Journal of Medical Entomology, XX(X), 2018, 1–7
doi: 10.1093/jme/tjy086
Research

Effects of Temephos, Permethrin, and *Eucalyptus nitens* Essential Oil on Survival and Swimming Behavior of *Aedes aegypti* and *Anopheles pseudopunctipennis* (Diptera: Culicidae) Larvae

Agustín Alvarez Costa, Paula V. Gonzalez, Laura V. Harburguer, and Héctor M. Masuh[1]

Centro de Investigaciones de Plagas e Insecticidas (UNIDEF-CONICET- CITEDEF), Juan Bautista de La Salle 4397, B1603ALO, Villa Martelli, Buenos Aires, Argentina and [1]Corresponding author, e-mail: hmasuh@gmail.com

Subject Editor: Timothy Lysyk

Received 5 March 2018; Editorial decision 10 May 2018

Abstract

An essential strategy to deal with mosquito-borne diseases is the control of larvae in their development sites. The mosquitoes *Anopheles pseudopunctipennis* (Theobald) (Diptera: Culicidae), a malaria vector, and *Aedes aegypti* (L.) (Diptera: Culicidae), vector of dengue, Zika, yellow fever, and chikungunya viruses, breed in very different habitats. Insecticide treatments of mosquito larvae focus mainly on their lethal effects. However, insecticide degradation or the poor dosage of larvicides will invariably lead to the sublethal exposure of a target (and nontarget) species, the nonlethal effects of these compounds may have important effects on vital insect activities, and therefore their evaluation is necessary. In this study, we assessed the survival and swimming behavior of larvae of *Ae. aegypti* and *An. pseudopunctipennis* exposed to increasing concentrations of three larvicides. We found that *Ae. aegypti*, was more sensitive to the larvicides than *An. pseudopunctipennis*, we also observed an overall decrease in the movement of those larvae of both species, which survive the treatments. This decrease might have ecological relevance in their natural habitats, increasing the chance to be predated and decreasing their ability to obtain food. Finally, this information will be valuable to assist authorities to make decisions in the implementation of further control programs.

Key words: sublethal effects, larvicides, larval behavior

Anopheles pseudopunctipennis (Theobald) (Diptera: Culicidae) is one of the most important malaria vectors in Central America and South America (Bruce-Chwatt 1985). The larvae of this mosquito are found in sun-exposed clean freshwater in association with floating plants and filamentous algae (Manguin et al. 1996). Larval habitats are located at altitudes between 200 to 3,200 m above sea level but are more abundant in the foothills (Fernandez-Salas et al. 1994). Experimental data are scarce for this mosquito because it is difficult to maintain under laboratory conditions owing to its eurygamic status (Lardeux et al. 2007). In striking contrast with *An. pseudopunctipennis*, *Aedes aegypti* (L.) (Diptera: Culicidae) larvae, the most important vector of dengue, Zika, yellow fever, and chikungunya viruses, develop in container habitats in tropical and subtropical urban and suburban areas (Scott et al. 2000, Valença et al. 2013, Ye et al. 2016). Controlling these vectors is regarded as essential for preventing epidemics. The most vulnerable stage in the life history of mosquitoes is the larva. Thus, among the control alternatives proposed, the control of larvae in their respective development sites is widely accepted and used for both species (Ordoñez Gonzalez et al. 2008, WHO 2018).

Neurotoxic insecticides, particularly organophosphate and pyrethroids, are the most frequently used compounds against these mosquitoes. The pyrethroid permethrin is the most widely used mosquito adulticide for both species and was recommended for treating drinking water containers for many years until 2011 (Herrera 2003, WHO 2011). In addition, the organophosphate temephos is the most common larvicidal treatment in water containers for *Ae. aegypti* (focal treatment) (Chavasse and Yap 1997). However, the continuous use of temephos contributes to select resistant strains of this vector, thus alternative new products need to be explored (WHO 1992, Osimitz and Murphy 1997, Abdel-Rahman et al. 2001, Braga and Valle 2007, Melo-Santos et al. 2010, Polson et al. 2011). In this way, essential oils delivered from plants exhibit good lethality against mosquito larvae (Pavela 2015). *Eucalyptus nitens* (Myrtaceae) is cultivated over the world and contained an important percentage of β-triketones, which presents high insecticidal activity (Cantrell et al. 2012, Park et al. 2017),

and a recent study performed in our laboratory showed that its oil have great repellent and larvicidal effect against *Ae. aegypti* and *Ae. albopictus* (Alvarez Costa et al. 2017).

Insecticide effects on insects in general, and mosquitoes in particular, focus mostly on their main purpose, their lethal effects (Paul et al. 2006, Pridgeon et al. 2008). However, insecticide degradation or the poor dosage of larvicides will invariably lead to the sublethal exposure of a target (and nontarget) species. The nonlethal effects of these compounds may have important effects on vital insect activities, and therefore their evaluation is necessary (Desneux et al. 2007, Cohnstaedt and Allan 2011, Shi et al. 2011, Guedes and Cutler 2014).

Several activities performed by mosquito juveniles, such as breathing, foraging, refuge seeking, and predator evasion, are strictly dependent on swimming, which emphasizes the importance of insecticide-induced changes in such behavioral patterns to the dynamics of the mosquito larvae population (Brackenbury 2001, Schulz and Dabrowski 2001, Kembro et al. 2009, Reynaldi et al. 2011, Janssens and Stoks 2012).

