Original Article

Effects of long-lasting pyriproxyfen formulation on biotic attractant factors for egg-laying in *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae)

Takashi TSUNODA^{*, 1)}, Dung Thi NGUYEN²⁾, Trang Vi QUYEN²⁾, Duoc Trong VU³⁾,
Phong Vu TRAN³⁾ and Noboru MINAKAWA¹⁾

* Corresponding author: scartick8019@gmail.com

¹⁾ *Laboratory of Vector Ecology and Environment, Department of Public Health and Environment,
Institute of Tropical Medicine (NEKKEN), Nagasaki University, Nagasaki, Japan*

²⁾ *Vietnam Research Station, Institute of Tropical Medicine (NEKKEN), Nagasaki University, Hanoi, Vietnam*

³⁾ *Department of Medical Entomology and Zoology, National Institute of Hygiene and Epidemiology (NIHE),
Hanoi, Vietnam*

(Received: 19 October 2020; Accepted: 12 August 2021)

Abstract: The attractive baited lethal ovitrap, an autocidal ovitrap, is an alternative tool to control mosquitoes by attracting ovipositing females to lay their eggs in artificial containers. Ovitrap attracts female mosquitoes by biotic factors, such as larval food and conspecific larvae. Long-lasting matrix-release formulations containing 2% pyriproxyfen, SumiLarv[®]2MR, are less harmful to the environment and can effectively reduce targeted mosquitoes for at least six months. We examined the effects of SumiLarv[®]2MR on attraction of *Aedes aegypti* (L.) and *Ae. albopictus* (Skuse) to biotic factors, such as larval food and conspecific larvae, by two-choice tests. For both species, no significant difference arose between water containing SumiLarv[®]2MR and water alone. Both species laid more eggs in cups with larval food prepared two days before, and the attraction to the food was not affected by pyriproxyfen. Conspecific larvae of both species did not show attraction clearly but pyriproxyfen might increase attraction to conspecifics in *Ae. albopictus*. SumiLarv[®]2MR did not deter gravid females of both species and did not reduce the effects of the larval food, suggesting that SumiLarv[®]2MR is a promising formulation for lethal ovitraps.

Key words: lethal ovitrap, insect growth regulator, oviposition

INTRODUCTION

Ultra-low volume aerosols targeted at adult mosquitoes are recommended for emergency control during arbovirus epidemics and their routine use as the principal response to epidemics has become widespread (Rigau-Perez et al., 1998). However, ultra-low volume aerosols containing such as pyrethroids have a limited impact on adults and no impact on immatures (Gubler, 1989; Newton and Reiter, 1992). Moreover, during the last few decades, mosquitoes have developed resistance to many insecticides (Nauen, 2007; Liu et al., 2015; Suesdek, 2019).

Artificial containers are an important habitat for immature yellow fever mosquito, *Aedes aegypti* (L.), and the Asian tiger mosquito, *Ae. albopictus* (Skuse). Ovitrap is a potentially sensitive and efficient tool for detecting the presence of *Aedes* mosquitoes (Fay and Perry, 1965; Fay and Eliason, 1966; Service, 1993). The attractive baited lethal ovitraps, i.e., autocidal ovitraps, are lure-and-kill traps that draw ovipositing

females with an attractive infusion and kill them (Lok et al., 1977; Service, 1993; Barrera et al., 2014; Johnson et al., 2017). In lethal ovitraps with insect growth regulators, however, ovipositing females are not killed, and their eggs develop into pupae. The pupae are then killed at a later stage of development, after the conspecific immature mosquitoes have attracted other gravid females. Therefore, a lethal trap with an insect growth regulator would enhance vector control by creating egg sinks; treated sites that exploit the attraction of ovipositing females to conspecific immature mosquitoes but reduce adult emergence (Wong et al., 2011).

As pyriproxyfen is an insect growth regulator with extremely low toxicity to mammals (Food and Agriculture Organization of the United Nations, 2000), it has been recommended by the WHO to control mosquito larvae (World Health Organization, 2001; World Health Organization, 2008). Pyriproxyfen has low mortality at the larval stage but markedly inhibits the emergence of adult mosquitoes (Estrada

and Mulla, 1986; Hirano et al., 1998). The preventing effect of the long-lasting matrix-release formulation containing 2% pyriproxyfen, SumiLarv[®]2MR, on the emergence of adults is stable for more than six months (Ohashi and Shono, 2015; Ohashi, 2017). Once SumiLarv[®]2MR is placed in water, the concentration of pyriproxyfen can be kept at a constant level for at least six months. Thus, heavy rainfall does not affect its efficacy within six months while the plastic disc remains in the container. As the formulation is compounded into a plastic disc, it is easy to check it with eyes in the field. Owing to its long-lasting efficacy and high degree of safety to non-target organisms, SumiLarv[®]2MR seems to be a suitable chemical compound to use in lethal ovitraps.

