Acta Tropica 158 (2016) 88–96

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Deltamethrin-mediated survival, behavior, and oenocyte morphology of insecticide-susceptible and resistant yellow fever mosquitos (*Aedes aegypti*)

Nadja Biondine Marriel[a], Hudson Vaner Ventura Tomé[b], Raul Carvalho Narciso Guedes[b], Gustavo Ferreira Martins[a,*]

[a] Departamento de Biologia Geral, Universidade Federal de Viçosa, Campus Universitário, Viçosa, Minas Gerais CEP 36570-900, Brazil
[b] Departamento de Entomologia, Universidade Federal de Viçosa, Campus Universitário, Viçosa, Minas Gerais CEP 36570-900, Brazil

ARTICLE INFO

Article history:
Received 24 December 2015
Received in revised form 26 February 2016
Accepted 27 February 2016
Available online 2 March 2016

Keywords:
Aedes aegypti
Behavior
Deltamethrin
Insecticide resistance
Oenocytes

ABSTRACT

Insecticide use is the prevailing control tactic for the mosquito *Aedes aegypti*, a vector of several human viruses, which leads to ever-increasing problems of insecticide resistance in populations of this insect pest species. The underlying mechanisms of insecticide resistance may be linked to the metabolism of insecticides by various cells, including oenocytes. Oenocytes are ectodermal cells responsible for lipid metabolism and detoxification. The goal of this study was to evaluate the sublethal effects of deltamethrin on survival, behavior, and oenocyte structure in the immature mosquitoes of insecticide-susceptible and resistant strains of *A. aegypti*. Fourth instar larvae (L4) of both strains were exposed to different concentrations of deltamethrin (i.e., 0.001, 0.003, 0.005, and 0.007 ppm). After exposure, L4 were subjected to behavioral bioassays. Insecticide effects on cell integrity after deltamethrin exposure (at 0.003 or 0.005 ppm) were assessed by processing pupal oenocytes for transmission electron microscopy or TUNEL reaction. The insecticide resistant L4 survived all the tested concentrations, whereas the 0.007-ppm deltamethrin concentration had lethal effects on susceptible L4. Susceptible L4 were lethargic and exhibited less swimming activity than unexposed larvae, whereas the resistant L4 were hyperexcited following exposure to 0.005 ppm deltamethrin. No sublethal effects and no significant cell death were observed in the oenocytes of either susceptible or resistant insects exposed to deltamethrin. The present study illustrated the different responses of susceptible and resistant strains of *A. aegypti* following exposure to sublethal concentration of deltamethrin, and demonstrated how the behavior of the immature stage of the two strains varied, as well as oenocyte structure following insecticide exposure.

1. Introduction

The mosquito *Aedes aegypti* is of great importance to public health as one of the main vectors of numerous arboviruses, including those causing dengue and the urban yellow fever viruses (Braga and Valle, 2007). To date, vector control is the principal method of preventing dengue and other diseases. Among the methods for the control of mosquito vectors, including those employed for *A. aegypti* control, the use of insecticides, such as deltamethrin, is widespread (Rose, 2001). However, the development of resistance to deltamethrin in vector populations has been reported (Gayathri and Murthy, 2006; Perera et al., 2008).

Insecticide resistance in insect vectors, including mosquitoes, occurs because of a strictly genetic mechanism. The main mutations affect the target proteins of insecticides, which may also be related to insecticide metabolism, and this may result in the reduction in insecticide exposure or uptake and sequestration of the insecticide away from the targeted proteins. The main pyrethroid sites of action are the neuron voltage-gated sodium channels (Narahashi, 1996, 2002; Soderlund, 2012), which affect the peripheral and central nervous system, stimulating cells to produce repetitive discharges and eventually causing paralysis and the *knock down* effect (Braga and Valle, 2007; Santos et al., 2007). However, there are mechanisms of resistance to pyrethroids that reduce neuronal sensitivity to the insecticide; one such mechanism is known as knockdown resistance (KDR) (Soderlund and Bloomquist, 1990). The KDR leads to approximately a 10- to 20-fold decrease in the sensitivity of the sodium channel insecticide (Davies et al., 2007a).

* Corresponding author.
E-mail address: gmartins@ufv.br (G.F. Martins).

http://dx.doi.org/10.1016/j.actatropica.2016.02.021
0001-706X/

In the house fly *Musca domestica,* for example, a change in a single nucleotide in the sodium channels results in a KDR mutation (Ingles et al., 1996; Knipple et al., 1994; Miyazaki et al., 1996; Williamson et al., 1993, 1996). In some cases, mutations represent a 100-fold increase in pyrethroid resistance what is known as super-KDR resistance (Davies et al., 2007a). Mutations in the sodium channel are responsible for KDR and have been documented in several agricultural pest and disease vector insects (Davies et al., 2007b; Dong, 2007). The selective pressure of insecticides, their frequency of use, and the inheritability of resistance to them contribute to the spread of insecticide resistance among insect populations, compromising control efforts (Hemingway and Ranson, 2000; Li et al., 2007).

The behavior of insects, as well as all animals, is governed by interactions between neurons and between the nervous system, and the environment. There are neurotoxic insecticides (such as deltamethrin, a type II pyrethroid) that act at specific sites in the insect nervous system while interacting with the sodium channels in neurons (Narahashi, 1996; Ray and Fry, 2006). Therefore, neurotoxic insecticides can affect the behavior of insects even at low concentrations (Guedes et al., 2016; Haynes, 1988). Sublethal exposure to insecticides can also alter predator-prey interactions (Clements and Newman, 2002; Dell'omo, 2002; Guedes et al., 2016; Teplitsky et al., 2005; Van Gossum et al., 2009), and interfere with development, survival, and mobility, as observed in *A. aegypti* (Tomé et al., 2014). In addition, various activities, such as seeking refuge, evasion, breathing, foraging, and locomotion, can be affected when insects are exposed to neurotoxic insecticides (Brackenbury, 2001; Guedes et al., 2016; Janssens and Stoks, 2012).

Insect behavioral responses to insecticides are diverse and may be related to insect mobility (Davidson, 1953; Guedes et al., 2016; Haubruge and Amichot, 1998; Lockwood et al., 1984), and these behavioral responses can be evaluated by excito-repellency tests (Roberts et al., 1997). Irritability by contact exposure takes place when adults of *A. aegypti* are directly exposed to insecticides, such as deltamethrin, and the mosquitoes quickly escape from insecticide-contaminated areas, which can have significant effects on the effectiveness of mosquito control and disease transmission (Kongmee et al., 2004). Therefore, other effects of deltamethrin should be evaluated to understand mosquito biology, including the behavioral effects of insecticide exposure.

