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RESEARCH ARTICLE

Insecticide resistance of *Stegomyia aegypti* (Diptera: Culicidae) population from Paranaguá a port city in southern Brazil

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ABSTRACT. *Stegomyia aegypti* (Linnaeus, 1762) vectors arboviruses of public health concern in urban areas of tropical countries, so it is necessary to reduce its population. Among the control methods used, chemically synthesized molecules have been widely employed, nonetheless, the over usage of the same mechanism of action can result in the resistance selection. Considering the influence of insect resistance with the success of chemical control of vectors, this study aims to assess the susceptibility to organophosphorus of a population of *S. aegypti* from Paranaguá (Paraná, Brazil), after intense use of malathion during a dengue outbreak. World Health Organization susceptibility tests and expression of Acetylcholinesterase ace-1, cytochrome P_{450} monooxygenase CYP6N12, and α-esterases CCEae3A genes were evaluated. The mortality rate of wild females (66.5%) indicated their resistance status, furthermore, a new discriminant concentration was detected in this population (3.41%). Exclusively CYP6N12 gene was overexpressed in malathion-resistant females indicating its possible contribution to the transformation of this insecticide. Constant monitoring of insecticide resistance of current and past molecules, mainly in port areas where there is a large flow of species, is crucial for effective use of insecticide in vector control programs.

KEY WORDS. Acetylcholinesterase, *Aedes*, cytochrome P_{450} monooxygenase, discriminant concentration, malathion, α-esterase.

INTRODUCTION

Stegomyia aegypti (=*Aedes aegypti*) (Linnaeus, 1762) is a globally distributed specie known for its highly anthropophilic and synanthropic preferences. Its proliferation has been favored by factors such as global warming and specific socioeconomic conditions (Kraemer et al. 2015, Alaniz et al. 2018, Rios et al. 2023). Notably, *S. aegypti* is a significant vector of viruses of human concern, including dengue, Zika, chikungunya, and yellow urban fever. The primary approach to preventing diseases caused by these arboviruses relies on effective vector control since vaccines, although effective, are often in limited supply (Valle et al. 2019). Successful vector control hinges on effective monitoring and understanding the vector's susceptibility to the chemical molecules used for its managment.

In 2016, Dengue outbreaks had a significant impact on the human populations of Paranaguá – Paraná (Brazil), resulting in approximately 15,538 cases and 29 deaths (SESA 2016). Additionally, there were nine autochthonous cases of chikungunya in 2016 and one case of Zika virus in 2017 (SESA 2021). To suppress mosquitos, several measures can be taken, including removing potential breeding sites, using bioinsecticides, and employing genetic tools. However, synthetic insecticides are still used to control the vector. Within conventional insecticides, organophosphorus based products

have raised concern in different regions of the world due to the development of selective resistance in *S. aegypti* (Campos et al. 2020, Hayd et al. 2020, Hidajat et al. 2020, Leandro et al. 2020, Cattel et al. 2021).

Organophosphorus insecticides are widely known for their effectiveness in inhibiting acetylcholinesterase (AChE) activity, disrupting neural impulses transmission. This disruption results in hyperexcitation of the nervous system, ultimately leading to the insect's death (Ware and Whitacre 2004, Araújo et al. 2016). During the malathion metabolism, the cytochrome P_{450} monooxygenases activate it to malaoxon, a more toxic compound than its parent and an irreversible AChE inhibitor (Feyereisen 1999, Scott 1999, Hemingway et al. 2004, Ware and Whitacre 2004). In addition to the cytochrome P_{450} monooxygenases (P_{450}), other enzymes such as esterases (EST) and glutathione-S-transferases (GST) play crucial roles in the biotransformation of insecticides to prevent them from reaching the target site (Hemingway et al. 2004, Li et al. 2007, Moyes et al. 2017).

The extensive use of insecticides to reduce temporary vector populations has resulted in increased resistance among specimens, posing risks to human, animal, and environmental welfare (Valle et al. 2019, Nunes et al. 2021, van den Berg et al. 2021). Resistance has been identified throughout assays, including bottle assays and filter paper impregnaion (WHO 2022a, 2022b). Insecticide resistance can be attributed to mechanisms such as penetration barriers, behavioral adaptation, enhanced metabolic detoxification, or target site insensitivity (Siegfried and Scharf 2001, Hemingway et al. 2004, Moyes et al. 2017, Nunes et al. 2021, van den Berg et al. 2021). Metabolic resistance occurs when enzymes like P_{450}, GST, and EST increase their capacity to metabolize or sequester the insecticide before it reaches its target (Hemingway and Ranson 2000). Among these enzymes, P_{450} notably overexpressed in individuals resistant to insecticides containing organophosphorus (Scott 1999). Certain P_{450} genes of the CYP6 subfamily have been recurrently associated with insecticide resistance in mosquitoes (Lima et al. 2015). Furthermore, metabolic resistance to organophosphorus insecticide is associated with the overexpression of α-esterases, such as CCEae3a (Goindin et al. 2017, Marcombe et al. 2019).

Acetylcholinesterase acts on insecticide resistance through molecular and metabolic mechanisms (Labbé et al. 2007, Djogbénou et al. 2008, Edi et al. 2014). In Culicidae, mutations in the ace-1 gene – which codes for the target site of organophosphorus – have been associated with the selection of resistance to these compounds (Weill et al. 2002, 2004, Hemingway et al. 2004). *Stegomyia aegypti*, however, rarely has mutations in the ace-1 gene that considerably affect resistance to organophosphorus (Bisset et al. 2006, Mori et al. 2007, dos Santos et al. 2020). Despite that, enzyme activity and active site insensitivity to insecticides are still commonly evaluated (Viana-Medeiros et al. 2018, Valle et al. 2019).

Recognizing that monitoring through susceptibility bioassays and the identification of molecular mechanisms are essential to prevent or delay the resistance selection, and to preserve the effectiveness of active ingredients (Hemingway and Ranson 2000), in Paranaguá, home to one of the most important ports in Brazil (ANTA 2018, Portos do Paraná 2022), the intensive use of a single chemical compound to control the vector during a dengue epidemic exerted selection pressure on mosquitoes, particularly with regard to malathion. The knowledge of the state of resistance in situations like this is extremely relevant for future decision-making. This study aimed to evaluate the susceptibility to malathion of *S. aegypti* from Paranaguá after a dengue outbreak when chemical control was an intensive method to control the mosquito population. Furthermore, the study intended to evaluate the expression of genes such as acetylcholinesterase, cytochrome P_{450} monooxygenase, and α-esterases, which are related to organophosphorus resistance.

MATERIAL AND METHODS

Study area

The municipality of Paranaguá is in the Southeastern of Paraná (PR), southern Brazil, at 25°31'12"S, 48°30'33"W, and 5 m asl, an area situated in the Atlantic Forest Biome (Mello et al. 2017) (Fig. 1A). It has an extension of 826,431 km² and an estimated population of 145,829 inhabitants with a demographic density of 177.23 inhabitants per km² (IBGE 2022). The city hosts the Dom Pedro II Port – also named Porto de Paranaguá – where national and international cargoes are transported. It is Paraná's main port and the third most important in Brazil (ANTA 2018, Portos do Paraná 2022). The study was carried out in this municipality in 2018, two years after the 2016 dengue epidemic, which had 15,538 confirmed cases that triggered 29 deaths. This public health situation led to the intensive use of malathion, a product recommended from 2010 to 2019 by the Ministry of Health, to carry out adult control of the vector (Brazil 2009).