Research on the swimming behavior of mosquito larvae is scarce, and the methodology is generally complex. Nevertheless, in previous studies from our laboratory, we developed a simple assay to evaluate the basal mosquito larvae behavior and characterized it for the two mosquito species used in this work (Gonzalez et al. 2017). In the present study, we assessed the survival and swimming behavior of *Ae. aegypti* and *An. pseudopunctipennis* larvae, exposed to increasing concentrations of the insecticides temephos, permethrin and the essential oil of *E. nitens*. This is the first report of the toxicity of these compounds on *An. pseudopunctipennis* larvae. We found that *Ae. aegypti* was more sensitive to larvicides than *An. pseudopunctipennis* and also a decrease in the overall movement of both larval species which survived to sublethal treatment exposures.

Materials and Methods

Chemicals

Temephos (97.5%, batch number: 130 A, ABATE, Supelco obtained from Sigma-Aldrich, Darmstadt, Germany) and permethrin (94.5%, 50:50 cis-trans, batch number: 50610, Dr. Ehrenstorfer GmbH, Augsburg, Germany) were used. The *E. nitens* essential oil was obtained and characterized according to a protocol previously used in our laboratory: samples of leaves were hydrodistillated for 90 min using a modified Clevenger-type apparatus, and the essential oil samples were diluted in hexane (1 mg/ml) and analyzed in a Shimadzu GC-17A interfaced to a Shimadzu quadrupole mass spectrometer, GC-MS QP 5050A (Alvarez Costa et al. 2017). The test concentrations of temephos and *E. nitens* were prepared by successive dilutions of 1 mg/ml absolute ethanol solutions. For permethrin acetone was used as a solvent. All solvents were analytical grade (Merck, Germany).

Biological Material

An. pseudopunctipennis larvae were collected, using a 0.05-mm mesh size sieve from natural breeding sites in a subtropical mountainous areas in San Ramón de la Nueva Orán, Salta, Argentina (23°08′10″S 64°19′20″W). Larvae were transported to the laboratory and were kept in bowls with dechlorinated water until used. An insecticide-susceptible strain of *Ae. aegypti* (Rockefeller strain, Venezuela) was used for these assays. The colony has been kept in the laboratory since 1996, free of exposure to pathogens, insecticides, or repellents, at 25–27°C, 50–60% of RH, and a 12:12 (L:D) h photoperiod (Lucia et al. 2007). All larval instars were fed on a mixture of rabbit pellets and yeast in a 3:1 proportion. Adult mosquitoes were fed on raisins, and a pigeon was offered for females to produce their eggs.

Larvicidal Activity

The larvicidal activity was performed by following a protocol previously used in our laboratory (Gómez et al. 2011, Alvarez Costa et al. 2017). Four or five concentrations for each larvicide were evaluated together with their respective control using the solvent without insecticide. One milliliter of the larvicide solution or solvent was added to 199 ml of dechlorinated water in a 500 ml plastic jar, 10 cm height and 8 cm in diameter, and shaken to obtain a homogeneous solution. Then, 50 ml of water with 20 late third or early four instar larvae, of *Ae. aegypti* or *An. pseudopunctipennis*, were added into the jar. Mortality of larvae was recorded after 24 h of exposure. Immobile larvae or those unable to raising the surface were considered as dead. At least five independent replicates were performed for each insecticide for both mosquito species. The assay was conducted and maintained in the same conditions used for rearing.

Larval Behavior Assay

Larvae of both species that survive to the larvicidal assay were used for this study (one larva per replicate). These larvae came from the control treatment and from three or four of the lowest concentrations used to evaluate of each larvicide. The larvae were picked and washed carefully in dechlorinated water to eliminate the larvicide residuals and kept at 27°C for 2 h. Each assay was performed in Petri dish microcosm, 9 cm in diameter. The larvae were transferred individually to separate Petri dishes filled with 40 ml of dechlorinated water. They were left for 10 min to acclimatize. Then, they were digitally recorded for 10 min with a video-camera (Panasonic Lumix DMS-LS 80). The tests were conducted in the same conditions used for rearing. Standardized hanging light (over experimental arena) was used to ensure sufficient contrast between insect and background of experimental arena.

The recorded videos were digitized and mosquito larvae activity was assessed using the video-tracking software (EthoVision XT10.1) to calculate behavioral variables (Noldus et al. 2001). We used dynamic subtraction to identify the larvae from their background.

In this study, the overall movement was expressed as spatial measurements (distance, speed, turning, etc.) that the human observer is unable to accurately estimate (Bureˇsová et al. 1986, Spruijt et al. 1998, Noldus et al. 2001).

Activity variables quantified were: 1) distance (distance swam by the larvae in the experimental arena, in cm), 2) velocity (distance swam by the larvae per unit time in the experimental arena, in cm per second), 3) absolute angular velocity (AAV, change in the direction of movement of the larvae between two consecutive samples, calculated per unit time, in radians per second), and 4) mobility state, the cumulative time, in seconds, of a discrete (state) variable with three possible states: highly mobile (HM), mobile (M) or immobile (I), depending on where the changed pixels of the detected subject between current sample and previous sample (referred to as changed area) lay relative to two defined thresholds (Grieco et al. 2010). The mobility state variable was established for each sample, according to the value running average mobility relative to the thresholds:

- Below the immobile threshold (20%), the state is *immobile* (It).
- Between the immobile threshold (20%) and the highly mobile threshold (60%), the state is *mobile* (Mt).
- Above the highly mobile threshold (60%), the state is *highly mobile* (HMt).

Statistical Analysis

Dose-mortality data from each pool were subjected to probit analysis (Litchfield and Wilcoxon 1949). The 50 and 95% lethal concentrations (LC_{50} and LC_{95}) with the 95% corresponding confidence limits were obtained using PoloPlus 2.0 (LeOra Software, CA, USA).