Organic materials, such as hay, are used as oviposition attractants in lethal ovitraps (Perich et al., 2003; Sithiprasasna et al., 2003; Mackay et al., 2013). However, as some insecticides in lethal ovitraps are less accessible or have a repellent effect on females (Williams et al., 2007; Quiroz-Martinez et al., 2012), it is crucial to evaluate whether pyriproxyfen affects female attraction before field testing. Larvae may hatch from eggs if the traps are checked at long intervals, for example, every other week. As conspecific immature mosquitoes strongly attract females (Soman and Reuben, 1970; Gubler, 1971; Allan and Kline, 1998), it is necessary to investigate whether pyriproxyfen affects female attraction in the presence of conspecific immature mosquitoes. In this study, we examined the effects of SumiLarv[®]2MR on the attractiveness of biotic factors, such as larval foods and conspecific larvae, in female *Ae. aegypti* and *Ae. albopictus*.

MATERIALS AND METHODS

Mosquitoes

About one thousand *Aedes* larvae were collected from water containers of households and gardens in suburban areas of Tuy Hoa City, Phu Yen Province, Vietnam, in April and May, 2016. Larvae were brought to the laboratory of the Department of Medical Entomology and Zoology, National Institute of Hygiene and Epidemiology, Vietnam. Emerged adults were identified by Rattanarithikul et al. (2005) and Stojanovich and Scott (1966). *Aedes aegypti* and *Ae. albopictus* adults were separated and put in the same species cage to establish the colony.

Eggs were hatched in a plastic tray (40×30×7 cm) at room temperature (25°C–29°C) under 14:10 h (light:dark) photoperiod. First-instar larvae ($n=200$) were transferred into a plastic container (24×17.5×10 cm) with dechlorinated water (5 cm depth) and powdered fish food (Hersteller, JBL GmbH4 Co. KG, Neuhausen, Germany). As the amount of food was dependent on the development of the larvae, a quarter tablet of powdered fish food per day was provided for 200 first and second instar larvae,

and a half tablet of the powdered fish food per day was given to 200 third or fourth instars. Pupae were placed in a transparent polyvinyl cup (11.5 cm diameter at the top, 9.5 cm diameter of the bottom, 8.5 cm height), and the cup was put in a cage (20.5×20.5×20.5 cm) for adult emergence. Adults were fed with 10% sugar soaked into cotton pads. Seven-day-old females were fed with mouse blood for oviposition. Rearing the larvae to adults and all experiments were conducted in a plant growth chamber (LH-60FL12-DT, Nippon Medical & Chemical Instruments Co. Ltd., Osaka, Japan) maintained at 27°C and 14:10 h (light:dark) conditions.

Two-choice tests

Preliminary experiments showed that *Ae. albopictus* laid eggs later and for a longer time than *Ae. aegypti*, we used *Ae. aegypti* gravid females on day 3 after taking a blood meal and *Ae. albopictus* at day 4. One female was kept in each cage with two transparent polyvinyl cups (Fig. 1). Each cup contained 300 mL water with two sheets of filter paper (Thanh Hien Co., Hanoi, Vietnam) for oviposition (17.5×4.5 cm, folded fit into the cup). The cage was composed of a wire frame (20.5×20.5×20.5 cm) covered with 16 mesh sized nylon cloth. The cages were numbered serially in the experiment. For example, control cups were placed to the right in the odd-numbered cages and to the left in the even-numbered cages so that the cups were not skewed to a particular position. A lump of cotton containing 10% sugar water was put on the cage. The cotton was covered with a transparent polyvinyl cup (7.5 cm diameter at the top, 4.5 cm diameter of the bottom, 4.0 cm height) to delay the evaporation of water. As one disc of SumiLarv[®]2MR is effective in 40 L water, we cut a disc into 30 mg sections and used one section submerged per treatment container. *Aedes albopictus* eggs were counted every day for four days, and *Ae. aegypti* eggs were counted for three days. Filter papers were changed every day.