Field collection

Stegomyia aegypti females were obtained from eggs collected in June 2018 using the ovitrap proposed by Fay and Eliason (1966), which consists of a 900 mL black container

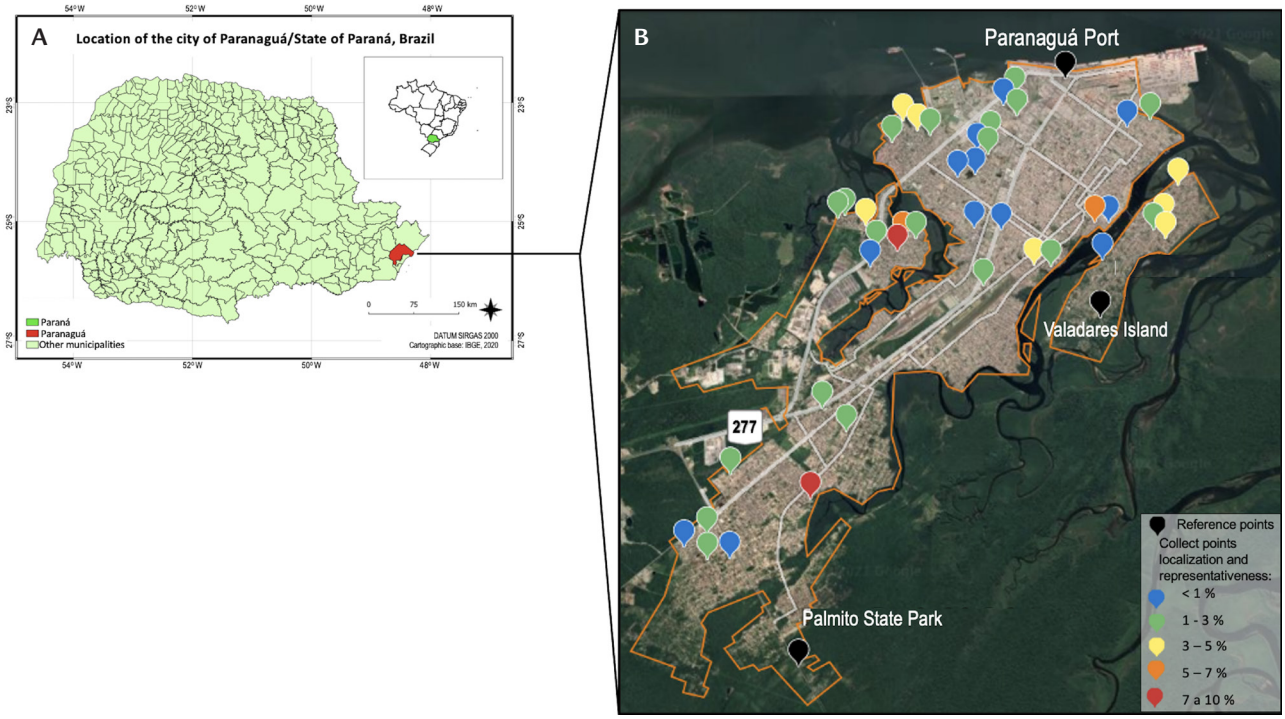

Figure 1. (A) Location of the city of Paranaguá, and (B) location of ovitraps containing the eggs obtained during the June 2018 monitoring cycle in the city. Colors depict the representativeness of each point in the establishment of the *S. aegypti* insectary used to obtain the F_1 and F_2 generations.

containing 250 mL of tap water and an oviposition substrate of wooden reed of 14 cm × 3 cm (Duratree). This field collect was part of the monthly monitoring cycles carried out in the municipality from 2017 to 2018 that included the evaluation of 331 ovitraps installed in house gardens, where they remained for four days. Thus, F_1 and F_2 generations were obtained from 30 ovitraps selected randomly in the June 2018 (Fig. 1B) aiming to include the genetic diversity of the population. This collection occurred after the application of malathion at ultra-low volume (approximately 4200 L) by the Ministry of State Health along with the Paranaguá Municipal Health Administration as an activity of the integrated vector management program to reduce the vector density since it was increasing in the city. Eggs were counted and fully grown in the Laboratory of Morphology and Physiology of Culicidae and Chironomidae (LAMFIC[2]) at the Departamento de Zoologia, Universidade Federal do Paraná.

Mosquito rearing

In order to maintain the genotypic variability and ensure spatial representation of the local population, eggs from more than 30 ovitraps were used to make an insectary, as recommended by the WHO (2016). To establish an insectary, adults of *S. aegypti* were placed in cages kept in a room at 25 ± 3 °C, 70% of RH, under natural light and fed a 10% honey sugar solution. In addition, they were supplied with blood from a Swiss strain of mice twice a week for 10 minutes under the authorization of the Ethics Committee on Animal Experimentation (UFPR #719). Females laid eggs on filter papers partially submerged in water. The filter papers were collected twice a week, dried at room temperature for 24 hours, and packed in paper bags.

Second generation adult females (F_2) were used to perform susceptibility bioassays and the gene expression study. As a reference, Rockefeller mosquitoes' strain was used in all experiments (Kuno 2010). This strain was obtained from the Laboratory of Physiology and Control of Arthropod Vector (Laficave) at Fundação Oswaldo Cruz, Rio de Janeiro, and has been kept in LAMFIC[2] under the aforementioned conditions for the establishment of the insectary.

WHO bioassays

Insecticide sensitivity tests were performed using the World Health Organization (WHO) filter paper method (WHO

2009). Whatman® grade 1 filter papers (12 × 12 cm) were impregnated with malathion 98%, diethyl 2-[(dimethoxyphosphorothioyl)sulfanyl]butanedioate – Pestanal® (CAS: 121-75-5, lot number BCBS8709V with expiration date August 2021 Sigma-Aldrich) and organic extra virgin olive oil (0.91 g L^{-1}) was used as a carrier and diluent to obtain the desired insecticide concentration. The olive oil and insecticide mixture were weighed for density adjustment, then, in order to achieve uniform distribution, the same volume of acetone was added before the paper impregnation. Filter papers impregnated exclusively with olive oil and acetone were used as a control group. After impregnation, which was performed using a pipette, the filter papers were dried in the dark for 24 hours, then stored individually in aluminum foil at 4 °C until use.

Discriminating concentration of malathion and bioassays

The discriminating concentration (DC) of 0.8%, as recommended by the WHO (2016) did not result in mortality of *S. aegypti* females of the Rockefeller strain. For this reason, a new DC was determined to be twice the LC_{99} (lethal concentration) of the susceptible strain, following the guidelines established by the WHO (2016). The concentration-response curve was estimated with the following quantities of malathion impregnated in the filter papers: 20 µg cm^{-2} (0.8%), 25 µg cm^{-2} (1.1%), 32.5 µg cm^{-2} (1.3%), 35 µg cm^{-2} (1.4%), and 35.5 µg cm^{-2} (1.42%). The DC was estimated by analyzing the mortality data through Probit regression using the "ecotoxicology" package (Gama 2015) in the R software (v.3.0.1) (R Core Team 2018).

