VECTOR BIOLOGY AND CONTROL DIVISION,
MINISTRY OF HEALTH AND WELLNESS,
Rte du Jardin, Curepipe, Mauritius
Tel: +230 6742111/6740019 Fax: +230 6742564
Research topic: Field evaluation of pyriproxyfen ovitraps STRICTLY CONFIDENTIAL

Reference: VB/MSC/21/28 **Date: 16 June 2021**

SCIENTIFIC REPORT

Title: Field evaluation of Aedes Tech Mosquito Home System ovitraps in Mauritius

Diana P. Iyaloo, Khouaildi B. Elahee, Nabiihah R. Munglee, Nilesh Latchooman, Srutee Ramprosand, Surendra Puryag, Varina Ramdonee-Mosawa, Ambicadutt Bheecarry

INTRODUCTION

Tourism being the third pillar of the economy of Mauritius (MoT, 2019), a lot of resources are invested annually by the Mauritian Government to ensure that the island remains free of mosquito borne diseases (Tatarsky *et al.*, 2011). However, because of extensive travel links with countries endemic for mosquito-borne diseases, several imported cases are reported every year (Health Statistics Unit, 2019). The country has also suffered five outbreaks of Dengue and one epidemic of Chikungunya since 2005 (Beesoon *et al.*, 2006; Ramchurn *et al.*,2009; Issack *et al.*, 2010; Jaddoo, 2019; GIS, 2020). The transmission of both diseases was solely attributed to *Ae. albopictus* which is widely distributed on the island. *Aedes aegypti*, a more competent vector, was in fact eliminated in the 1950s (Dowling, 1953) and has since then, not been detected on the island. The country's main line of action against *Ae. albopictus* includes: biweekly larviciding of permanent or semi-permanent potential breeding sites, spraying of ports of entry every four months with a residual pyrethroid-based insecticide and focalized treatment in the vicinity of a confirmed or suspected case of mosquito borne disease which involves contact tracing and house to house surveys for active case detection, yard inspections, larviciding of breeding sites and thermal fogging with an adulticide (MoHQL, 2009). Despite these plethora of measures, the control of *Ae. albopictus* still remains a challenge mainly because of the species' ability to breed in a variety of habitats which are often cryptic or hard to reach by control agents. In urbanized areas where all householders usually go to work during the day, yards are often closed and hence cannot be inspected by the authorities.

Autodissemination lethal ovitraps can overcome the limitations of traditional control methods by targeting cryptic or logistically inaccessible larval habitats (Ponlawat *et al.*, 2013). A systematic use of autodissemination lethal ovitrap as a component of an Integrated Vector Management (IVM) strategy, has the potential to control *Ae. albopictus* because of the peri-domestic nature of the species and its tendency to skip-oviposit (*i.e.* lay eggs formed during one gonotrophic cycle in several places) and breed in a large varieties of artificial breeding sites (Hawley, 1988).

Autodissemination is in fact a vector control method that utilizes a 'pull' (attraction and transfer) and 'push' (dispersal and transfer to target habitats) technology. It involves the contamination of insects with a biological or chemical insecticide and the subsequent horizontal or vertical transfer of lethal concentration of the product to other insects via mating, oviposition, aggregation and other behaviours (Gaugler *et al.*, 2011). In essence, an autodissemination lethal ovitrap is a standard ovitrap containing a larvicide or an Insect Growth Regulator which is slow acting and hence does not rapidly kill female mosquitoes coming to

oviposit or rest on its water surface. Because of the skip ovipositing nature of *Aedes* mosquitoes, it is likely that females impregnated with the lethal solution, will visit and disperse the product to other breeding sites before dying.

Pyriproxyfen is an insect growth regulator that hinders molting and has been used in several autodissemination traps to control mosquito populations (for review see, Devine, 2016; Faraji and Unlu, 2016; Maoz *et al.*, 2017; McKemey A. and Adey R., 2018). It is a juvenile hormone analog with very low mammalian toxicity (Miyamoto *et al.*, 1993), has no deleterious effects to wildlife, has residual properties of up to six months in different kinds of mosquito breeding sites (Sihuincha *et al.*, 2005; Yapabandara and Curtis, 2002; Vythilingam *et al.*, 2005; Chavasse *et al.*, 1995) and is recommended by the World Health Organization for the treatment of potable water against mosquitoes (WHO, 2008).

Specifically for *Aedes* mosquitoes, pyriproxyfen has been found to decrease female fertility and fecundity (Dash and Ranjit, 1992; Ponlawat *et al.*, 2013; Seccacini *et al.*, 2014, Harris *et al.*, 2013). It also negatively affects the development of the aquatic life stages of the mosquito at extraordinarily low concentrations - LC_{50} in *Ae. albopictus* and *Ae. aegypti* are 0.012 and 0.023ppb respectively (Gaugler *et al.*, 2012; Itoh *et al.*, 1994). Its potential effects on the aquatic life stages includes a decrease in hatch rate among exposed eggs and the inability of larvae to develop to the pupal or adult stages (Itoh *et al.*, 1994; Ohba *et al.*, 2013; Sihuincha *et al.*, 2005; Ponlawat *et al.*, 2013; Juri *et al.*, 2013; Darriet *et al.*, 2010; Devine *et al.*, 2009; Doud *et al.*, 2014; Ocampo *et al.*, 2014; Seccacini *et al.*, 2014; Seng *et al.*, 2008; Suman *et al.*, 2014; Gaugler *et al.*, 2012).