All experiments were conducted on both *Ae. aegypti* and *Ae. albopictus* with $n=29$ to 40 *Ae. aegypti* and *Ae. albopictus* females for each experiment. In each cage, the gravid female laid their eggs in two cups freely. All cups contained water and filter paper. The following were experimental conditions: Experiment 1. For testing whether Sumilarv[®]2MR attracts/repels ovipositing females or not, treatment cups contained 30 mg SumiLarv[®]2MR added two days before the start of the experiment. The control cup contained only water without SumiLarv[®]2MR; Experiment 2. For examining the effects of food and timing of administration, 50 mg of powdered fish food was placed in the treatment cups at the start of the experiment or two days before, and the control cup had no food; Experiment 3. For testing whether SumiLarv[®]2MR interferes with food attraction, 50 mg

Fig. 1. Schematic drawing of the experimental cage. Wire frames were covered with 16 mesh sized nylon cloth. Two transparent polyvinyl cups (treatment and control) and one mosquito female were put in each cage.

powdered fish food and 30 mg SumiLarv[®]2MR were put in treatment cups at the start of the experiment or two days before. The control cups had neither food nor SumiLarv[®]2MR; Experiment 4. To examine the effect of conspecific larvae, 50 mg of powdered fish food were placed in treatment cups two days before the start of the experiment, and 20 third-instar larvae were added at the start of the experiment. The control cup contained 50 mg of powdered fish food; Experiment 5. For testing whether SumiLarv[®]2MR interferes with attraction of conspecific larvae, 50 mg of powdered fish food and 30 mg SumiLarv[®]2MR were placed in treatment cups two days before the start of the experiment, and 20 third-instar larvae were added at the start of the experiment. The control cup contained 50 mg of powdered fish food.

Statistical Analysis

Statistical analysis was performed after summing the number of eggs laid daily for three (*Ae. aegypti*) or four days (*Ae. albopictus*). Wilcoxon signed-rank test with continuity correction was used to compare the number of eggs between treatment and control cups. Non-inferiority trial was conducted to identify that the attractiveness to water with SumiLarv[®]2MR was not inferior 10% or more than water without SumiLarv[®]2MR, when water containing food or conspecific larvae without SumiLarv[®]2MR showed significant attraction. One-sided test for the difference in proportion as ratio of the number of eggs in treatment cups with/without SumiLarv[®]2MR was performed and 90% confidence intervals (90% CIs) was estimated to determine the lower 5% limit (Farrington and Manning, 1990). Water with SumiLarv[®]2MR was considered not inferior to water without SumiLarv[®]2MR, in case the lower limit of the confidence interval was greater than -0.1 (10% non-

A) *Ae. aegypti*

B) *Ae. albopictus*

Fig. 2. Boxplot of the number of eggs laid in the cup containing SumiLarv[®]2MR (IGR) in water compared with the control cup containing only water (Water). (A) *Aedes aegypti* ($n=32$), (B) *Aedes albopictus* ($n=32$). Horizontal bar in the middle of the box indicates the median value. The tops and bottoms of the box indicate the 75th and 25th quartiles, respectively. Whiskers denote the maximum and the minimum values without outliers. ns: no significant difference.

Fig. 3. Boxplot of the number of eggs laid in treatment cup of food and control cup (Water). Food was added to the treatment cups (A) at the start of the experiment with *Ae. aegypti* (Food 0 day; $n=29$), (B) two days before *Ae. aegypti* (Food 2 day; $n=34$), (C) at the start of the experiment with *Ae. albopictus* (Food 0 day; $n=32$), or (D) two days before *Ae. albopictus* (Food 2 day; $n=32$) were introduced. Horizontal bar in the middle of the box indicates the median value. The tops and bottoms of the box indicate the 75th and 25th quartiles, respectively. Whiskers denote the maximum and the minimum values without outliers. ns: no significant difference. *** $P<0.001$.

inferiority margin). All analyses were conducted in R software (R Core Team, 2016).

RESULTS

Experiment 1. There was no difference between SumiLarv[®]2MR-treated water and control water for *Ae. aegypti* ($V=344$, $P>0.05$) and in *Ae. albopictus* ($V=297.5$, $P>0.05$) (Fig. 2A, B).

Experiment 2. The choice of oviposition site was influenced by the timing of food administration for *Ae. aegypti*. There was no difference between treatment cup with powdered fish food at the start of the experiment and control in *Ae. aegypti* ($V=251$, $P>0.05$) (Fig. 3A). However, when powdered fish food was added two days before the start of the experiment, *Ae. aegypti* females laid significantly more eggs in treatment cups ($V=491.5$, $P<0.001$) (Fig. 3B). *Aedes albopictus* females laid significantly more eggs in the treatment cups than control cups at the start of the experiment ($V=491$, $P<0.001$) and two days before ($V=388.5$, $P<0.001$) (Fig. 3C, D).