The mortality bioassay was carried out according to the WHO (2016) guidelines. Groups of 20 to 25 susceptible 3–4 days-old, non-blood fed females were inserted into tubes containing the malathion-impregnated paper and held for one hour. The tubes were placed horizontally in a dark chamber. After insecticide exposure, the females were transferred by being gently blown into a resting tube containing a clean filter paper, which was placed vertically into a BOD-type chamber under a photoperiod of 12:12, room temperature of 26 ± 2 °C, and average relative humidity of 72 ± 3%. The mosquitoes were fed a 10% honey sugar solution. Mortality rate was evaluated after 24 hours of exposure. At least 100 females, including the control groups, were exposed to each concentration.

Susceptibility bioassays with the *S. aegypti* population from Paranaguá were carried out both with the new DC of 85.25 µg cm^{-2} (3.41%) and a concentration of 125 µg cm^{-2} (5%), in compliance with the WHO (2016) guidelines. A total of 1,197 F_2 females were exposed to the new DC and 321 to the control. Similarly, 55 females of the Rockefeller strain were exposed to the new DC and 23 to the control. For the second bioassay, 290 females from the Paranaguá F_2 colony were exposed to the 5% concentration of malathion (125 µg cm^{-2}), whereas 75 were exposed to the control paper. In the case of the Rockefeller strain, 81 females were exposed to the same concentration. Finally, 154 of them were exposed to the control. Paranaguá and Rockefeller females that survived after 24 hours of exposure were frozen and stored at -80 °C to ensure the integrity of the nucleic acids to be later processed in the molecular experiments.

CCEae3A, CYP6N12, and ace-1 expression levels

Total RNA was extracted from female of *S. aegypti* individual using the RNeasy® Mini Kit (Qiagen) extraction kit, which was eluted in 30 µL of RNase-free water and treated with Deoxyribonuclease I (DNase I) (Sigma Aldrich), according to the instructions provided by the manufacturer. RNA quantification and purity analysis were carried out using a Nanodrop™ 2000 spectrophotometer, and integrity was evaluated using a 1% agarose gel. After treatment, the samples were quantified again by fluorescence in a Quantus™ Fluorometer (Promega) using the QuantiFluor® dsDNA System kit (Promega), as recommended by the manufacturer. Complementary DNA (cDNA) synthesis was performed using the High-Capacity cDNA Reverse Transcription Kit® (Thermo Fisher Scientific) also in accordance with the manufacturer's instructions. The samples were stored in a freezer at -20 °C.

The expression levels of α-esterase (CCEae3A), P_{450} (CYP6N12), and acetylcholinesterase (ace-1) genes of *S. aegypti* females were evaluated using the oligonucleotides shown in Table 1. Their access codes in GenBank are AAEL005112 (α-esterase), AAEL009124 (P_{450}), AAEL000511 (ace-1), as well as AAEL011197 (actin 5C) and AAEL004175 (Ribossomal Protein S17 40S) for the housekeeping genes.

The gene expression of 24 females that survived exposure to malathion DC (T1) was compared with those of the control groups. One of the control groups consisted of 10 unexposed females of the Rockefeller strain (C1_R). The second group comprised 14 unexposed females from Paranaguá (C2_Pr). Lastly, the third control group included 10 females from Paranaguá that were exposed to the filter paper impregnated with acetone and olive oil (C3_Pr).

Reactions of qPCR were performed in 10 µL of final volume, using 1 ng of cDNA, 5 µL of PowerUp SYBR Green Master Mix® solution (Thermo Fisher Scientific), 0.8 µM of forward primer, 0.8 µM of reverse primer, and 1.4 µL of ultrapure water (Milli-Q). Samples were analyzed using a Rotor Gene Q5 Plex thermocycler (Qiagen). The temperature

Table 1. Forward and reverse primers are used to quantify *S. aegypti* gene expression by real-time polymerase chain reaction with amplicon size, hybridation region, efficiency, and R^2 values.

Enzyme	Gene	Primers (5'-3')	Amplicon (pb)	Hybridation	Efficiency (%)	R^2
Acetilcolinesterase	ace-1	F-GCAATCGGGATGCATTGGAC	81	Exon 7	101.8	0.98
		R-CTGCATATCGCTGGGCAAAC		Exon 7		
Cytochrome P_{450} monooxygenase	CYP6N12	F-GATCAATGTATCAACGAGTCCC	165	Exon 1-2	96.6	0.99
		R-GGGATAGTATTCTGCGTCGT		Exon 2		
α-esterase	CCEae3A	F-GCAATATGTCAGTCGGGAGT	129	Exon 1	100.6	0.99
		R-GAGGGTTTCGTAAATTTCATCATCG		Exon 1-2		
Actin 5C	Act-5C	F-CGTTCGTGACATCAAGGAAA	175	Exon 2	97.3	0.99
		R-GAACGATGGCTGGAAGAGAG		Exon 2		
Ribosomal Protein S17 40S	RPS17	F-AAGAAGTGGCCATCATTCCA	200	Exon 2	100.4	0.98
		R-GGTCTCCGGGTCGACTTC		Exon 3		

ramp was applied as follows: two minutes of waiting at 50 °C, two minutes of initial denaturation at 95 °C, 40 cycles of 15 seconds at 95 °C, hybridization for 15 seconds at 53 °C, and extension for 60 seconds at 72 °C. The efficiency of the qPCR reaction was evaluated using a dissociation curve, 0.8 μM of each primer, and a gradient of cDNA concentrations with a 1:5 serial dilution. All qPCR reactions were performed in triplicate, including a control reaction (ultrapure water). The acceptable difference in cycle threshold (Cts) between the replicates was at most 0.5 (Bio-Rad 2013). Relative gene expression was estimated using the $2^{-\Delta\Delta CT}$ comparative method between target genes and assuming a reaction efficiency of 100% between genes (Livak and Schmittgen 2001). The method was adapted to the housekeeping genes actin-1 and ribosomal protein S17 (Riedel et al. 2014, Dzaki et al. 2017).

Outliers from the gene expression data were removed using the Outlier calculator GraphPad software and a significance level of $\alpha = 0.05$. Statistical analyses were performed using the GraphPad Prism v. 5.0 software. The distributions of the expression data were observed using the Kolmogorov-Smirnov test, which considers the residual value of the regression results (Massey 1951). Comparison between groups was performed using the nonparametric Kruskal-Wallis test to identify any significant differences between median values of the groups (Kruskal and Wallis 1952). And Dunn's post hoc test was used to detect which specific median value was significant from the others according to a pair-wise comparison (Dunn 1961). The level of significance was $\alpha = 0.05$.

RESULTS

Malathion discriminating concentration.

The mortality rate of the Rockefeller strain females is shown in Table 2.

Table 2. Mortality rate of *S. aegypti* females of the Rockefeller strain exposed to malathion concentrations ranging from 0.8% to 1.42%.

Malathion quantity		Female response			
μg cm^{-2}	%	Alive	Dead	Total	Mortality rate (%)
20.0	0.80	109	26	135	19.3
25.0	1.10	139	131	270	48.5
32.5	1.30	31	94	125	75.2
35.0	1.40	6	141	147	95.9
35.5	1.42	7	135	142	95.1
Control	0	119	0	119	0

The amount of malathion necessary to kill 99% (LC_{99}) Rockefeller females was 42.7 μg a.i cm^{-2} (1.7%) (Table 3). The mortality curve and the upper and lower limits of the response to malathion concentrations are shown in Fig. 2. The control group mortality rate was less than 5%, hence no data were adjusted to perform the susceptibility test (Silva et al. 2007).

