

# Lufenuron can be transferred by gravid *Aedes aegypti* females to breeding sites and can affect its fertility, fecundity and blood intake capacity

**CURRENT STATUS:** UNDER REVIEW

Parasites & Vectors  BMC

Paula V Gonzalez  
Consejo Nacional de Investigaciones Cientificas y Tecnicas

Laura Harburguer  
Consejo Nacional de Investigaciones Cientificas y Tecnicas

 lharburguer@citedef.gob.ar *Corresponding Author*

## DOI:

10.21203/rs.2.20032/v2

## SUBJECT AREAS

*Parasitology*

## KEYWORDS

*Aedes aegypti, lufenuron, auto-dissemination, fertility, fecundity.*

## Abstract

Background: *Aedes aegypti* (L.) is the main vector of dengue, yellow fever, Zika and chikungunya viruses. A new method for controlling this mosquito has been developed based on the possibility that wild adult mosquitoes exposed to artificial resting sites contaminated with a larvicide, can disseminate it to larval breeding sites, is named "auto-dissemination". The present study was undertaken to evaluate if a chitin synthesis inhibitor like lufenuron can be disseminated to larval breeding sites and prevent adult emergence and also if forced contact of *Ae. aegypti* females with treated surfaces can affect its fertility, fecundity and blood intake capacity. Methods: Larval susceptibility to lufenuron was measured through EI 50 and EI 90. On the other hand gravid females were exposed by tarsal contact to lufenuron-treated papers, we used the WHO susceptibility test kit tube to line the papers, and 1, 3 or 5 females for the transference. We also evaluated if the exposure of mosquito females to lufenuron-treated papers (0.4 and 1 mg a.i./cm<sup>2</sup>) has an effect on their fertility, fecundity or in the ability to feed on blood. In each assay 12-15 mosquito females were exposed to lufenuron for 1 hour; 24 h before (before blood meal - BBM) or 24 h after a blood meal (ABM). Results: Lufenuron proved to be very active against *Ae. aegypti* larvae with an EI 50 of 0.164 ppb and EI 90 of 0.81 ppb. We also found that lufenuron can be transferred by females from treated surfaces to clean containers causing the inhibition of emergence of the larvae (between 30 and 50%). This effect was dependent on the concentration applied on the paper and also the number of females added to each cage. Conclusions: This paper introduces an innovation by first exploring the possibility that an IGR belonging to the group of benzoylphenyl ureas, such as lufenuron, can be transferred by gravid females to breeding sites and that at the same time can have an effect on fertility, fecundity and blood intake capacity of adult mosquitoes. Keywords: *Aedes aegypti*, lufenuron, auto-dissemination, fertility, fecundity.

## Background

*Aedes aegypti* (L.) is the main vector of dengue, yellow fever, Zika and chikungunya viruses in many parts of the world affecting millions of people worldwide each year. The most commonly utilized strategy to reduce *Ae. aegypti* densities is aimed at the larval stages (removal of breeding sites,

larvicide and community education) to reduce the population of new adults. Also, adult control using spatial sprays with adulticides is recommended when dengue outbreaks occurs [1]. Because adult emergence from container habitats is continuous, conventional adult insecticides spraying generally achieves inadequate and merely transient control [2-6].

The use of larvicides in containers that can result as potential breeding places and cannot be eliminated is the main alternative in control programs, but this only targets an unknown percentage of the overall aquatic habitat. Application of larvicides to containers used as oviposition sites requires a house-to-house search to find and treat containers, which may not be feasible in large communities. In addition, treating larval production sites has produced insecticide resistant populations especially to the principal larvicide used the last years, the organophosphorus temephos; pointing out the need of new larvicides for mosquito control [7-11].

During the past two decades, considerable progress has been made in the development of natural and synthetic compounds that are capable of interfering with the growth, development and reproduction processes of insects [12]. These substances are classified as insect growth regulators (IGRs) and compared with other insecticides, are safer for the environment and non-target organisms, including mammals, at the recommended doses [13-15]. There are three major groups of IGRs: the juvenile hormone analogues, the ecdysone agonists and the chitin synthesis inhibitors [12, 16]. A common property of this last group of IGRs, also called benzoylureas (BPU), is that they result in abortive molting and egg hatching as a consequence of chitin synthesis inhibition in the course of cuticle formation. The first chitin synthesis inhibitor introduced in markets was diflubenzuron [17]. This IGR was used successfully against various pest insects, including mosquitoes [14, 18, 19]. Among the most successful benzoylurea compounds next to diflubenzuron are triflumuron, hexaflumuron, lufenuron and novaluron. Lufenuron is one of the most newly introduced synthetic benzoylurea (CibaGeigy in 1998) used for the control of lepidopteran and coleopteran larvae. It is a compound found to be nontoxic to mammals and other vertebrates at the doses required against insects. Furthermore, it has been reported that lufenuron is suitable for integrated pest management programs because of its long residual action and safety to adult beneficial insects, mites and spiders

[20]. Although several reports describe the effects of different benzoylureas, against disease vectors [21–25], there is only one study that evaluates the toxicity of lufenuron on *Ae. aegypti* larvae, nothing is known about its effect in the biology and reproductive fitness of the adult.

*Aedes aegypti* is a diurnal species that displays skip-oviposition behavior, i.e. lays small numbers of eggs in multiple sites [26]. These sites are often small and difficult to locate, which makes effective larvae elimination difficult. A novel method of control for this mosquito species was suggested from laboratory research results reported by Itoh and colleagues [27]. They found that bloodfed females of *Ae. aegypti* that had been forced into contact with surfaces treated with the IGR, pyriproxyfen (a juvenile hormone analogue), transported sufficient amounts to disrupt larval development in untreated oviposition sites. This approach, named “auto-dissemination”, is based on the possibility that wild adult mosquitoes exposed to artificial resting sites contaminated with pyriproxyfen, can disseminate insecticide to larval breeding sites, thus preventing adult emergence. Extraordinarily low doses of pyriproxyfen are needed to interfere with the metamorphosis of juvenile stages [28], and/or to cause morphological and functional aberrations in emerging adults, such as decreased fertility [29–31].

Since the work of Itoh and colleagues [27] a lot of evidence has been collected in laboratory and field works that shows that mosquito females either forced to walk on pyriproxyfen-treated surface or topically contaminated can contaminate larval sites and significantly inhibit adult emergence [28, 32–35]. However, it has never been evaluated if an IGR belonging to chitin synthesis inhibitors group can be disseminated, which doses are needed on a contaminated surface to have an effect in untreated oviposition sites and if they can affect the adult fecundity and fertility.