Principal component analyses (PCA) were performed using the standardized values of the behavioral variables that present significant regressions with the concentration of the larvicide. We performed independent PCA for the three larvicides in each species. PCA was realized to condense the information of the six variables mentioned above into a new variable, the first principal component (PC 1), which is a lineal combination of the variables that retained the majority of its information. Since PCA generates theoretical axis, the biological significance for each PC 1 obtained is always independent for each treatment and depends of their correlations with the original variables. Then, Spearman correlations were used to quantify the association between the variables and the PC 1 to characterize it. In the case of our study almost all PC1 obtained, except for *E. nitens* on *An. pseudopunctipennis* treatment, resulted in an 'inactivity axis'. Linear regressions analyses were made with the PC 1 obtained and the concentration of the larvicide. These analyses were made for each larvicide and each species. For the entire analyses the threshold for significance was set at $P < 0.05$. Statistical software R (v.3.3.1; R Core Team 2016) was used for all analysis.

Results

Larvicidal Activity

The larval mortality produced by the three larvicides was dose-dependent for both mosquito species. *Ae. aegypti* larvae were more sensitive than *An. pseudopunctipennis* in all of the cases. No mortality was registered in the control treatments (C) (Table 1).

Larval Behavior

Mean and standard error for the behavioral variables studied of each treatment for both species are presented in Supp. Table 1.

The results of the PCA for the larval behavior experiments using the three larvicides in both mosquito species are shown in (Table 2, Supp. Fig. 1 and Supp. Table 2). The first PC 1 obtained from the PCA explained a great percentage of the variability, between 64.3 and 90.1%. For *Ae. aegypti*, the variables distance, velocity, time in the HMt and time in the Mt presented negative significant correlations with the PC 1 but time in the It and AAV presented a positive significant correlation (Table 2). Therefore, in these cases, the PC 1 was defined as inactivity axis, in which positive values indicate lower swimming activity and negative values indicates higher swimming activity. In the case of *An. pseudopunctipennnis* the relationships between the behavioral variables and the PC 1 depended on the larvicide. The behavioral variables obtained from larvae exposed to permethrin followed the same pattern that for *Ae. aegypti*, distance, velocity, HMt, and Mt presented negative significant correlations with the PC 1 and It and AAV presented a positive significant correlation (Table 2). A similar pattern was observed when temephos was used although in this case, the correlation between It and PC 1 was not significant (Table 2). Hence, again these PC 1 were also defined as inactivity axis. In contrast, the correlations between the variables and the PC 1 obtained for *An. pseudopunctipennis* larvae exposed to *E. nitens* were different, the variables distance, velocity, HMt, and Mt presented positive significant correlations with the PC 1 and It and AAV presented negative significant correlation

Table 1. Percentage of mortality of each concentration (cc), LC_{50} and LC_{95} (±95% confidence interval) of temephos, permethrin and *E. nitens* essential oil for *Ae. aegypti* and *An. pseudopunctipennis*

Species	Larvicide	cc (ppm)	Mortality (%)	CL_{50}	CL_{95}	X^2	GL	H	*n*
Ae. aegypti	Temephos	C	0.00 ± 0.00	2.222 ppb	4.224 ppb	28.429	30	0.943	640
		0.001	2.50 ± 0.94	(2.091–2.361)	(3.833–4.775)				
		0.002	38.13 ± 5.08						
		0.004	93.75 ± 2.45						
	Permethrin	C	0.00 ± 0.00	3.731 ppb	11.669 ppb	51.24	34	1.507	720
		0.001	8.75 ± 4.27	(3.330–7.179)	(9.727–14.75)				
		0.002	11.88 ± 2.49						
		0.004	53.13 ± 2.98						
		0.012	95.63 ± 1.99						
	E. nitens	C	0.00 ± 0.00	43.846 ppm	91.121 ppm	185.97	26	7.153	560
		20	1.25 ± 1.25	(35.895–51.537)	(72.903–142.02)				
		60	24.00 ± 3.35						
		80	88.33 ± 1.44						
An. pseudopuncti pennis	Temephos	C	0.00 ± 0.00	3.576 ppb	11.875 ppb	25.853	10	2.585	240
		0.001	8.52 ± 3.24	(2.760–4.797)	(7.706–31.327)				
		0.002	31.67 ± 16.91						
		0.004	44.81 ± 2.89						
		0.008	91.67 ± 4.41						
	Permethrin	C	0.00 ± 0.00	9.117 ppb	57.738 ppb	5.604	10	0.56	240
		0.002	10.00 ± 2.89	(7.425–12.204)	(32.935–164.51)				
		0.004	23.33 ± 1.67						
		0.008	38.33 ± 6.01						
		0.012	65.00 ± 2.89						
	E. nitens	C	0.00 ± 0.00	87.881 ppm	663.31 ppm	4.332	10	0.433	240
		20	13.89 ± 2.00	(67.965–150.94)	(292.73–5313.7)				
		40	23.33 ± 3.33						
		60	31.67 ± 1.67						
		80	53.33 ± 3.33						

X^2, Chi-squared parameter; H, heterogeneity; *n*, number of larvae used in the assays; C, control.

Table 2. Spearman correlation index resulted between the behavioral variables measurements and the PC 1 obtained of the PCA

First PC1	Variability explained (%)	Distance	Velocity	HMt	Mt	It	AAV
Ae. aegypti							
Temephos	80.5	−0.44	−0.44	−0.38	−0.40	0.41	0.37
Permethrin	74.1	−0.42	−0.41	−0.40	−0.43	0.38	0.41
E. nitens	90.1	−0.42	−0.42	−0.40	−0.37	0.43	0.41
An. pseudopunctipennis							
Temephos	64.3	−0.46	−0.46	−0.47	−0.43	0.04 (NS)	0.41
Permethrin	69.8	−0.48	−0.48	−0.44	−0.42	0.25	0.33
E. nitens	66.1	0.43	0.44	0.45	0.42	−0.36	−0.32

(Table 2). Therefore, in this case, the PC 1 was defined as 'activity axis' in which positive values indicate higher swimming activity and negative values indicates lower swimming activity. In summary, since PCA generates theoretical axis, the biological significance for each PC 1 obtained is always independent of each treatment. In the case of our study, all PC 1 were defined as inactivity axis, except for the treatment of *E. nitens* in *An. pseudopunctipennis* where PC1 were defined as activity axis.

