initial LC_{95} values of permethrin ranged from 4.2 (Guadalajara) to 20.9 mg l^{-1} (La Paz) (Table 2). For La Paz, Culiacan and Guadalajara, the LC_{50} and LC_{95}, the confidence limits of the F_1 larvae did not overlap with the New Orleans strain. The initial relative response (F_1) at the 50% mortality (RR_{50}) varied from 14.8× (Guadalajara) to 25.2× (La Paz), and the RR_{95} ranged from 53.8 (Guadalajara) to 267.9× (La Paz). At LC_{95}, higher rate of resistance decline (RV_{95}) occurred from F_1 to F_2: La Paz (−98.6%), Culiacan (−95.4%), and Guadalajara (−94.2%). In all cases, in 7 generations, the preexisting level of resistance at the LC_{95} (RR_{95}) reversed (R_{95}) to 0.34× (La Paz), 0.25× (Culiacan), and 0.22× (Guadalajara), respectively; indicating that resistance to permethrin is unstable (Table 2).

With malathion, the initial LC_{95} in the F_1 larvae of *Ae. aegypti* ranged from 1.06 (Guadalajara) to 1.99 mg l^{-1} (Culiacan) (Table 3). At both the LC_{50} and LC_{95}, the response of the F_1 of field-collected populations did not overlap with the New Orleans Strain. In the same order, the RR_{50} (RR_{95}) values were between 14.7 (5.6)× to 27.6 (10.5)× for Guadalajara and Culiacan, respectively. With malathion, at RR_{95} level, the most important percent of the decline was observed from F_1 to F_2: La Paz (−45.7%), Culiacan (−37.6%), and Guadalajara (−22.6%). However, in the Guadalajara population, the higher insecticide reversion occurred from F_2 to F_4 (−41.5%) (Table 3). In all cases, in 7 generations, the preexisting level of resistance at the LC_{95} (RR_{95}) reversed (R_{95}) to 0.05× (La Paz), 0.09× (Culiacan), and 0.09× (Guadalajara), indicating that resistance to permethrin is unstable (Table 3).

According to Mazzarri and Georghiou (1995), in the case of organophosphates, carbamates, and pyrethroids, values of RR_{50} and RR_{95} <5× obtained in laboratory bioassays indicate that the field population remains susceptible; thus, the biological effectiveness of the insecticides is unlikely to be affected. Moderate field resistance is suspected when these values range between 5 and 10×. If $RR_{50(95)}$ is >10×, then high field resistance is expected. Therefore, the evaluated field-collected populations may be considered resistant to permethrin and malathion.

The rate of insecticide resistance decline through generations that are no longer exposed to the insecticide depends on the interactions of several factors, such as the negative impact on fitness and the initial frequency of alleles for resistance (Abbas et al. 2015, Djihinto et al. 2013, Grossman et al. 2018, Jan et al. 2015, Roush and Croft 1986). Chang et al. (2012) found that a population of *Ae. aegypti* from Taiwan that was highly resistant to permethrin (400×) responded similarly to the susceptible one after 15 generations without selection pressure. Grossman et al. (2018) monitored

Table 2. Response to permethrin in fourth-instar larvae of *Ae. aegypti,* reared during 7 generations without insecticide selection pressure.

Population	Generation	n^a	df	b ± EE	LC_{50} (mg l^{-1}) (CL 95%)	LC_{95} (mg l^{-1}) (CL 95%)	$Pr > \chi^2$	RR_{50}[b] (RV_{50}[c])	RR_{95}[b] (RV_{95}[c])
New Orleans (Susceptible)		800	6	3.5 ± 0.22	0.027 (0.025–0.028)	0.078 (0.069–0.09)	0.96		
La Paz	F1	720	10	1.1 ± 0.09	0.68 (0.55–0.85)	20.9 (12.0–44.0)	0.4	25.2	267.9
	F2	600	4	2.1 ± 0.15	0.046 (0.04–0.05)	0.28 (0.22–0.39)	0.35	1.7 (−93.2)	3.6 (−98.6)
	F4	700	5	2.1 ± 0.13	0.07 (0.06–0.08)	0.43 (0.34–0.57)	0.14	2.6 (3.5)	5.5 (0.7)
	F5	700	5	2.1 ± 0.13	0.06 (0.05–0.07)	0.41 (0.32–0.54)	0.3	2.2 (−1.4)	5.3 (−0.1)
	F6	900	7	2.7 ± 0.16	0.032 (0.029–0.034)	0.12 (0.11–0.15)	0.95	1.2 (−4.1)	1.5 (−1.3)
	F7	800	6	3.1 ± 0.2	0.025 (0.023–0.027)	0.085 (0.074–0.10)	0.29	0.9 (−1.0)	1.1 (−0.1)
				R_{95} = average rate of reduction in resistance at 95% mortality = 0.34					
Culiacan	F1	540	7	1.3 ± 0.13	0.42 (0.33–0.52)	8.42 (5.05–18.08)	0.98	15.6	107.9
	F2	500	3	2.8 ± 0.21	0.096 (0.086–0.107)	0.38 (0.3–0.5)	0.26	3.6 (−77.1)	4.9 (−95.4)
	F4	600	4	2.4 ± 0.17	0.06 (0.05–0.06)	0.28 (0.23–0.37)	0.24	2.2 (−8.5)	3.6 (−1.1)
	F5	600	4	2.1 ± 0.15	0.032 (0.028–0.036)	0.19 (0.14–0.28)	0.98	1.2 (−6.6)	2.4 (−1.0)
	F6	1000	8	2.2 ± 0.15	0.041 (0.036–0.045)	0.22 (0.18–0.27)	0.9	1.5 (2.1)	2.8 (0.3)
	F7	800	6	2.4 ± 0.21	0.028 (0.024–0.032)	0.13 (0.11–0.17)	0.97	1.0 (−3.1)	1.7 (−1.0)
				R_{95} = average rate of reduction in resistance at 95% mortality = 0.25					