The present study was undertaken to evaluate if a BPU like lufenuron can be disseminated to larval breeding sites and prevent adult emergence and also if forced contact of *Ae. aegypti* females with treated surfaces can affect its fertility, fecundity and blood intake capacity.

## Methods

### **Mosquitoes**

A susceptible strain of *Ae. aegypti* (CIPEIN) was used. This strain was originated from a Rockefeller

strain from Venezuela and had been kept in the laboratory since 1996, reared at  $26 \pm 2^\circ\text{C}$  under a 12:12 h light: dark photoperiod. This colony is maintained free of exposure to pathogens, insecticides or repellents. Eggs were collected over wet cotton then dehydrated at room temperature and stored at least 30 days. They were rehydrated in dechlorinated water (about 500 eggs per 2 liters of water) and 24 h after rehydration, first instar larvae were observed. Larvae were fed on a mixture of rabbit pellet and yeast. For this study, late third-instar or early fourth-instar larvae were used, and 5-7 days old adults females.

### **Chemicals**

Lufenuron (N-[(2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)phenyl)carbonyl]-2,6-difluorobenzamide), (technical grade 97.8%; Zhejiang Segate Science and Technology Co., Ltd, China) was used.

Silicone oil Dow Corning 556 was purchased from Daltosur SRL, Buenos Aires, Argentina.

All solvents used (acetone) were analytical grade.

### **Determination of larval susceptibility to lufenuron**

The larvicidal bioassay was completed following the protocol by Bisset and colleagues [36]. One milliliter of the insecticide solution to be assayed was added to 224 mL of water in a 500 mL plastic container, and then was shaken lightly to ensure a homogeneous test solution. Then, 25 mL of water with 20 late third or early fourth instar *Ae. aegypti* larvae were added to the container. Five different concentrations of lufenuron were tested, ranged from 0.016 to 1 mg/L (ppb). One milliliter of solvent (acetone) was added to other cup and used as a control. Each concentration, including the control, was replicated five times. Cups containing the treated larvae were placed in a regulated chamber ( $26 \pm 2^\circ\text{C}$ , 60-70% RH and 12:12 h photoperiod) with 100 mg of food mixture supplied once.

Bioassays were monitored for several days, until all insects either died or reached adulthood. Adult emergence inhibition (EI) data were registered as soon as all control group specimens emerged. The results were used to calculate the  $\text{EI}_{50}$  and  $\text{EI}_{90}$  values using the Probit method [37]. These values correspond to the lufenuron doses necessary to inhibit adult development of 50 and 90% of the specimens treated, respectively.

## **Transfer of lufenuron by treated females to clean containers with larvae**

Gravid females were exposed by tarsal contact to lufenuron-treated papers to determine if mosquitoes, after walking on the treated paper, could transport sufficient amounts to disrupt the development of larvae held in containers with water.

Methods modified from Itoh and colleagues [27] were used. Technical grade lufenuron was diluted with a 9:1 mixture of acetone : silicone oil and applied to Whatman® N°1 filter papers (12 x 15 cm) at an application rate of 0.2, 0.4 and 1 mg active ingredient per cm<sup>2</sup> (mg a.i./cm<sup>2</sup>). These doses were chosen based on previous work done with pyriproxyfen [32] where similar doses were used; also knowing that lufenuron has a higher EI<sub>50/90</sub> and therefore is less effective, a slightly higher dose was also added (1 mg/cm<sup>2</sup>). After acetone-silicone oil solutions of lufenuron were applied, paper discs were air dried in darkness for 1 h and used to line the inside wall of a WHO susceptibility test kit tube and held in place by a wire ring.

Fifteen females, 5 to 7 days old, were confined to the test kit for 1h. They had been blood fed 3 days before and the day of the treatment. After exposure to treated paper 1, 3, or 5 treated females were released into a mosquito cardboard cage of 5 kg (13 cm height x 21.5 cm diameter) with gauze as lid. A plastic container (8 cm top diameter, 7 cm bottom diameter x 5 cm high) containing 20 late 3<sup>rd</sup>/early 4<sup>th</sup> instar larvae in 100 ml of water and lined with filter paper was added to the cage. The females were allowed to lay eggs for five days on the filter paper. The filter paper was then removed for observation of egg deposition. If any of the females in the cages retained eggs the assay was discarded. Larvae in the plastic container were fed daily and reared at 26 ± 2°C until adults emerged or all individuals die. Adult emergence inhibition (EI) data were calculated as described in the larvicidal bioassay.

Each combination of lufenuron dose and number of IGR-exposed females was replicated 5 times. Control papers were treated with 1 ml of the acetone-oil mixture, and used as described for IGR-treated papers.

Mosquito cardboard cages were discarded once finished the assay and new ones were used for the

next replicates.

### **Effect of lufenuron on female fertility and fecundity**

As described before females were exposed by tarsal contact to lufenuron-treated papers (0.4 and 1 mg a.i./cm<sup>2</sup>) to determine if it has an effect on their fecundity (increase or decrease of the number of eggs laid), fertility (reduction of hatchability or viability of eggs) or in the ability to feed on blood. In each assay 12-15 mosquito females were exposed to lufenuron for 1 hour; 24 h before (before blood meal - BBM) or 24 h after a blood meal (ABM). Then females were released into a mosquito cardboard cage in which a container with 100 ml of water lined with Whatman<sup>®</sup> N°1 filter paper (20 x 6 cm) as oviposition substrate was placed.

The females were allowed to lay eggs for 5 days and the number of eggs laid per female was counted, both on the filter paper and in the water. Females were removed, dissected and the assay was discarded if retained eggs were found. The filter papers were left to dry and stored in sealed, plastic bags for 15 days to allow eggs to complete embryogenesis. After this period a known number of eggs were placed in water to evaluate egg hatch and therefore, fecundity. As *Ae. aegypti* eggs usually hatch erratically or asynchronous depending on the environmental conditions [38], the number of larvae was counted after two, four and seven days.

For those females that were first exposed to lufenuron and then fed (BBM), we also evaluate mortality 24 h after exposure and the percentage of females that fed on blood.

### **Statistical analysis.**

In the determination of larval susceptibility to lufenuron bioassays, data was subjected to log-dose, probit-mortality analysis (PoloPlus 2.0 software LeOra Software Company, Petaluma, CA). Prior to these analyses, the percentage inhibition of emergence of adults in treated container was corrected for mortality in control containers [39].