The metabolism of xenobiotics is one of the main detoxification mechanisms of pesticides in insects. Several insect tissues and cells are involved in the detoxification processes, including the integument, midgut, fat bodies, and oenocytes. Oenocytes are cells of ectodermal origin that are allegedly involved in insect protection against insecticides (Clark and Dahm, 1973; Lycett et al., 2006). Therefore, as detoxification enzymes present on oenocytes are potentially induced by chemical stress, these cells are a promising target to elucidate insecticide action and insect response to insecticide exposure. Herein we report on a comparative study of two populations (one susceptible and the other resistant to pyrethroids) of the yellow fever mosquito *A. aegypti.* The goals of our study were to determine their survival, larval behavior, and (pupal) oenocyte morphology following exposure to various sublethal concentrations of deltamethrin.

2. Material and methods

2.1. Mosquitoes

Fourth instar larvae (L4) and pupae of *A. aegypti* were obtained from two different strains: the pyrethroid-susceptible PPCampos strain (Campos dos Goytacazes), and the pyrethroid-resistant F2 Oiapoque strain (Rio de Janeiro). Mosquitoes were maintained in the insectary of the Department of General Biology, Federal University of Viçosa (Viçosa, MG, Brazil) under controlled temperature (25 ± 2 °C), relative humidity (60 ± 2%), and photoperiod (12:12 L:D).

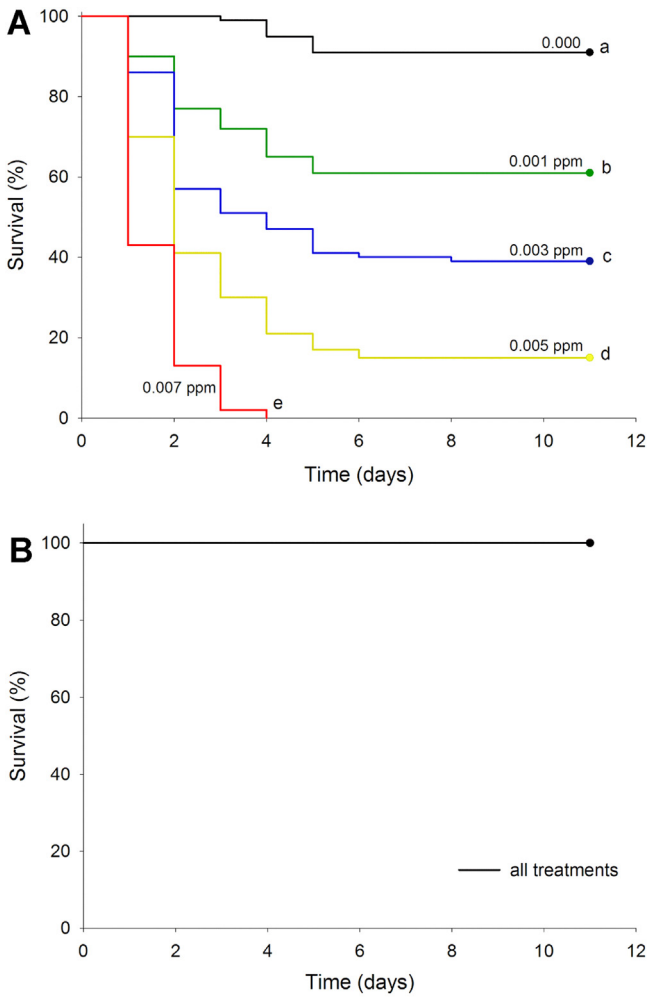

Fig. 1. Survival curves for L4 of pyrethroid-susceptible *A. aegypti* (A) and resistant (B) strains subjected to increasing sublethal concentrations of the pyrethroid insecticide deltamethrin. Survival curves of different colors different by Holm-Sidakís test ($P > 0.05$).

2.2. Survival

One hundred L4 of both populations were maintained in 500-mL glass containers with 200 mL of water and 5 mg of turtle food (ReptoLife) and were subjected to different concentrations of deltamethrin (0.001, 0.003, 0.005, and 0.007 ppm). The control group underwent the same treatment, except for exposure to the insecticide. Deltamethrin (25 g ia L^{-1} emulsifiable concentrate, Bayer CropScience, São Paulo, SP, Brazil) was diluted with distilled water to obtain the desired concentrations. After exposure, the larvae were kept in the same environment, monitored, and evaluated until emergence of all adults 12 days following exposure. The survival curves were estimated by the Kaplan-Meyer design using the software SigmaPlot v. 12.0 (Systat, Jan Jose, CA, US).

2.3. Swimming behavior

For the behavioral assays, six independent replicates were performed as follows: six glass vials containing 25 L4 of both strains – pyrethroid susceptible and resistant – were exposed to 0.003 and 0.005-ppm of the pyrethroid insecticide deltamethrin.

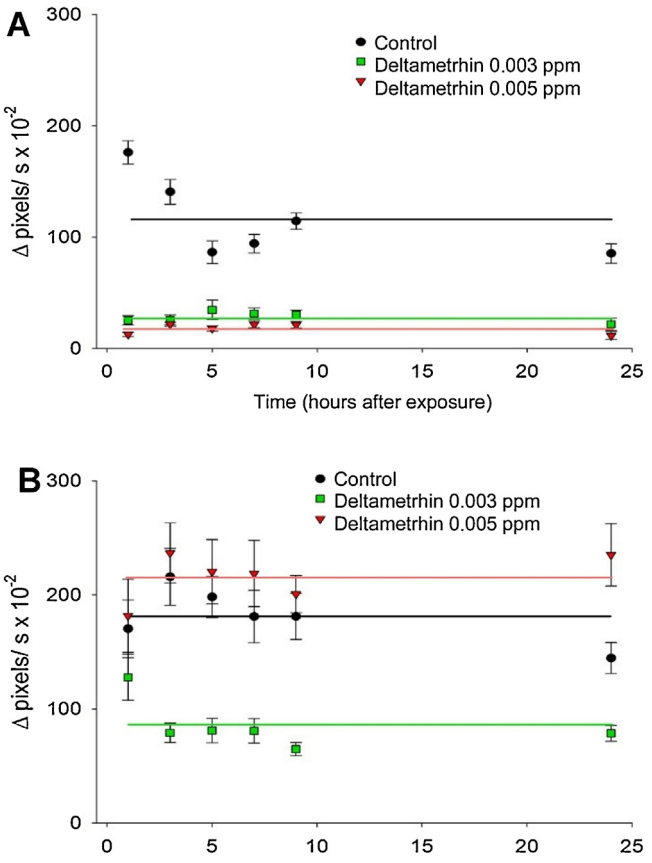

Fig. 2. Linear regressions illustrating the variation of pixels over time in susceptible (A) and resistant (B) L4 *Aedes aegypti* at 1, 3, 5, 7, 9, and 24 h after the exposure to 0.003 or 0.005 ppm deltamethrin.

Unexposed individuals of the two strains were used as separate controls. Exposed and unexposed individuals were monitored at different time intervals (1 h, 3 h, 5 h, 7 h, 9 h, and 24 h after insecticide exposure). L4 were transferred briefly to arenas consisting of Petri dishes placed on a white surface and containing 20 mL of distilled water. After each recording, larvae were returned to their respective vials (Tomé et al., 2014).