Table 2. Continued.

Population	Generation	n^a	df	b ± EE	LC_{50} (mg l^{-1}) (CL 95%)	LC_{95} (mg l^{-1}) (CL 95%)	Pr $> \chi^2$	$RR_{50}{}^b$ ($RV_{50}{}^c$)	$RR_{95}{}^b$ ($RV_{95}{}^c$)
Guadalajara	F1	480	6	2.7 ± 0.18	0.40 (0.32–0.49)	4.2 (2.9–6.8)	0.96	14.8	53.8
	F2	600	4	2.5 ± 0.17	0.054 (0.048–0.061)	0.24 (0.2–0.3)	0.2	2.0 (−86.5)	3.1 (−94.2)
	F4	480	4	3.1 ± 0.26	0.035 (0.031–0.039)	0.12 (0.1–0.15)	0.97	1.3 (−4.7)	1.5 (−2.8)
	F5	700	5	1.9 ± 0.13	0.052 (0.045–0.06)	0.35 (0.28–0.47)	0.41	1.9 (4.2)	4.5 (5.4)
	F6	700	5	3.8 ± 0.25	0.039 (0.036–0.041)	0.1 (0.09–0.12)	0.63	1.4 (−3.2)	1.3 (−5.9)
	F7	800	6	2.0 ± 0.22	0.018 (0.013–0.022)	0.11 (0.09–0.15)	0.95	0.7 (−5.2)	1.4 (0.2)
					R_{95} = average rate of reduction in resistance at 95% mortality = 0.22				

[a] n = *Total* of treated larvae.

[b] RR = Relative response = $LC_{50(95)}$ field population/$LC_{50(95)}$ susceptible population.

[c] RV = Resistance variation = $[(RR_{50(95)Fn} - RR_{50(95)Fn\text{-}1})/RR_{50(95)F1}]\times 100$, positive and negative values for $RV_{50(95)}$ indicate the increase or decrease in resistance, respectively.

Table 3. Response to malathion in early fourth-instar larvae of *Ae. aegypti* reared during 7 generations without insecticide selection pressure.

Population	Generation	n^a	df	b ± EE	LC_{50} (mg l^{-1}) (CL 95%)	LC_{95} (mg l^{-1}) (CL 95%)	$Pr > \chi^2$	RR_{50}^b (RV_{50}^c)	RR_{95}^b (RV_{95}^c)
New Orleans (Susceptible)		600	4	1.5 ± 0.18	0.017 (0.014–0.022)	0.19 (0.11–0.45)	0.22		
La Paz	F1	700	5	2.7 ± 0.18	0.33 (0.3–0.36)	1.29 (1.07–1.64)	0.59	19.4	6.8
	F2	700	5	3.7 ± 0.27	0.25 (0.24–0.27)	0.7 (0.61–0.84)	0.62	14.7 (−24.2)	3.7 (−45.7)
	F4	700	5	2.4 ± 0.16	0.18 (0.16–0.2)	0.84 (0.7–1.06)	0.54	10.6 (−21.2)	4.4 (10.8)
	F5	420	5	2.8 ± 0.38	0.1 (0.08–0.13)	0.4 (0.26–0.93)	0.07	5.9 (−24.2)	2.1 (−34.1)
	F6	800	6	2.7 ± 0.17	0.15 (0.13–0.16)	0.6 (0.51–0.75)	0.9	8.8 (15.1)	3.2 (15.5)
	F7	900	7	2.2 ± 0.15	0.091 (0.083–0.10)	0.51 (0.41–0.66)	0.65	5.4 (−17.8)	2.7 (−6.9)
R_{95} = average rate of reduction in resistance at 95% mortality = 0.05									
Culiacan	F1	500	3	2.6 ± 0.23	0.47 (0.42–0.53)	1.99 (1.55–2.81)	0.52	27.6	10.5
	F2	480	6	2.57 ± 0.2	0.28 (0.25–0.32)	1.24 (0.98–1.69)	0.19	16.5 (−40.4)	6.5 (−37.6)
	F4	600	4	3.1 ± 0.22	0.25 (0.23–0.27)	0.83 (0.7–1.04)	0.21	14.7 (−6.3)	4.4 (−20.6)
	F5	600	4	3.1 ± 0.21	0.2 (0.18–0.22)	0.67 (0.57–0.83)	0.93	11.8 (−10.6)	3.5 (−8.0)
	F7	900	7	3 ± 0.17	0.12 (0.11–0.13)	0.44 (0.37–0.53)	0.22	7.1 (−17.0)	2.3 (−11.5)
R_{95} = average rate of reduction in resistance at 95% mortality = 0.09									

Table 3. Continued.