Results of forced-contact experiments were analyzed by MANOVA (Sigmaplot 11.0, Systat Software Inc., San Jose, CA) to determine if EI% varied significantly between trials, the number of females per bioassay cage or application rates. To compare the number of eggs/female, the percentage of

hatched eggs and the females that feed on blood after exposure to lufenuron between the treated and control group a Student t test was used or a Wilcoxon test (Mann-Whitney) when the assumption of normality was not met. The level of significance was set at  $p \leq 0.05$  (Sigmaplot 11.0, Systat Software Inc., San Jose, CA).

## Results

### **Transfer of lufenuron by treated females to clean containers with larvae**

Lufenuron proved to be very active against *Ae. aegypti* larvae with an  $EI_{50}$  of 0.164 ppb ( $CI_{95}$  0.039-0.486 ppb) and  $EI_{90}$  of 0.81 ppb ( $CI_{95}$  0.32-33.3 ppb) (Table 1).

We also found that lufenuron can be transferred by females from treated surfaces to clean containers causing the inhibition of emergence of the larvae. This effect was dependent on the concentration applied on the paper ( $F = 4.92$ ,  $df = 2$ ,  $P = 0.013$ ) and also the number of females ( $F = 4.75$ ,  $df = 2$ ,  $P = 0.015$ ) added to each cage. When the lowest dose of lufenuron was applied ( $0.2 \text{ mg/cm}^2$ ) there were no differences in the number of females used with a mean  $EI\%$  between 17 and 30% (Fig. 1). On the other hand, no differences in  $EI\%$  were found between the doses applied to the paper when only one female was used to transfer lufenuron, indicating that there is a maximum amount that a female can transfer regardless of the dose with which she comes in contact (17, 23 and 30%  $EI$  for doses of 0.2, 0.4 and  $1 \text{ mg/cm}^2$  respectively). At the highest doses (0.4 and  $1 \text{ mg/cm}^2$ ) no differences were found between using 3 or 5 females. A slight tendency of higher larvae mortality values is observed in the dose of  $1 \text{ mg/cm}^2$  although on average they do not present significant differences with the dose of  $0.4 \text{ mg/cm}^2$ .

### **Effect of lufenuron on female fertility and fecundity**

The effect of lufenuron on the immature stages of insects is well known, however little is known about its direct effect on adults. In this trial the effect of lufenuron on fertility, fecundity and blood intake capacity of adult *Ae. aegypti* females was evaluated.

When females were exposed to lufenuron 24 h after blood meal (ABM) no differences were found in the number of eggs laid per female between the treated ( $0.4$  or  $1 \text{ mg/cm}^2$ ) or the control group (Table

2). No significant differences were found in the hatching percentage between the control group (82.7%) and the females that were exposed to a dose of 0.4 mg/cm<sup>2</sup> (78.5%). However, significant differences were found in the group in which females contacted a surface with 1 mg/cm<sup>2</sup> of lufenuron (83.1% in control group vs. 44.8% in treated,  $T = 3.42$ ,  $df = 4$ ,  $P = 0.026$ ).

When the females were first exposed to lufenuron and fed after 24 h of this exposure (BBM) no differences were found in the number of eggs laid per female between the treated (0.4 or 1 mg/cm<sup>2</sup>) or the control group (Table 2). Also no significant differences were found in the hatching percentage between the control group (89.8%) and the females that were exposed to a dose of 0.4 mg/cm<sup>2</sup> (79.5%). However, significant differences were found in the group in which females contacted a surface with 1 mg/cm<sup>2</sup> of lufenuron (88.3% in the control group vs. 46.6% in the treated one;  $W = 26$ ,  $P = 0.029$ ).

Also for the BBM treatment, no differences were found in the percentage of mortality after 24 h between the females who had contacted a lufenuron surface before blood meal in both the 0.4 mg/cm<sup>2</sup> (5.1%) or 1 mg/cm<sup>2</sup> (7%) dose and the control females (3%). Regarding the ability to blood feed of those females who had previously contacted a lufenuron-treated surface, we found that 63.6% of the females who contacted a surface with 0.4 mg/cm<sup>2</sup> fed while in the control group this percentage was 89.2%, although the difference between groups was greater than 20%, it was not statistically significant.

On the other hand, 56.2% of the females who contacted a surface with 1 mg/cm<sup>2</sup> of lufenuron fed while in the control group this percentage was 97%, with significant differences between both values ( $W = 37.5$ ,  $P = 0.039$ ).

## Discussion

Future challenges in integrated pest management require the development of selective and environmentally safe pesticides along with new strategies to apply them. The more specifically these insecticides act, and the less are their adverse side effects on beneficial insects and the environment, the more they are appropriated to control arthropod pests. Insecticides with novel modes of action

such as chitin synthesis inhibitors disrupt cuticle formation [40]; suppression of chitin deposition in treated insect often causes high mortality during molting [41].

The survivorship of mosquitoes is very important for production of progeny, development, and transmission of pathogens among the hosts. Several studies reported the effects of sublethal exposure of IGRs on survival, fecundity, fertility and blood intake capacity of mosquito females [28-31, 42-45]. However, very few studies have evaluated the direct effect of IGRs, mainly pyriproxyfen, on adult mosquitoes [46-49].

Among the new strategies that are trying to be implemented to control vector mosquitoes is the approach called "auto-dissemination", based on the possibility that wild adult mosquitoes exposed to artificial resting sites contaminated with IGRs (so far pyriproxyfen, a juvenile hormone analogue), can disseminate insecticide to larval breeding sites, thus preventing adult emergence [28, 30, 32, 33]. This strategy is facilitated by the oviposition behaviour of *Ae. aegypti*, that typically distribute the eggs from a single gonothrophic cycle among several temporary sites. The "auto-dissemination" approach can be proposed as a 'pull' (i.e attraction of wild mosquitoes to contaminated sites) and 'push' (i.e. dispersal of contaminated mosquitoes and dissemination of IGR to larval habitats) control strategy with the potential to target the myriad of cryptic larval breeding sites that cannot be reached by traditional larvicidal applications.

The objective of our work was to evaluate for the first time if an IGR belonging to the chitin synthesis inhibitors group can be transferred by mosquito females who contact a treated surface to larval breeding sites. On the other hand, it was also studied whether the contact with a lufenuron-treated surface can have effects on female's fertility, fecundity and their blood intake capacity.

