Resistance to Permethrin in *Aedes aegypti* (L.) in Northern Mexico

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Resumen. Se determino la dosis diagnostico (DD) del insecticida permetrina en poblaciones del mosquito *Aedes* aegypti (L.) de tres estados del norte de Mexico: Coahuila, Sonora y Tamaulipas. Se determinaron enzimas detoxificativas para cada una de las poblaciones y los resultados fueron comparados con la cepa susceptible Nueva Orleáns de *Ae. aegypti*. La población de Coahuila mostró iAChE elevada en comparación con la cepa de referencia NO y aunque este mecanismo no está relacionado a la resistencia a piretroides, su presencia en niveles tan elevados sugiere resistencia a organofosforados y carbamatos. Las oxidasas estuvieron presentes en la población de Sonoita, estado de Sonora como el mecanismo principal de resistencia a permetrina.

Abstract. Diagnostic dose (DD) of permethrin was determined in *Aedes aegypti* (L.), from three states (Coahuila, Sonora, and Tamaulipas) in northern Mexico. After exposing 10 groups of 100 females to the DD obtained and producing 50% mortality, individuals were divided into two categories: survivors and dead. Mosquitoes in each of these categories were dissected to separate the head, thorax, and abdomen. Biochemical tests were done on the head and thorax to determine activity by resistance-related enzymes including: α and β-esterases, mixed-function oxidases (MFOs), glutathione-S-transferase (GST) and insensitive acetyl cholinesterase (iAChE). Results were compared with those for a susceptible strain of *Ae. aegypti* from New Orleans. A population from Coahuila showed iAChE as the only enzyme activity that surpassed the threshold established with the susceptible strain, however, this mechanism is not associated with resistance to pyrethroids, but rather with resistance to organophosphate and carbamate insecticides. For the populations from Tamaulipas, none of the mechanisms studied were important in conferring resistance to permethrin. MFOs were present at elevated levels of activity, appearing as the main detoxifying mechanism, in the population from Sonoita, Sonora state.

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Introduction

During and after World War II, epidemics of dengue fever and dengue hemorrhagic fever began to occur in Southeast Asia (Gubler 1997). During the last 25 years, these have intensified with the increased spread of the virus and its four serotypes, as well as the spread of its principal vector, the yellowfever mosquito, *Aedes aegypti* (L.), resulting in epidemics affecting large populations and with greater frequency. With the exception of sporadic outbreaks in the Caribbean Islands, dengue and yellow fever were controlled effectively in the Americas from 1946 until the 1970s as a result of an eradication program by the Pan American Health Organization (Schliessman and Calheiros 1974). Because vaccinations are not available for dengue fever, yellowfever mosquito must be controlled through labor-intensive home-to-home destruction of larval habitat or by insecticides that were increasingly used after World War II. Frequent use of insecticide, however, has led to chemical resistance in many arthropods and this led to operational problems in vector control programs. In 1947, the first case of mosquitoes resistant to insecticide was observed in Florida where the mosquitoes *Ae. taeniorhynchus* (Wiedmann) and *Ae. sollicitans* (Walker) showed resistance to DDT (Brown 1986). The problem increased during the following decades.

The World Health Organization (WHO) defines resistance to insecticides as the "development of the ability" of a population of insects to tolerate doses of toxins that would be lethal for the majority of the individuals of a normal population of the same species (Anonymous 1957). Populations of insects can survive the effect of chemical compounds by different physiological mechanisms, including modification of the target site and elevated production of detoxifying enzymes (Martinez-Torres et al. 1998, Brooke et al. 1999). Many species of mosquitoes have developed resistant populations. These can include populations resistant to more than one active ingredient (multiple resistance). This can lead to economic consequences with greater costs (increased doses of the toxins or number of applications), and with the re-emergence of mosquito-related diseases. Thus, control programs are confronted with serious problems (Brogdon and McAllister 1998a). Four main mechanisms of resistance associated with pyrethroids have been reported: over-expression and increased production of monooxygenases (in particular, multi-function oxidases), non-specific esterases, GST, and reduced sensitivity of sodium channels in neuron membranes ("kdr," knock down resistance), the target site of action for DDT and pyrethroids (Oppenoorth 1985, Georghiou 1986, Grant and Matsumura 1988, Nelson et al. 1996, Chandre et al. 1999).

