A New Ovitrap Made of Slow Release Natural Materials Containing Pyriproxyfen for *Aedes aegypti* (Diptera:Culicidae) Control

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ABSTRACT This initial study is aimed to measure the performance of incorporating pyriproxyfen in natural materials with low environmental impact to obtain slow release formulations that can be used as larvicidal or autocidal ovitraps avoiding hatched *Aedes aegypti* (L.) eggs to emerge as adults. Hollow candles made of beeswax or paraffin:stearin 1:1 mixture containing pyriproxyfen 0.01 and 0.05% were prepared and used as holding water containers for larval bioassay. Pyriproxyfen was released quickly into the larvae-breeding water. *Ae. aegypti* larvae were introduced immediately after the addition of tap water to the hollow candles ($t = 1$ min) or after 1, 4, and 8 h. More than 40% of the larvae did not emerge as adults for $t = 1$ min, reaching 80–100% when the larvae were added after 1 or 4 h, respectively. The hollow candles were kept at room temperature, and water was replaced every 15 d. Bioassays performed every 30 d showed that the residual activity obtained for both matrices and both concentrations of pyriproxyfen was higher than 360 d, with 100% inhibition of adult emergence.

KEY WORDS *Aedes aegypti* control, dengue, larvicide ovitrap, pyriproxyfen

Dengue is an illness spread through the bites of virus-infected mosquitoes. It is endemic in 100 countries where an estimated 2.5 billion people live, representing more than one-third of the world's population (Gubler and Clark 1995). The primary global vector of dengue is the mosquito *Aedes aegypti* (L.), which is found in Argentina since 1996 (Vezzani and Carbajo 2008). As no vaccines are available, mosquito control is the most important measure to prevent this disease.

The elimination of *Ae. aegypti* oviposition sites, as well as the application of larvicides in containers that cannot be eliminated (WHO 1995), are the principal preventive activities in control programs as recommended by the World Health Organization (WHO). Current WHO research strategies for dengue control promotes an Integrated Vector Management that includes the use of new tools for vector control such as lethal, autocidal, or sticky ovitraps. (http://www.who.int/denguecontrol/research/en/).

Ovitraps first were described by Fay and Perry (1965) and have been widely used around the world as surveillance tools, for detecting and monitoring *Ae. aegypti* populations. The ovitrap first was used for *Ae. aegypti* control in 1969 at Singapore International Airport, with a significant reduction of the mosquito population (Chan 1973). Different studies demonstrated the feasibility of using modified ovitraps for the control of *Ae. aegypti* (Antonio-Arreola et al. 2011). As an extension, new lethal ovitraps were designed in which the rough egg-laying strip was treated with a pyrethroid insecticide and used as a lure-and-kill device (Perich et al. 2003;; Williams et al. 2007; Ritchie et al. 2008, 2009). Considering the increasing use of lethal ovitraps for *Aedes* control (Zeichner 2011) and the success of nets impregnated with insecticides in controlling malaria vectors, more recently measured in *Aedes* control (Lenhart et al. 2008), our laboratory began to study the incorporation of insecticides into different materials acting as slow release formulations.

In this study, we began to measure natural materials such as stearin, paraffin wax, or beeswax, containing pyriproxyfen as larvicide, to be used as constitutive materials in slow release devices for larvae control.

An *Ae. aegypti* insecticide-susceptible strain (originated from the Rockefeller strain) was reared at 25 ± 2°C, 80–90% RH, and a photoperiod of 12:12 (L:D) h. Larvae were fed on a mixture of rabbit pellets and yeast. Late third- or early fourth-instar larvae were used for bioassays (WHO 2005) according to previous studies.