In our work, lufenuron proved to be highly effective on *Ae. aegypti* larvae with an  $El_{50}$  of 0.164 ppb and an  $El_{90}$  of 0.81 ppb. These values were significantly lower than those found by Salokhe and colleagues [50], they obtained a value of 6 ppb; although they do not specify if used lufenuron technical grade or a formulation, so we cannot make a direct comparison with our results. The values obtained in our work are similar to those obtained for other IGRs of this group such as triflumuron [24]

with a value of  $El_{50}$  and  $El_{90}$  of 0.8 and 1.8 ppb, respectively. Also for diflubenzuron a value of 0.5 ppb and 3.5 ppb was obtained for the  $El_{50}$  and  $El_{90}$  [44]. Although the  $El_{50}$  values for pyriproxyfen, the IGR used in the “auto-dissemination” assays, are approximately 10 times smaller ( $El_{50} = 0.011$  ppb, [27]), we believe that the values obtained for lufenuron indicate that it would be a good candidate to explore in future field trials.

Regarding the “auto-dissemination” or horizontal transfer of lufenuron, our work shows that mosquito females contaminated with lufenuron can transfer enough material to water containers to exert a significant lethal effect on larvae developing there. This effect was dependent on the concentration applied on the paper and also the number of females added to each cage. This is the first work where it is demonstrated that an IGR of the BPU group can be transferred to larval breeding sites by mosquito females. On the other hand there is numerous evidence of this phenomenon for pyriproxyfen [20, 30, 31] where mortality is almost exclusively limited to the pupal stage [27]. However, the use of IGR of the BPU group could mean an advantage since mortality would occur earlier in development, that is, between larval molts or molting from larvae to pupa.

The doses of lufenuron used in our work were slightly higher than those used in other works with pyriproxyfen. This is due on one hand to the difference in effectiveness between both active ingredients (measured as  $El_{50}$ ), and on the other hand because the surfaces used in the works using pyriproxyfen were different from the paper used in our study. For example, in the work of Itoh and colleagues [27] a film of polyethylene terephthalat was used while Chism and Apperson [32] used seed germination papers. These surfaces could allow a greater bioavailability of the IGR therefore it would be interesting to repeat our work using different surfaces and evaluate if this could have any differential effect.

Itoh and colleagues [27] reported equivalent effects were achieved using 1, 3 or 5 *Ae. aegypti* females, however Chism and Apperson [32], using *Ae. albopictus*, found that percentage mortality was significantly lower at a density of one female per cage and increased to higher and equivalent levels of mortality at 3 and 5 females, they argue this difference to a variation in sensitivity between

both species. In the case of our work, the results were similar to those of Chism's work, probably because as lufenuron is less potent as a larvicide than pyriproxyfen, the amount of females that are involved in the transfer of the IGR is important.

In the last part of our work the effect of lufenuron on fertility, fecundity and blood intake capacity of adult *Ae. aegypti* females directly exposed to a surface treated with lufenuron was evaluated. When females were exposed to lufenuron 24 hours after blood meal (ABM) a reduction in the hatching percentage close to 40% was observed of those females that had contacted a surface with 1 mg/cm<sup>2</sup>. Also, when the contact with the surface treated with the IGR was before blood feeding (BBM), a 40% reduction in egg hatching was observed at the dose of 1 mg/cm<sup>2</sup>. This would indicate that the moment at which contact with lufenuron occurs (before or after blood feeding) is not decisive in the effect on fertility and fecundity but the dose used does; since the same results were obtained with both ABM and BBM regimes. On the other hand, the studies developed with pyriproxyfen indicate that the moment of contact with this IGR is decisive for its effect on the fertility and fecundity of the adult's mosquitoes. Aiku and colleagues [51] found significant effect on egg hatching when *An. stephensi* females were exposed to bednet treated with 2% pyriproxyfen 24 hours after blood feeding. Also Gaugler and colleagues [34] found the same results with *Ae. albopictus*, suggesting that mosquitoes are most susceptible when bloodfed one day prior to pyriproxyfen exposure and therefore exposed while developing their eggs. Itoh and colleagues [27] reported that tarsal contact of *Ae. aegypti* with 0.1 mg/cm<sup>2</sup> pyriproxyfen before a bloodmeal induced a large decrease in the number of eggs hatch compared with contact after a bloodmeal.

Finally, our work shows a reduction in the percentage of females that fed on blood for the dose of 1 mg/cm<sup>2</sup> of lufenuron, from 97% in the control group to less than 60% in the treated one. However, there was no effect of previous exposure to lufenuron on female mortality. There are no other works that evaluate the direct effect of IGRs on the blood intake capacity of mosquito females. Only the work of Vasuki [45] found that *Ae. aegypti* larvae and pupae exposed to sublethal doses of the IGR hexaflumuron significantly reduced the quantity of blood ingested as adult females with a

corresponding reduction in egg laying.

## Conclusion

During the past decades, a larger number of chemically unrelated insecticidal compounds have been developed and commercialized that interfere with chitin synthesis, which is essential to reproduction, growth, and development of insects [52]. This paper introduces an innovation by first exploring the possibility that an IGR belonging to the group of BPUs, such as lufenuron, can be transferred by gravid females to breeding sites and that at the same time can have an effect on fertility, fecundity and blood intake capacity of adult mosquitoes. Although lufenuron is effective against many insects, the proposed approach targets container breeding species with such tiny amounts of compound disseminated exclusively to their breeding sites, that impacts on non-target species are likely to be minimal. In the future it might be interesting to explore lufenuron ability to replicate the results found here in field conditions.

## Abbreviations

BPU: Benzoylureas

EI: Adult emergence inhibition

EI%: Adult emergence inhibition percentage

EI<sub>50</sub>: Adult emergence inhibition 50%

EI<sub>90</sub>: Adult emergence inhibition 90%

CI<sub>95</sub>: Confidence limits 95%

ABM: After blood meal

BBM: Before blood meal

## Declarations

### **Consent for publication**

Not applicable

### **Availability of data and materials**

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

## **Funding**

This investigation received support from Bunge y Born Foundation and the Agencia Nacional de Promoción Científica y Tecnológica (Argentina) through PICT 1729-2015.

## **Ethics approval**

No human participants, human data or human tissue were used in the present study. Insects were fed on pigeon blood once per week according to a protocol approved by the Institutional Animal Care and Use Committee of CIPEIN (IACUC/CICUAL 1531/13).

## **Acknowledgment**

Not applicable

## **Competing interests**

The authors declare that they have no competing interests.

## **Authors' contributions**

PVG and LH designed, performed the study, and analysis of the data. PVG and LH wrote the manuscript. All authors read and approved the final manuscript.

