Comparison of the insecticide susceptibilities of laboratory strains of *Aedes aegypti* and *Aedes albopictus*

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A susceptible strain of Aedes albopictus *derived from the Gainesville strain (Florida, USA) was established in our laboratory. The larvicidal efficacies of the neurotoxic insecticides temephos, permethrin and the pure* cis *and* trans-*permethrin isomers and the microbial insecticide* Bacillus thuringiensis israelensis *(*Bti*) against* Ae. albopictus *were estimated and compared to a susceptible strain of* Aedes aegypti*. The larvicidal effect of insect growth regulator pyriproxyfen was also evaluated in both mosquito strains. The median lethal concentration/median emergency inhibition values for* Ae. aegypti *and* Ae. albopictus*, respectively, were: temephos, 3.058 and 6.632 ppb, permethrin, 3.143 and 4.933 ppb,* cis-*permethrin, 4.457 and 10.068 ppb,* trans-*permethrin, 1.510 and 3.883 ppb,* Bti*, 0.655 and 0.880 ppb and pyriproxyfen, 0.00774 and 0.01642 ppb.* Ae. albopictus *was more tolerant than* Ae. aegypti *to all six larvicides evaluated. The order of susceptibility for* Ae. aegypti *was pyriproxyfen* > Bti > trans-*permethrin > temephos > permethrin >* cis-*permethrin and for* Ae. albopictus *was pyriproxyfen* > Bti > trans-*permethrin > permethrin > temephos >* cis-*permethrin. Because both species can be found together in common urban, suburban and rural breeding sites, the results of this work provide baseline data on the susceptibility of* Ae. albopictus *to insecticides commonly used for controlling* Ae. aegypti *in the field.*

Key words: *Aedes albopictus* - *Aedes aegypti* - insecticide susceptibility

Dengue fever (DF) and dengue haemorrhagic fever are vector-borne diseases of public health importance in tropical, subtropical and temperate regions of the world (Gubler 1998). It is a popular belief that *Aedes aegypti* (Linnaeus) is the sole vector of the four distinct serotypes of dengue virus that causes the spectrum of disease symptoms collectively known as "dengue" (including DF, dengue haemorrhagic fever and dengue shock syndrome). However, other *Aedes* species, such as *Aedes albopictus* (Skuse), have been incriminated as DF vectors in epidemics in Southeast Asia and other parts of the world (Knudsen 1995) and could be responsible for the occurrence of these viruses in places where *Ae. aegypti* is absent (Shroyer 1986). *Ae. albopictus* is also a potential vector of several additional arboviruses, some of which have considerable medical importance, such as Chikungunya virus (Mangiafico 1971).

Ae. aegypti and *Ae. albopictus* are able to coexist in man-made containers in urban, suburban and rural areas (Vezzani & Carbajo 2008). Additionally, *Ae. albopictus* larvae inhabit natural containers such as bromeliads, bamboo stumps and tree-holes close to human habitats and can survive throughout a broad range of temperatures and relative humidity (RH) levels (Hawley 1988).

In North America, *Ae. albopictus* was first discovered in Texas (USA) in 1985 (Francy et al. 1990). In South America, it was discovered for the first time in Brazil during 1986 (Rai 1991). In Argentina, it was initially detected in Misiones near the Brazilian border, in early 1998 (Rossi et al. 1999, Schweigmann et al. 2004).

The continuous expansion of the geographic range of *Ae. albopictus* in Latin America, including Argentina, the recent findings regarding its vector potential (Mitchell et al. 1987) and the observations made indicating a competitive advantage for *Ae. albopictus* over *Ae. aegypti* (Braks et al. 2004), have increased the public awareness of this mosquito and the attempts to control it.

In the absence of a dengue vaccine, controlling dengue vectors is regarded as essential in preventing epidemics. The application of larvicides to containers that cannot be eliminated is still considered a priority by control programmes. However, this activity is both labour intensive and time consuming and not all containers can be treated because there are certain locations in and around urban areas that offer permanent breeding sites. In addition, the continuous application of insecticides in vector control strategies can result in the development of insecticide resistance.

To contribute to the knowledge on the susceptibility of dengue vectors to insecticides, we determined a baseline susceptibility of laboratory strains of *Ae. aegypti* and *Ae. albopictus* larvae to the insecticides commonly used in Argentina for vector control. This information will be valuable in the implementation of further resistance monitoring programmes. According to our past

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experience, the baseline susceptibility to insecticides was similar for laboratory and field strains of *Ae. aegypti* in areas without insecticide treatments (Seccacini et al. 2008b, Albrieu Llinás et al. 2010). The results of this work will allow us to determine the most effective compound for the less susceptible mosquito species and to suggest the best strategy control authorities if both species coexist in urban and periurban breeding containers.

Susceptible strains of *Ae. aegypti* (derived from the Rockefeller strain from Venezuela) and *Ae. albopictus* (derived from the strain from Gainesville, FL, USA) were reared at 25 ± 2ºC at 80-90% RH and with a photoperiod of 12:12 h. Larvae of both species were fed on a mixture of rabbit pellets and yeast and were used for the bioassays according to the methods of previous studies (Lucia et. al. 2007).