Resistance by these mechanisms has been reported to pyrethroids in populations of *Aedes* in various countries in the Caribbean and South America. These include Venezuela (Field et al. 1984, Mazarri and Georghiou 1995), Puerto Rico (Hemingway et al. 1989), and the Dominican Republic (Mekuria et al. 1991).

Yellowfever mosquito was eliminated in Mexico in 1963; however, the mosquito was re-introduced in 1975 along the northern border (Gomez-Dantes et al. 1993). In December 1978, the first outbreak of 36 cases of dengue fever occurred in Tapachula, Chiapas. Between 1978 and 1984, dengue produced multiple epidemics in large areas of the country (Gomez-Dantes et al. 1993). Recently, an increase in the number of hurricanes and tropical storms has created environmental conditions favorable to increase in abundance of mosquitoes.

Control of vectors in Mexico relies mainly on the reduction of human-vector contact through the use of specific chemical compounds. Ultra-low volume

applications of permethrin for adult mosquitoes and temephos for mosquito larvae are standard tools to control the vector of dengue in Mexico (Norma Oficial Mexicana 2002). Recent reports have shown detoxifying enzymes, especially esterases, as the principal mechanism of resistance to permethrin in populations of *Ae. aegypti* in northern (Baja California Norte and South) (Flores et al. 2005) and southern Mexico (Quintana Roo) (Flores et al. 2006).

Two of the most important components in vector control campaigns are: 1) establishment of the diagnostic dose (this allows monitoring of populations over time) and 2) biochemical assays (information on specific problems of resistance in the field) (Brogdon 1984). These components help establish appropriate measures of control in areas where the mosquito populations are abundant throughout the year. Therefore, the main objectives of this investigation were to provide information about susceptibility of mosquito populations from northern Mexico to commonly used adulticide and to determine the main enzymatic mechanisms of resistance to permethrin.

Materials and Methods

Study Area. Yellowfever mosquito larvae were collected in the: community of Valle Verde, municipality of Torreon in Coahuila; Sonoita and Hermosillo, municipalities in Sonora; Nuevo Laredo, Matamoros, and Reynosa in Tamaulipas, Mexico (Fig. 1).

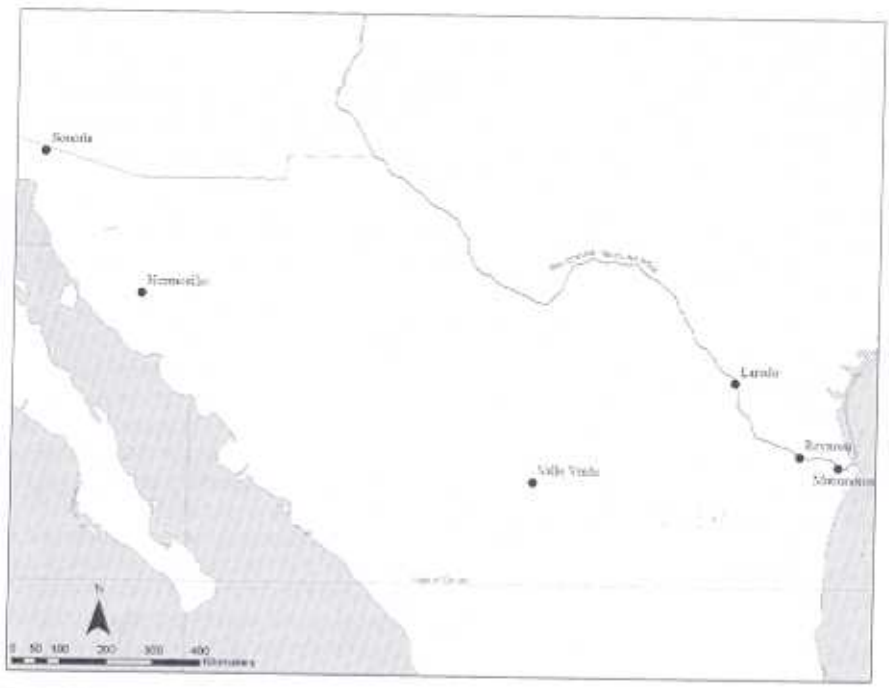

Fig. 1. Collection sites of *Aedes aegypti* in three states in northern Mexico.