The possible impacts of pyriproxyfen on *Aedes* mosquitoes may therefore vary considerably depending notably on the formulation and concentration of pyriproxyfen as well as the stage (developmental and/or physiological) of the mosquito at the time of exposure (Itoh *et al.*, 1994; Sihuincha *et al.*, 2015; Devine, 2016; Maoz *et al.*, 2017). While it is clear that ovitrap with pyriproxyfen has a direct negative effect on the mosquito, mixed results were obtained among studies investigating the autodissemination properties of pyriproxyfen (Sihuincha *et al.*, 2005; Suman *et al.*, 2014; Ponlawat *et al.*, 2013; Gaugler *et al.*, 2012; Caputo *et al.*, 2012; Devine *et al.*, 2009; Itoh *et al.*, 1994; Snetselaar *et al.*, 2014; Sithiprasasna *et al.*, 2003, Dell Chism and Apperson, 2003; McKemey and Adey, 2018). This depended mostly on the trap design including the formulation and concentration of the pyriproxyfen; and the experimental design such as trap coverage and the availability of natural breeding sites in the study site.

As a first part of this study, the Aedes Tech Mosquito Home System (One Team Networks Sdn. Bhd., Malaysia), an ovitrap containing an aqueous solution of 0.004 % (w/w) pyriproxyfen, was investigated for its autocidal and autodissemination properties in field conditions. The second part of the study evaluated the attractiveness of the trap.

MATERIALS AND METHODS

Study design

Week 1 to Week 22 (26 June to 20 November 2020)

Field experiment was carried out in two forested zones (Zone A and B) in Pamplemousses close to SSRN Hospital, Mauritius (Fig. 1). Pointe des Lascars, a village 20 Km away from Pamplemousses, was selected as a control area (Fig. 1).

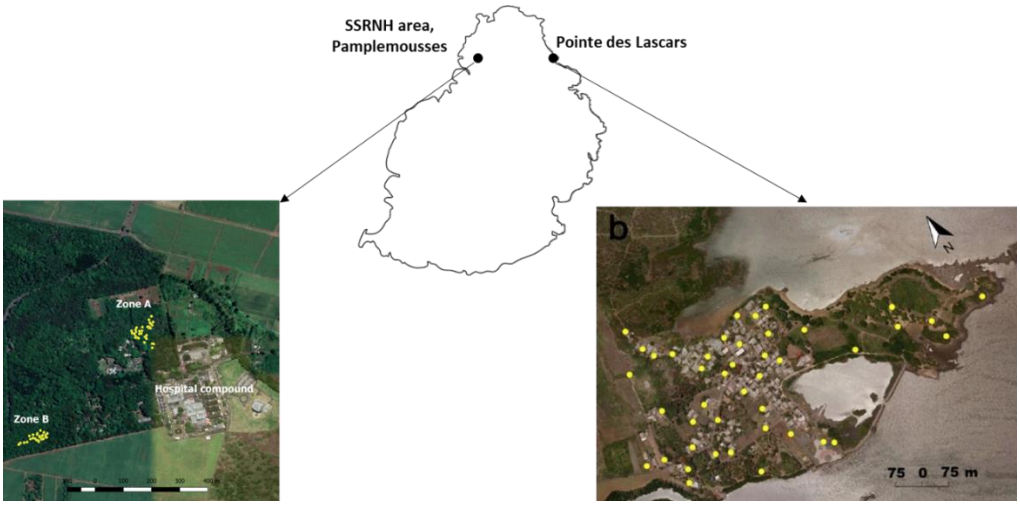

Fig. 1: Two regions in the north of Mauritius – Pamplemousses and Pointe des Lascars, were respectively selected as treatment and control sites for investigating the effect of P_P ovitraps.

From Week 1 to Week 6 (26 June to 31 July 2020), 50 normal ovitraps containing water (NT_W) were set up and monitored weekly in both Zones. On Week 7 (07 August 2020), 15 unproductive ovitraps in each Zone was removed and an Aedes Tech Mosquito Home System ovitrap containing pyriproxyfen (P_P) was added to each of the 35 remaining normal ovitraps (Fig. 2). 43 normal ovitraps containing water were also set up in Pointe des Lascars, the control area. From Week 7 to Week 22 (20 November 2020), ovitraps in the three sites were serviced on the same day on a weekly basis by replacing the water and strip in the latter. Ovistrips collected were brought back to the VBCD laboratory for processing.

A pyriproxyfen ovitrap (P_P) consisted of an Aedes Tech Mosquito Home System ovitrap unit lined with its ovistrip (One Team Networks Sdn. Bhd., Malaysia) containing 500 ml of 0.004 % (w/w) PP solution (One Team Networks Sdn. Bhd., Malaysia; PP ovitrap). A normal ovitrap (NT_W) consisted of a black cylindrical plastic container (15 cm in height and 12 cm in diameter) containing 500 ml tap water, lined on the inside with an ovistrip (34 cm x 9 cm, 145 g/m^2, Sartorius Stedium Biotech, Göttingen GmbH, Germany) and covered with a plastic cap bearing 23 holes 1 cm in diameter (Iyaloo *et al.*, 2014; 2019) (Fig. 3).