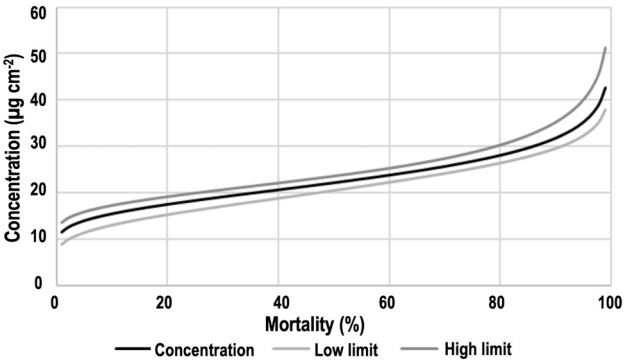

Figure 2. Mortality curve showing the upper and lower limits for each concentration of malathion exposure for *S. aegypti* of the Rockefeller strain.

Table 3. Response to malathion concentration in virgin Rockefeller females. (N) Number of females used in the bioassay, (SD) standard deviation, (LC) lethal concentration, (CI) confidence interval; (χ^2) chi-square, (DF) degree of freedom.

Strain	N	Slope (SD)	$LC_{99}(CI_{95})$ µg cm^{-2} %		χ^2	DF	p-value
Rockefeller	819	8.184 ± 0.64	42.70 (38.00–51.20)	1.70 (1.50–2.00)	3.22	3	0.64

The estimated discriminating concentration was 3.41%, which corresponds to 85.25 µg cm^{-2} of active ingredient.

Resistance of *S. aegypti* from Paranaguá to malathion

In total, 1,518 adult females collected in 2018 in Paranaguá were exposed to malathion at concentrations of 3.41% and 5%, which caused a mortality rate of 66.5% and 96.89%, respectively (Table 4). This result indicates that, considering the WHO criteria, the *S. aegypti* population in Paranaguá is resistant to malathion (WHO 2016). Rockefeller females exhibited a 100% mortality rate, as expected.

Table 4. Mortality of virgin females of *S. aegypti* of both the Rockefeller strain and the Paranaguá population (F_2) after exposure to 3.41% and 5% of malathion using the WHO filter paper method.

Female origin	Concentration	Female response	N	Total	Mortality (%)
Paranaguá (F_2)	3.41 %	Alive	401	1197	66.5
		Dead	796		
	0 %	Control	317	317	0.01
Rockefeller	3.41%	Alive	0	55	100.0
		Dead	55		
	0 %	Control	23	23	0
Paranaguá (F_2)	5.00 %	Alive	9	290	96.9
		Dead	281		
	0 %	Control	75	75	0
Rockefeller	5.00 %	Alive	0	81	100.0
		Dead	81		
	0 %	Control	154	154	0.01

Gene expression

Gene expression levels of ace-1 and α-esterase from females in the resistant population (T1) showed no difference when compared to those in the control groups (C1_R, C2_Pr, and C3_Pr) (Fig. 3A, 3B), indicating that these enzymes remained unchanged after malathion exposure. Gene expression in the control groups of Paranaguá females (C2_Pr and C3_Pr) and the Rockefeller strain (C1_R) did not differ.

The gene expression of cytochrome P_{450} monooxygenase from females exposed to malathion (T1) was higher when compared to unexposed females (C2_Pr) (Fig. 3C). The gene expression of females of the Paranaguá control groups (C2_Pr and C3_Pr) and the Rockefeller strain (C1_R) were similar.

DISCUSSION

This study shows a new discriminating concentration established under laboratory conditions for malathion and the insecticide susceptibility results for the *S. aegypti* females from Paranaguá collected in 2018. The DC obtained (3.41%) was 4.25-fold higher than that recommended by the WHO (0.8% and 1.5%) (WHO 1992, 2016, 2022a). The low mortality rate of *S. aegypti* females when exposed to a 0.8% concentration of malathion was also observed by Macoris (Macoris et al. 2007). Probably due to the low or null response, several studies have used a DC of 5%, which is recommended for monitoring malathion susceptibility in *Anopheles* females (Ocampo et al. 2011, Arslan et al. 2016, Kamgang et al. 2017, Bharati and Saha 2018, Soni et al. 2018). In any case, the estimated DC in this study was different from that of others (Karunaratne et al. 2013, Hayd et al. 2020).

In addition to presenting suitable weather conditions for the vector development, (Vanhoni and Mendonça 2008), Paranaguá has a constant flow of national and international transport by land and sea, which allows the genetic flow of the species – including those with some degree of resistance to malathion (Diaz-Nieto et al. 2016, De Sá et al. 2019, Díaz-Maitra et al. 2019, Schmidt et al. 2019), as already described in other cities in the state of Paraná (Campos et al. 2020, Leandro et al. 2020).

Although this study did not aim to evaluate *S. aegypti*'s gene flow, it may have influenced the resistance phenotype of this vector in Paranaguá, as well as the selection pressure resulted from the chemical control practices implemented to prevent the transmission of the arbovirus (Valle et al. 2019). Likewise in 2015 and 2016, the chemical control practices in Paranaguá increased due to the occurrence of a dengue epidemic that caused many infections and deaths in the local population (SESA 2016). This epidemic event confirms the challenges faced in effectively controlling vector populations (Smith et al. 2012, Achee et al. 2015), leading to intensive insecticide application (Garcia et al. 2018, Leandro et al. 2020).

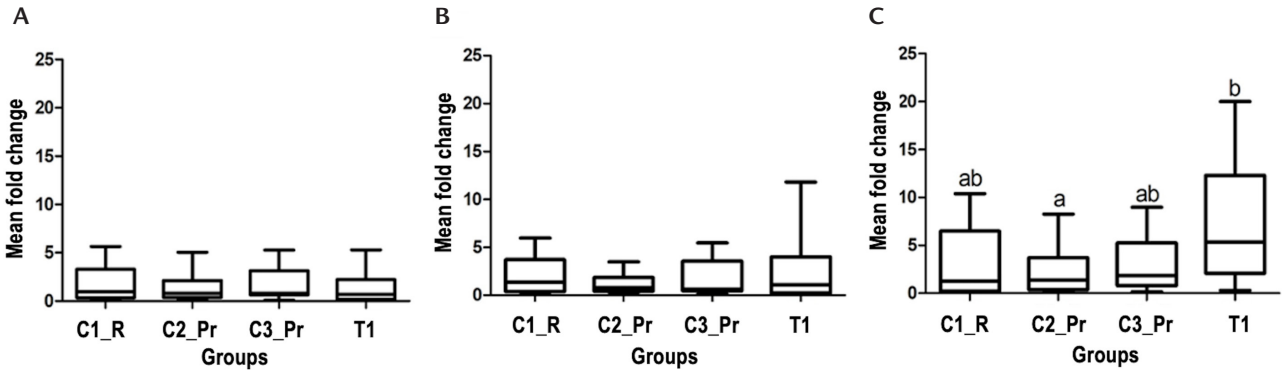

Figure 3. Relative gene expression of the (A) acetylcholinesterase (ace-1), (B) α-esterase (CCEae3A), and (C) cytochrome P_{450} (CYP6N12) genes in *S. aegypti* females. C1_R: Rockefeller control group (n=10); C2_Pr: Paranaguá control group (n=14); C3_Pr: Paranaguá solvent control group (n=10); T1: Paranaguá resistant females after exposure to malathion at a concentration of 3.41% (n=24). Data are presented as median ± interquartile range. Different letters indicate significant difference at $p < 0.05$, according to Dunn's post hoc tests.