Collection of Mosquitoes. Mosquitoes were collected from containers of water that usually are productive regardless of rainfall. These containers include flower pots in cemeteries, 200-liter drums, used tires, and other objects discarded by humans. The larvae were transported in Whirlpak® bags to an insectary, placed in plastic trays (35 x 25 cm), and fed finely ground AquaCrece® fish food. Pupae were transferred in 250-ml receptacles to screened cages (30 x 30 x 30 cm). Adult mosquitoes were allowed to feed on cotton soaked in a 10% sugar solution. Females were fed rat blood to promote production of eggs. Colonies were maintained at 24 ± 2°C and 70% relative humidity. Containers with water and filter paper were provided for egg laying. The eggs were used to establish F_1 and F_2 generations.

Adult Bioassays. Bioassays were done using a bottle bioassay described by Brogdon and McAllister (1998a), exposing adults (F_1 and F_2) for 1 hour and consisted of four doses of permethrin and one check without insecticide. For treatment (coating) of bottle interiors, technical-grade solutions of permethrin (Chem Service, West Chester, PA) were diluted in acetone. A 1-ml portion of diluted toxicant was transferred to a 250-ml Wheaton bottle. The bottle was shaken, rolled, and inverted such that all surfaces were exposed to the solution. The bottles were allowed to dry for 24 hours in a dark place. All treatments and checks were replicated three times. A total of 20 unfed adult females, two days after emerging, was used for each replication. Checks were prepared using 1 ml of acetone. DD was determined according to the method proposed by Brogdon and McAllister (1998b).

Using DD of Permethrin for Selecting Individuals. Groups of 25 females were exposed to the DD of permethrin. When 50% died, they were separated into two categories, survivors and dead, and stored at -70°C. This procedure was repeated until 1,000 mosquitoes were obtained per population. This included the susceptible New Orleans strain. The head and thorax of each mosquito was removed and saved in 1.5-ml tubes for the biochemical assays done individually.

Biochemical Assays. The head and thorax of each mosquito collected from the survivor and dead categories were homogenized individually in 100 µl of 0.01 M potassium phosphate buffer, pH 7.2. The homogenate was mixed in 2 ml of the same buffer. Aliquots of 100 µl were transferred to microplate wells. Thirty adult females were analyzed per plate in triplicate. Five enzymes were determined for each mosquito: α- and β-esterases, multi-function oxidases, glutathione S-transferase and insensitive acetylcholinesterase. Absorbance was read with a Benchmark spectrophotometer (Bio-Rad Laboratories, Hercules, CA).

Analysis of Results. Results of biochemical assays were expressed as a mean absorbance values for each enzyme, survivor and dead to DD of permethrin and population of yellowfever mosquito. The susceptible New Orleans strain was used as a reference. ANOVA (p = 0.05) was used to determine significant differences in enzyme activities between the individuals classified as survivors or dead after exposure to permethrin at the DD in each of the populations.

The maximum absorbance value for the New Orleans strain was selected as the threshold of susceptibility (or resistance) and the percentage of resistance was calculated according to the mosquitoes that surpassed the threshold established by this strain. The concentration of protein was determined according to the method established by Brogdon (1984), in case variation might be attributed to the size of the mosquitoes.

Results and Discussion

Adult Bioassays. DD values of permethrin obtained for the populations from Coahuila, Sonora, and Tamaulipas (Table 1) were less than those reported by Flores et al. (2005) for the populations from Tecate and Tijuana in Baja California Norte and from Ciudad Constitucion and Loreto in Baja California Sur (172 µg of a.i/bottle).