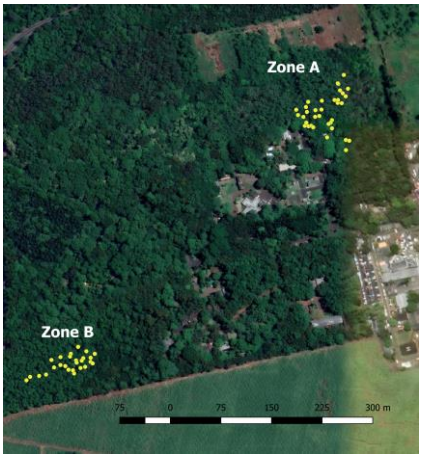

Fig. 2: Ovitrap location (yellow dots) in two forested zones (Zone A and B) in SSRN Hospital area, Pamplemousses

Week 23 to Week 34 (27 November 2020 to 12 February 2021)

On Week 23 (27 November 2020), ovitraps were removed in Zone B and only 24 of the most productive ovitrap locations in Zone A were maintained. At each ovitrap location in Zone A, a set of the following 5 types of ovitrap were set up: (1) NT_W (A normal ovitrap as described in the previous section containing 500 ml water and covered with a plastic cap bearing 23 holes 1 cm in diameter); (2) NL_W (A normal ovitrap with water covered with a plastic cap without any holes. Instead 15 holes were bored on the lateral surface of the trap close to its opening); (3) NL_P (A similar ovitrap to NL_W except that water was replaced with 500 ml 0.004 % (w/w) pyriproxyfen solution); (4) P_P (An Aedes Tech Mosquito Home System ovitrap unit containing 500 ml 0.004 % (w/w) Pyriproxyfen solution as described in the previous section) and (5) P_W (A similar ovitrap to P_P except that the Pyriproxyfen solution was replaced with water) (Fig. 3).

As a control, 20 NT_W ovitraps were set up in a Botanical Garden 2.8 km away from Zone A in Pamplemousses (Fig. 4). Ovitraps in both sites were monitored on the same day on a weekly basis.

Fig. 3: 3 ovitrap designs used in this study (from left to right): NT (black plastic container covered with a plastic cap bearing 23 holes 1 cm in diameter); NL (black plastic container with 15 holes on the lateral side and covered with a plastic cap without any holes), P (an Aedes Tech Mosquito Home System ovitrap, covered on the top and with 10 vertical slits on the lateral side)

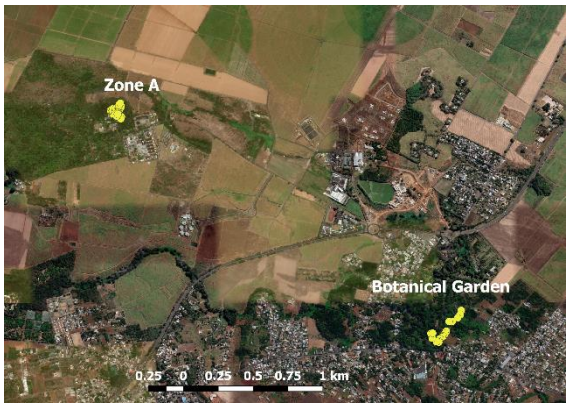

Fig. 4: Ovitrap location (yellow dots) in a treatment (Zone A) and control (Botanical Garden) areas in Pamplemousses

Processing of ovistrips

As soon as ovistrips were brought back to the VBCD laboratory, they were air dried (26 ± 2°C, 70 ± 10 % RH, 12:12 (L:D) h) for four days to allow the eggs to maturate. Eggs on the ovistrips were subsequently counted. To evaluate the attractiveness of each trap for *Ae. albopictus*, ovitrap index (frequency of traps with eggs per week) and mean ovitrap productivity (mean number of eggs per ovitrap per week) were calculated.

During the first part of this study (i.e. from Weeks 1 to 22), for each Zone, all papers from the P_P ovitraps, 10 randomly selected papers from NT_W ovitraps and 20 randomly selected papers from control ovitraps in Pointe des Lascars, were processed to determine the adult emergence rate. During the second part of this study (i.e. from Weeks 23 to 34), all papers from the P_P ovitraps, NL_P ovitraps and control ovitraps from Pamplemousses Botanical Garden, were processed to determine hatch rate and adult emergence rate.

The number of hatch and unhatched eggs on each of the selected ovistrips were counted before the latter were put to hatch. From Week 7 to Week 15, ovistrips from ovitraps containing pyriproxyfen (i.e. P_Ps) were inserted in a solution consisting of 28 % tuna meal, 36 % bovine liver powder and 36 % brewer's yeast for hatching and larval development (Iyaloo and Facknath, 2017) while from Week 16 to Week 35, ovistrips from ovitraps containing pyriproxyfen (i.e. P_Ps and NL_Ps) were inserted in a 0.004 % (w/w) pyriproxyfen solution. Ovistrips collected from ovitraps containing water (i.e. NT_W, NL_W, P_W and control ovitraps) throughout the study were inserted in a solution consisting of 28 % tuna meal, 36 % bovine liver powder and 36 % brewer's yeast for hatching and larval development (Iyaloo and Facknath, 2017). Hatch rate was calculated by expressing the number of hatched eggs after 72 h as a percentage of the total number of eggs on the ovistrip. Larvae that hatched from each ovistrip were fed daily (Iyaloo *et al.*, 2019). Adult emergence rate for each ovistrip was calculated by expressing the total number of adult mosquitoes that developed as a percentage of the initial number of first instar larvae.