Experiment 3. When food and SumiLarv[®]2MR were put in a treatment cup at the start of the experiment, there was no significant difference between treatment and control cups for *Ae. aegypti* ($V=239$, $P>0.05$) (Fig. 4A). However, when powdered

fish food and SumiLarv[®]2MR were added two days before, *Ae. aegypti* females laid significantly more eggs in treatment cups ($V=428.5$, $P<0.001$) (Fig. 4B). *Aedes albopictus* females laid significantly more eggs in treatment cups at the start of the experiment ($V=414$, $P<0.01$) and two days before ($V=452$, $P<0.001$) (Fig. 4C, D).

Experiment 4. When food and larvae were put in a treatment cup, there was no significant difference between treatment and control cups for *Ae. aegypti* ($V=382$, $P>0.05$) and *Ae. albopictus* ($V=299.5$, $P>0.05$) (Fig. 5A, B).

Experiment 5. The response to the presence of conspecific larvae was different between *Ae. aegypti* and *Ae. albopictus* mosquitoes. When powdered fish food and SumiLarv[®]2MR were placed in treatment cups two days before the experiment, and 20 larvae were added at the start of the experiment, there was no significant difference between treatment and control cups containing foods in *Ae. aegypti* ($V=337$, $P>0.05$) (Fig. 6A). However, *Ae. albopictus* females laid significantly more eggs in the treatment cups under the same conditions ($V=450.5$, $P<0.001$) (Fig. 6B).

No inferiority of Sumilarv[®]2MR. To test whether the addition of Sumilarv[®]2MR affects the effect of the food, the results of Experiments 2 and 3 were

Fig. 4. Boxplot of the number of eggs laid in treatment cup of food and IGR (SumiLarv®2MR), and control cup (Water). Fish food and IGR were added (A) at the start of the experiment (Food 0 day+IGR; $n=31$), or (B) two days before with *Aedes aegypti* (Food 2 day+IGR; $n=30$), (C) at the start of the experiment (Food 0 day+IGR; $n=32$), or (D) two days before (Food 2 day+IGR; $n=30$) with *Aedes albopictus*. Horizontal bar in the middle of the box indicates the median value. The tops and bottoms of the box indicate the 75th and 25th quartiles, respectively. Whiskers denote the maximum and the minimum values without outliers. ns: no significant difference. ** $P<0.01$, *** $P<0.001$.

compared, except for *Ae. aegypti* with 0 day food. The proportion of eggs in the treatment cup for food two days before was 0.764 in Exp. 2 and 0.774 in Exp. 3. Attractiveness to water with food two days before and Sumilarv®2MR was not inferior to that without Sumilarv®2MR in *Ae. aegypti*, as the lower limits of 90% confidence interval (-0.007 , 0.028) were larger than -0.1 ($Z=10.14$, $P<0.001$). In *Ae. albopictus*, the proportion of eggs in the treatment cup containing food at the start of experiment was 0.795 in Exp. 2 and 0.662 in Exp. 3. The lower limit of 90% CIs (-0.148 , -0.117) for the cup containing food at the start of experiment and Sumilarv®2MR was lower than -0.1 ($Z=-3.35$, $P>0.05$), which indicated that addition of Sumilarv®2MR was inferior to water including food at the start of experiments without Sumilarv®2MR. On the contrary, the proportion of eggs in the treatment cup containing food for food two days before was 0.684 in Exp. 2 and 0.807 in Exp. 3. The lower limit of 90% CIs (0.106 , 0.140) for food two days before and Sumilarv®2MR was much larger than -0.1 in *Ae. albopictus* ($Z=22.71$, $P<0.001$), which showed that the addition of Sumilarv®2MR was not inferior to water without Sumilarv®2MR.

DISCUSSION

Our results showed that pyriproxyfen did not deter gravid females of both species and did not reduce the effects of larval food as attractant. As the vapor pressure and Henry's Law constant of pyriproxyfen are moderate, the volatilization from water into the atmosphere is slight (Sullivan, 2000). Moreover, since 98% of SumiLarv®2MR was composed of resin, it is unlikely that components other than pyriproxyfen had dissolved in water. In a previous study, *Ae. aegypti* females laid the same number of eggs in pyriproxyfen-treated tap water and untreated tap water (Sihuinch et al., 2005). Our study also showed that the water containing pyriproxyfen from SumiLarv®2MR did not affect the attraction or deterrence of *Ae. aegypti* or *Ae. albopictus* females. Even when SumiLarv®2MR was added, gravid females of both species laid more eggs in cups containing powdered fish food added two days before.