For the control treatments larvae presented the highest swimming activity in *Ae. aegypti* for all the larvicides and in *An. pseudopunctipennis* for temephos and permethrin, as presented the lowest values of the inactivity axis (Fig. 1). The regression analyses indicated a concentration-dependent increase of the inactivity axis of the *Ae. aegypti* larvae exposed to the three larvicides, temephos, permethrin, and *E. nitens* essential oil ($P < 0.05$, Fig. 1A–C, Table 3). Considering that positive values of the inactivity axis represents lower activity of the larvae, we observed a concentration-dependent decrease in their activity. Furthermore, the same concentration-dependent reduction of the activity was observed in *An. pseudopunctipennis* larvae exposed to temephos and permethrin ($P < 0.05$, Fig. 1D–E, Table 3). However, no relation was observed between the *An. pseudopunctipennis* larvae activity axis and the concentration of *E. nitens* essential oil ($P > 0.05$, Fig. 1F, Table 3).

Discussion

In this study, we determined the effects of temephos, permethrin and *E. nitens* essential oil on the survival and swimming behavior of two mosquito species, *An. pseudopunctipennis* and *Ae. aegypti*. We found a concentration-dependent decrease in the swimming activity of the surviving larvae exposed to larvicides. Here we also reported the first determination of toxicological parameters for the three larvicides in *An. pseudopunctipennis*.

In all the cases, evaluated larval mortality was dose dependent. For *Ae. aegypti*, the LC_{50} obtained for the three larvicides were similar to the values obtained in other studies from our laboratory (Gómez et al. 2011, Alvarez Costa et al. 2017). Furthermore, this is the first study reporting the LC_{50} and LC_{95} of temephos, permethrin, and *E. nitens* essential oil on *An. pseudopunctipennis*. For both species, the LC_{50} of the essential oil was similar to the values obtained for other *Eucalyptus* (Lucia et al. 2008, Alvarez Costa et al. 2017) but were several times greater compared with the values obtained for the synthetic larvicides. However, the essential oils of cultivated plants are easily available at reasonable costs and usually are more environmental friendly than the synthetic larvicides (Batish et al. 2008, Campolo et al. 2016). In addition, The LC values for *Ae. aegypti* were lower than those for *An. pseudopunctipennis* indicating its higher sensitivity to the larvicides. This higher sensitivity for *Ae. aegypti* larvae could be because it belongs to a laboratory strain, which has been kept for decades not being under selection pressure, meanwhile *An. pseudopunctipennis* larvae were collected from the field. However, this higher sensitivity for *Ae. aegypti* was also reported by several authors in comparison with other *Anopheles* species both rearing under laboratory conditions (Amer and Mehlhorn 2006, Arredondo-Jiménez and Valdez-Delgado 2006, Kumar et al. 2012, Kemabonta and Nwankwo 2013, Senthilkumar et al. 2013, Elumalai et al. 2016). Nevertheless, in this work we could not discriminate if the difference in sensitivity to these larvicides is an intrinsic characteristic of the species or a consequence of the source of the biological material. Finally, the LC_{50} values of *An. pseudopunctipennis* will be valuable in the implementation of further Malaria control programs because will allow to determine the most cost-effective compound and assist authorities to make decisions based on scientific evidence.

We identified a concentration-dependent decrease of the swimming activity for the larvae exposed. In *Ae. aegypti* this decrease was observed for the three larvicides evaluated and in *An. pseudopunctipennis* for temephos and permethrin. A decrease in the swimming activity of mosquito larvae exposed to other synthetic and natural insecticides was registered in other studies (Kembro et al. 2009, Tomé et al. 2014, Marriel et al. 2016). The results obtained in our study could be due to the neurotoxic mode of action of temephos and permethrin (Narahashi 1996, Mileson et al. 1998, Ray and Fry 2006). As a consequence, this inactivity would decrease the chances of survival, affecting the time spent on feeding and respiration behavior (Brackenbury 2001). Furthermore, could influence the behavior of predator avoidance, e.g., affecting their performance in the searching for refuge or affecting their alarm response to them (Reynaldi et al. 2011, Janssens and Stoks 2012). However, further studies regarding how the inactivity as consequence of the exposing to larvicides affects the ecological traits of the larvae are required.

The swimming activity of the larvae exposed to the *E. nitens* essential oil decreased in the case of *Ae. aegypti* but did not change for *An. pseudopunctipennis*. This difference could be due to several reasons. The essential oils are a complex mixture of compounds and a combination of different modes of action is probably present and could affect differentially to each species (Pavela 2015, Dambolena et al. 2016). On the other hand, *An. pseudopunctipennis* larvae with no stimulus tend to spend more time immobile compared with *Ae. aegypti* larvae (Gonzalez et al. 2017). This behavior could mask the decrease on the swimming activity produced by the essential oil, especially considering that the maximum essential oil concentration evaluated for *An. pseudopunctipennis* was below the LC_{50} obtained in this study. Then, probably using concentrations with higher levels of lethality would produce a significant change in the larval behavior. However, we used 80 ppm as the top concentration due to the limitation of the water solubility of two main components of the essential oil, *p*-cymene, and flavesone (Banerjee et al. 1980, TGSC 2018).