The locomotion activity of the larvae in the arena was recorded for 15 min by an automated video monitoring system equipped with a CCD camera (ViewPoint Life Sciences Inc., Montreal, Canada) connected to a computer. The recorded parameter in each arena was the level of activity, measured in pixels. For each replicate, the control and treatment groups were randomly placed and interspersed in four different positions to prevent any possible position effects, for example, subtle differences in light intensity or temperature gradient (Tomé et al., 2014).

2.4. Oenocytes

Oenocytes were dissected from pupae of the two strains (treatment and control) whose L4 were previously treated with 0.003 and 0.005 ppm deltamethrin. To obtain large amounts of cells, oenocytes were dissected from pupae 48 h after insecticide exposure. At this stage, the oenocytes are loosely clustered cells that have not yet spread within the fat body, making them easier to isolate (Martins et al., 2011c). With the aid of a stereoscope microscope, pupae were dissected on glass slides in phosphate buffer (PBS 0.1 M, pH 7.2). The clusters of oenocytes were collected with the aid of a micropipette (1–10 μL) and fixed (Martins et al., 2011c).

2.5. Transmission electron microscopy (TEM)

Dissected oenocytes (treatment and control) were transferred to microcentrifuge tubes containing 1 mL of 2.5% glutaraldehyde in 2% sucrose and 0.1 M cacodylate buffer pH 7.2 for at least 2 h. After fixation, cells were washed three times in PBS, and post-fixed in 1% osmium tetroxide for 2 h in the dark. The cells were then placed in LRWhite resin (London Resin Company Ltd.) in gelatin capsules. Before each solution exchange, the microtubes containing the oenocytes were subjected to a short spin (up to 5000 rpm) to obtain pellets (Martins et al., 2011c). Ultrathin sections were stained with uranyl acetate and lead citrate for 15 and 8 min, respectively. The material was analyzed under the TEM Zeiss EM109 at the Núcleo de Microscopia e Microanálise at Universidade Federal de Viçosa (NMM/UFV), or at the Plataforma de Microscopia Eletrônica Rudolf Barth do Instituto Oswaldo Cruz (FIOCRUZ, RJ) under the TEM JEOL-JEM-1011.

2.6. TUNEL reaction

Oenocytes were dissected and transferred to previously cleaned glass slides and allowed to rest for 30 min for cell adhesion at room temperature. Adherent cells were fixed with 4% paraformaldehyde (pH 7.4) for 30 min, washed three times with PBS, and stored in distilled water at 4 °C (Martins et al., 2011c). For the detection of cleavage of the nuclear DNA, fixed oenocytes were subjected to TUNEL (the terminal deoxynucleotidyl transferase dUTP nick end labeling) reaction for 1 h at 37 °C using the kit *In Situ* Cell Death Fluorescein (Roche Molecular Biochemicals, Mannheim, Germany) according to the manufacturer's instructions. The slides with adhered cells were mounted in Mowiol antifading medium (Fluka, Steinheim, Germany), analyzed, and photographed using the fluorescence microscope Zeiss plus Axiostar with CCD camera AxioCam MRM at NMM/UFV. For the negative control for the TUNEL reaction, one slide with oenocytes of each experimental group (total of 4) was incubated without the transferase enzyme.

2.7. Statistical analyses

Larval activity data of the two strains were subjected to repeated measure analyses of variance to determine the effects of deltamethrin concentrations. Differences were evaluated by Fisher's exact test (PROC ANOVA; SAS Institute, 2008). Survival curves were compared using the Holm-Sidak's test using SigmaPlot.

3. Results

3.1. Survival

The survival of *A. aegypti* was recorded for susceptible and resistant strains subjected to different concentrations of deltamethrin (0.001, 0.003, 0.005, and 0.007 ppm). According to Kaplan-Meyer estimators, the survival of the susceptible strain differed significantly among the tested concentrations ($\chi^2 = 301.97$, df = 4; $P < 0.001$) (Fig. 1). For the susceptible strain, 100% of exposed larvae died at 0.007-ppm deltamethrin by seven days after exposure. Thus, 0.007 ppm is a lethal concentration and prevents the insecticide susceptible larvae from reaching the adult stage. For the other concentrations, the insecticide-susceptible individuals exhibited different survival rates (Fig. 1A).

The resistant strain responded differently to the deltamethrin than did the susceptible strain. Both deltamethrin-exposed and -unexposed individuals of the resistant strain had a constant survival rate of 100%, being unaffected by the four concentrations ($\chi^2 = 3.78$, df = 4; $P = 0.43$) (Fig. 1B). Deltamethrin concentrations of 0.003 ppm and 0.005 ppm were chosen for subsequent sublethal assessments

of behavior and pupal oenocytes of *A. aegypti* because they led to the average and highest mortality values obtained, respectively.

3.2. Swimming behavior

Behavior of insecticide-susceptible and resistant L4 was recorded at 1, 3, 5, 7, 9, and 24 h after deltamethrin exposure. Repeated measures performed for locomotor activity of L4 indicated significant differences between treatments and controls (control vs. deltamethrin concentrations: $F_{2,15} = 125.54$, $P < 0.0001$), time after exposure (Wilk's $\gamma > 0.11$; $F > 16.69$; df num/den = 5/11; $P < 0.0001$), and interactions between sources of variation (Wilk's $\gamma > 0.05$; $F > 7.36$; df num/den = 10/22; $P < 0.0001$) for the susceptible strain. Repeated measures analyses also indicated significant differences between treatments (control vs. deltamethrin concentrations: $F_{2,15} > 17.28$, $P < 0.0001$), time after exposure (Wilk's $\gamma > 0.33$; $F > 4.32$; df num/den = 5/11; $P = 0.02$), and interaction between the sources of variation (Wilk's $\gamma > 0.08$; $F > 5.33$; df num/den = 10/22; $P = 0.0005$) for the resistant strain.

For both insecticide-susceptible and resistant strains, linear regressions were not significant ($P > 0.005$); therefore, there was no time-dependent variation in locomotor activity for exposed and unexposed individuals. Although there was no variation over time, the activity (measured in pixels) of susceptible L4 was lower in the deltamethrin-exposed insects than in the unexposed insects (Fig. 2A). In resistant L4, hyperexcitation was observed 24 h after exposure with 0.005 ppm deltamethrin in comparison to the control. On the other hand, resistant L4 treated with 0.003 ppm showed a decrease in activity (Fig. 2B). Apparently, the unexposed individuals of the resistant strain exhibit higher activity than do the susceptible individuals. However, when the experiment was repeated for the same parameters to compare the susceptible and resistant controls, there were no significant differences between their activity levels.

3.3. Cell damage

There were very few oenocytes positive (~1.5% of cells) for the TUNEL reaction in either susceptible (Fig. 3A–F) or resistant individuals (Fig. 3G–L). Approximately 600 cells were analyzed for each strain, and there were no differences regarding positive cells (with a green fluorescent nucleus) of L4 treated with the concentrations of 0.003 ppm or 0.005 ppm.