To investigate the occurrence of autodissemination, from Weeks 7 to 22, 5 ml water from each of the 35 normal ovitraps (N_Ws) in each zone were sampled every week, pooled into a jar and

brought to the VBCD laboratory. The missing volume in each ovitrap was immediately topped up with tap water. Once in the laboratory, water in each jar was equally aliquoted into 5 containers. Twenty-five laboratory-reared third instar larvae were subsequently inserted in each container and allowed to develop till the adult stage. Adult emergence rate was calculated for each container. Controls were set up following the same procedure except that dechlorinated tap water (for Experiments 1 and 2) and water from control ovitraps in Pamplemousses Botanical Garden (For Experiment 2) were used.

Evaluating lethal concentration of pyriproxifen solution on eggs and larvae under laboratory condition

Early stage 3 larvae (n=30) raised from eggs collected from PDL were immersed in 100 ml Pyriproxifen solutions diluted at 0.63, 1.25, 2.5, 5, 10, 20 and 40 (original stock) ppm. Thirty eggs, checked to be apparently sound under stereomicroscope were similarly placed in diluted Pyriproxifen solutions of 1, 4, 20 and 40 ppb. Tests were set in triplicate and included a control blank of distilled water.

Data analysis

Data were tested for normality and for homogeneity of variances using respectively the Anderson-Darling and the Levene's tests. If data were non-parametric, the latter were either angle transformed (arcsine sqrt) for frequency data or log transformed (Log_{10} (n+1)) for count data and tested again for normality and for homogeneity of variances prior to statistical analysis. Trap attractiveness was evaluated by comparing ovitrap index and weekly mean of egg density from treatment ovitraps using ANOVA and Tukey's post-hoc test. The difference in hatch rate and adult emergence rate among the different treatments were evaluated using ANOVA and Tukey's post-hoc test for parametric data and Kruskal-Wallis and Dunn's multiple comparisons tests for non-parametric data. The effect of autodissemination was investigated by comparing mean adult production rate in contaminated and control containers using Student's *t*-tests. To evaluate lethal concentration of pyriproxifen solution in laboratory condition, probit analysis at 95% fiducial confidence level, were carried out on the number of potential eggs and larvae failing to reach adulthood using a logistic regression model in both cases. All statistical analyses were performed using Minitab 16 (Minitab Inc., State College, PA) or GraphPad Prism version 6.05 (GraphPad Software, San Diego, USA), with alpha level of 0.05. To aid interpretability, raw data (mean ± SE) are presented in the tables and figures, unless otherwise stated.

RESULTS AND DISCUSSION

Hatch rate

Hatch rate (Fig. 5) differed significantly among treatment ovitraps (One-Way ANOVA: df = 2, 33; F = 4.541; P = 0.018). Pyriproxyfen had a slight but significant suppressive effects on hatch rate (approximately 7 %) when compared to control ovitraps (Tukey Post Hoc < 0.05).

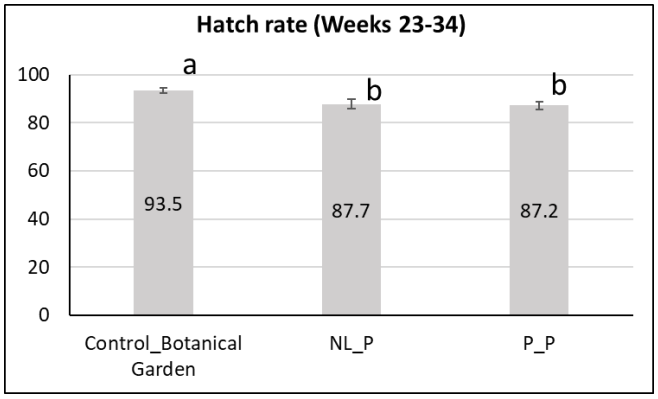

Fig. 5: Hatch rate (mean ± SE) of eggs on ovistrips from control ovitraps containing water in Pamplemousses Botanical Garden (Control_Botanical Garden), normal ovitraps containing pyriproxyfen solution (NL_P), and Aedes Tech Mosquito Home System ovitrap containing pyriproxyfen solution (P_P) in Zone Aduring Weeks 23- 34. Different letters represent statistical differences between treatments (*Post hoc* Tukey tests, $P < 0.05$).

Adult emergence rate of eggs on ovistrips

Adult emergence rate (Figs. 6 & 7) differed significantly among ovitrap treatments during Weeks 7 to 22 (Kruskal-Wallis = 67.97; $P < 0.0001$) and Weeks 23 to 34 (Kruskal-Wallis = 31.02; $P < 0.0001$). Pyriproxyfen had a major significant suppressive effect on adult emergence rate; ranging from a suppression of 65.5 % when P_P ovistrips were processed in water for hatching and larval development to complete suppression of adult emergence in Zones A and B when hatching and larval development took place in the pyriproxyfen solution.