Paraffin wax, stearin (triglyceryl stearate), and beeswax were purchased from Parafarm (Saporiti, Argentina). Pyriproxyfen (2-[1-methyl-2-(4-phenoxyphenoxy) ethoxy] pyridine) (97.8%, China Kelinon Agrochemical Co., Ltd., China) at concentrations of 0.01 and 0.05% was incorporated with continuous magnetic stirring into the fused paraffin:stearin (1:1) mixture or beeswax, in a water bath heated at 65°C. "Ad hoc" aluminum molds were used to design the hollow

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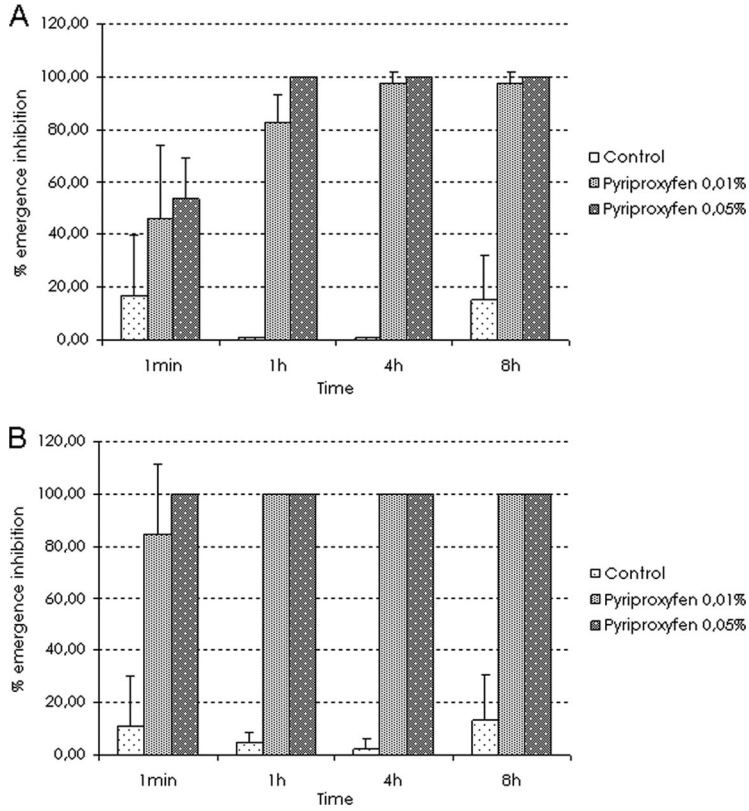

Fig. 1. Percentage of *Ae. aegypti* EI for pyriproxyfen 0.01 and 0.05% released into the water at different times: (A) from beeswax hollow candles, (B) from paraffin:stearin (1:1) hollow candles.

candles. Once cold, they were unmold and used as bioassay jars.

The larvicidal bioassay was performed according to a protocol previously used in our laboratory (Bisset et al. 2005). Tap water (250 ml) was added to the hollow candles and next, 20 late third- or early fourth-instar *Ae. aegypti* larvae. After addition of 100 mg of the food mixture, the jars were maintained in a regulated chamber at 27 ± 2°C, 80–90% RH, and a photoperiod of 12:12 (L:D) h. As in previous studies, the jars were examined daily, and larval and pupal cumulative mortality and adult emergence were recorded until adult emergence was completed in all the control jars (Seccacini et al. 2008, Harburguer et al. 2009). Larvae mortality was expressed as Adult Emergence Inhibition (EI%) according to Mulla et al. (1986).

Identical hollow candles without pyriproxyfen were used as control jars; each assay was replicated three times.

To measure residual activity, the tap water contained in the jars and used in the previous larval bioassay was replaced with clean water every 15 d up to the end of the assay. The bioassay was repeated as stated before every 30 d up to 360 d. The hollow candles were kept inside the laboratory and maintained in natural conditions at a temperature of 20 ± 5°C.

In a separate bioassay, the initial rate of larvicide activity was calculated by taking off aliquots of 100 ml from the upper part of independent hollow candles at different intervals of time since the moment of adding the tap water (t = 1 min, 1 h, 4 h, 8 h). The 100-ml aliquots were introduced in clean jars, and the bioassay was performed as described before.