3.4. Transmission electron microscopy

Pupal oenocytes of susceptible or resistant individuals exhibited no changes in their ultrastructure compared to their respective unexposed controls (Figs. 4 and 5). These cells had extensive membrane invaginations (Figs. 4 A, 5 B) and electron-dense cytoplasm, with lipid droplets in the cytoplasm and mitochondria (Figs. 4 A–F and 5 A–F). Oenocytes have the characteristics of a metabolically active cell, with a central nucleus and a well-developed nucleolus, and predominant non-condensed chromatin (Figs. 4 B,C,F, and 5 A,C,F). Some treated cells (at 0.005 ppm deltamethrin) of the two strains had damaged mitochondria (Figs. 4 H and 5 H) and nuclei (Fig. 4G). In these cases, the complete disorganization of the nuclear chromatin and membrane was observed, and there were changes in the morphology of mitochondrial cristae (Figs. 4 G–H; 5 H).

4. Discussion

The high mortality of susceptible *A. aegypti* L4 after deltamethrin exposure at different concentrations is in accordance with the observations of Tomé et al. (2014), who showed the lethal effects of deltamethrin in the same population and stage, suggesting that this insecticide can be very toxic to the mosquito larvae. In general, despite the potentially low pyrethroid penetration through the larval integument, the pyrethroid could be quickly absorbed via the digestive tract after ingestion or via the respiratory tract (Soderlund et al., 2002). After absorption, the insecticide caused immediate paralysis and mortality (knock down) (Santos et al., 2007). Unlike susceptible individuals, all of the resistant-individuals exposed reached the adult stage. The deltamethrin is effective and broadly recommended as adulticide in the control of *A. aegypti* (WHO, 2005; Kumar et al., 2011). However, this pyrethroid insecticide is also effective as a larvicide with reported effects on mosquito development and behavior (e.g., Gayathri and Murthy, 2006; Perera et al., 2008; Tomé et al., 2014). Although we did not identify the mechanism involved in mosquito resistance, it has been broadly confirmed that resistance in *A. aegypti* populations may be associated with mutations in sodium channels or by enhanced detoxification of xenobiotics (Kumar et al., 2002; Martins et al., 2009; Montella et al., 2007; Rodríguez et al., 2005; Saavedra-Rodriguez et al., 2007).

The exposure of susceptible *A. aegypti* L4 to 0.003 or 0.005 ppm deltamethrin led to reduced locomotor activities when compared with those of the unexposed controls. This lethargic effect was probably associated with the neurotoxic mechanism of action of pyrethroids (Narahashi, 1996; Ray and Fry, 2006). The lethargic L4 had difficulty in swimming, which in turn could lead to the impairment of their foraging activity. In addition, because of the difficulty in swimming and moving, L4 may be more frequently attacked by predators and would have difficulty in searching for refuge, thereby decreasing their chances of survival (Brackenbury, 2001; Janssens and Stoks, 2012). For example, it was observed that the pyrethroid treatment of *Culex pipiens molestus* larvae compromised their alarm response to predators, increasing the likelihood of larval mortality caused by predation (Reynaldi et al., 2011).

The behavior responses related to the pyrethroid resistant strain of *A. aegypti* differed from those observed in the susceptible strain. The resistant L4 exposed to 0.003 ppm deltamethrin exhibited reduced locomotor activity when compared to the respective control; however, they did not demonstrate the lethargic effect observed in susceptible L4. Moreover, resistant L4 exhibited hyperexcitability when compared with the control 24 h after exposure to 0.005 ppm deltamethrin. In addition to acting on the sodium channels of nerve cells, the type II pyrethroids, such as deltamethrin, may also interact with the γ-aminobutyric acid (GABA) receptors, an important inhibitory neurotransmitter in the central nervous system, as observed in *Anopheles gambiae* adults treated with deltamethrin (Bradberry et al., 2005; Santos et al., 2007; Taylor-Wells et al., 2015; Velisek et al., 2006). GABA leads to hyperpolarization of the post-synaptic terminal, and hyperexcitation in resistant L4 probably may be a consequence of the blockage of GABA receptors by deltamethrin (Macdonald and Olsen, 1994).

Not surprisingly, the negative effects of exposure to deltamethrin were more evident for the susceptible individuals of *A. aegypti*, once the insecticide impaired swimming movements of the L4. On the other hand, in the resistant individuals, the treatment led to hyperexcitability, which apparently did not have significant negative implications because all of the exposed L4 were moving normally, with no difficulty swimming or foraging.

The *A. aegypti* oenocytes allegedly participate in the detoxification of exogenous molecules by expressing protein transcripts of the cytochrome P450 superfamily (over 8%) (Martins et al., 2011a) or by the superexpression of cytochrome P450 reductase enzyme in *A. gambiae* and *Drosophila melanogaster* (Lycett et al., 2006). Our work focused on the analysis of possible side effects of deltamethrin in the oenocytes caused by their alleged role in xenobiotic detoxification (Martins et al., 2011a; Lycett et al., 2006). However, no significant cell damage was detected using the TUNEL