Lethal ovitraps containing pyriproxyfen lead to 100% inhibition of the emergence of both species over 30 weeks (Harburguer et al., 2016). The presence of organic material in the water greatly increased the number of eggs laid by *Ae. aegypti*, even in the

Fig. 5. Boxplot of the number of eggs laid in treatment cup containing fish food and 20 larvae (Food+Larva), compared with control cups containing fish food (Food). (A) *Aedes aegypti* ($n=35$), (B) *Aedes albopictus* ($n=30$). Horizontal bar in the middle of the box indicates the median value. The tops and bottoms of the box indicate the 75th and 25th quartiles, respectively. Whiskers denote the maximum and the minimum values without outliers. ns: no significant difference.

presence of pyriproxyfen (Sihuinchá et al., 2005). Our results support the effectiveness of lethal ovitraps with pyriproxyfen against *Ae. aegypti* and *Ae. albopictus*, when organic matter would be added as food. Organic material such as hay is often used to enhance the attractiveness to mosquitoes in field surveillance (Reiter et al., 1991). The addition of such organic materials would contribute to the long-term residual activity of pyriproxyfen in water because the active ingredient is (1) retained in organic materials, (2) released gradually from organic materials to the water, and (3) ingested by larvae when they feed on the organic materials (Ohashi, 2017).

Because the water is meant to be left unattended, microbes are expected to grow in the water. Gravid females of *Ae. aegypti* are attracted to oviposition sites by bacteria-associated chemical cues from bamboo and white-oak leaf infusions (Ponnusamy et al., 2008) and geosmin produced by cyanobacteria (Melo et al., 2020). *Aedes albopictus* gravid females display significantly higher oviposition in water containing bacteria (Trexler et al., 2003). The attractant of *Ae. albopictus* females to cups containing SumiLarv[®]2MR

Fig. 6. Boxplot of the number of eggs laid in treatment cup containing fish food, 20 larvae, and SumiLarv[®]2MR (Food+Larva+IGR), compared with control cups containing fish food (Food). (A) *Aedes aegypti* ($n=40$), (B) *Aedes albopictus* ($n=31$). Horizontal bar in the middle of the box indicates the median value. The tops and bottoms of the box indicate the 75th and 25th quartiles, respectively. Whiskers denote the maximum and the minimum values without outliers. ns: no significant difference. *** $P < 0.001$.

and powdered fish food at the start of the experiment was inferior to cups without SumiLarv[®]2MR in our study. However, water containing food prepared two days before the experiment attracted gravid females to lay greater number of eggs, which suggest that pyriproxyfen does not suppress the proliferation of microbes. When we use SumiLarv[®]2MR in a lethal trap, water including foods and pyriproxyfen would gradually become more attractive for gravid females.

The oviposition response of gravid females to the presence of conspecific larvae was different between *Ae. aegypti* and *Ae. albopictus* in our study. Though *Ae. albopictus* gravid females laid more eggs in cups containing conspecific larvae than cups without larvae, *Ae. aegypti* did not. Oviposition assays indicated that female mosquitoes select larval habitats with conspecifics (Yoshioka et al., 2012). As the density of conspecific larvae increases, the oviposition activity peaks and then decreases both in *Ae. aegypti* and *Ae. albopictus* (Wasserberg et al., 2014; Gonzalez et al., 2016). According to Gonzalez et al (2016), the

maximal oviposition response is observed in 1562.2 conspecific larvae per liter in *Ae. aegypti* and 156.3 in *Ae. albopictus*. The density of both species was 66.7 conspecific larvae per liter in our study, which is much lower than the reported values for maximal attractiveness in *Ae. aegypti*. As we examined the effect of pyriproxyfen on the presence of conspecifics in 20 larvae, we need to investigate the effect of pyriproxyfen at other densities.