Pyriproxyfen (97.8%) (China Kelinon Agrochemical Co, Ltd, China), temephos (90%) (Ningguo Jiahua Chemistry Co, Ltd, China) and *Bti* [*Bacillus thuringiensis israelensis*, Bactivec® - 1200 International Toxic Units (ITU)/mg, Valent BioSciences, USA] were used as insecticides. Technical grade permethrin (*cis*:*trans* mixture 1:1), *cis*-permethrin and *trans*-permethrin were provided by Chemotecnica SA, Argentina.

The test concentrations of all insecticides, except temephos and *Bti*, were prepared by successive dilutions of 1 mg/mL acetone solutions. Temephos was diluted in absolute ethanol and *Bti* was diluted in water. All solvents used were for analysis (Merck, Germany).

The larvicidal bioassay was performed according to a protocol previously used in our laboratory (Bisset et al. 2005). One millilitre of the insecticide solution to be tested was added to 224 mL of water in a 500 mL plastic jar, which was shaken lightly to ensure a homogeneous test solution. Then, 20 late third or early forth instar *Ae. aegypti* or *Ae. albopictus* larvae previously acclimated for 2-3 h in 25 mL of water were added to the jar. Five different concentrations of each insecticide were tested and untreated cups were used as a control. Each concentration was replicated three-five times. No food was offered to the larvae except in the pyriproxyfen assay, in which approximately 100 mg of rabbit pellets were added to each jar. All bioassays were conducted in a regulated chamber (25 ± 2ºC, 80-90% RH and 12:12 h photoperiod) and larvae mortality was recorded after 24 h of exposure to temephos, *Bti*, permethrin and the *cis/trans* isomers. As in previous studies (Seccacini et al. 2008a) of pyriproxyfen, the jars were examined daily and cumulative larval and pupal mortality and adult emergence were recorded until adult emergence was complete in all of the control jars.

Dose-mortality data from each pool were subjected to probit analysis (Litchfield & Wilcoxon 1949). The 50% and 95% lethal concentrations (LC_{50} and LC_{95}) with the corresponding confidence limits were obtained using PoloPlus 2.0 (LeOra Software, USA) and were expressed as the final concentration in parts per billion. For pyriproxyfen, the effective concentration to inhibit adult emergence by 50% (EI_{50}) observed on the day all of the control mosquitoes emerged as adults was calculated. The values of LC_{50} and EI_{50} were adjusted for the mortality of the controls (Mulla et al. 1974) and were considered to be significantly different if the 95% confidence intervals (CI) did not overlap (Robertson & Preisler 1992). A tolerance index is defined as the ratio between the LC_{50} or the EI_{50} values for *Ae. albopictus* and those for *Ae. aegypti* was calculated for each insecticide assayed. Table shows the larvae mortality data for *Ae. aegypti* and *Ae. albopictus* expressed as LC_{50}/EI_{50} and LC_{95}/EI_{95} with the corresponding CIs and the tolerance index between species. Pyriproxyfen was the most ef-

TABLE

Comparative susceptibility of *Aedes aegypti* and *Aedes albopictus* to larvicides used in Argentina

	Ae. aegypti		*Ae. albopictus*		
Insecticide	LC_{50} (95% CI) pbb	LC_{95} (95% CI) pbb	LC_{50} (95% CI) pbb	LC_{95} (95% CI) pbb	Tolerance index
Temephos	3.058 (2.533-3746)	6.546 (4.998-11.126)	6.632 (5.975-7.339)	10.746 (9.352-13.543)	2.169 (1.853-2.540)
Permethrin	3.143 (2.609-3.703)	9.381 (7.330-13.827)	4.933 (4.080-6.033)	13.578 (10.121-22.239)	1.569 (1.249-1.972)
Cis-permethrin	4.457 (3.984-4.992)	10.015 (8.419-12.819)	10.068 (8.888-11.407)	24.942 (20.654-32.480)	2.260 (1.910-2.674)
Trans-permethrin	1.510 (1.233-1.853)	5.202 (3.777-8.752)	3.883 (3.432-4.384)	7.010 (5.901-9.428)	2.572 (2.180-3.035)
Bti	0.655 (0.528-0.796)	1.364 (1.053-2.391)	0.880 (0.765-1.014)	1.919 (1.565-2.640)	1.267 (1.095-1.467)
Pyriproxyfen[a]	0.008 (0.005-0.011)	0.067 (0.040-0.148)	0.016 (0.008-0.032)	0.217 (0.089-1.219)	2.122 (1.209-3.725)

a: the effective concentration for pyriproxyfen is measured as adult emergence inhibition (EI_{50}/EI_{95}) not as lethal concentration (LC_{50}/LC_{95}). *Bti*: *Bacillus thuringiensis israelensis*; CI: confidence interval.

fective insecticide against *Ae. aegypti* and *Ae. albopictus*. A tolerance index is defined as the ratio between the LC_{50} or the EI_{50} values for *Ae. albopictus,* and those for *Ae. aegypti* was calculated for each insecticide assayed that of *cis*-permethrin (LC_{50} = 4.457 and 10.068 ppb), the less active isomer of permethrin. *Bti* was also effective, with LC_{50} = 0.655 and 0.880 ppb for *Ae. aegypti* and *Ae. albopictus*, respectively, followed by *trans*-permethrin (LC_{50} = 1.510 and 3.883 ppb, respectively), which was more effective than permethrin (*cis*:*trans* 1:1 mixture with 3.143 and 4.933 ppb, respectively) and temephos (3.058 and 6.632 ppb, respectively).