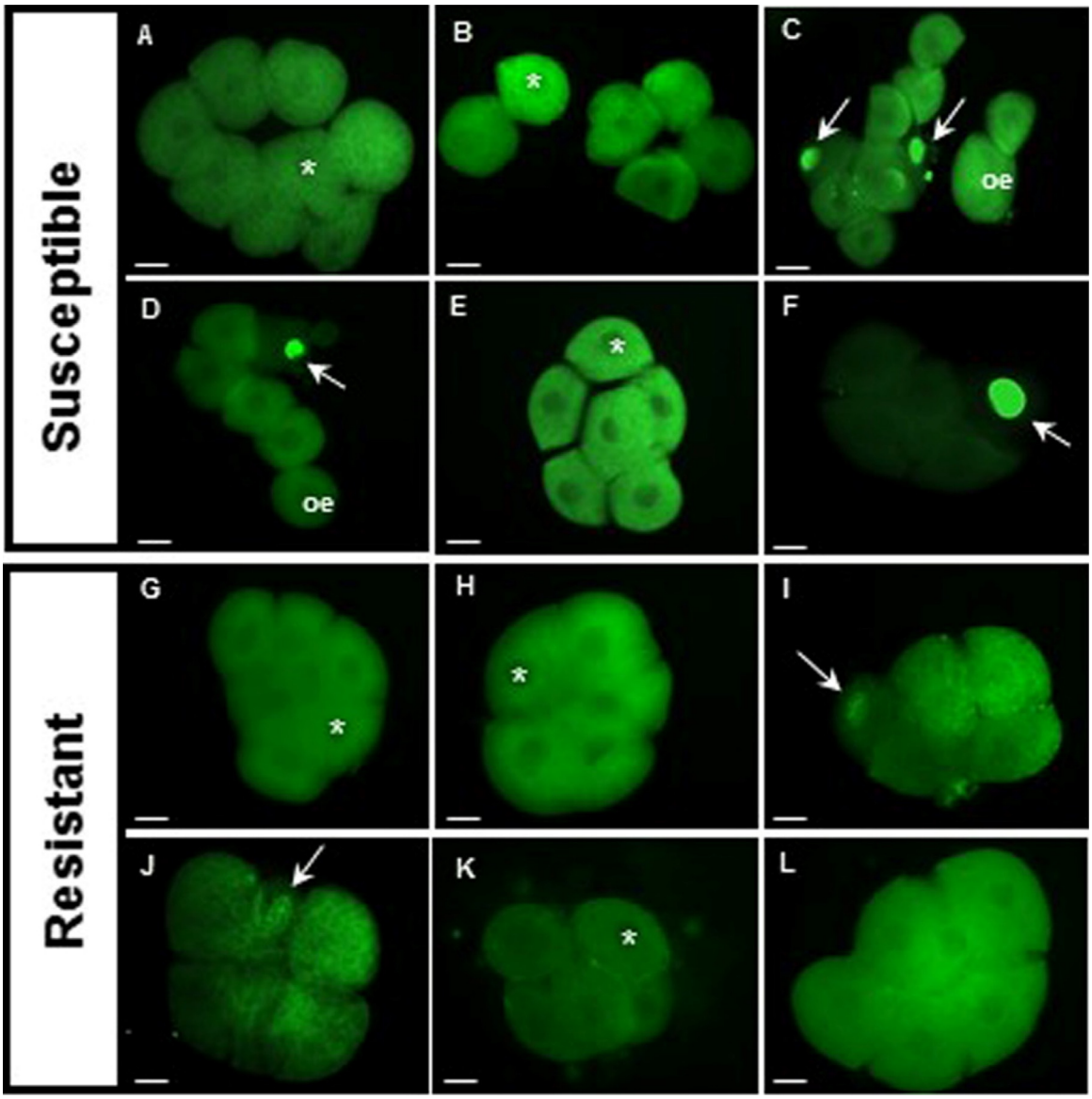

Fig. 3. Pupal oenocytes of *A. aegypti* deltamethrin susceptible (A–F) and resistant (G–L) strains subjected to TUNEL reaction: (A, B, G, and H): clusters of oenocytes (oe) of control (unexposed individuals) with no nuclear fluorescence (*). (C, D, J, and I): clusters of oenocytes after 0.003 ppm deltamethrin treatment. (E, F, K, and L): clusters of oenocytes from treated individuals with 0.005 ppm deltamethrin. The nucleus of oenocytes with DNA damage (arrows) are green-fluorescent. Arrows indicate cells. Bars = 20 μm.

assay and TEM analysis. There were few TUNEL-positive cells in the pupae of susceptible and in the resistant individuals in comparison to their respective unexposed controls. Oenocyte ultrastructure remained unaltered in exposed individuals of the two strains compared with unexposed controls. Even in exposed individuals, the cells maintained their original characteristics and preserved metabolically active cells with the cytoplasm filled with translucent vesicles, which correspond to the accumulation of lipids (Gutierrez et al., 2007) and invaginations of the plasma membrane, which are responsible for the increase of the cell surface (Martins et al., 2011a,b).

To obtain a larger amount of oenocytes, the cells were dissected from pupae, where they were grouped, more conspicuous, and either loosely integrated or not integrated into fat body (Martins et al., 2011c). The deltamethrin exposure started at L4, and the individuals were continuously exposed to insecticide until pupation and dissection of oenocytes. It was not possible to search for any deltamethrin effect on larval oenocytes because they disappear during pupation. Therefore, any eventual indirect effect on oenocytes formation should appear later, once oenocyte differentiation occurs *de novo* during pupation (Wigglesworth, 1933). Deltamethrin exposure appears to affect cells differently. In this case, different targets (oenocytes or the nervous system) were not affected in the same way. Contrary to the expected results, deltamethrin exposure did not lead to oenocyte death in the susceptible strain, or even altered oenocyte ultrastructure, indicating that cell integrity is not related to insecticide resistance. Furthermore, serious impairment was observed of motor activities of the susceptible strain and hyperexcitation in resistant L4 treated with 0.005 ppm deltamethrin.

5. Conclusion

Deltamethrin damage to DNA integrity and oenocyte ultrastructure was very limited in the immature larva of both susceptible and resistant strains of *A. aegypti*. This confirmed that there were no