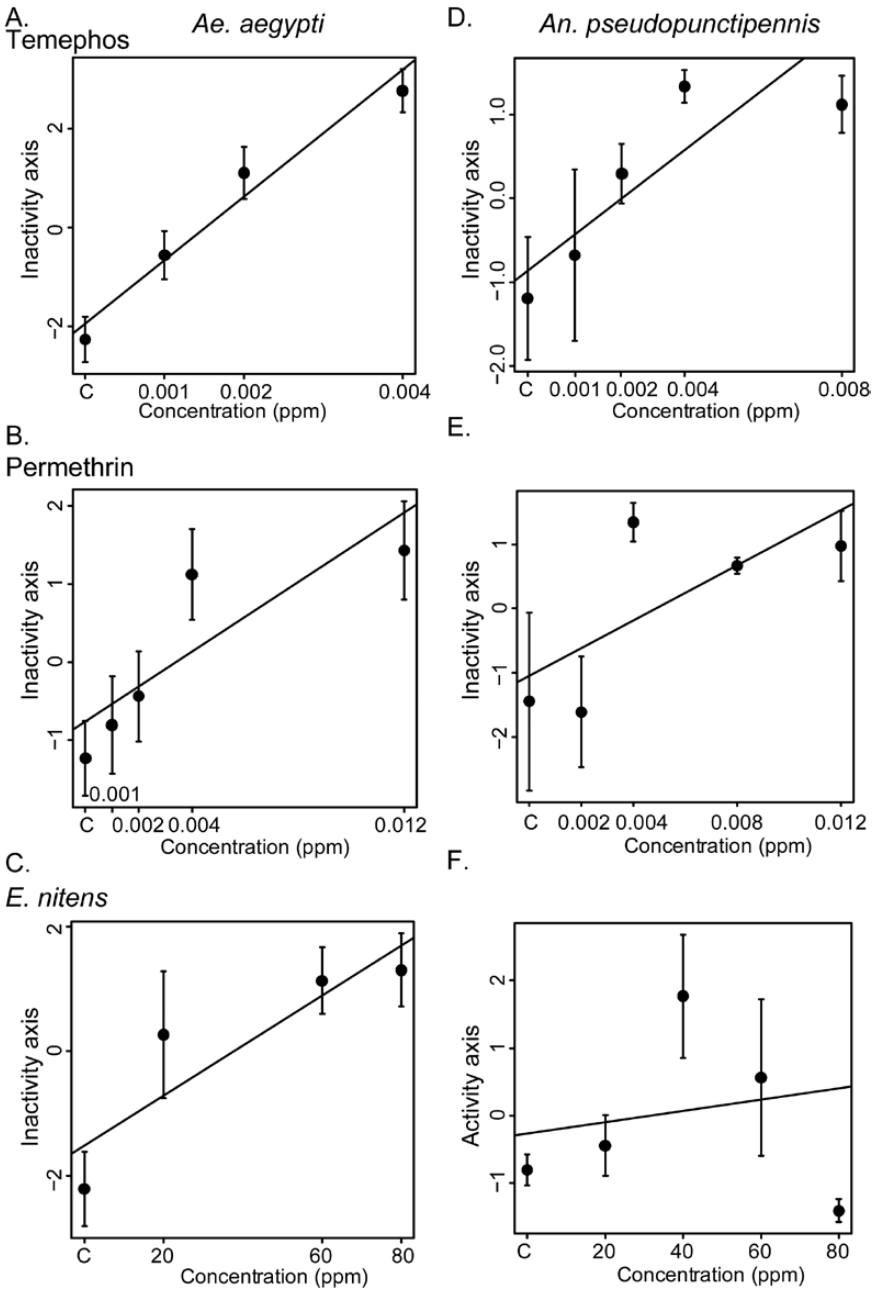

Fig. 1. Regression analyses between the inactivity or activity axis obtained from the PCA and the concentration of larvicide temephos (A and D), permethrin (B and E) and *E. nitens* essential oil (C and F) on *Ae. aegypti* (A, B and C) and *An. pseudopunctipennis* (D, E and F) larvae. C: Control treatments.

Table 3. Regression parameters of the PC 1 and the concentration of larvicide temephos, permethrin and *E. nitens* essential oil on *Ae. aegypti* and *An. pseudopunctipennis*

Specie	Larvicide	Intercept	Slope	R^2	*F*	df	*P*-value
Ae. aegypti							
	Temephos	−1.94 ± 0.37	1281.12 ± 182.33	0.65	49.37	1	1.5E-7
	Permethrin	−0.76 ± 0.35	223.54 ± 68.42	0.17	10.67	1	0.002
	E. nitens	−1.52 ± 0.57	0.04 ± 0.01	0.32	12.4	1	0.0016
An. pseudopunctipennis							
	Temephos	−0.87 ± 0.47	361.27 ± 142.65	0.18	6.41	1	0.016
	Permethrin	−1.05 ± 0.59	215.13 ± 92.06	0.19	5.46	1	0.028
	E. nitens	−0.27 ± 0.54	0.008 ± 0.013	0.012	0.42	1	0.52 (NS)

Therefore, the use of a higher concentration of the whole essential oil will affect the proportions of the main components in water.