Pyriproxyfen is considered one of the most promising larvicidal and pupacidal products currently available and accepted by WHO for drinking water treatments (WHO 2008).

Considering field studies with a granular formulation containing 0.5% pyriproxyfen (Sumilarv, Sumitomo Chemical Co., Japan), our previous screening bioassays, and that field dose rates must be higher than EI_{50} values, we chose in this study 0.01 and 0.05% as the concentrations for our slow release devices. Currently, we are measuring lower concentrations of pyriproxyfen up to 10^{-5}%.

In Fig. 1A and B, immature stages mortality, measured as the EI%, in beeswax and paraffin:stearin mixture hollow candles containing 0.01 and 0.05% pyriproxyfen, respectively, and performed with the water taken from the jars at different contact times, are shown. Standard deviation for each measure is given. It can be seen that pyriproxyfen is immediately released from the matrix into the water. Because EI

increased from 40% for $t = 1$ min to 80–100% for 1 and 4 h, respectively, it can be supposed that the concentration of pyriproxyfen in the water increases with the time of contact with the jar until a plateau is reached. It can also be seen that at the initial time, EI was higher for paraffin:stearin mixture than for beeswax. This can be explained considering that the liberation rate of pyriproxyfen may be different in both matrices or that more pyriproxyfen is initially bio available in the internal surface for the paraffin:stearin mixture than for beeswax. The mean value for EI in control hollow candles was below 20%. The assay was performed with late third- or early fourth-instar *Ae. aegypti* larvae, as previous studies of our laboratory have shown that larvae mortality after pyriproxyfen treatments occurs mainly at the pupal stage (Harburguer et al. 2009). In addition, pyriproxyfen does not inhibit oviposition, and eggs immersed in a water solution of pyriproxyfen hatch normally to larvae (unpublished observations).

Regarding the residual activity bioassays performed with the beeswax and paraffin:stearin mixture hollow candles containing 0.01 and 0.05% pyriproxyfen, we found 100% EI up to 360 d for both matrices and for both concentrations of pyriproxyfen studied. Because the bioassays for residual activity were performed every 30 d, the water in the hollow candles was changed twice in that time, showing that pyriproxyfen was released continuously into the water.

According to previous studies of our laboratory, adult emergence was achieved between days 10 and 14, with no differences between control and pyriproxyfen-treated groups. The main factor responsible for EI was mortality at different instances of the pupal stage (Harburguer et al. 2009).

The results presented in this initial study show that natural materials such as beeswax or paraffin:stearin containing pyriproxyfen in low concentrations represent a new long-lasting material with a long residual activity and low environmental impact.

It can be concluded that the hollow candles impregnated with pyriproxyfen could be used as larvicidal or autocidal ovitraps for *Ae. aegypti* control, in which hatched eggs would be unable to emerge as adults. Because these ovitraps only kill larvae, pupae, or both, their use might be associated with adulticide treatments to avoid reinfestation.

Our laboratory is also working on pieces of different sizes and shapes, made of the same natural materials containing from 10^{-5} to 0.5% pyriproxyfen, to be sunken or sticked on the wall under the water surface of different mosquito-breeding containers (water storage tanks, pots, wells, or drains). The long lasting release of pyriproxyfen from these pieces of natural materials can convert any breeding container in an autocidal ovitrap.

In another preliminary study, modified ovitraps were designed in which pyriproxyfen was incorporated into a black high density polyethylene matrix and assayed for EI (Lorenzo et al. 2011) with excellent results and are going to be assayed in a field trial (Argentine patent presented N° 20110104153). Because a large number and well-trained vector control officers were required to inspect and maintain ovitraps as a surveillance tool, a modified ovitrap in which eggs that have hatched would be unable to emerge as adults may provide a new tool to diminish mosquito populations.

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