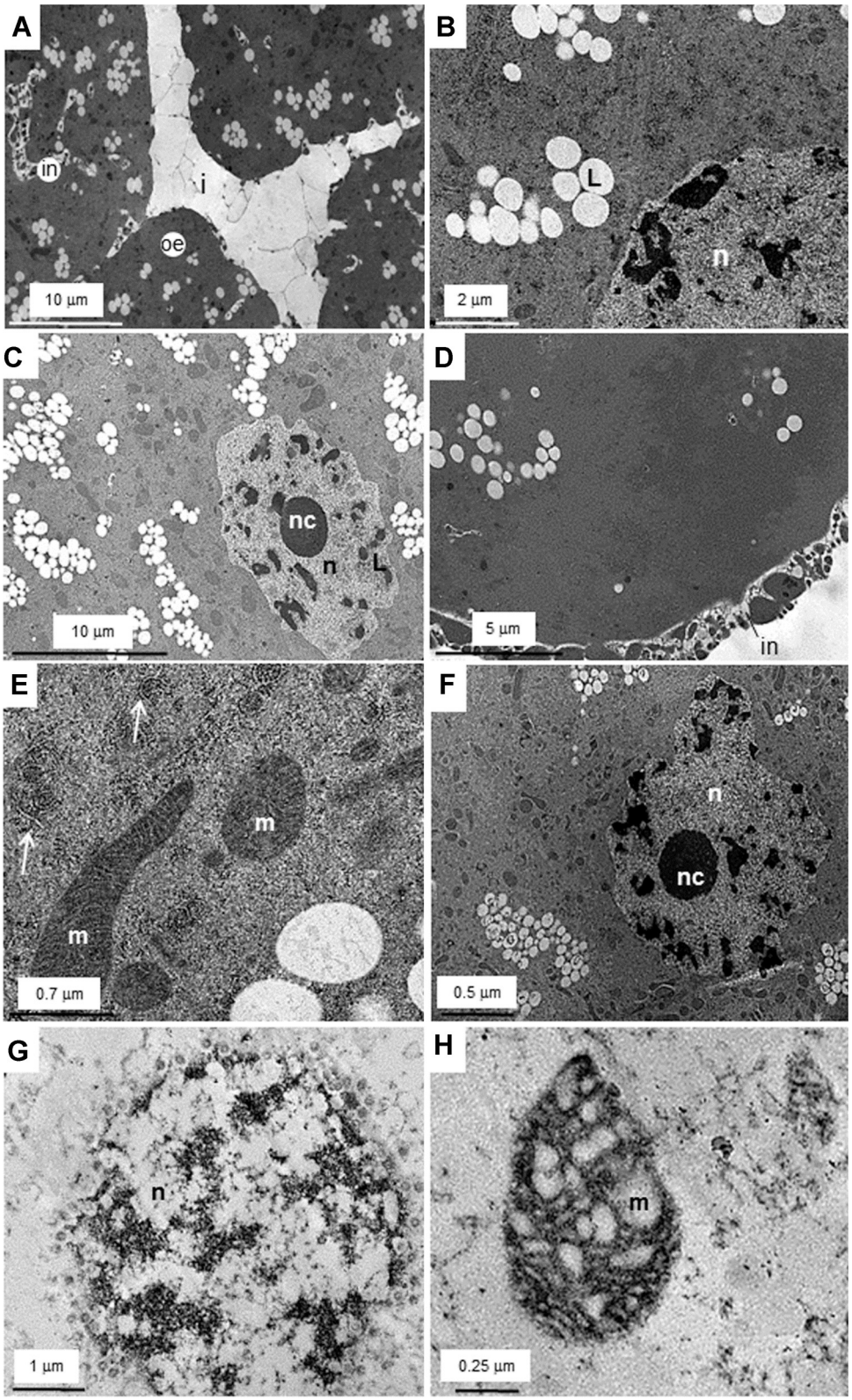

Fig. 4. Transmission electron micrographs of pupal oenocytes of *A. aegypti* of the insecticide-susceptible strain. (A and B): Oenocytes (e) of the control (unexposed individuals). (C, D, and E): oenocytes of individuals exposed to 0.003 ppm deltamethrin. (F, G, and H): oenocytes of individuals exposed to 0.005 ppm deltamethrin. (A): Four grouped oenocytes with membrane invaginations (in). (I): intercellular space. (B, C, and F): General view of oenocytes with no sign of cell damage with a large nucleus (n) with non-condensed chromatin (nc), a translucent cytoplasm filled with vesicles resembling lipid droplets (L), and mitochondria (M). (D): Details of intact mitochondria in a cell of an individual exposed to 0.003 ppm deltamethrin. Arrows indicate the rough endoplasmic reticulum. (E): cell cortex with membrane invaginations (in). (G–H): Abnormal nucleus (n) and mitochondrion (m) in cells of treated individuals with 0.005 ppm deltamethrin. Note the changes in the patterns of chromatin and nuclear membrane and mitochondrial crests.

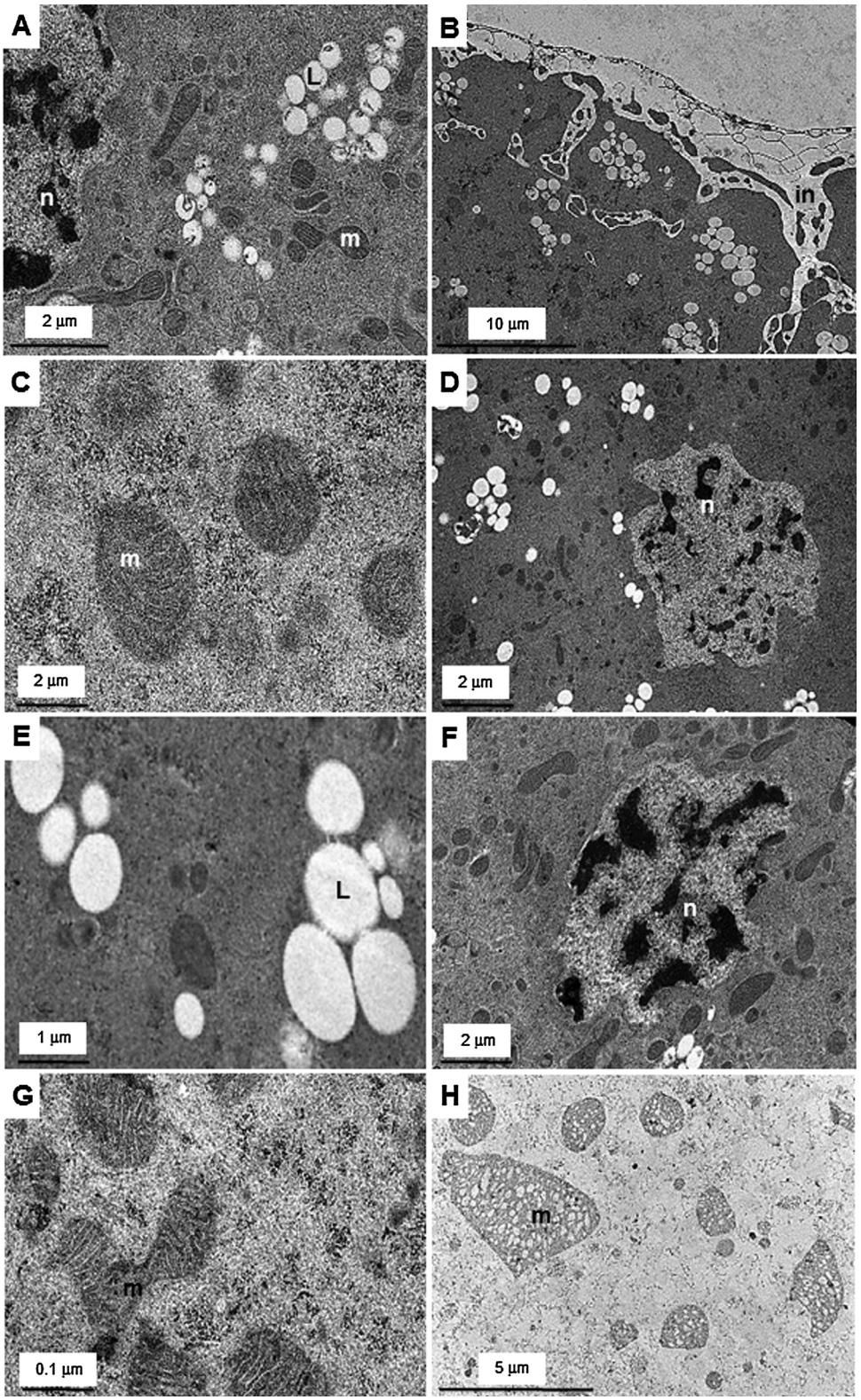

Fig. 5. Transmission electron micrographs of pupal oenocytes of the insecticide-resistant strains of *A. aegypti*. (A and B): oenocytes (e) of control (unexposed group). (C, D, and E): oenocytes of an individual exposed to 0.003 ppm deltamethrin. (F, G, and H): oenocytes of an individual exposed to 0.005 ppm deltamethrin. (A, C, and F): General view of oenocytes with no sign of cell damage with a large nucleus (n) with non-condensed chromatin (nc), a translucent cytoplasm filled with vesicles resembling lipid droplets (L), and mitochondria (M). (B): Invaginations (in) of the cell membrane. (D): mitochondria of intact cells of an individual exposed to 0.003 ppm deltamethrin. (E): Lipid droplets (L). (G): mitochondria in intact cells of an individual exposed to 0.005 ppm. (H): Abnormal mitochondria (m) with abnormal crests in a cell of an individual exposed to 0.005 ppm deltamethrin.

significant secondary effects of deltamethrin on oenocytes under the conditions tested in this study that would contribute to pyretroid resistance. Therefore, oenocytes preserve their functions even in susceptible individuals, confirming that the difference in the susceptible and resistant strains is probably caused by target site differences in the nervous system instead of damage on oenocytes. In contrast, survival and swimming under deltamethrin exposure differ between the mosquito strains, indicating that the resistance insects are able to better cope with the insecticide. Finally, our work contributed to the elucidation of the responses related to deltamethrin exposure and the understanding of the various sublethal effects of the insecticide in susceptible and resistant strains of *A. aegypti* and their relationship to neurological and physiological processes that directly affect mosquito behavior and ecology.