The lack of data on the susceptibility status of the *S. aegypti* population in Paranaguá before and during the epidemic of dengue did allow us to establish a correlation between the effect of chemical control and the frequency of resistant mosquitoes in the city. Thus, this is the first time that the malathion susceptibility of the *S. aegypti* population was studied after the epidemic that occurred in Paranaguá during 2015 and 2016 (SESA 2016, Brazil 2020).

Stegomyia aegypti resistance to malathion has been reported in other cities in the state of Paraná, including Foz do Iguaçu, Londrina, and Maringá (Campos et al. 2020, Leandro et al. 2020). This resistance has also been suggested for the cities of Paranavaí and Francisco Beltrão (Campos et al. 2020). This situation reinforces the need to reduce the vector's chemical control and to prioritize other prevention and control methods that reduce risks to the animal, human, and environmental welfare (Nunes et al. 2021).

The gene expression in resistant females exhibited an increase in cytochrome P_{450} monooxygenase (CYP6N12) as a response to malathion exposure, whereas α-esterase and ace-1 showed no significant alterations. The over expression of CYP6N12 may be related to a metabolic alteration either in the desulfuration reaction to form an active oxon metabolite that is the anticholinesterase compound, or in the dearylation reaction to form an inactive metabolite (Phosphorothioate) (Buratti et al. 2005, Krieger 2010). Also, increased CYP6N12 expression could be associated with gene amplification (Bariami et al 2012, Vlogiannitis et al. 2021).

Increased P_{450} expression was observed following exposure to organophosphorus, pyrethroids, and neonicotinoids (Strode et al. 2012, Vontas et al. 2012, Riaz et al. 2013, Reid et al. 2014, Saavedra-Rodriguez et al. 2019). In contrast to our findings, a malathion-resistant *S. aegypti* population from Recife, northeastern Brazil, exhibited no changes in P_{450} expression (Thornton et al. 2020). However, a malathion susceptible and temephos resistant population – previously selected in laboratory for 20 generations – exhibited overexpression of the same P_{450} gene evaluated in our study (Strode et al. 2012). The expression of other P_{450} genes – such as CYP6M11, CYP9J28, and CYP6BB2 – has also been associated with organophosphorus resistance (Goindin et al. 2017, Thornton et al. 2020).

Considering the involvement of the cytochrome P_{450} monooxygenase in the resistance to pyrethroids, neonicotinoids, and organophosphorus (Strode et al. 2012, Vontas et al. 2012, Riaz et al. 2013, Reid et al. 2014, Saavedra-Rodriguez et al. 2019), the overexpression of this enzyme in the *S. aegypti* population from Paranaguá can also be related to pyrethroid resistance, which is reinforced by the domestic use of pyrethroids. Pyrethroids and neonicotinoids are commercially available, which makes their domestic use for vector control accessible (SESA 2019, Valle et al. 2019, Paraná 2020).

In the current study was not identified an overexpression of α-esterase, although resistance due to increased esterase metabolism caused by point mutations in the gene are very frequent in malathion-resistant mosquitoes (Hemingway 2000, Hemingway et al. 2004). Considering the overexpression mechanism, our results suggest low or no α-esterase participation in malathion resistance in the Paranaguá population. However, it is possible that another

esterase-related mechanism may be related to the studied population's resistance.

Paranaguá resistant females showed no variations in ace-1 expression, contrary to the results observed in *Culex* and *Anopheles* mosquitoes (Labbé et al. 2007, Djogbénou et al. 2008, Edi et al. 2014). Mutations in the ace-1 gene involved in resistance mechanisms to organophosphorus in *S. aegypti* are seldom reported (Bisset et al. 2006, Mori et al. 2007, dos Santos et al. 2020).

This study highlights the need to monitor insecticide resistance as a strategy to mitigate the adverse effects of chemical control, as well as the need to prevent the occurrence of health problems caused by arboviruses.

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LITERATURE CITED

Achee NL, Gould F, Perkins TA, Reiner RC, Morrison AC, Ritchie SA, Gubler DJ, Teyssou R, Scott TW (2015) A Critical Assessment of Vector Control for Dengue Prevention. Plos Neglected Tropical Diseases 9: 1–19. https://doi.org/10.1371/journal.pntd.0003655

ANTA (2018) Movimentação portuária cresce 2,7% em 2018. Agência Nacional de Transportes Aquaviários, Brasil. Available online at https://www.gov.br/antaq/pt-br/noticias/copy2_of_2021/movimentacao-portuaria-cresce-2-7-em-2018 [Accessed: 13/08/2022]

Alaniz AJ, Carvajal MA, Bacigalupo A, Cattan PE (2018) Global spatial assessment of *Aedes aegypti* and *Culex quinquefasciatus*: a scenario of Zika virus exposure. Epidemiology & Infection: 1–11. https://doi.org/10.1017/S0950268818003102

Araújo CRM, Santos VLA, Gonsalves AA (2016) Acetylcholinesterase – AChE: A pharmacological interesting enzyme. Revista Virtual de Química 8(6): 1818–1834. https://doi.org/10.21577/1984-6835.20160122

Arslan A, Rathor HR, Mukhtar MU, Mushtaq S, Bhatti A, Asif M, Arshad I, Ahmad JF (2016) Spatial distribution and insecticide susceptibility status of *Aedes aegypti* and *Aedes albopictus* in dengue affected urban areas of Rawalpindi, Pakistan. Journal of Vector Borne Diseases 53(2): 136–143.

Bariami V, Jones CM, Poupardin R, Vontas J, Ranson H (2012) Gene Amplification, ABC Transporters and Cytochrome P450s: Unraveling the Molecular Basis of Pyrethroid Resistance in the Dengue Vector, *Aedes aegypti*. Plos Neglected Tropical Diseases 6(6): e1692. https://doi.org/10.1371/journal.pntd.0001692

Bharati M, Saha D (2018) Multiple insecticide resistance mechanisms in primary dengue vector, *Aedes aegypti* (Linn.) from dengue endemic districts of sub-Himalayan West Bengal, India. Plos One 13: 1–13. https://doi.org/10.1371/journal.pone.0203207

Bio-Rad (2013) Reagent Comparison Guide for Real-Time PCR. BioRad Bulletin 6252.

Bisset J, Rodríguez MM, Fernández D (2006) Selection of insensitive acetylcholinesterase as a resistance mechanism in *Aedes aegypti* (Diptera: Culicidae) from Santiago de Cuba. Journal of Medical Entomology 43: 1185–1189. https://doi.org/10.1603/0022-2585(2006)43[1185:SOIAAA]2.0.CO;2

Brazil (2009) Diretrizes Nacionais para a Prevenção e Controle de Epidemias de Dengue. Ministério da Saúde, Brasília, 160 pp.