In this study, we have chosen to evaluate behavioral variables that were directly related with the swimming activity of the larvae: the distance swam, the mean velocity, the time on each mobility state (HMt, Mt, and It) and the AAV. The last variable, AAV, is related with changes in the swimming direction of the larvae. The positive relationship of this variable with the PC 1 and in consequence with the concentration exposed could imply an undirected or uncontrolled swimming behavior. The relationship with this behavior generated by sublethal concentrations of pesticides was already observed in other organisms (Little and Finger 1990). On the other hand, the high percentage of variability explained by the PC 1 in all the cases indicates that each variable presented similar information but not identical. Therefore, analyzing the effects of insecticides using each variable alone would result in a redundant analysis, difficult to understand and incomplete, because the interaction between the variables is not considered. Finally, the larvae swimming activity was evaluated only by registering their horizontal movements. We chose to evaluate the horizontal movements to quantify activity because they are very simple to measure and are used by several authors (Liu et al. 2010, Tomé et al. 2014, Marriel et al. 2016, Gonzalez et al. 2017). In addition, the larvae, which were not able to do the vertical movements to breathe, were considered dead. Despite this, further studies could be directed to evaluate the effects of the larvicides in the vertical swimming activity to complement and verify the results of this work.

In conclusion, survival and behavioral effects of temephos, permethrin and *E. nitens* essential oil on *Ae. aegypti* and *An. pseudopunctipennis* were studied in this work. We determined for the first time toxicological values of the three larvicides for *An. pseudopunctipennis*. In almost all the cases, we identify a decrease on the swimming activity of the larvae exposed to the larvicides, and therefore it would lead to a higher mortality of the larvae in their natural habitats. Further studies would be necessary to verify and quantify the effects of the larvicides in the feeding behavior and the anti-predator response of the mosquito larvae. The current work has only examined the sublethal effects on the swimming behavior of those larvae that survived to an exposure to larvicides; future research may concentrate in the study of the sublethal effects of the larvicides in other life cycle stages as pupae or emerged adults.

Supplementary Material

Supplementary data are available at *Journal of Medical Entomology* online.

Acknowledgments

This work was supported by project PICT 2012-1857 from the Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT-Argentina) and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). We are very grateful to Laci C., Laci E., and Carrizo of the National Coordination of Vector Control, Argentinean Ministry of Health, for their assistance with fieldwork. A.A.C., P.V.G., L.H., and H.M. are members of the CONICET. We thank two anonymous reviewers and editor for their valuable comments and suggestions, which helped improve the quality of this work. Finally, we thank T. Comber for the English writing corrections.

References Cited

Abdel-Rahman, A., A. K. Shetty, and M. B. Abou-Donia. 2001. Subchronic dermal application of N,N-Diethyl m-toluamide (DEET) and permethrin to adult rats, alone or in combination, causes diffuse neuronal cell death and cytoskeletal abnormalities in the cerebral cortex and the hippocampus, and Purkinje neuron loss i. Exp. Neurol. 172: 153–171.

Alvarez Costa, A., C. V. Naspi, A. Lucia, and H. M. Masuh. 2017. Repellent and larvicidal activity of the essential oil from *Eucalyptus nitens* against *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae). J. Med. Entomol. 54: 670–676.

Amer, A., and H. Mehlhorn. 2006. Repellency effect of forty-one essential oils against *Aedes*, *Anopheles*, and *Culex* mosquitoes. Parasitol. Res. 99: 478–490.

Arredondo-Jiménez, J. I., and K. M. Valdez-Delgado. 2006. Effect of Novaluron (Rimon® 10 EC) on the mosquitoes *Anopheles albimanus*, *Anopheles pseudopunctipennis*, *Aedes aegypti*, *Aedes albopictus* and *Culex quinquefasciatus* from Chiapas, Mexico. Med. Vet. Entomol. 20: 377–387.

Banerjee, S., S. H. Yalkowsky, and C. Valvani. 1980. Water solubility and octanol/water partition coefficients of organics. Limitations of the solubility-partition coefficient correlation. Environ. Sci. Technol. 14: 1227–1229.

Batish, D. R., H. P. Singh, R. K. Kohli, and S. Kaur. 2008. *Eucalyptus* essential oil as a natural pesticide. For. Ecol. Manage. 256: 2166–2174.

Brackenbury, J. 2001. Locomotion through use of the mouth brushes in the larva of *Culex pipiens* (Diptera: Culicidae). Proc. R. Soc. B Biol. Sci. 268: 101–106.

Braga, I. A., and D. Valle. 2007. *Aedes aegypti*: inseticidas, mecanismos de ação e resistência. Epidemiol. e Serviços Saúde. 16: 279–293.

Bruce-Chwatt, L. J. 1985. Essential malariology, 1st ed. J. Wiley, New York, NY.

Bureˇsová, O., J. J. Bolhuis, and J. Bureˇs. 1986. Differential effects of Cholinergic Blockade on performance of rats in the water tank navigation task and in a radial water maze. Behav. Neurosci. 100: 476–482.

Campolo, O., F. V. Romeo, G. M. Algeri, F. Laudani, A. Malacrinó, N. Timpanaro, and V. Palmeri. 2016. Larvicidal effects of four citrus peel essential oils against the arbovirus vector *Aedes albopictus* (Diptera: Culicidae). J. Econ. Entomol. 109: 360-365.

Cantrell, C. L., F. E. Dayan, and S. O. Duke. 2012. Natural products as sources for new pesticides. J. Nat. Prod. 75: 1231–1242.

Chavasse, D. C., and H. H. Yap. 1997. Chemical methods for the control of vectors and pests of public health importance. World Healt Organization (WHO), Geneva, Switzerland.

Cohnstaedt, L. W., and S. A. Allan. 2011. Effects of sublethal pyrethroid exposure on the host-seeking behavior of female mosquitoes. J. Vector Ecol. 36: 395–403.

Dambolena, J. S., M. P. Zunino, J. M. Herrera, R. P. Pizzolitto, V. A. Areco, and J. A. Zygadlo. 2016. Terpenes: natural products for controlling insects of importance to human health—a structure-activity relationship study. Psyche A J. Entomol. 2016: 1–17.