Table 1. Diagnostic Dose of Permethrin for *Ae. aegypti* Populations from Coahuila, Sonora, and Tamaulipas, Mexico

Population	DD (µg of a.i/bottle)
Coahuila	86
Sonora	86
Tamaulipas	110

Fig. 2 shows the absorbance means for the five enzyme assays of the New Orleans strain (susceptible reference strain) and the population from Torreon, Coahuila treated with the DD of 86 µg of permethrin per bottle. Means were analyzed by Tukey's test for multiple comparison of means ($p = 0.05$), to determine differences.

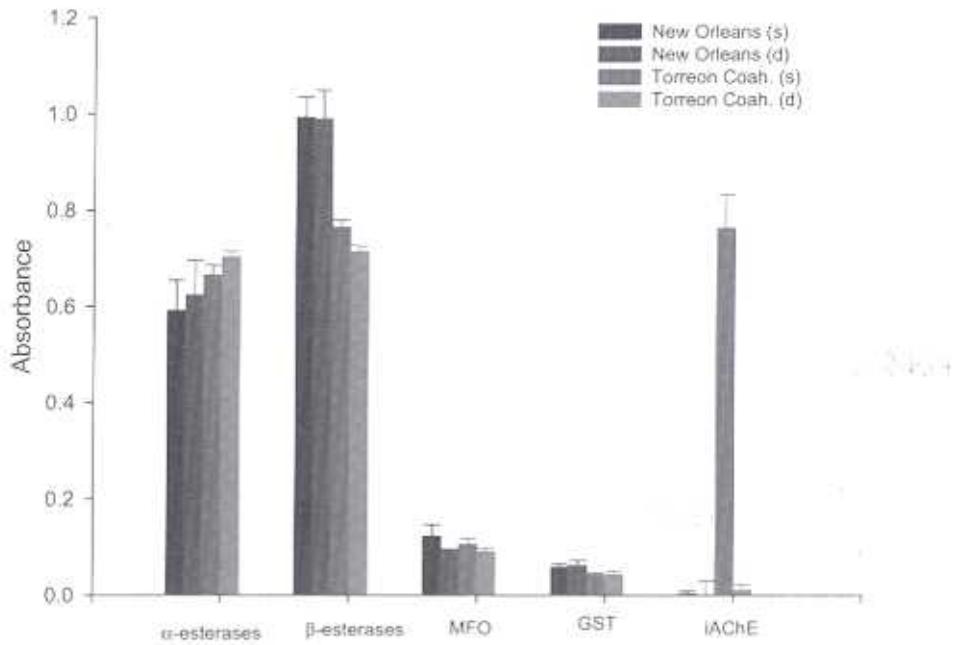

Fig. 2. Mean absorbance values of adult females of *Aedes aegypti* from Coahuila state and the New Orleans strain obtained in biochemical assays.

For the population from Torreon, Coahuila (survivors and dead), only two enzyme activities, α esterases and iAchE, showed higher absorbance values compared to the New Orleans strain, suggesting resistance in the population to insecticides associated with these mechanisms (Kadous et al. 1983, Hemingway and Georghiou 1983). In the assays for β esterases, MFO and GST, the absorbance means in the Torreon population (survivors and dead) were less than those of New Orleans mosquitoes, which could characterize a population susceptible to insecticides inactivated by these enzymes (Hemingway and Georghiou 1983, Hemingway and Ranson 2002).

Table 2 shows the percentage of the mosquitoes from Torreon, Coahuila, selected with permethrin DD that surpassed the threshold established by the susceptible strain. High levels of iAChe in the strain of yellowfever mosquito from Coahuila could suggest a later appearance of resistance to temephos, which could worsen problems with vector control.