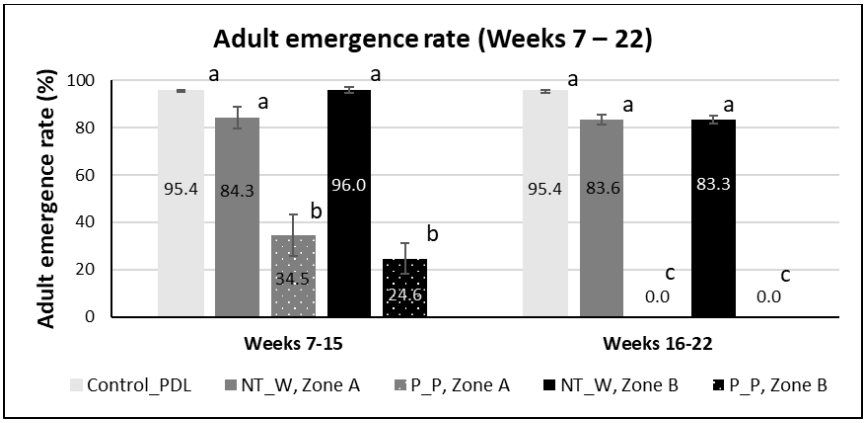

Fig. 6: Adult emergence rate (mean ± SE) of hatched eggs on ovistrips from control ovitraps containing water in Pointe des Lascars (Control_PDL), normal ovitraps containing water in Zone A (NT_W_Zone A), Aedes Tech Mosquito Home System ovitrap containing pyriproxyfen in Zone A (P_P_Zone A), normal ovitraps containing water in Zone B (NT_W_Zone B) and Aedes Tech Mosquito Home System ovitraps containing pyriproxyfen in Zone B (PP_Zone B) during Weeks 1- 9 and Weeks 10-16, when hatching and larval development of eggs from P_P ovistrips respectively occurred in water and a pyriproxyfen solution. Different letters represent statistical differences between treatments (*Dunn's multiple comparisons test*, $P < 0.05$).

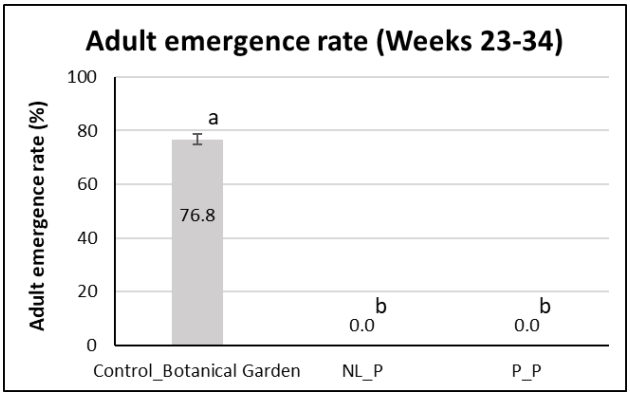

Fig. 7: Adult emergence rate (mean ± SE) of hatched eggs on ovistrips from control ovitraps containing water in Pamplemousses Botanical Garden (Control_Botanical Garden), normal ovitraps containing pyriproxyfen (NL_P) and Aedes Tech Mosquito Home System ovitraps containing pyriproxyfen (P_P) in Zone A in Pamplemousses. Different letters represent statistical differences between treatments (*Dunn's multiple comparisons test*, $P < 0.05$).

Adult emergence rate of larvae in water collected from field ovitraps

Adult emergence rate of larvae bred in tap water (negative control) did not differ significantly from those bred in water collected from normal ovitraps in Zone A (T = -0.11, df = 30, P = 0.92) and Zone B (T = 0.00, df = 30, P = 1.0) (Fig. 5). Hence, autodissemination could not be demonstrated in this study.

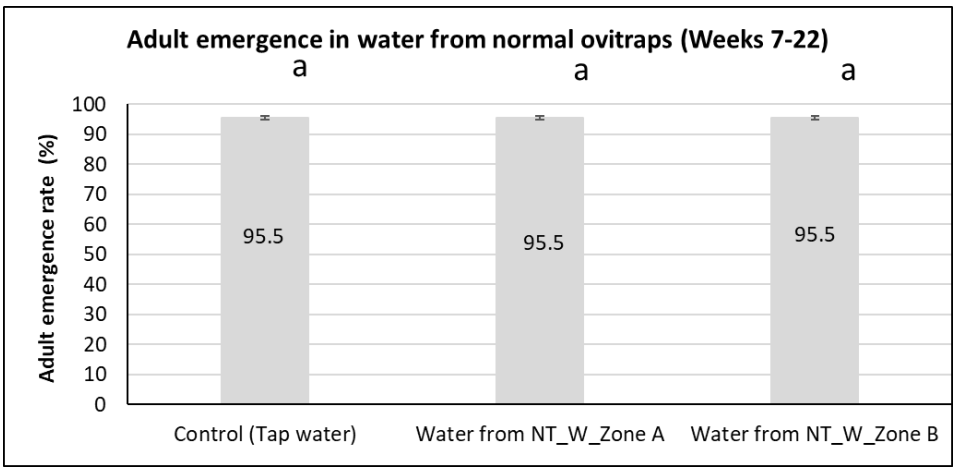

Fig. 8: Adult emergence rate (mean ± SE) of L3 larvae immersed in tap water (control) and water collected from normal ovitraps in Zone A (NT_W_Zone A) and Zone B (NT_W_Zone B). Different letters represent statistical differences between treatments (Student t- tests, $P < 0.05$).