Acknowledgments

We thank Dr. Ademir de Jesus Martins Júnior and José Bento Pereira Lima (Instituto Oswaldo Cruz, IOC-Fiocruz, Rio de Janeiro) for kindly providing the mosquito eggs of the F2 Oiapoque strain, NMM/UFV and IOC/Fiocruz for technical assistance with TEM, and FAPEMIG and CAPES (PVE 390 88881.030429/2013-01) for financial support.

References

Brackenbury, J., 2001. Locomotion through use of the mouth brushes in the larva of *Culex pipiens* (Diptera: Culicidae). Proc. R. Soc.f Lond. B: Biol.Sci. 268 (1462), 101–106.
Bradberry, S.M., Cage, S.A., Proudfoot, A.T., Vale, J.A., 2005. Poisoning due to pyrethroids. Toxicol. Rev. 24 (2), 93–106.
Braga, I.A., Valle, D., 2007. Aedes aegypti: inseticidas, mecanismos de ação e resistência. Epidemiol. Serviços Saúde 16 (4), 279–293.
Clark, M.K., Dahm, P.A., 1973. Phenobarbital-induced, membrane-like scrolls in the oenocytes of *Musca domestica* Linnaeus. J. Cell Biol. 56 (3), 870.
Clements, W.H., Newman, M.C., 2002. Community Ecotoxicology. John Wiley & Sons.
Davidson, G., 1953. Experiments on the effect of residual insecticides in houses against *Anopheles gambiae* and *A. funestus*. Bull. Entomol. Res. 44 (02), 231–254.
Davies, T.G., Field, L.M., Usherwood, P.N., Williamson, M.S., 2007a. DDT, pyrethrins, pyrethroids and insect sodium channels. IUBMB Life 59 (3), 151.
Davies, T.G.E., Field, L.M., Usherwood, P.N.R., Williamson, M.S., 2007b. A comparative study of voltage-gated sodium channels in the Insecta: implications for pyrethroid resistance in Anopheline and other Neopteran species. Insect Mol. Biol. 16 (3), 361–375.
Dell'omo, G. (Ed.), 2002. Behavioural Ecotoxicology. John Wiley & Sons.
Dong, K., 2007. Insect sodium channels and insecticide resistance. Invert. Neurosci. 7 (1), 17–30.
Gayathri, V., Murthy, P.B., 2006. Reduced susceptibility to deltamethrin and kdr mutation in *Anopheles stephensi* Liston, a malaria vector in India. J. Am. Mosq. Control Assoc. 22 (4), 678–688.
Guedes, R.N.C., Smagghe, G., Starl, J.D., Desneux, N., 2016. Pesticide-induced stress in arthropod pests for optimized integrated pest management programs. Annu. Rev. Entomol. 61, 3.1–3.20.
Gutierrez, E., Wiggins, D., Fielding, B., Gould, A.P., 2007. Specialized hepatocyte-like cells regulate *Drosophila* lipid metabolism. Nature 445 (7125), 275–280.
Haubruge, É., Amichot, M., 1998. Les mécanismes responsables de la résistance aux insecticides chez les insectes et les acariens. Biotechnologie, Agronomie Société et Environnement 2 (3), 161–174.
Haynes, K.F., 1988. Sublethal effects of neurotoxic insecticides on insect behavior. Annu. Rev. Entomol. 33 (1), 149–168.
Hemingway, J., Ranson, H., 2000. Insecticide resistance in insect vectors of human disease. Annu. Rev. Entomol. 45 (1), 371–391.
Ingles, P.J., Adams, P.M., Knipple, D.C., Soderlund, D.M., 1996. Characterization of voltage-sensitive sodium channel gene coding sequences from insecticide-susceptible and knockdown-resistant house fly strains. Insect Biochem. Mol. Biol. 26 (4), 319–326.
Janssens, L., Stoks, R., 2012. How does a pesticide pulse increase vulnerability to predation?: Combined effects on behavioral antipredator traits and escape swimming. Aquat. Toxicol. 110, 91–98.
Knipple, D.C., Doyle, K.E., Marsella-Herrick, P.A., Soderlund, D.M., 1994. Tight genetic linkage between the kdr insecticide resistance trait and a voltage-sensitive sodium channel gene in the house fly. Proc. Natl. Acad. Sci. 91 (7), 2483–2487.
Kongmee, M., Prabaripai, A., Akratanakul, P., Bangs, M.J., Chareonviriyaphap, T., 2004. Behavioral responses of *Aedes aegypti* (Diptera: Culicidae) exposed to deltamethrin and possible implications for disease control. J. Med. Entomol. 41 (6), 1055–1063.
Kumar, S., Thomas, A., Sahgal, A., Verma, A., Samuel, T., Pillai, M.K.K., 2002. Effect of the synergist, piperonyl butoxide, on the development of deltamethrin resistance in yellow fever mosquito, *Aedes aegypti* L. (Diptera: Culicidae). Arch. Insect Biochem. Physiol. 50 (1), 1–8.
Kumar, S., Thomas, A., Pillai, M.K.K., 2011. Deltamethrin: promising mosquito control agent against adult stage of *Aedes aegypti* L. Asian Pac. J.Trop. Med. 4 (6), 430–435.
Li, X., Schuler, M.A., Berenbaum, M.R., 2007. Molecular mechanisms of metabolic resistance to synthetic and natural xenobiotics. Ann. Rev. Entoml. 52, 231–253.
Lockwood, J.A., Sparks, T.C., Story, R.N., 1984. Evolution of insect resistance to insecticides: a reevaluation of the roles of physiology and behavior. Bull. Entomol. Soc. Am. 30 (4), 41–51.
Lycett, G.J., McLaughlin, L.A., Ranson, H., Hemingway, J., Kafatos, F.C., Loukeris, T.G., Paine, M.J.I., 2006. Anopheles gambiae P450 reductase is highly expressed in oenocytes and in vivo knockdown increases permethrin susceptibility. Insect Mol. Biol. 15 (3), 321–327.
Macdonald, R.L., Olsen, R.W., 1994. GABAA receptor channels. Annu. Rev. Neurosci. 17 (1), 569–602.
Martins, A.J., de Andrade Lins, R.M.M., Linss, J.G.B., Peixoto, A.A., Valle, D., 2009. Voltage-gated sodium channel polymorphism and metabolic resistance in pyrethroid-resistant *Aedes aegypti* from Brazil. Am. J. Trop. Med. Hyg. 81 (1), 108–115.
Martins, G.F., Ramalho-Ortigão, J.M., Lobo, N., Severson, D.W., McDowel, M.A., Pimenta, P.F.P., 2011a. Insights into the transcriptome of oenocytes from *Aedes aegypti* pupae. Mem. Inst. Oswaldo Cruz 106, 308–315.
Martins, G.F., Serrão, J.E., Ramalho-Ortigão, J.M., Pimenta, P.F.P., 2011b. Histochemical and ultrastructural studies of the mosquito *Aedes aegypti* fat body: effects of aging and diet type. Microsc. Res. Tech. 74 (11), 1032–1039.
Martins, G.F., Guedes, B.A.M., Silva, L.M., Serrão, J.E., Fortes-Dias, C.L., Ramalho-Ortigão, J.M., Pimenta, P.F.P., 2011c. Isolation, primary culture and morphological characterization of oenocytes from *Aedes aegypti* pupae. Tissue Cell 43 (2), 83–90.
Miyazaki, M., Ohyama, K., Dunlap, D.Y., Matsumura, F., 1996. Cloning and sequencing of thepara-type sodium channel gene from susceptible and kdr-resistant German cockroaches (*Blattella germanica*) and house fly (*Musca domestica*). Mol. Gen. Genet. 252 (1–2), 61–68.
Montella, I.R., Martins, A.J., Viana-Medeiros, P.F., Lima, J.B.P., Braga, I.A., et al., 2007. Insecticide resistance mechanisms of Brazilian *Aedes aegypti* populations from 2001 to 2004. Am. J. Trop. Med. Hyg. 77 (12), 467–477.
Narahashi, T., 1996. Neuronal ion channels as the target sites of insecticides. Pharmacol. Toxicol. 79 (1), 1–14.
Narahashi, T., 2002. Nerve membrane ion channels as the target site of insecticides. Mini Rev. Med. Chem. 2 (4), 419–432.
Perera, M.D.B., Hemingway, J., Karunaratne, S.P., 2008. Multiple insecticide resistance mechanisms involving metabolic changes and insensitive target sites selected in anopheline vectors of malaria in Sri Lanka. Malar. J. 7 (1), 168.
Ray, D.E., Fry, J.R., 2006. A reassessment of the neurotoxicity of pyrethroid insecticides. Pharmacol. Ther. 111 (1), 174–193.
Reynaldi, S., Meiser, M., Liess, M., 2011. Effects of the pyrethroid fenvalerate on the alarm response and on the vulnerability of the mosquito larva *Culex pipiens molestus* to the predator *Notonecta glauca*. Aquat. Toxicol. 104 (1), 56–60.
Roberts, D.R., Chareonviriyaphap, T., Harlan, H.H., Hshieh, P., 1997. Methods of testing and analyzing excito-repellency responses of malaria vectors to insecticides. J. Am. Mosq. Control Assoc. 13 (1), 13–17.
Rodríguez, M.M., Bisset, J.A., De Armas, Y., Ramos, F., 2005. Pyrethroid insecticide-resistant strain of *Aedes aegypti* from Cuba induced by deltamethrin selection. J. Am. Mosq. Control Assoc. 21 (4), 437–445.
Rose, R.I., 2001. Pesticides and public health: integrated methods of mosquito management. Emerg. Infect. Dis. 7 (1), 17.
SAS Institute, 2008. SAS/STAT User's Guide. S.A.S. Institute, USA.
Saavedra-Rodriguez, K., Urdaneta-Marquez, L., Rajatileka, S., Moulton, M., Flores, A.E., et al., 2007. A mutation in the voltage-gated sodium channel gene associated with pyrethroid resistance in Latin American *Aedes aegypti*. Insect Mol. Biol. 16 (6), 785–798.
Santos, M.D., Areas, M.A., Reyes, F.G.R., 2007. Piretroides—uma visão geral. Alimentos e Nutrição Araraquara 18 (3), 339–349.
Soderlund, D.M., Bloomquist, J.R., 1990. Molecular mechanisms of insecticide resistance. In: Roush, R.T., Tabashnik, B.E. (Eds.), Pesticide Resistance in Arthropods. Chapman and Hall, New York and London, p. 58.
Soderlund, D.M., Clark, J.M., Sheets, L.P., Mullin, L.S., Piccirillo, V.J., Sargent, D., Weiner, M.L., 2002. Mechanisms of pyrethroid neurotoxicity: implications for cumulative risk assessment. Toxicology 171 (1), 3–59.
Soderlund, D.M., 2012. Molecular mechanisms of pyrethroid insecticide neurotoxicity: recent advances. Arch. Toxicol. 86 (2), 165–181.
Taylor-Wells, J., Brooke, B.D., Bermudez, I., Jones, A.K., 2015. The neonicotinoid imidacloprid, and the pyrethroid deltamethrin, are antagonists of the insect Rdl GABA receptor. J. Neurochem. 135 (4), 705–713.
Teplitsky, C., Piha, H., Laurila, A., Merilä, J., 2005. Common pesticide increases costs of antipredator defenses in *Rana temporaria* tadpoles. Environ. Sci. Technol. 39 (16), 6079–6085.
Tomé, H.W., Tales, V., Pascini, T.V., Dângelo, R.A., Guedes, R.N., Martins, G.F., 2014. Survival and swimming behavior of insecticide-exposed larvae and pupae of the yellow fever mosquito *Aedes aegypti*. Parasites Vectors 7, 195.