Brazil (2020) Monitoramento dos casos de Arboviroses urbanas transmitidas pelo *Aedes* (dengue, chikungunya e Zika), semanas epidemiológicas 01 a 52 Boletim epidemiológico 2. Ministério da Saúde, Secretaria de Vigilância em Saúde. Available online at: https://www.gov.br/saude/pt-br/centrais-de-conteudo/publicacoes/boletins/epidemiologicos/edicoes/2020/boletim-epidemiologico-vol-51-no-02.pdf [Accessed: 20/08/2020]

Buratti FM, D'Aniello A, Volpe MT, Meneguz A, Testai E (2005) Malathion bioactivation in the human liver: the contribution of different cytochrome p450 isoforms. Drug Metabolism and Disposition: The Biological Fate of Chemicals 33(3): 295–302. https://doi.org/10.1124/dmd.104.001693

Campos KB, Martins AJ, Rodovalho CM, Bellinato DF, Dias LS, Macoris MLG, Andrighetti MTM, Lima JBP, Obara MT (2020) Assessment of the susceptibility status of *Aedes ae-*

gypti (Diptera: Culicidae) populations to pyriproxyfen and malathion in a nation-wide monitoring of insecticide resistance performed in Brazil from 2017 to 2018. Parasites & Vectors 1: 531. https://doi.org/10.1186/s13071-020-04406-6

Cattel J, Haberkorn C, Laporte F, Gaude T, Cumer T, Renaud J, Sutherland IW, Hertz JC, Bonneville J, Arnaud V, Fustec B, Boyer S, Marcombe S, David J (2021) A genomic amplification affecting a carboxylesterase gene cluster confers organophosphate resistance in the mosquito *Aedes aegypti*: From genomic characterization to high-throughput field detection. Evolutionary Applications 14(4): 1009–1022. https://doi.org/10.1111/eva.13177

De Sá ELR, Rodovalho CDM, De Sousa NPR, De Sá ILR, Bellinato DF, Dias LDS, Da Silva LC, Martins AJ, Lima JBP (2019) Evaluation of insecticide resistance in *Aedes aegypti* populations connected by roads and rivers: The case of Tocantins state in Brazil. Memórias do Instituto Oswaldo Cruz 114: 1–10. https://doi.org/10.1590/0074-02760180318

Diaz-Maitra A, Cunha-Machado AS, Souza LA, Costa FM, Scarpassa VM (2019) Exploring deeper genetic structures: *Aedes aegypti* in Brazil. Acta Tropica 195: 68–77. https://doi.org/10.1016/j.actatropica.2019.04.027

Díaz-Nieto LM, Chiappero MB, Díaz-de-Astarloa C, Maciá A, Gardenal CN, Berón CM (2016) Genetic Evidence of Expansion by Passive Transport of *Aedes (Stegomyia) aegypti* in Eastern Argentina. Plos Neglected Tropical Diseases 10: e0004839. https://doi.org/10.1371/journal.pntd.0004839

Djogbénou L, Chandre F, Berthomieu A, Dabiré R, Koffi A, Alout H, Weill M (2008) Evidence of Introgression of the ace-1R Mutation and of the *ace*-1 Duplication in West African *Anopheles gambiae* s. s. Plos One 3: e2172. https://doi.org/10.1371/journal.pone.0002172

dos Santos CR, de Melo-Rodovalho C, Jablonka W, Martins AJ, Lima JBP, dos Santos-Dias L, da Silva-Neto MAC, Atella GC (2020) Insecticide resistance, fitness and susceptibility to Zika infection of an interbred *Aedes aegypti* population from Rio de Janeiro, Brazil. Parasites & Vectors 13: 293. https://doi.org/10.1186/s13071-020-04166-3

Dunn OJ (1961) Multiple comparisons among means. JASA 56: 54–64.

Dzaki N, Ramli KN, Azlan A, Ishak IH, Azzam G (2017) Evaluation of reference genes at different developmental stages for quantitative real-time PCR in *Aedes aegypti*. Scientific Reports 7: 43618. https://doi.org/10.1038/srep43618

Edi CV, Djogbénou L, Jenkins AM, Regna K, Muskavitch MAT, Poupardin R, Jones CM, Essandoh J, Kétoh GK, Paine MJI, Koudou BG, Donnelly MJ, Ranson H, Weetman D (2014) CYP6 P450 Enzymes and ACE-1 Duplication Produce Extreme and Multiple Insecticide Resistance in the Malaria Mosquito *Anopheles gambiae*. Plos Genetics 10: e1004236. https://doi.org/10.1371/journal.pgen.1004236

Fay RW, Eliason DA (1966) A preferred oviposition site as surveillance method for *Aedes aegypti*. Mosquito News 26: 531–535.

Feyereisen R (1999) Insect P450 enzymes. Annual Review of Entomology 44: 507–533. https://doi.org/10.1146/annurev.ento.44.1.507

Gama J (2015) Ecotoxicology: Methods for Ecotoxicology. Available online at https://cran.r-project.org/web/packages/ecotoxicology/index.html [Accessed: 03/03/18]

Garcia GA, David MR, Martins AJ, Maciel-de-Freitas R, Linss JGB, Araújo SC, Lima JBP, Valle D (2018) The impact of insecticide applications on the dynamics of resistance: The case of four *Aedes aegypti* populations from different Brazilian regions. Plos Neglected Tropical Diseases 12: e0006227. https://doi.org/10.1371/journal.pntd.0006227

Goindin D, Delannay C, Gelasse A, Ramdini C, Gaude T, Faucon F, David JP, Gustave J, Vega-Rua A, Fouque F (2017) Levels of insecticide resistance to deltamethrin, malathion, and temephos, and associated mechanisms in *Aedes aegypti* mosquitoes from the Guadeloupe and Saint Martin islands (French West Indies). Infectious Diseases of Poverty 6: 38. https://doi.org/10.1186/s40249-017-0254-x

Hayd RLN, Carrara L, de Melo Lima J, de Almeida NCV, Lima JBP, Martins AJ (2020) Evaluation of resistance to pyrethroid and organophosphate adulticides and kdr genotyping in *Aedes aegypti* populations from Roraima, the northernmost Brazilian State. Parasites & Vectors 13: 264. https://doi.org/10.1186/s13071-020-04127-w

Hemingway J (2000) The molecular basis of two contrasting metabolic mechanisms of insecticide resistance. Insect Biochemistry and Molecular Biology 30: 1009–1015. https://doi.org/10.1016/S0965-1748(00)00079-5

Hemingway J, Hawkes NJ, McCarroll L, Ranson H (2004) The molecular basis of insecticide resistance in mosquitoes. Insect Biochemistry and Molecular Biology 34: 653–665. https://doi.org/10.1016/j.ibmb.2004.03.018

Hemingway J, Ranson H (2000) Insecticide resistance in insect vectors of human disease. Annual Review of Entomology 45: 371–391. https://doi.org/10.1146/annurev.ento.45.1.371

Hidajat MC, Dharmana E, Prihatin MT, Martini, Ambargarjito T (2020) Molecular Resistance Status of *Aedes aegypti* to the Organophosphate and Pyrethroid Insecticides in Central Sulawesi and East Nusa Tenggara Provinces, Indonesia. Proceedings of the 5th Universitas Ahmad Dahlan Public Health Conference: 122–127. https://doi.org/10.2991/ahsr.k.200311.023

IBGE (2022) Paranaguá. Instituto Brasileiro de Geografia e Estatística, available online at: https://cidades.ibge.gov.br/brasil/pr/paranagua/panorama [Accessed: 13/08/2023]

Kamgang B, Yougang AP, Tchoupo M, Riveron JM, Wondji C (2017) Temporal distribution and insecticide resistance profile of two major arbovirus vectors *Aedes aegypti* and *Aedes albopictus* in Yaoundé, the capital city of Cameroon. Parasites & Vectors 10: 469. https://doi.org/10.1186/s13071-017-2408-x

Karunaratne SHPP, Weeraratne TC, Perera MDB, Surendran SN (2013) Insecticide resistance and, efficacy of space spraying and larviciding in the control of dengue vectors *Aedes aegypti* and *Aedes albopictus* in Sri Lanka. Pesticide Biochemistry and Physiology 107: 98–105. https://doi.org/10.1016/j.pestbp.2013.05.011

Kraemer MUG, Sinka ME, Duda KA, Mylne AQN, Shearer FM, Barker CM, Moore CG, Carvalho RG, Coelho GE, Van Bortel W, Hendrickx G, Schaffner F, Elyazar IR, Teng HJ, Brady OJ, Messina JP, Pigott DM, Scott TW, Smith DL, Wint GW, Golding N, Hay SI (2015) The global distribution of the arbovirus vectors *Aedes aegypti* and *Ae. albopictus*. Elife 4: 1–18. https://doi.org/10.7554/eLife.08347

Krieger RI (2010) Hayes' handbook of pesticide toxicology. Amsterdam, Elsevier, vol. 1, 3rd ed., 2342 pp. https://doi.org/10.1016/C2009-1-03818-0

Kruskal WH, Wallis WA (1952) Use of ranks in one-criterion variance analysis. Journal of the American Statistical Association 47: 583–621.