Weekly Ovitrap Index and ovitrap productivity

From 07 August to 20 November 2020, there were no significant differences in weekly ovitrap index and weekly ovitrap productivity between normal ovitraps containing water (NT_W) and Aedes Tech Mosquito Home System ovitraps containing pyriproxyfen (P_P) in Zone A (Ovitrap Index: T = 1.33, df = 29, P = 0.195; Ovitrap productivity: T = 1.81, df = 29, P = 0.081) and Zone B (Ovitrap Index: T = 0.53, df = 29, P = 0.600; Egg density: T = 1.33, df =

29, P = 0.194). However from 27 November 2020 onwards, probably with acclimatization of the mosquito to the P_P ovitraps, weekly Ovitrap index and ovitrap productivity were significantly higher in P_P ovitraps than in NT_W ovitraps in Zone A (Ovitrap Index: T = -4.30, df = 19, P = 0.000; Ovitrap productivity: T = -3.16, df = 21, P = 0.005). Ovitrap productivity and the frequency of P_P ovitraps with eggs were approximately two to three times higher than in NT_W ovitraps (Figs. 9 & 10).

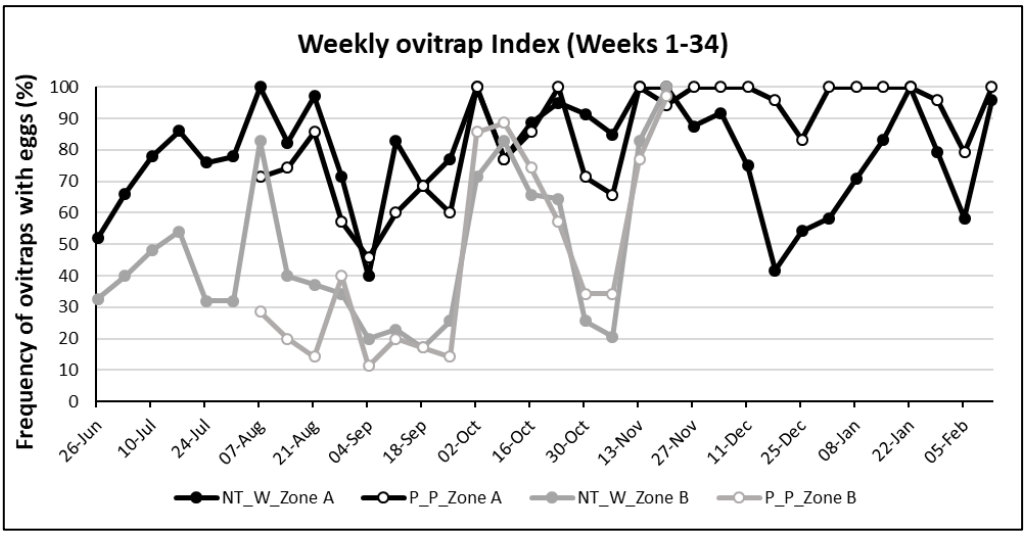

Fig. 9: Weekly frequency of normal and pyriproxyfen ovitraps positive for eggs of *Ae. albopictus* egg density in Zone A (respectively NT_W_Zone A and P_P_Zone A) and Zone B (respectively NT_W_Zone B and P_P_Zone B) in Pamplemousses

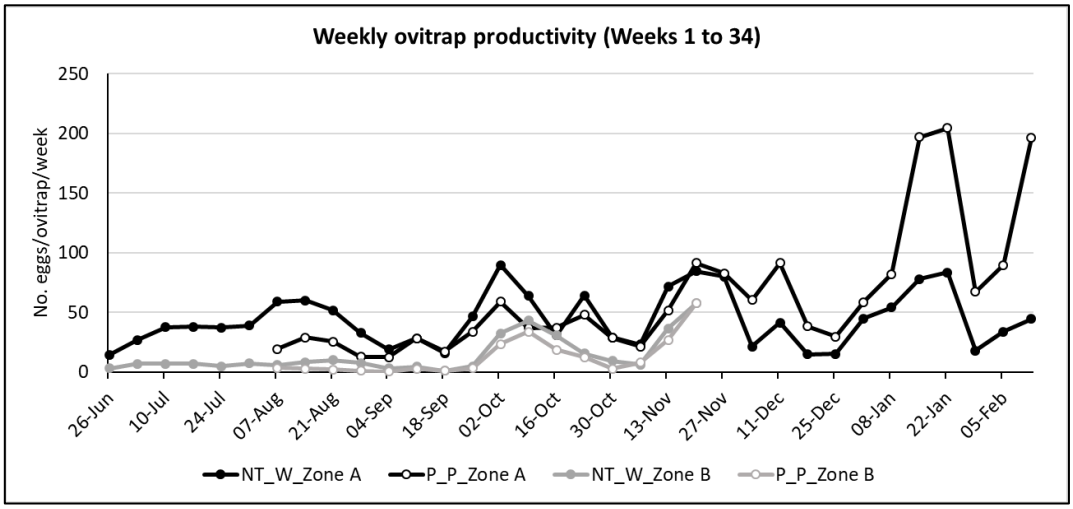

Fig. 10: Weekly mean of *Ae. albopictus* egg density in normal and pyriproxyfen ovitraps in Zone A (respectively NT_W_Zone A and P_P_Zone A) and Zone B (respectively NT_W_Zone B and P_P_Zone B) in Pamplemousses , **(c)** daily recapture rate of *Ae. albopictus* females from BGS traps in PDL, PAN

Ovitrap attractiveness: ovitrap design vs oviposition solution

Ovitrap Index (Kruskal-Wallis = 24.92; $P < 0.0001$) and ovitrap productivity (One-Way ANOVA: df = 4, 55; F = 6.45; P <0.0001) differed significantly among the five types of ovitraps (Figs. 11 & 12). Trap design was the most important determining factor in trap attractiveness. The Aedes Tech Mosquito Home System ovitrap attracted significantly more *Ae. albopictus* mosquitoes than the other ovitraps with respectively 96.2 and 92.7 % of P_P and P_W ovitraps positive for eggs of *Ae. albopictus*. P_P and P_W ovitraps were also 1.5 to 3.5 times more productive than the other types of ovitrap. Ovitrap productivity did not significantly differ between water-containing and pyriproxyfen-containing ovitraps of the same design (i.e. P_W vs P_P and NL_W vs NL_P) which indicates that pyriproxyfen did not significantly affect the oviposition activities of *Ae. albopictus*.