Desneux, N., A. Decourtye, and J.-M. Delpuech. 2007. The sublethal effects of pesticides on beneficial arthropods. Annu. Rev. Entomol. 52: 81–106.

Elumalai, D., M. Hemavathi, P. Hemalatha, C. V. Deepaa, and P. K. Kaleena. 2016. Larvicidal activity of catechin isolated from *Leucas aspera* against *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus* (Diptera: Culicidae). Parasitol. Res. 115: 1203–1212.

Fernandez-Salas, I., D. R. Roberts, M. H. Rodriguez, and C. F. Marina-Fernandez. 1994. Bionomics of larval populations of *Anopheles pseudopunctipennis* in the Tapachula foothills area, southern Mexico. J. Am. Mosq. Control Assoc. 10: 477–486.

Gómez, A., E. Seccacini, E. Zerba, and S. Licastro. 2011. Comparison of the insecticide susceptibilities of laboratory strains of *Aedes aegypti* and *Aedes albopictus*. Mem. Inst. Oswaldo Cruz. 106: 993–996.

Gonzalez, P. V., A. A. Costa, H. M. Masuh, and L. Eisen. 2017. A video-tracking analysis-based behavioral assay for larvae of *Anopheles pseudopunctipennis* and *Aedes aegypti* (Diptera: Culicidae). J. Med. Entomol. 54: 793–797.

Grieco, F., L. Loijens, O. E. Krips, S. G, A. J. Spink, and P. Zimmerman. 2010. EthoVision XT reference manual. Noldus Information Technology, Wageningen, Netherlands.

Guedes, R. N. C., and G. C. Cutler. 2014. Insecticide-induced hormesis and arthropod pest management. Pest Manag. Sci. 70: 690–697.

Herrera, J. V. 2003. Prevención y control de la Malaria y otras enfermedades trasmitidas por vectores en el Perú. Rev. Peru. Epidemiol. 11: 18.

Janssens, L., and R. Stoks. 2012. How does a pesticide pulse increase vulnerability to predation? Combined effects on behavioral antipredator traits and escape swimming. Aquat. Toxicol. 110: 91–98.

Kemabonta, K., and A. Nwankwo. 2013. Larvicidal effectiveness of spinosad and temephos on *Anopheles gambiae* & *Aedes aegypti*. Int. J. Sci. Nat. 4: 214–222.

Kembro, J. M., R. H. Marin, J. A. Zygadlo, and R. M. Gleiser. 2009. Effects of the essential oils of *Lippia turbinata* and *Lippia polystachya* (Verbenaceae) on the temporal pattern of locomotion of the mosquito *Culex quinquefasciatus* (Diptera: Culicidae) larvae. Parasitol. Res. 104: 1119–1127.

Kumar, S., R. Warikoo, M. Mishra, A. Seth, and N. Wahab. 2012. Larvicidal efficacy of the Citrus limetta peel extracts against Indian strains of *Anopheles stephensi* Liston and *Aedes aegypti* L. Parasitol. Res. 111: 173–178.

Lardeux, F., V. Quispe, R. Tejerina, R. Rodríguez, L. Torrez, B. Bouchité, and T. Chávez. 2007. Laboratory colonization of *Anopheles pseudopunctipennis* (Diptera: Culicidae) without forced mating. Comptes Rendus - Biol. 330: 571–575.

Litchfield, J. T. J., and F. Wilcoxon. 1949. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther. 96: 99–113.

Little, E. E., and S. E. Finger. 1990. Swimming behavior as an indicator of sublethal toxicity in fish. Environ. Toxicol. Chem. 9: 13–19.

Liu, C., R. J. Pitts, J. D. Bohbot, P. L. Jones, G. Wang, and L. J. Zwiebel. 2010. Distinct olfactory signaling mechanisms in the malaria vector mosquito *Anopheles gambiae*. PLoS Biol. 8: 27–28.

Lucia, A., P. Gonzalez Audino, E. Seccacini, S. Licastro, E. Zerba, and H. Masuh. 2007. Larvicidal effect of *Eucalyptus Grandis* essential oil and turpentine and their major components on *Aedes Aegypti* larvae. J. Am. Mosq. Control Assoc. 23: 299–303.

Lucia, A., S. Licastro, E. Zerba, and H. Masuh. 2008. Yield, chemical composition, and bioactivity of essential oils from 12 species of *Eucalyptus* on *Aedes aegypti* larvae. Entomol. Exp. Appl. 129: 107–114.

Manguin, S., D. R. Roberts, E. L. Peyton, E. Rejmankova, and J. Pecor. 1996. Characterization of *Anopheles pseudopunctipennis* larval habitats. J. Am. Mosq. Control Assoc. 12: 619–626.

Marriel, N. B., H. V. V. Tomé, R. C. N. Guedes, and G. F. Martins. 2016. Deltamethrin-mediated survival, behavior, and oenocyte morphology of insecticide-susceptible and resistant yellow fever mosquitos (*Aedes aegypti*). Acta Trop. 158: 88–96.

Melo-Santos, M. A. V., J. J. M. Varjal-Melo, A. P. Araújo, T. C. S. Gomes, M. H. S. Paiva, L. N. Regis, A. F. Furtado, T. Magalhaes, M. L. G. Macoris, M. T. M. Andrighetti, et al. 2010. Resistance to the organophosphate temephos: mechanisms, evolution and reversion in an *Aedes aegypti* laboratory strain from Brazil. Acta Trop. 113: 180–189.