Table 2. Percentage of Females of *Ae. aegypti* from Torreon, Coahuila, Selected with Permethrin DD that Surpassed the Threshold of Resistance Established with the Susceptible Strain from New Orleans

Enzyme	Survivors	Dead
α esterases	46.0	45.0
β esterases	0.4	0.0
MFO	18.6	19.0
GST	14.0	12.6
iAChE	100.0	0.0

Studies by Vaughan et al. (1998) in *Culex* strains from the Caribbean Island of Trinidad suggested resistance to organophosphates -- the group of insecticides that includes temephos used by health programs to control larval stages in Mexico -- is usually mediated by two mechanisms: 1) esterases, as a result of gene amplification and 2) insensitive acetylcholinesterase. These results demonstrated that selection pressure exerted by the insecticides (depending on the quantity and frequency of application), as well as inherent characteristics of the species selected, result in a focal resistance to insecticides (Hemingway and Ranson 2002, Brogdon 2003).

Populations from Tamaulipas (Fig. 3, Table 3) also showed the presence of the insensitive acetylcholinesterase mechanism in surviving individuals of the populations from the state, albeit of lesser importance compared to the results from Coahuila. Based on this tendency we could encounter in the near future resistance to organophosphates and carbamates.

However, it should not be discounted that other non-enzymatic mechanisms are working to produce resistance in those populations where we did not find a significant presence of resistance-associated enzyme activity. Still, early detection of enzymatic resistance mechanisms in populations of yellowfever mosquito could prompt an important change in control programs and prolong the effective use of insecticides.

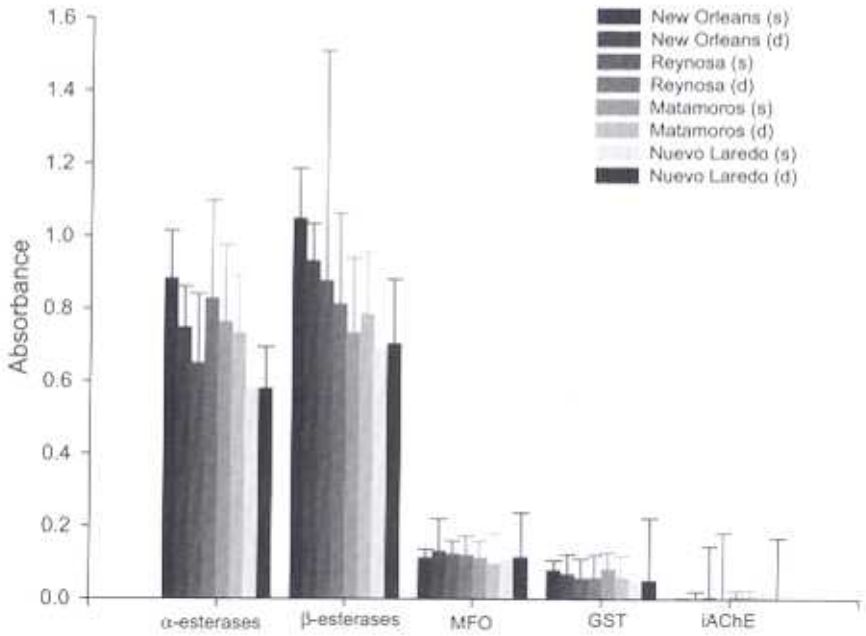

Fig. 3. Mean absorbance values of adult females of *Aedes aegypti* from Tamaulipas state and the New Orleans strain obtained in biochemical assays.

Table 3. Percentage of *Aedes aegypti* Females from Tamaulipas Selected with Permethrin DD that Surpassed the Threshold of Resistance Established by the Susceptible New Orleans Strain

	Reynosa		Matamoros		Nuevo Laredo	
Enzyme	Survivors	Dead	Survivors	Dead	Survivors	Dead
α esterases	3.6	30.4	6.6	7.6	0.0	0.0
β esterases	6.0	7.8	0.0	0.4	0.0	0.8
MFO	6.8	1.2	8.2	0.8	3.4	0.0
GST	6.4	0.8	8.2	5.6	0.2	0.2
iAChE	15.0	0.2	22.0	0.0	21.0	1.2

Fig. 4 presents the mean absorbance values of the five enzymes from the New Orleans strain (susceptible reference) and populations from Sonoita and Hermosillo. Mean absorbance values from Hermosillo and Sonoita populations were greater than those of the New Orleans strain in comparing the results for α esterase of individuals that survived, indicating an enzymatic activity significantly greater than that of the susceptible strain and a warning of focal resistance.