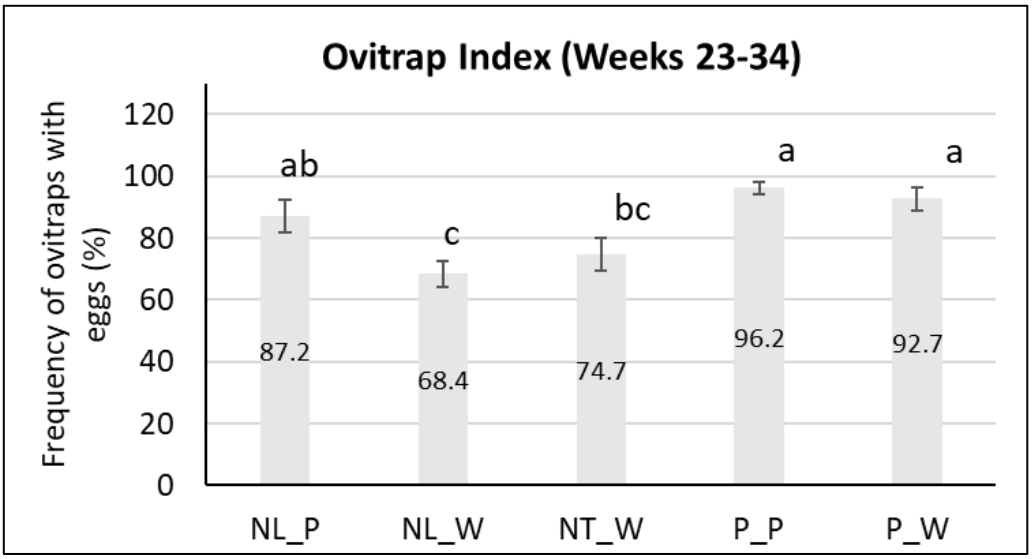

Fig. 11: Average of weekly Ovitrap Index in 5 types of ovitraps (NL_P, normal ovitrap with lateral holes and pyriproxyfen solution; NL_W, normal ovitrap with lateral holes and water; NT_W, normal ovitrap with holes on the lid and water; P_P, Aedes Tech Mosquito Home System ovitrap with pyriproxyfen solution; P_W, Aedes Tech Mosquito Home System ovitrap with water) from Weeks 23 to 34. Different letters represent statistical differences between treatments (*Dunn's multiple comparisons test*, $P < 0.05$).

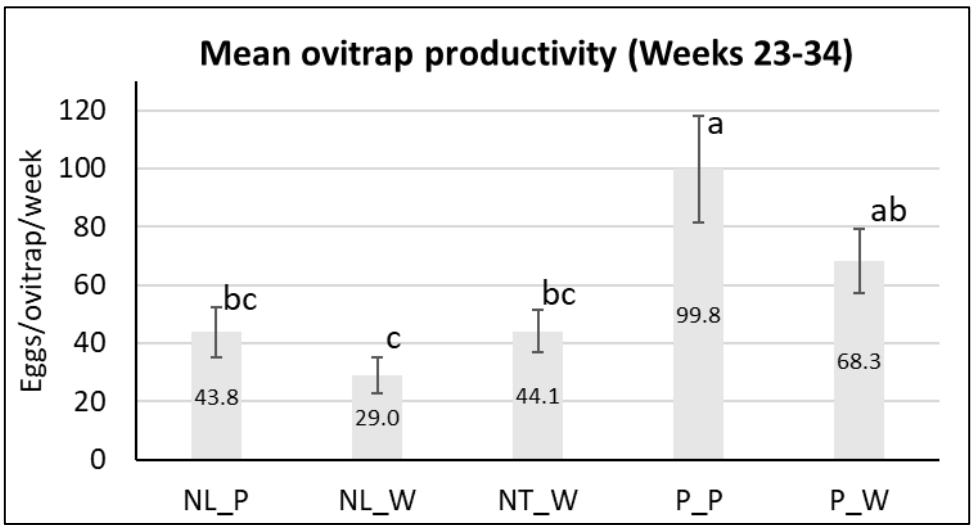

Fig. 12: Average of weekly ovitrap productivity in 5 types of ovitraps (NL_P, normal ovitrap with lateral holes and pyriproxyfen solution; NL_W, normal ovitrap with lateral holes and water; NT_W, normal ovitrap with holes on the lid and water; P_P, Aedes Tech Mosquito Home System ovitrap with pyriproxyfen solution; P_W, Aedes Tech Mosquito Home System ovitrap with water) from Weeks 23 to 34. Different letters represent statistical differences between treatments (*Post hoc* Tukey tests, $P < 0.05$).