Kuno G (2010) Early History of laboratory breeding of *Aedes aegypti* (Diptera: Culicidae) focusing on the origins and use of selected strains. Journal of Medical Entomology 47: 957–971. https://doi.org/10.1603/ME10152

Labbé P, Berthomieu A, Berticat C, Alout H, Raymond M, Lenormand T, Weill M (2007) Independent Duplications of the Acetylcholinesterase Gene Conferring Insecticide Resistance in the Mosquito *Culex pipiens*. Molecular Biology and Evolution 24(4): 1056–1067. https://doi.org/10.1093/molbev/msm025

Leandro AS, Rios JA, Britto AS, Galvão SR, Lopes RD, Rivas AV, Martins CA, Silva I, Delai RM, Gonçalves DD, Silva MAN, Palacio-Cortés AM, Schuartz V, Sibim AC, Castro WAC (2020) Malathion insecticide resistance in *Aedes aegypti*: laboratory conditions and in situ experimental approach through adult entomological surveillance. Tropical Medicine and Health 25: 1271–1282. https://doi.org/10.1111/tmi.13474

Li X, Schuler MA, Berenbaum MR (2007) Molecular mechanisms of metabolic resistance to synthetic and natural xenobiotics. Annual Review of Entomology 52: 231–253. https://doi.org/10.1146/annurev.ento.51.110104.151104

Lima VS, Pinto AC, Rafael MS (2015) Effect of isodillapiole on the expression of the insecticide resistance genes GSTE7 and CYP6N12 in *Aedes aegypti* from central Amazonia. Genetics and Molecular Research 14: 16728–16735. https://doi.org/10.4238/2015.December.11.20

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. Methods 25: 402–408. https://doi.org/10.1006/meth.2001.1262

Macoris MDLDG, Andrighetti MTM, Otrera VCG, Carvalho LR, de-Caldas-Júnior AL, Brogdon WG (2007) Association of insecticide use and alteration on *Aedes aegypti* susceptibility status. Memórias do Instituto Oswaldo Cruz 102: 895–900. https://doi.org/10.1590/S0074-02762007000800001

Marcombe S, Fustec B, Cattel J, Chonephetsarath S, Thammavong P, Phommavanh N, David JP, Corbel V, Sutherland IW, Hertz JC, Brey PT (2019) Distribution of insecticide resistance and mechanisms involved in the arbovirus vector *Aedes aegypti* in Laos and implication for vector control. Plos Neglected Tropical Diseases 13: e0007852. https://doi.org/10.1371/journal.pntd.0007852

Massey Jr FJ (1951) The Kolmogorov-Smirnov test for goodness of fit. Journal of the American Statistical Association 46: 68–78. https://doi.org/10.1080/01621459.1951.10500769

Mello YR, Lopes FCA, Roseghini WFF (2017) Características climáticas e análise rítmica aplicada a episódios extremos de precipitação e temperatura no município de Paranaguá, PR. Revista Brasileira de Climatologia 20: 313–336.

Mori A, Lobo NF, DeBruyn B, Severson DW (2007) Molecular cloning and characterization of the complete acetylcholinesterase gene (Ace1) from the mosquito *Aedes aegypti*. Insect Molecular Biology 37: 667–674. https://doi.org/10.1016/j.ibmb.2007.03.014

Moyes CL, Vontas J, Martins AJ, Ng LC, Koou SY, Dusfour I, Raghavendra K, Pinto J, Corbel V, David JP, Weetman D (2017) Contemporary status of insecticide resistance in the major *Aedes* vectors of arboviruses infecting hu-

mans. Plos Neglected Tropical Diseases 11: e0005625. https://doi.org/10.1371/journal.pntd.0005625

Nunes A, Schmitz C, Moura S, Maraschin M (2021) The use of pesticides in Brazil and the risks linked to human health. Brazilian Journal of Development 7: 37885–37904. https://doi.org/10.34117/bjdv7n4-311

Ocampo CB, Salazar-Terreros MJ, Mina NJ, McAllister J, Brogdon W (2011) Insecticide resistance status of *Aedes aegypti* in 10 localities in Colombia. Acta Tropica 118: 37–44. https://doi.org/10.1016/j.actatropica.2011.01.007

Paraná (2020) Força-tarefa contra dengue apresenta primeiros resultados positivos. Governo do Estado do Paraná, available online at http://www.aen.pr.gov.br/modules/noticias/article.php?storyid=105310&tit=Forca-tarefa-contra-dengue-apresenta-primeiros-resultados-positivos [Accessed: 26/09/2020]

Portos do Paraná (2022) Plano de desenvolvimento e zoneamento do Porto de Paranaguá. Available online at https://www.documentador.pr.gov.br/documentador/pub.do?action=d&uuid=@gtf-escriba-appa@f5f0e399-a186-4586-a4a8-59c1229e2d04&emPg=true [Accessed: 13/08/2023]

R Core Team (2018) The R Project for Statistical Computing. Available online at https://www.r-project.org/ [Accessed: 08/11/2018]

Reid WR, Thornton A, Pridgeon JW, Becnel JJ, Tang F, Estep A, Clark GG, Allan S, Liu N (2014) Transcriptional analysis of four family 4 P450s in a Puerto Rico strain of *Aedes aegypti* (Diptera: Culicidae) compared with an Orlando strain and their possible functional roles in permethrin resistance. Journal of Medical Entomology 51: 605–615. https://doi.org/10.1603/ME13228

Riaz MA, Chandor-Proust A, Dauphin-Villemant C, Poupardin R, Jones CM, Strode C, Régent-Kloeckner M, David JP, Reynaud S (2013) Molecular mechanisms associated with increased tolerance to the neonicotinoid insecticide imidacloprid in the dengue vector *Aedes aegypti*. Aquatic Toxicology 126: 326–337. https://doi.org/10.1016/j.aquatox.2012.09.010

Riedel G, Rüdrich U, Fekete-Drimusz N, Manns MP, Vondran FWR, Bock M (2014) An Extended ΔCT-Method Facilitating Normalisation with Multiple Reference Genes Suited for Quantitative RT-PCR Analyses of Human Hepatocyte-Like Cells. Plos One 9: e93031. https://doi.org/10.1371/journal.pone.0093031