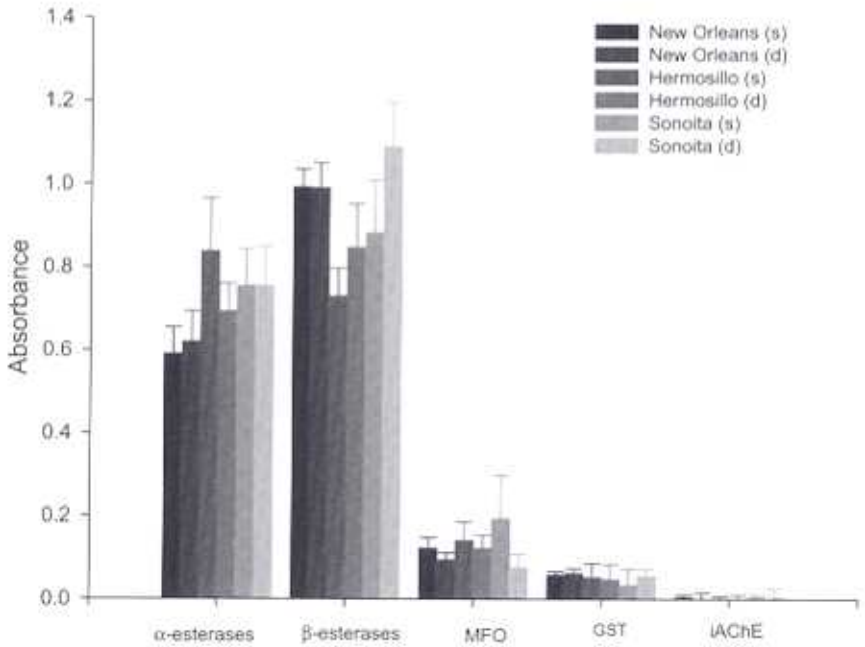

Fig. 4. Mean absorbance values of adult females of *Aedes aegypti* from Sonora state and the New Orleans strain obtained in biochemical assays.

In regard to the percentage of individuals from Sonora populations that surpassed the threshold of each assay (Table 4), it is established that α-esterases represent the mechanism present in both populations. As with MFO, it can represent an important detoxifying mechanism in the population from Sonoita.

Table 4. Percentage of *Aedes aegypti* Females Selected with Permethrin DD from Sonora that Surpassed the Threshold of Resistance Established by the Susceptible New Orleans Strain

	Hermosillo		Sonoita	
Enzyme	Survivors	Dead	Survivors	Dead
α esterases	94.2	83.3	37.5	70.8
β esterases	0.0	17.5	0.0	15.8
MFO	23.2	44.2	70.0	4.2
GST	18.3	15.8	20.0	13.3
iAChE	4.1	3.3	24.0	59.1

Conclusions

The DD for permethrin for the populations from the states of Coahuila (0.0086%), Sonora (0.0086%), and Tamaulipas (0.0110%) were less than those for DD established by the WHO (0.25% of the a.i.). For populations from the states of Baja California Norte and Sur, a DD of 0.0172% had been established (Flores et al. 2005).

For the population from Torreon, Coahuila, individuals surviving exposure to the DD of permethrin showed slightly more enzymatic activity compared to the susceptible strain, albeit not significantly. However, this population had activity levels significantly greater than those of other populations studied. Results demonstrated that the enzymatic mechanism involving α- esterase is present, but is not the principal means of detoxification, because it is found in both survivors and killed after exposure to the DD of permethrin. In enzyme assays for oxidases and GST, some individuals surpassed, albeit at non-significant percentages ($p > 0.05$), the threshold of resistance established with the New Orleans strain. Insensitive acetylcholinesterase was significantly present in this population, because it was found in 100% of the survivors of the diagnostic dose. However, this mechanism is not associated with resistance to pyrethroids, but rather with resistance to organophosphate and carbamate insecticides.