Mosquito population control methods should be changed according to mosquito species because differences in preferred breeding sites and habitat-seeking behavior lead to differences in mosquito prevalence after SumiLarv[®]2MR disc treatment (Lamaningao et al., 2020). A study in Lao PDR determined that *Ae. aegypti* females prefer large containers and *Ae. albopictus* prefer smaller ones (Ohashi et al., 2018), therefore, the lethal trap size should be tailored to the target mosquito. Furthermore, as gravid females of *Ae. aegypti* and *Ae. albopictus* display skip-oviposition behavior by laying small numbers of eggs to hedge risks (Chadee et al., 1990; Corbet and Chadee, 1993; Trexler et al., 1998; Davis et al., 2015), the number and placement of lethal traps per intervention area, in addition to the trap size, must be considered. To attain successful control, we should evaluate whether as many small containers containing pyriproxyfen (SumiLarv[®]2MR) as possible or putting SumiLarv[®]2MR disc in large containers, depending on mosquito species.

ACKNOWLEDGEMENTS

We would like to appreciate Nguyen Vi Anh, Nguyen Thi Tra Giang, and Nguyen Thi Yen of the Department of Medical Entomology and Zoology, National Institute of Hygiene and Epidemiology, Vietnam, for helping us in preparation of the experiments. We thank Kazunori Ohashi of Sumitomo Chemical Co., Ltd., in Tokyo and Hyogo, Japan, for giving us helpful comments on the manuscript. We also thank Sumitomo Chemical Co., Ltd., in Tokyo and Hyogo, Japan, to support our use of the SumiLarv[®]2MR discs. This research was supported by the Japan Agency for Medical Research and Development under Grant Number JP20wm0125006.