Ríos C, Rosas N, Delgadillo-Iglesias MÁ, Solis-Soto MT (2023) Presence of *Aedes aegypti* in a high-altitude area in Bolivia. BioRxiv, Preprint, https://doi.org/10.1101/2023.08.07.552199

Saavedra-Rodriguez K, Campbell CL, Lenhart A, Penilla P, Lozano-Fuentes S, Black WC (2019) Exome-wide association of deltamethrin resistance in *Aedes aegypti* from Mexico. Insect Molecular Biology 28: 591–604. https://doi.org/10.1111/imb.12575

Schmidt TL, van Rooyen AR, Chung J, Endersby-Harshman NM, Griffin PC, Sly A, Hoffmann AA, Weeks AR (2019) Tracking genetic invasions: Genome-wide single nucleotide polymorphisms reveal the source of pyrethroid-resistant *Aedes aegypti* (yellow fever mosquito) incursions at international ports. Evolutionary Applications 12: 1136–1146. https://doi.org/10.1111/eva.12787

Scott JG (1999) Cytochromes P450 and insecticide resistance. Insect Molecular Biology 29: 757–777. https://doi.org/10.1016/S0965-1748(99)00038-7

SESA (2016) Situação da Dengue, Chikungunya e Zika Vírus no Paraná, 2015/2016. Informe técnico 36 – Período 2015/2016 – Semana Epidemiológica (SE) 31/2015 a 30/2016. Secretaria de Estado da Saúde do Paraná, available online at: https://www.dengue.pr.gov.br/sites/dengue/arquivos_restritos/files/documento/2020-11/dengueinformetcnico36_2015_2016atse30201_zika_chikungunya_novatabelachikun_zika_2016_09_08divulgado.pdf [Accessed: 10/05/2022]

SESA (2019) Inseticidas destinados ao controle de *Aedes aegypti*. Secretaria de Estado da Saúde do Paraná, Nota técnica 07/CVIA/DAV/SESA, available online at: https://www.saude.pr.gov.br/sites/default/arquivos_restritos/files/documento/2020-03/07CVIADAVSESA.pdf [Accessed: 31/05/2021]

SESA (2021) Boletins da dengue. Secretaria de Estado da Saúde do Paraná, available online at: http://www.dengue.pr.gov.br/Pagina/Boletins-da-Dengue [Accessed: 25/05/2021]

Siegfried BD, Scharf ME (2001) Mechanisms of organophosphate resistance in insects. In: Ishaaya I (Ed.) Biochemical sites of insecticide action and resistance. Springer, Berlin, 269–291. https://doi.org/10.1007/978-3-642-59549-3_13

Silva WC, Ribeiro JD, Souza HEM, Corrêa RS (2007) Atividade inseticida de *Piper aduncum* L. (Piperaceae) sobre *Aetalion* sp. (Hemiptera: Aetalionidae), praga de importância econômica no Amazonas. Acta Amazonica 37: 293–298. https://doi.org/10.1590/S0044-59672007000200017

Smith DL, Battle KE, Hay SI, Barker CM, Scott TW, McKenzie FE (2012) Ross, Macdonald, and a Theory for the Dynamics and Control of Mosquito-Transmitted Pathogens.

Plos Pathogens 8: e1002588. https://doi.org/10.1371/journal.ppat.1002588

Soni M, Bhattacharya C, Sharma J, Dutta P (2018) Bioassay and molecular study for detection of insecticide resistance dengue causing mosquito vectors. Journal of Medical Microbiology 36: 435–438.

Strode C, de Melo-Santos M, Magalhães T, Araújo A, Ayres C (2012) Expression profile of genes during resistance reversal in a temephos selected strain of the dengue vector, *Aedes aegypti*. Plos One 7: e39439. https://doi.org/10.1371/journal.pone.0039439

Thornton J, Gomes B, Ayres C, Reimer L (2020) Insecticide resistance selection and reversal in two strains of *Aedes aegypti*. Wellcome Open Research 5: 183. https://doi.org/10.12688/wellcomeopenres.15974.2

Valle D, Bellinato DF, Viana-Medeiros PF, Lima JBP, Martins-Junior ADJ (2019) Resistance to temephos and deltamethrin in *Aedes aegypti* from Brazil between 1985 and 2017. Memórias do Instituto Oswaldo Cruz 114: 1–17. https://doi.org/10.1590/0074-02760180544

van den Berg H, Velayudhan R, Yadav RS (2021) Management of insecticides for use in disease vector control: Lessons from six countries in Asia and the Middle East. Plos Neglected Tropical Diseases 15: e0009358. https://doi.org/10.1371/journal.pntd.0009358

Vanhoni F, Mendonça F (2008) O clima do litoral do estado do Paraná. Revista Brasileira de Climatologia 3: 49–63. https://doi.org/10.5380/abclima.v3i0.25423

Viana-Medeiros PF, Bellinato DF, Valle D (2018) Laboratory selection of *Aedes aegypti* field populations with the organophosphate malathion: Negative impacts on resistance to deltamethrin and to the organophosphate temephos. Plos Neglected Tropical Diseases 12: e0006734. https://doi.org/10.1371/journal.pntd.0006734

Vlogiannitis S, Mavridis K, Dermauw W, Snoeck S, Katsavou E, Morou E, Harizanis P, Swevers L, Hemingway J, Feyereisen R, van Leeuwen T, Vontas J (2021) Reduced proinsecticide activation by cytochrome P450 confers coumaphos resistance in the major bee parasite *Varroa destructor*. Proceedings of the National Academy of Sciences 118(6): e2020380118. https://doi.org/10.1073/pnas.2020380118

Vontas J, Kioulos E, Pavlidi N, Morou E, della Torre A, Ranson H (2012) Insecticide resistance in the major dengue vectors *Aedes albopictus* and *Aedes aegypti*. Pesticide Biochemistry and Physiology 104: 126–131. https://doi.org/10.1016/j.pestbp.2012.05.008

Ware GW, Whitacre DM (2004) The Pesticide Book. MeisterPro Information Resources, Ohio, 488 pp.

Weill M, Berthomieu A, Berticat C, Lutfalla G, Nègre V, Pasteur N, Philips A, Leonetti J, Fort P, Raymond M (2004) Insecticide resistance: a silent base. Current Biology 14: 552–553.

Weill M, Fort P, Berthomieu A, Dubois MP, Pasteur N, Raymond M (2002) A novel acetylcholinesterase gene in mosquitoes codes for the insecticide target and is non-homologous to the ace gene *Drosophila*. Proceedings of the Royal Society B: Biological Sciences 269: 2007–2016. https://doi.org/10.1098/rspb.2002.2122

WHO (1992) Vector resistance to pesticides: Fifteenth report of the WHO Expert Committee on Vector Biology and Control. World Health Organization, Geneva, Technical Report Series #818, 62 pp. https://doi.org/10.1016/0035-9203(93)90514-q

WHO (2009) Guidelines for efficacy testing of insecticides for indoor and outdoor ground-applied space spray applications. World Health Organization, Geneva, 53 pp.

WHO (2016) Test procedures for insecticide resistance monitoring in malaria vector mosquitoes. World Health Organization, Geneva, 55 pp.

WHO (2022a) Determining discriminating concentrations of insecticides for monitoring resistance in mosquitoes: report of a multicentre laboratory study and WHO expert consultations. World Health Organization, Geneva, 94 pp.

WHO (2022b) Standard operating procedure for testing insecticide susceptibility of adult mosquitoes in WHO bottle bioassays. World Health Organization, Geneva, 16 pp.

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