Van Gossum, H., Bots, J., Snijkers, T., Meyer, J., Van Wassenbergh, S., De Coen, W., De Bruyn, L., 2009. Behaviour of damselfly larvae (*Enallagma cyathigerum*) (Insecta, Odonata) after long-term exposure to PFOS. Environ. Pollut. 157 (4), 1332–1336.

Velisek, J., Wlasow, T., Gomulka, P., Svobodova, Z., Dobsikova, R., Novotny, L., Dudzik, M., 2006. Effects of cypermethrin on rainbow trout (*Oncorhynchus mykiss*). Vet. Med. 51 (10), 469.

(WHO) Safety of pyrethroids for public health use, 2005. Document WHO/CDS/WHOPES/GCDPP/2005.10. WHO, Geneva.

Wigglesworth, V.B., 1933. Memoirs: the physiology of the cuticle and of ecdysis in *Rhodnius prolixus* (Triatomidae, Hemiptera); with special reference to the function of the oenocytes and of the dermal glands. Q. J. Microsc. Sci. 2 (302), 269–318.

Williamson, M.S., Denholm, I., Bell, C.A., Devonshire, A.L., 1993. Knockdown resistance (kdr) to DDT and pyrethroid insecticides maps to a sodium channel gene locus in the housefly (*Musca domestica*). Mol. Gen. Genet. 240 (1), 17–22.

Williamson, M.S., Martinez-Torres, D., Hick, C.A., Devonshire, A.L., 1996. Identification of mutations in the houseflypara-type sodium channel gene associated with knockdown resistance (kdr) to pyrethroid insecticides. Mol. Gen. Genet. 252 (1–2), 51–60.