Population	Generation	n^a	df	b ± EE	LC_{50} (mg l^{-1}) (CL 95%)	LC_{95} (mg l^{-1}) (CL 95%)	$Pr > \chi^2$	RR_{50}^b (RV_{50}^c)	RR_{95}^b (RV_{95}^c)
Guadalajara	F1	700	5	2.6 ± 0.17	0.25 (0.23–0.28)	1.06 (0.89–1.34)	0.53	14.7	5.6
	F2	560	5	2.94 ± 0.2	0.22 (0.2–0.25)	0.82 (0.69–1.03)	0.92	12.9 (−12.0)	4.3 (−22.6)
	F4	420	5	2.9 ± 0.28	0.1 (0.09–0.11)	0.38 (0.29–0.55)	0.18	5.9 (−48.0)	2.0 (−41.5)
	F5	600	4	3.0 ± 0.21	0.1 (0.09–0.11)	0.35 (0.29–0.43)	0.6	5.9 (0.0)	1.8 (−2.8)
	F7	800	6	3.4 ± 0.2	0.083 (0.077–0.089)	0.24 (0.21–0.29)	0.61	4.9 (−6.8)	1.3 (−10.3)
				R_{95} = average rate of reduction in resistance at 95% mortality = 0.09					

[a] n = *Total* of treated larvae.

[b] RR = Relative response = $LC_{50(95)}$ field population/$LC_{50(95)}$ susceptible population.

[c] RV = Resistance variation = $[(RR_{50(95)Fn} - RR_{50(95)Fn\text{-}1})/RR_{50(95)F1}] \times 100$, positive and negative values for $RV_{50(95)}$ indicate the increase or decrease in resistance, respectively.

the frequency of resistance alleles in a population of *Ae. aegypti* from Yucatan, Mexico, resistant to permethrin. They found that after 15 breeding generations without selection pressure, the resistance gene frequency decreased from 0.75 to 0.2. They indicated that a cost in fitness was associated with pyrethroid resistance; then, susceptibility can be restored after an absence of selection pressure.

Strode et al. (2012) found that *Ae. aegypti* developed resistance to temephos after 13 generations of selection with the insecticide, and it took another 13 generations without selection pressure to re-establish susceptibility. Melo-Santos et al. (2009) found that a temephos-resistant *Ae. aegypti* population (7X), declines resistance in only 9 generations without selection pressure. This decline was associated with a possible cost in fitness. According to Chang et al. (2012), insecticide resistance reverses without selection pressure because natural selection is unfavorable to resistance genes.

One of the premises in managing resistance to insecticides is supported in the interruption of selection pressure with insecticides so that the susceptibility is restored (Schechtman and Souza 2015). Roush and Croft (1986) indicated that the stability of insecticide resistance is influenced by factors such as differences in fitness, initial frequencies of resistance genes, and the dominance relationships between susceptibility alleles and resistance. However, the cost of fitness may disappear with time when resistance to insecticides increases and stabilizes in the population (Djihinto et al. 2013). In this case, we estimate that relaxation in selection pressure during 7 generations is sufficient for the evaluated populations to reverse the existing resistance levels to permethrin and malathion. However, the number of generations without selection, with these and related insecticides required to revert resistance, may vary over time. Thus, the rate of decline in response to the insecticides used to fight this vector under field conditions must be systematically evaluated to estimate the length of the relaxation window.

Until now, malathion has been applied to decrease the density of *Ae. aegypti* adults. Several pyrethroids (phenothrin, deltamethrin, transfluthrin, lambda-cyhalothrin, alpha-cypermethrin, and bifenthrin), except permethrin, are also used against adults (CENAP-RECE 2023). These pyrethroids are related to mechanisms of resistance (Kasai et al. 2014). According to Georghiou (1972), when an insect population remains resistant to insecticides, it increases the time to reverse susceptibility. Thus, the steady use of malathion and pyrethroids against *Ae. aegypti* may threaten their usefulness and reduce the advantage of rotating them to mitigate the evolution of resistance.

Conclusions. Resistance to permethrin and malathion was detected in the populations of *Ae. aegypti* from La Paz, Culiacan, and Guadalajara in Mexico. These levels of resistance were unstable. In the absence of selection pressure, in most cases, the higher decline of resistance was observed from F_1 to F_2. Under the tested conditions, 7 generations free of selection pressure with permethrin or malathion were required to reverse the existing resistance levels.

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