Reynosa, Matamoros, and Nuevo Laredo, the populations from Tamaulipas, clearly demonstrated that the mechanisms involving α and β esterases were not important in conferring resistance to permethrin at the three locations studied. Contrasting results were reported by Flores et al (2005, 2006) who found α esterases as a main resistance mechanism in populations from Baja California north and south and elevated both, α and β esterases in surviving populations exposed DD of permethrin from Quintana Roo, south part of Mexico. The values obtained for the oxidases at Nuevo Laredo and Matamoros did not significantly surpass the threshold of tolerance established by the susceptible strain from New Orleans. On the other hand, the mechanism involving insensitive acetylcholinesterase seemed to be operating in surviving individuals after exposure to the DD of permethrin and at a significant level compared with the rest of the detoxifying mechanisms at the three locations studied. However, these findings indicate this mechanism is not associated with resistance to pyrethroids but to organophosphates and carbamates.

The populations from Sonora, Hermosillo, and Sonoita showed levels of α-esterases greater than that of the threshold but were not significantly different in the killed individuals. Surviving mosquitoes from Sonoita showed levels of β-esterases greater than that of the threshold, but were not significantly different from those obtained in the killed individuals. These results indicated that none of these mechanisms were involved in the resistance by these populations to permethrin. However, MFOs were present in the population from Sonoita, at elevated levels of activity, appearing as the main detoxifying mechanism for this population.

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References Cited

Anonymous. 1957. World Health Expert Committee on Insecticides 7th Report. WHO Technical Report Series No. 125.

Brogdon, W. G. 1984. Mosquito protein microassay. I. Protein determinations from small portions of single-mosquito homogenates. Comp. Biochem. Physiol. B. 79: 457-459.

Brogdon, W. G. 2003. Vector resistance, pp. 88-93. *In* L. Stacy, M. L. Stanley, N. Marjan, and T. Burroughs [eds.] The Resistance Phenomenon in Microbes and Infectious Disease Vectors. National Academy of Sciences, Washington, DC.

Brogdon, W. G., and J. C. McAllister. 1998a. Insecticide Resistance and Vector Control. CDC, Atlanta, GA.

Brogdon, W. G., and J. C. McAllister. 1998b. Simplification of adult mosquito bioassays through use of time-mortality determinations in bottles. J. Am. Mosq. Control Assoc. 14: 159-164.

Brooke, B. D., H. H. Richard, L. K. Lizette, J. Dossou-Yovo, and C. Maureen. 1999. Evaluation of polymerase chain reaction assay for detection of pyrethroid insecticide resistance in a malaria vector species of *Anopheles gambiae* complex. J. Am. Mosq. Contr. Assoc. 15: 565-568.

Brown, A. W. A. 1986. Insecticide resistance in mosquitoes: a pragmatic review. J. Am. Mosq. Assoc. 2: 123-140.

Chandre, F., F. Darriet, S. Manguin, C. Brengues, P. Carnevale, and P. Guillet. 1999. Pyrethroid cross resistance spectrum among populations of *Anopheles gambiae* from Cote D'Ivoire. J. Am. Mosq. Contr. Assoc. 15: 53-59.

Field, W. N., J. M. Hitchen, and A. T. Rees. 1984. Esterase activity in strains of *Aedes aegypti* (Diptera: Culicidae) tolerant and susceptible to the organophosphate insecticide malathion. J. Med. Entomol. 21: 412-418.

Flores, A. E., W. Albedaño-Vázquez, I. Fernández-Salas, M. H. Badii, H. Loaiza-Becerra, G. Ponce-Garcia, S. Lozano-Fuentes, W. G. Brogdon, W. C. Black IV, and B. Beaty. 2005. Elevated α-esterase levels associated with permethrin tolerance in *Aedes aegypti* (L.) from Baja California, Mexico. Pesticide Biochem. Physiol. 82: 66-78.

Flores, A. E., J. Salomon Grajales, I. Fernandez Salas, G. Ponce Garcia, S. Lozano Fuentes, W. G. Brogdon, W. C. Black IV, and B. Beaty. 2006. Mechanisms of insecticide resistance in field populations of *Aedes aegypti* (L.) from Quintana Roo, Southern Mexico. J. Am. Mosq. Control Assoc. 22: 672-677.