## **References**

1. Pilger D, De Maesschalck M, Horstick O, San Martin JL. Dengue outbreak response: documented effective interventions and evidence gaps. *TropIKA 1. net* 2010;1:1-31.
2. Perich MJ, Tidwell MA, Williams DC, Sardelis MR, Pena CJ, Mandeville RD, et al. Comparison of ground and aerial ultra-low volume applications of malathion against *Aedes aegypti* in Santo Domingo, Dominican Republic. *J Am Mosq Control Assoc* 1990;6:1-6.
3. Newton EA, Reiter P. A model of the transmission of dengue fever with an evaluation of the impact of ultra-low volume (ULV) insecticide applications on dengue epidemics. *Am J Trop Med Hyg* 1992;47(6):709-20.
4. Yap HH, Chong AS, Adanan CR, Chong NL, Rohaizat B, Malik YA, et al. Performance of ULV formulations (Pestguard 102 / Vectobac AS 12) against three mosquito species. *J*

- Am Mosq Control Assoc 1997;13:384-88.
5. Perich MJ, Davila G, Turner A, Garcia A, Nelson M. Behavior of resting *Aedes aegypti* (Culicidae: Diptera) and its relation to ultra-low volume adulticide efficacy in Panama City, Panama. J Med Entomol 2000;37:541-46.
  6. Harburguer L, Seccacini E, Licastro S, Zerba E, Masuh H. Droplet size and efficacy of an adulticide-larvicide ultra low volume formulation on *Aedes aegypti* using different solvents and spray application methods. Pest Manag Sci 2012;68:137-41.
  7. Rawlins SC. Spatial distribution of insecticide resistance in Caribbean populations of *Aedes aegypti* and its significance. Rev Panam Salud Publica 1998;4(4):243-51.
  8. Braga IA, Lima JBP, Soares SS, Valle D. *Aedes aegypti* resistance to temephos during 2001 in several municipalities in the states of Rio de Janeiro, Sergipe and Alagoas, Brazil. Mem Inst Oswaldo Cruz 2004;99(2):199-203.
  9. Jirakanjanakit N, Saengtharatip S, Rongnoparut P, Duchon S, Bellec C, Yoksan S. Trend of temephos resistance in *Aedes (Stegomyia)* mosquitoes in Thailand during 2003-2005. Environment Entomol 2007;36(3):506-11.
  10. Seccacini E, Lucia A, Zerba E, Licastro S, Masuh H. *Aedes aegypti* (L.) resistance to temephos in Argentina. J Am Mosq Control Assoc 2008;24(4):608-9.
  11. Araújo AP, Araujo Diniz DF, Helvecio E, Arruda de Barros R, Fontes de Oliveira CM, Junqueira Ayres CF, et al. The susceptibility of *Aedes aegypti* populations displaying temephos resistance to *Bacillus thuringiensis israelensis*: a basis for management. Parasites Vectors 2013;6:297-315.
  12. Tunaz H, Uygun N. Insect growth regulators for insect pest control. Turk J Agric For 2004;28:377-87.
  13. Mulla MS, Darwazeh HA, Kennedy B, Dawson DM. Evaluation of new insect growth regulators against mosquitoes with notes on non target organisms. J Am Mosq Control

- Assoc 1986;2:314-20.
14. Mulla MS. The future of insect growth regulators in vector control. J Am Mosq Control Assoc 1995;11:269-73.
  15. Suman DS, Parashar BD, Prakash S. Efficacy of various insect growth regulators on organophosphate resistant immatures of *Culex quinquefasciatus* (Diptera: Culicidae) from different geographical areas of India. J Entomol 2010;7(1):33-43.
  16. Graf JF. The role of insect growth regulators in arthropod control. Parasitol Today 1993;9(12):471-4.
  17. Miyamoto J, Hirano M, Takimoto Y, Hatakoshi M. Insect growth regulators for pest control, with emphasis on juvenile hormone analogs: present status and future prospects. ACS Symp Ser, ACS Washington, D.C. 1993;524:144-68.
  18. Ali A, Chowdhury MA, Hossain MI, Mahmud-ul-Ameen, Habiba DB, Aslam AF. Laboratory evaluation of selected larvicides and insect growth regulators against field-collected *Culex quinquefasciatus* larvae from urban Dhaka, Bangladesh. J Am Mosq Control Assoc 1999;15:43-7.
  19. Cetin H, Yanikoglu A, Cilek JE. Efficacy of diflubenzuron, a chitin synthesis inhibitor, against *Culex quinquefasciatus* larvae in septic tank water. J Am Mosq Control Assoc 2006;22:343-5.
  20. Match® Reliable Lepidoptera control in maize, vegetables and potatoes. Novartis AG Basel, Switzerland, 1998.
  21. Vasuki V. Adult longevity of certain mosquito species after larval and pupal exposure to sublethal concentration of an insect growth regulator, hexaflumuron. Southeast Asian J Trop Med Public Health 1992;23:121-4.
  22. Vasuki V, Rajavel AR. Influence of short time exposure to an insect growth regulator, hexaflumuron, on mortality and adult emergence of vector mosquitoes. Mem Inst

- Oswaldo Cruz 1992;87(2):275-83.
23. Martins AJ, Belinato TA, Lima JBP, Valle D. Chitin synthesis inhibitor effect on *Aedes aegypti* populations susceptible and resistant to organophosphate temephos. Pest Manag Sci 2008;64(6): 676-80.
  24. Belinato TA, Martins AJ, Pereira-Lima JB, de Lima Camara TN, Peixoto AA, Valle D. Effect of the chitin synthesis inhibitor triflumuron on the development, viability and reproduction of *Aedes aegypti*. Mem Inst Oswaldo Cruz 2009;104:43-7.
  25. Belinato TA, Martins AJ, Pereira Lima JB, Valle D. Effect of triflumuron, a chitin synthesis inhibitor, on *Aedes aegypti*, *Aedes albopictus* and *Culex quinquefasciatus* under laboratory conditions. Parasites Vectors 2013;6:83.
  26. Reiter P. Oviposition, dispersal, and survival in *Aedes aegypti*: Implications for the efficacy of control strategies. Vector Borne Zoonotic 2007;7:261-73.
  27. Itoh T, Kawada H, Abe A, Eshita Y, Rongsriyam Y, Igarashi A. Utilization of bloodfed females of *Aedes aegypti* as a vehicle for the transfer of the insect growth regulator pyriproxyfen to larval habitats. J Am Mosq Control Assoc 1994;10:344-7.
  28. Caputo B, Ienco A, Cianci D, Pombi M, Petrarca V, Baseggio A, et al. The “auto-dissemination” approach: a novel concept to fight *Aedes albopictus* in urban areas. PLoS Negl Trop Dis 2012;6(8):e1793.
  29. Iwanaga K, Kanda T. The effects of a juvenile hormone active oxime ether compound on the metamorphosis and reproduction of an anopheline vector, *Anopheles balabacensis* (Diptera: Culicidae). Appl Entomol Zool 1988;23:186-93.
  30. Sihuincha M, Zamora-Perea E, Orellana-Rios W, Stancil JD, López-Sifuentes V, Vidal-Oré C, et al. Potential use of pyriproxyfen for control of *Aedes aegypti* (Diptera: Culicidae) in Iquitos, Perú. J Med Entomol 2005;42:620-30.
  31. Harburguer L, Zerba E, Licastro S. Sublethal effect of pyriproxyfen released from a