REFERENCES

- Allan, S. A. and Kline, D. L. 1998. Larval rearing water and preexisting eggs influence oviposition by *Aedes aegypti* and *Ae. albopictus* (Diptera: Culicidae). *J. Med. Entomol.*, 35: 943–947.
- Barrera, R., Amador, M., Acevedo, V., Hemme, R. R. and Felix, G. 2014. Sustained, area-wide control of *Aedes aegypti* using CDC autocidal gravid ovitraps. *Am. J. Trop. Med. Hyg.*, 91: 1269–1276.
- Chadee, D. D., Corbet, P. S. and Greenwood, J. J. D. 1990. Egg-laying yellow fever mosquitoes avoid sites containing eggs laid by themselves or by conspecifics. *Entomol. Exp. Appl.*, 57: 295–298.
- Corbet, P. S. and Chadee, D. D. 1993. An improved method for detecting substrate preferences shown by mosquitoes that exhibit “skip-oviposition”. *Physiol. Entomol.*, 18: 114–118.
- Davis, T. J., Kaufman, P. E., Hogsette, J. A. and Kline, D. L. 2015. The effects of larval habitat quality on *Aedes albopictus* skip oviposition. *J. Am. Mosq. Control Assoc.*, 31: 321–328.
- Estrada, J. G. and Mulla, M. S. 1986. Evaluation of two new insect growth regulators against mosquitoes in the laboratory. *J. Am. Mosq. Control Assoc.*, 2: 57–60.
- Farrington, C. P. and Manning, G. 1990. Test statistics and sample size formulae for comparative binomial trials with null hypothesis of non-zero risk difference or non-unity relative risk. *Stat. Med.*, 9: 1447–1454.
- Fay, R. W. and Eliason, D. A. 1966. A preferred oviposition site as a surveillance method for *Aedes aegypti*. *Mosq. News*, 26: 531–535.
- Fay, R. W. and Perry, A. S. 1965. Laboratory studies of ovipositional preferences of *Aedes aegypti*. *Mosq. News*, 25: 276–281.
- Food and Agriculture Organization of the United Nations. World Health Organization, FAO Panel of Experts on Pesticide Residues in Food and the Environment, WHO Core Assessment Group on Pesticide Residues 2000. Pesticide residues in food: 1999: toxicological evaluations. Joint Meeting of the FAO Panel of Experts on Pesticides Residues in Food and the Environment and the WHO Core Assessment Group, Rome, 20–29 September 1999, World Health Organization.
- Gonzalez, P. V., Gonzalez Audino, P. A. and Masuh, H. M. 2016. Oviposition behavior in *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in response to the presence of heterospecific and conspecific larvae. *J. Med. Entomol.*, 53: 268–272.
- Gubler, D. J. 1971. Studies on the comparative oviposition behavior of *Aedes (Stegomyia) albopictus* Skuse and *Aedes (Stegomyia) polynesiensis* Marks. *J. Med. Entomol.*, 6: 675–682.
- Gubler, D. J. 1989. *Aedes aegypti* and *Aedes aegypti*-borne disease control in the 1990s: top down or bottom up. Charles Franklin Craig Lecture. *Am. J. Trop. Med. Hyg.*, 40: 571–578.
- Harburguer, L., Licastro, S., Masuh, H. and Zerba, E. 2016. Biological and chemical characterization of a new larvicide ovitrap made of plastic with pyriproxyfen incorporated for *Aedes aegypti* (Diptera: Culicidae) control. *J. Med. Entomol.*, 53: 647–652.
- Hirano, M., Hatakoshi, M., Kawada, H. and Takimoto, Y. 1998. Pyriproxyfen and other juvenile hormone analogues. *Rev. Toxicol.*, 2: 357–394.
- Johnson, B., Ritchie, S. A. and Fonseca, D. M. 2017. The state of the art of lethal oviposition trap-based mass interventions for arboviral control. *Insects*, 8: e5.
- Lamaningao, P., Kanda, S., Shimono, T., Inthavongsack, S., Xaypangna, T. and Nishiyama, T. 2020. *Aedes* mosquito surveillance and the use of a larvicide for vector control in a rural area of the Lao People's Democratic Republic. *Trop. Med. Health*, 48: e54.
- Liu, H. M., Yang, P. P., Cheng, P., Wang, H. F., Liu, L. J., Huang, X., Zhao, Y. Q., Wang, H. W., Zhang, C. X. and Gong, M. Q. 2015. Resistance level of mosquito species (Diptera: Culicidae) from Shandong Province, China. *Int. J. Insect Sci.*, 7: 47–52.
- Lok, C. K., Kiat, N. S. and Koh, T. K. 1977. An autocidal ovitrap for the control and possible eradication of *Aedes aegypti*. *Southeast Asian J. Trop. Med. Public Health*, 8: 56–62.
- Mackay, A. J., Amador, M. and Barrera, R. 2013. An improved autocidal gravid ovitrap for the control and surveillance of *Aedes aegypti*. *Parasit. Vectors*, 6: 225.
- Melo, N., Wolff, G. H., Costa-da-Silva, A. L., Arribas, R., Triana, M. F., Gugger, M., Riffell, J. A., DeGennaro, M. and Stensmyr, M. C. 2020. Geosmin attracts *Aedes aegypti* mosquitoes to oviposition sites. *Curr. Biol.*, 30: 127–134.
- Nauen, R. 2007. Insecticide resistance in disease vectors of public health importance. *Pest Manag. Sci.*, 63: 628–633.
- Newton, E. A. and Reiter, P. 1992. 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.*, 47: 709–720.
- Ohashi, K. 2017. Control of mosquito larvae in catch basins using pyriproxyfen and the mechanism underlying residual efficacy. *Med. Entomol. Zool.*, 68: 127–135.
- Ohashi, K. and Shono, Y. 2015. Recent progress in the research and development of new products for malaria and dengue vector control. *Sumitomo Kagaku*, 2015: 4–14.