Georghiou, G. P. 1986. The magnitude of resistance problem, pp. 14-43. *In* Pesticide Resistance: Strategies and Tactics for Management. National Academy Press, Washington, DC.

Gomez-Dantes, H., R. Tapia-Conyer, O. Velazques-Monroy, and M. L. Zarate-Aquino. 1993. Monografia sobre la epidemiología del dengue. Direccion General de Epidemiologia. Secretaria de Salud.

Grant, D. F., and F. Matsumura. 1988. Glutathione S-transferase in *Aedes aegypti* larvae. Purification and properties. Insect Biochem. 18: 615-622.

Gubler, D. J. 1997. Dengue and dengue hemorrhagic fever: its history and resurgence as a global public health problem, pp. 1-22. *In* D. J. Gubler and G. Kuno [eds.] Dengue and Dengue Hemorrhagic Fever. CAB International, London.

Hemingway, J., and G. P. Georghiou. 1983. Studies on malathion on the acetylcholinesterase of *Anopheles albimanus* resistant and susceptible to organophosphate and carbamate insecticides. Pest. Biochem. Physiol. 19: 167-171.

Hemingway J., and H. Ranson. 2000. Insecticide resistance in insect vectors of human disease. Ann. Rev. Entomol. 45: 371-391.

Hemingway, J., R. G. Boddington, and J. Harris. 1989. Mechanisms of insecticide resistance in *Aedes aegypti* (L.) (Diptera: Culicidae) from Puerto Rico. Bull. Entomol. Res. 79: 123-130.

Kadous, A. A., S. M. Ghiasuddin, F. Matsumura, J. G. Scott, and K. Tanaka. 1983. Difference in the picrotoxinin receptor between the cyclodiene resistant and susceptible strains of the German-cockroach. Pest. Biochem. Physiol. 19: 157–166.

Martinez-Torres, D., F. Chandre, M. S. Williamson, F. Darriet, J. B. Serge, A. L. Devonshire, P. Guillet, N. Pasteur, and D. Pauron. 1998. Molecular characterization of pyrethroid knockdown resistance (kdr) in the major malaria vector *Anopheles gambiae s.s.* Insect Molec. Biol. 7: 179-184.

Mazarri, M. B., and G. R. Georghiou. 1995. Characterization of resistance to organophosphate, carbamate, and pyrethroide insecticides in field populations of *Aedes aegypti* from Venezuela. J. Am. Mosq. Control Assoc. 11: 315-322.

Mekuria, Y., T. A. Gwinn, D. C. Williams, and T. A. Tidwell. 1991. Insecticide susceptibility of *Aedes aegypti* from Santo Domingo, Dominican Republic. J. Am. Mosq. Control Assoc. 7: 69-72.

Nelson, D. R., L. Koymans, T. Kamataki, J. J. Stegeman, R. Feyereisen, D. J. Waxman, M. R. Waterman, O. Gotoh, M. J. Coon, R. W. Eastbrook, I. C. Gunsalus, and D. W. Nebert. 1996. P450 superfamily: update on new sequences, gene mapping, accession numbers and nomenclature. Pharmacogenetics 6: 1-42.

Norma Oficial Mexicana. 2002. NOM-032-SSA2-2002, Para la vigilancia epidemiológica, prevención y control de enfermedades transmitidas por vector.

Oppenoorth, F. J. 1985. Biochemistry and genetics of insecticide resistance, pp. 731-773. *In* G. A. Kerkut and L. I. Gilbert [eds.] Comprehensive Insect Physiology Biochemistry and Pharmacology 12. Pergamon Press, Oxford.

Schiliessman, D. J., and L. B. Calheiros. 1974. A review of the status of yellow fever and *Aedes aegypti* eradication programs in the Americas. Mosq. News 34: 1-9.

Vaughan, A., D. D. Chadee, and R. Frrench-Constant. 1998. Biochemical monitoring of organophosphorus and carbamate insecticide resistance in *Aedes aegypti* mosquitoes from Trinidad. Med. Vet. Entomol. 12: 318-321.