- fumigant formulation on fecundity, fertility, and ovicidal action in *Aedes aegypti* (Diptera: Culicidae). J Med Entomol 2014;51(2): 436-43.
32. Dell Chism B, Apperson C. Horizontal transfer of the insect growth regulator pyriproxyfen to larval microcosms by gravid *Aedes albopictus* and *Ochlerotatus triseriatus* mosquitoes in the laboratory. Med Vet Entomol 2003;17:211-20.
33. Devine GJ, Zamora Perea E, Killeen GF, Stancil JD, Clark SJ, Morrison AC. Using adult mosquitoes to transfer insecticides to *Aedes aegypti* larval habitats. Proc Natl Acad Sci USA 2009;106(28):11530-34.
34. Gaugler R, Suman D, Wang Y. An autodissemination station for the transfer of an insect growth regulator to mosquito oviposition sites. Med Vet Entomol 2012;26:37-45.
35. Chandel K, Suman DS, Wang Y, Unlu I, Williges E, Williams GM, et al. Targeting a hidden enemy: pyriproxyfen autodissemination strategy for the control of the container mosquito *Aedes albopictus* in cryptic habitats. PLoS Negl Trop Dis 2016;10(12): e0005235.
36. Bisset J, Blanco S, Braga I, Coto H, Masuh H, Moncayo A, et al. Protocolo para determinar la susceptibilidad o resistencia a insecticidas de mosquitos de la especie *Aedes aegypti*. (Protocol to evaluate the susceptibility or resistance to insecticides by *Aedes aegypti* mosquitoes). Documento propuesto por la Red Latinoamericana de Control de Vectores (RELCOV) 2005; Fundación Mundo Sano Eds. Buenos Aires, Argentina 13 pp.
37. Lichtfield JT, Wilcoxon F. A simplified method of evaluating dose-effect experiments. J Pharmacol Exp Ther 1949;2:99-113.
38. Gillett JD, Roman EA, Phillips V. Erratic hatching in *Aedes* eggs: A new interpretation. Proc R Soc Lond 1977;1123(196):223-232.

39. Abbott WS. A method of computing the effectiveness of an insecticide. *J Econ Entomol* 1925;18:265-7.
40. Cohen E. Chitin biochemistry: synthesis and inhibition. *Annual Review of Entomology* 1987;32: 71-93.
41. . Dean SR, Meola RW, Meola SM, Sittertz-Bhatkar H, Schenker R. Mode of action of lufenuron on laval cat fleas (Siphonaptera: Pulicidae). *J Med Entomol* 1998;35(5):720-4.
42. . Miura T, Schaefer CH, Takahashi RM, Mulligan FS. Effects of the insect growth inhibitor, dimilin, on hatching of mosquito eggs. *J Econ Entomol* 1976;69:655-8.
43. Kelada NL, Gaaboub IA, Rawash IA. The effect on reproduction and morphometrics of females of *Culex pipiens* of treatment with six insect growth regulators. *J Agric Sci* 1981; 96:611-8.
44. Fournet F, Sannier C, Monteny N. Effects of the insect growth regulators OMS 2017 and diflubenzuron on the reproductive potential of *Aedes aegypti*. *J Am Mosq Control Assoc* 1993;9(4):426-30.
45. Vasuki V. Influence of IGR treatment on oviposition of three species of vector mosquitoes at sublethal concentrations. *Southeast Asian J Trop Med Public Health* 1999;30:200-03.
46. . Ohashi K, Nakada K, Ishiwatari T, Miyaguchi J, Shono Y, Lucas JR, et al. Efficacy of pyriproxyfen-treated nets in sterilizing and shortening the longevity of *Anopheles gambiae* (Diptera: Culicidae). *J Med Entomol* 2012;49:1052-8.
47. . Harris C, Lwetoijera DW, Dongus S, Matowo NS, Lorenz LM, Devine GJ, et al. Sterilising effects of pyriproxyfen on *Anopheles arabiensis* and its potential use in malaria control. *Parasites Vectors* 2013;6:144.
48. . Ohba S, Ohashi K, Pujiyati E, Higa Y, Kawada H, Mito N, et al. The effect of

pyriproxyfen as a “population growth regulator” against *Aedes albopictus* under semi-field conditions. PLoS ONE 2013; 8(7): e67045.

49. Mbare O, Lindsay SW, Fillinger U. Pyriproxyfen for mosquito control: female sterilization or horizontal transfer to oviposition substrates by *Anopheles gambiae* sensu stricto and *Culex quinquefasciatus*. Parasites Vectors 2014;7:280.
50. . Salokhe SG, Mukherjee SN, Deshpande SG, Ghule VP, Mathad JR. Effect of sub-lethal concentrations of insect growth regulator, lufenuron on larval growth and development of *Aedes aegypti*. Current Science 2010;99(9).
51. Aiku AO, Yates A, Rowland M. Laboratory evaluation of pyriproxifen treated bednets on mosquito fertility and fecundity. A preliminary study. West African Journal of Medicine 2006;25:22-6.
52. Muthukrishnan S, Merzendorfer H, Arakane Y, Kramer KJ. Chitin metabolism in insects. In: Gilberts LI editors. Comprehensive Molecular Insect Science. London: Academic Press; 2012. p. 193-235.

## Tables

### Table 1.

Title: Toxicity of lufenuron to laboratory-reared late 3rd and early 4th instar *Ae. aegypti* exposed continuously in the laboratory.