- Ohashi, K., Takano, M., Xaypangna, T. and Nishiyama, T. 2018. Difference in larval habitat size between *Aedes aegypti* and *Aedes albopictus* in a dengue-endemic village, Lao People's Democratic Republic. *Southeast Asian J. Trop. Med. Public Health*, 49: 761–769.
- Perich, M., Kardec, A., Braga, I., Portal, I., Burge, R., Zeichner, B. W. B. and Wirtz, R. 2003. Field evaluation of a lethal ovitrap against dengue vectors in Brazil. *Med. Vet. Entomol.*, 17: 205–210.
- Ponnusamy, L., Xu, N., Nojima, S., Wesson, D. M., Schal, C. and Apperson, C. S. 2008. Identification of bacteria and bacteria-associated chemical cues that mediate oviposition site preferences by *Aedes aegypti*. *Proc. Natl. Acad. Sci. U.S.A.*, 105: 9262–9267.
- Quiroz-Martinez, H., Garza-Rodriguez, M. I., Trujillo-Gonzalez, M. I., Zepeda-Cavazos, I. G., Siller-Aguillon, I., Martinez-Perales, J. F. and Rodriguez-Castro, V. A. 2012. Selection of oviposition sites by female *Aedes aegypti* exposed to two larvicides. *J. Am. Mosq. Control Assoc.*, 28: 47–49.
- R Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available from: <https://www.R-project.org/>
- Rattanarithikul, R., Harrison, B. A., Panthusiri, P. and Coleman, R. E. 2005. Illustrated keys to the mosquitoes of Thailand. I. Background; geographic distribution; lists of genera, subgenera, and species; and a key to the genera. *Southeast Asian J. Trop. Med. Public Health*, 36: 1–80.
- Reiter, P., Amador, M. A. and Colon, N. 1991. Enhancement of the CDC ovitrap with hay infusions for daily monitoring of *Aedes aegypti* populations. *J. Am. Mosq. Control Assoc.*, 7: 52–55.
- Rigau-Perez, J. G., Clark, G. G., Gubler, D. J., Reiter, P., Sanders, E. J. and Vorndam, A. V. 1998. Dengue and dengue haemorrhagic fever. *Lancet*, 352: 971–977.
- Service, M. W. 1993. Mosquito Ecology. Field Sampling Methods. Second edition, 988 pp., Chapman & Hall, London.
- Sihuinchu, M., Zamora-Perea, E., Orellana-Rios, W., Stancil, J. D., Lopez-Sifuentes, V., Vidal-Ore, C. and Devine, G. J. 2005. Potential use of pyriproxyfen for control of *Aedes aegypti* (Diptera: Culicidae) in Iquitos, Peru. *J. Med. Entomol.*, 42: 620–630.
- Sithiprasasna, R., Mahapibul, P., Noigamol, C., Perich, M. J., Zeichner, B. C., Burge, B., Norris, S. L., Jones, J. W., Schleich, S. S. and Coleman, R. E. 2003. Field evaluation of a lethal ovitrap for the control of *Aedes aegypti* (Diptera: Culicidae) in Thailand. *J. Med. Entomol.*, 40: 455–462.
- Soman, R. S. and Reuben, R. 1970. Studies on the preference shown by ovipositing females of *Aedes aegypti* for water containing immature stages of the same species. *J. Med. Entomol.*, 7: 485–489.
- Stojanovich, C. J. and Scott, H. G. 1966. Illustrated key to mosquitoes of Vietnam. US Department of Health Education and Welfare, Public Health Service, 158 pp. Atlanta, GA.
- Suesdek, L. 2019. Microevolution of medically important mosquitoes - A review. *Acta Trop.*, 191: 162–171.
- Sullivan, J. 2000. Environmental fate of pyriproxyfen D. o. P. Regulation, Sacramento, CA. Available from: <http://www.cdpr.ca.gov/docs/empr/pubs/fatememo/pyrprxfn.pdf>
- Trexler, J. D., Apperson, C. S. and Schal, C. 1998. Laboratory and field evaluations of oviposition responses of *Aedes albopictus* and *Aedes triseriatus* (Diptera: Culicidae) to oak leaf infusions. *J. Med. Entomol.*, 35: 967–976.
- Trexler, J. D., Apperson, C. S., Zurek, L., Gemenio, C., Schal, C., Kaufman, M., Walker, E., Watson, D. W. and Wallace, L. 2003. Role of bacteria in mediating the oviposition responses of *Aedes albopictus* (Diptera: Culicidae). *J. Med. Entomol.*, 40: 841–848.
- Wasserberg, G., Bailes, N., Davis, C. and Yeoman, K. 2014. Hump-shaped density-dependent regulation of mosquito oviposition site-selection by conspecific immature stages: theory, field test with *Aedes albopictus*, and a meta-analysis. *PLoS One*, 9: e92658.
- Williams, C. R., Ritchie, S. A., Long, S. A., Dennison, N. and Russell, R. C. 2007. Impact of a bifenthrin-treated lethal ovitrap on *Aedes aegypti* oviposition and mortality in north Queensland, Australia. *J. Med. Entomol.*, 44: 256–262.
- Wong, J., Stoddard, S. T., Astete, H., Morrison, A. C. and Scott, T. W. 2011. Oviposition site selection by the dengue vector *Aedes aegypti* and its implications for dengue control. *PLoS Negl. Trop. Dis.*, 5: e1015.
- World Health Organization. 2001. Report of the fourth WHOPES working group meeting. WHOPES working group meeting, 4th, WHO.
- World Health Organization. 2008. Pyriproxyfen in drinking-water: use for vector control in drinking-water sources and containers. Background document for development of WHO guidelines for drinking water quality, Geneva: WHO.
- Yoshioka, M., Couret, J., Kim, F., McMillan, J., Burkot, T. R., Dotson, E. M., Kitron, U. and Vazquez-Prokopec, G. M. 2012. Diet and density dependent competition affect larval performance and oviposition site selection in the mosquito species *Aedes albopictus* (Diptera: Culicidae). *Parasit. Vectors*, 5: 225.