Mileson, B. E., J. E. Chambers, W. L. Chen, W. Dettbarn, M. Ehrich, A. T. Eldefrawi, D. W. Gaylor, K. Hamernik, E. Hodgson, A. G. Karczmar, et al. 1998. Common mechanism of toxicity: a case study of organophosphorus pesticides. Toxicol. Sci. 41: 8–20.

Narahashi, T. 1996. Neuronal ion channels as the target sites of insecticides. Phurmacology Toxicol. 18: 1–14.

Noldus, L. P. J. J., A. J. Spink, and R. A. J. Tegelenbosch. 2001. EthoVision: a versatile video tracking system for automation of behavioral experiments. Behav. Res. Methods Instruments Comput. 33: 398–414.

Ordoñez Gonzalez, J. G., M. G. Vazquez Martinez, K. M. Valdez Delgado, R. P. Penilla Navarro, J. L. Torres Estrada, and A. D. Rodriguez Ramirez. 2008. Fundamentos y métodos de control químico integrado. Manual para la vigilancia y el control del paludismo en Mesoamérica, 1st ed. Inst. Nac. Salud Pública., Cuernavaca, México.

Osimitz, T. G., and J. G. Murphy. 1997. Neurological effects associated with use of the insect repellent N,N-diethyl-m-toluamide (DEET). J. Toxicol. Clin. Toxicol. 35: 435–441.

Park, C. G., M. Jang, E. Shin, and J. Kim. 2017. Myrtaceae plant essential oils and their β-triketone components as insecticides against Drosophila suzukii. Molecules. 22: 1050–1060.

Paul, A., L. C. Harrington, and J. G. Scott. 2006. Evaluation of novel insecticides for control of dengue vector *Aedes aegypti* (Diptera : Culicidae). J. Med. Entomol. 43: 55–60.

Pavela, R. 2015. Essential oils for the development of eco-friendly mosquito larvicides: a review. Ind. Crops Prod. 76: 174–187.

Polson, K. A., W. G. Brogdon, S. C. Rawlins, and D. D. Chadee. 2011. Characterization of insecticide resistance in Trinidadian strains of *Aedes aegypti* mosquitoes. Acta Trop. 117: 31–38.

Pridgeon, J. W., R. M. Pereira, J. J. Becnel, S. A. Allan, G. G. Clark, and K. J. Linthicum. 2008. Susceptibility of *Aedes aegypti*, *Culex quinquefasciatus* Say, and *Anopheles quadrimaculatus* Say to 19 pesticides with different modes of action. J. Med. Entomol. 45: 82–87.

R Core Team. 2016. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Ray, D. E., and J. R. Fry. 2006. A reassessment of the neurotoxicity of pyrethroid insecticides. Pharmacol. Ther. 111: 174–193.

Reynaldi, S., M. Meiser, and M. Liess. 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: 56–60.

Schulz, R., and J. M. Dabrowski. 2001. Combined effects of predatory fish and sublethal pesticide contamination on the behavior and mortality of mayfly nymphs. Environ. Toxicol. Chem. 20: 2537–2543.

Scott, T. W., A. C. Morrison, L. H. Lorenz, G. G. Clark, D. Strickman, P. Kittayapong, H. Zhou, and J. D. Edman. 2000. Longitudinal studies of *Aedes aegypti* (Diptera: Culicidae) in Thailand and Puerto Rico: population dynamics. J. Med. Entomol. 37: 77–88.

Senthilkumar, A., M. Jayaraman, and V. Venkatesalu. 2013. Chemical constituents and larvicidal potential of Feronia limonia leaf essential oil against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*. Parasitol. Res. 112: 1337–1342.

Shi, X., L. Jiang, H. Wang, K. Qiao, D. Wang, and K. Wang. 2011. Toxicities and sublethal effects of seven neonicotinoid insecticides on survival, growth and reproduction of imidacloprid-resistant cotton aphid, *Aphis gossypii*. Pest Manag. Sci. 67: 1528–1533.

Spruijt, B. M., M. O. S. Buma, P. B. A. van Lochem, and J. B. I. Rousseau. 1998. Automatic behavior recognition: what do we want to recognize and how do we measure it?, pp. 264–266. In L. P. J. J. Noldus (ed.), Proceedings of Measuring Behavior. University of Groningen, Groningen, Netherlands.

TGSC, T. G. S. C. 2018. Flavesone. Physical proprieties. http://www.thegoodscentscompany.com/data/rw1567781.html

Tomé, H. V., T. V. Pascini, R. A. Dângelo, R. N. Guedes, and G. F. Martins. 2014. Survival and swimming behavior of insecticide-exposed larvae and pupae of the yellow fever mosquito *Aedes aegypti*. Parasit. Vectors. 7: 195.

Valença, M. A., L. S. Marteis, L. M. Steffler, A. M. Silva, and R. L. C. Santos. 2013. Dynamics and characterization of *Aedes aegypti* (L.) (Diptera: Culicidae) key breeding sites. Neotrop. Entomol. 42: 311–316.

WHO. 1992. Vector resistance to pesticides. Fifteenth report of the WHO expert committee on vector biology and control. World Heal. Organ. - Tech. Rep. Ser. 818: 1–62.

WHO. 2011. Guidelines for drinking-water quality. WHO Chronicle. 38: 104–108. http://apps.who.int/iris/bitstream/10665/44584/1/9789241548151_eng.pdf

WHO. 2018. Chemical control. Programme of dengue control. http://www.who.int/denguecontrol/control_strategies/chemical_control/en

Ye, Y. H., S. F. Chenoweth, A. M. Carrasco, S. L. Allen, F. D. Frentiu, A. F. van den Hurk, N. W. Beebe, and E. A. McGraw. 2016. Evolutionary potential of the extrinsic incubation period of dengue virus in *Aedes aegypti*. Evolution (N. Y). 70: 2459–2469.