Specie	N	El <sub>50</sub> (ppb)	CI <sub>95</sub> (95% fiducial limits )	El <sub>90</sub> (ppb)	(95%
<i>Ae. aegypti</i>	600	0.164	0.039-0.486	0.81	

Legend: El<sub>50</sub> and El<sub>90</sub> (CI<sub>95</sub> limits) for lufenuron expressed in mg/L (ppb). Abbreviations: *El*<sub>50-90</sub>, Adult emergence inhibition 50-90%; CI<sub>95</sub>, 95% confidence interval.

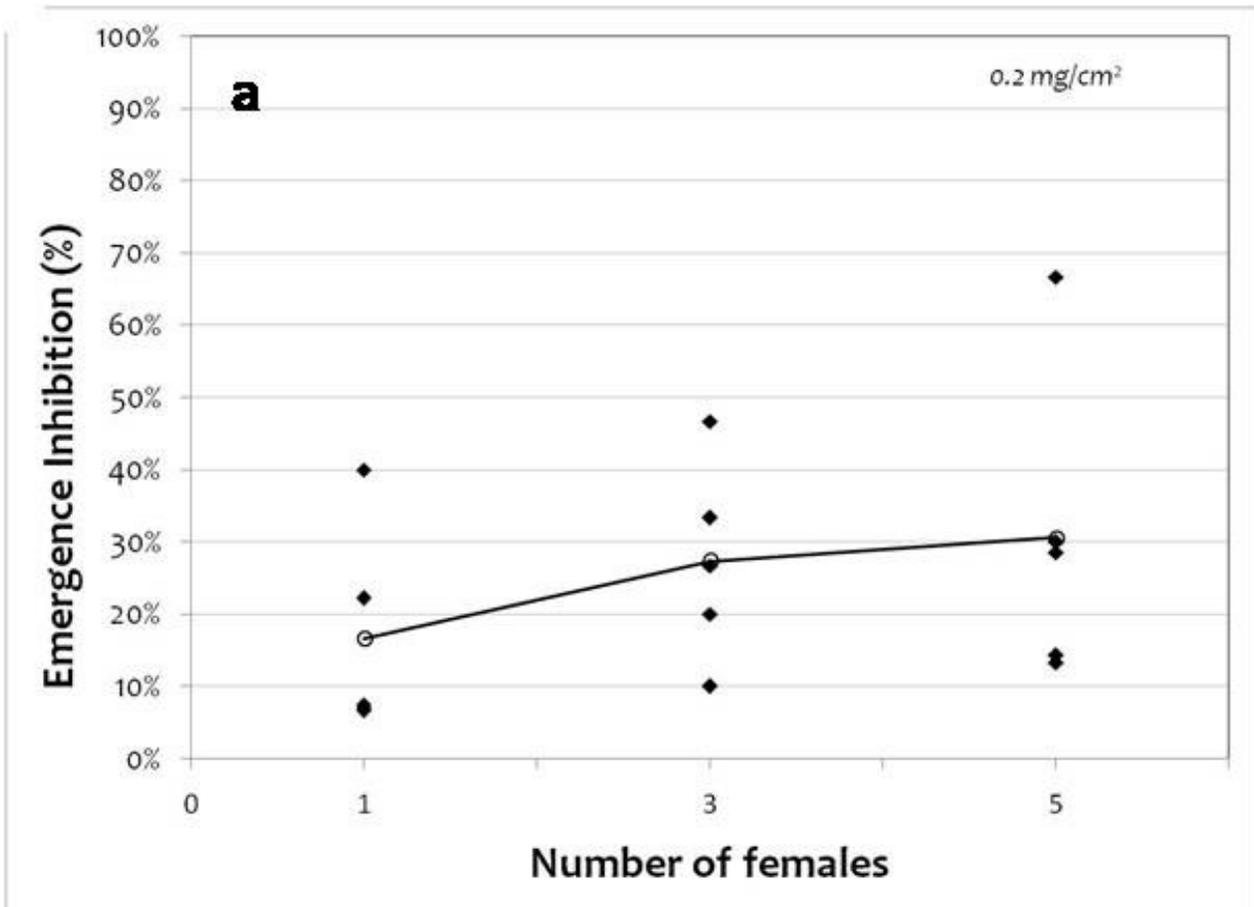
### Table 2.

Title: Effects of IGRs lufenuron on egg production, hatchability of eggs, mortality and blood fed of *Ae. aegypti* females.

		Number eggs / female		Hatching (%)		
		( $\pm$ SE)		( $\pm$ SE)		
		Control	Treated	Control	Treated	Cont
BBM (Exposition to lufenuron Before Blood Meal)	0.4 mg/cm <sup>2</sup>	54.2 $\pm$ 4.4	48.2 $\pm$ 2.5	89.9 $\pm$ 4.4	79.5 $\pm$ 7.5	2.9 $\pm$
	1 mg/cm <sup>2</sup>	46.9 $\pm$ 3.8	38.4 $\pm$ 10.9	88.3 $\pm$ 4.6	46.6 $\pm$ 20*	2.9 $\pm$
ABM (Exposition to lufenuron After Blood Meal)	0.4 mg/cm <sup>2</sup>	49.6 $\pm$ 6.5	49.2 $\pm$ 3.3	82.7 $\pm$ 2.1	78.5 $\pm$ 4.5	---
	1 mg/cm <sup>2</sup>	54.8 $\pm$ 7.6	47.7 $\pm$ 4.2	83.1 $\pm$ 1.1	44.8 $\pm$ 11.2*	---

Legend: \*significant differences between treated and control group (P < 0.05). Abbreviations: *ABM*: After blood meal; *BBM*: Before blood meal; SE: standard error.