For temephos, the mortality data we obtained for *Ae. albopictus* (Table) were similar to the data obtained by other authors (Ali et al. 1995, Romi et al. 2003, Ponlawat et al. 2005), but for permethrin there were differences that could be due to the fact that in most of the cases the exact composition of permethrin (ratio of *cis*/*trans* isomers) is unknown (Sulaiman et al. 1991, Ali et al. 1995). We found that for both mosquito species *trans*-permethrin was more effective than the 1:1 *cis*-*trans* mixture and *cis*-permethrin alone. These results are in agreement with the results obtained by Pap et al. (1996), who found that the *trans* isomers of some pyrethroids were more active than the *cis* isomers against *Aedes* mosquitoes. For mammals and most insect species, it has been reported that the *cis* isomer is more active than the *trans* isomer (Naumann 1990), because the *trans* isomer is metabolised faster by hydrolytic esterases (Perry et al. 1998). As can be seen in Table, the inverse situation was observed for *Ae. aegypti* and *Ae. albopictus*.

The small difference observed between *Bti* and pyriproxyfen in both mosquito species (Table) could be related to the particular mode of action of these larvicides. *Bti* endotoxin acts on the midgut epithelium, which is presumably similar in these two species. Pyriproxyfen is a mimic of a juvenile hormone that regulates metamorphosis. We also found a higher toxicity value for *Bti* than other authors have (Ali et al. 1995, Duque & Navarro-Silva 2005). When *Bti* is used, the active ingredient concentration is expressed as ITU compared to a known standard. Our laboratory found differences between the nominal and real ITU values for *Bti* formulations of different origins (unpublished observations). Larvae mortality is strongly dependent on the *Bti* formulation, being affected by factors such as the bacteria strain, way of fermentation and shell stability. An important factor affecting the performance of a particular *Bti* formulation is time elapsed since it was manufactured.

Table also shows the tolerance index for *Ae. albopictus* relative to *Ae. aegypti*. The susceptibilities of *Ae. aegypti* and *Ae. albopictus* to insecticides were different, but based on the CIs, these differences were only significant ($p < 0.05$) for the neurotoxic insecticides (temephos, permethrin, *cis*-permethrin and *trans*-permethrin), but not for pyriproxyfen and *Bti*, most likely due to its different mode of action.

There is very little published information on the susceptibilities to insecticides of *Ae. albopictus* compared to *Ae. aegypti* under laboratory conditions. A review of insecticide resistance in dengue vectors revealed that there is a great deal of information regarding the insecticide susceptibility of *Ae. aegypti*; some on information on the susceptibility of *Ae. albopictus* and few studies that have compared both species (Ranson et al. 2010). Another fact to consider is that there is no available reference strain for *Ae. albopictus*. The reference strain for *Ae. aegypti* in most laboratories is the Rockefeller strain or the Bora-Bora strain. However, almost all the studies on *Ae. albopictus* use a local field strain that was collected and reared in the laboratory for different periods of time and then the most susceptible strain is taken as the reference. In our case, we used the Gainesville strain, which has been reared in the laboratory since 1992 and is strongly believed to be susceptible.

Based on our results, we conclude that, for laboratory strains, *Ae. albopictus* is more tolerant than *Ae. aegypti* to the six compounds studied, but this difference was only significant for neurotoxic insecticides. The most effective insecticide was pyriproxyfen and *trans*-permethrin was more effective than the *cis*-*trans* mixture or the *cis* isomer. LC_{50} values obtained in the laboratory are useful to compare the insecticidal effects between insect strains, although it is known that the field concentrations used for control strategies are higher than the LC_{50} values obtained in the laboratory.

For vector control strategies, the Ministry of Health of Argentina currently uses temephos and *Bti* as larvicides and *cis*-permethrin as an adulticide. New formulations based on pyriproxyfen and permethrin are needed in Argentina.

This comparative study of the susceptibility of *Ae. aegypti* and *Ae. albopictus* to larvicides with different modes of action under laboratory conditions could provide baseline susceptibility data for the insecticides used in vector control programmes and these data could be useful for further studies on field strains and in the monitoring of resistance. *Ae. aegypti* and, to an increasing extent, *Ae. albopictus* are species that do not respect country borders and because both mosquito species can be found in the same man-made containers in urban and periurban areas, information on the insecticide susceptibilities of both species could help to improve future control activities.

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