Figures



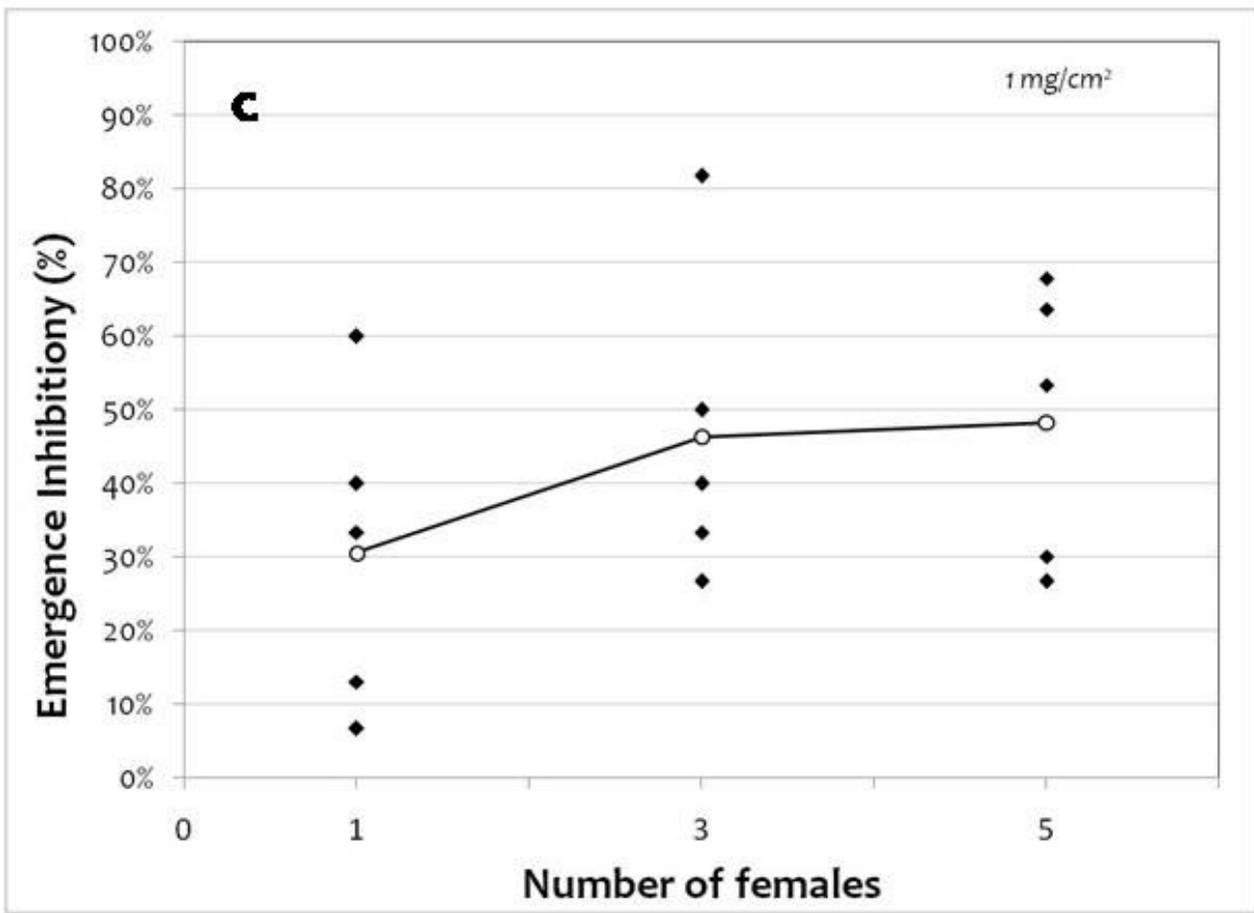
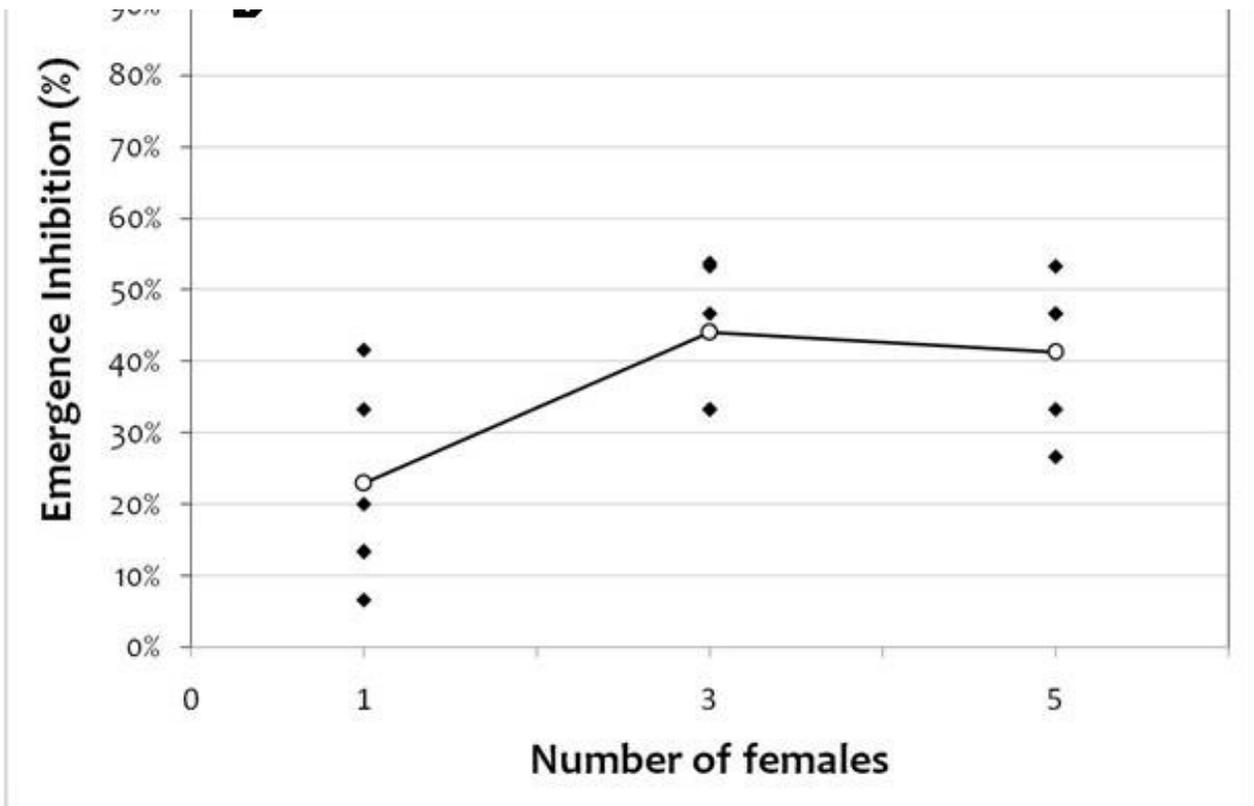


Figure 1

Lufenuron dissemination by gravid *Ae. aegypti* females to larval breeding sites measured as Emergence Inhibition (%) Legend: Emergence Inhibition (%) achieved in larval microcosms through horizontal movement of lufenuron by gravid *Ae. aegypti* females that were forced to contact lufenuron-treated paper: 0.2 (A), 0.4 (B) or 1 (C) mg/cm<sup>2</sup>. Diamonds (◆) data points, circles (○) means of five replicates.