Lethal concentration of pyriproxifen solution on eggs and larvae in laboratory condition

Probit analysis indicate that the concentration of pyriproxifen applied to eggs and larvae were significantly correlated with the failure of mosquitoes to reach adulthood stage according to a logistic regression model (P= 0.000; Pearson Goodness of fit; p = 0.183 & 0.980, respectively for eggs and larval exposure). For stage III larval exposure to pyriproxifen, the 99th percentile concentration for failure of larvae to reach the adult stage was within the range of 5.76 to 12.4 ppm. Conversely, when exposed at the egg stage, the lethal concentration ranged from 75 to 131 ppb.

CONCLUSION

In this study, Aedes Tech Mosquito Home System ovitraps with its 0.004 % (w/w) pyriproxyfen solution (P_Ps) were two to three times more productive for eggs of *Ae. albopictus* than the other ovitraps. Furthermore, the pyriproxyfen solution of the Aedes Tech Mosquito Home System unit had a strong suppressive effect on adult emergence with complete adult suppression (100 ± 0 %, mean ± SE) when ovipositied eggs were left to hatch and develop in the pyriproxyfen solution. The autocidal potency of the pyriproxyfen solution did not decrease over time (28 weeks in this study), making the Aedes Tech Mosquito Home System unit a very attractive tool for surveying and controlling *Ae. albopictus*. In comparison to normal water-containing ovitraps, the Aedes Tech Mosquito Home System units do not have to be serviced on a weekly basis thereby requiring relatively less man power, resources and transport logistics for its large-scale deployment and maintenance in the field.

Autodissemination of pyriproxyfen from Aedes Tech Mosquito Home System units to other ovitraps containing 500 ml water could not be demonstrated in the field in this study. However

under laboratory conditions, a pyriproxyfen concentration ranging from 5.76 to 12.4 ppm (i.e. a dilution of roughly 5000 fold of the stock pyriproxyfen solution) and from 75 to 131 ppb (i.e. a dilution of roughly 421000 fold of the stock pyriproxyfen solution) was required to have a 99 % suppressive effect on adult emergence for Stage III larvae and eggs respectively. This implies that the suppressive effect of pyriproxyfen through autodissemination by the Aedes Tech Mosquito Home System units could still occur if contaminated females visit relatively smaller breeding sites such as water accumulation in tin cans, flower saucers, small containers, rubber tyres and bottle caps which are often found to be positive for *Ae. albopictus* larvae by the Vector Biology and Control Division mosquito surveillance team in Mauritius.

REFERENCES

1. Beesoon, S., Funkhouser, E., Kotea, N., Spielman, A. & Robich, R.M. Chikungunya fever, Mauritius, 2006. *Emerg. Infect. Dis.* **14**, 337 (2008).
2. Caputo B, Ienco A, Cianci D, Pombi M, Petrarca V, Baseggio A, et al. (2012) The "Auto-Dissemination" Approach: A Novel Concept to Fight *Aedes albopictus* in Urban Areas. PLoS Negl Trop Dis 6(8): e1793.
3. Chavasse, D.C., Lines, J.D., Ichimori, K., Majala, A.R., Minjas, J.N. and Marijani, J., 1995. Mosquito control in Dar es Salaam. II. Impact of expanded polystyrene beads and pyriproxyfen treatment of breeding sites on *Culex quinquefasciatus* densities. *Medical and veterinary entomology*, *9*(2), pp.147-154.
4. Darriet, F., Marcombe, S., Etienne, M., Yébakima, A., Agnew, P., Yp-Tcha, M.M. and Corbel, V., 2010. Field evaluation of pyriproxyfen and spinosad mixture for the control of insecticide resistant Aedes aegypti in Martinique (French West Indies). *Parasites & vectors*, *3*(1), p.88.
5. Dash AP, Ranjit MR. Comparative efficacy of aphid extracts and some juvenoids against the development of mosquitoes. *J Am Mosq Control Assoc* 1992; 8: 247-51.
6. Dell Chism B, Apperson CS (2003) Horizontal transfer of the insect growth regulator pyriproxyfen to larval microcosms by gravid *Aedes albopictus* and *Ochlerotatus triseriatus* mosquitoes in the laboratory. Medical and Veterinary Entomology 17: 211–220.
7. Devine GJ, Perea EZ, Killeen GF, Stancil JD, Clark SJ, et al. (2009) Using adult mosquitoes to transfer insecticides to Aedes aegypti larval habitats. Proceedings of the National Academy of Science USA 106: 11530–11534 doi:10.1073/pnas.0901369106.
8. Devine, G. (2016) Auto-Dissemination of Pyriproxyfen for the Control of Container-inhabiting Mosquitoes - a Progress Review. Outlooks on Pest Management, 27, 164-167.
9. Devine, G.J., Perea, E.Z., Killeen, G.F., Stancil, J.D., Clark, S.J. and Morrison, A.C., 2009. Using adult mosquitoes to transfer insecticides to Aedes aegypti larval habitats. *Proceedings of the National Academy of Sciences*, *106*(28), pp.11530-11534.
10. Doud, C.W., Hanley, A.M., Chalaire, K.C., Richardson, A.G., Britch, S.C. and Xue, R.D., 2014. Truck-Mounted Area-Wide Application of Pyriproxyfen Targeting Aedes aegypti and Aedes albopictus in Northeast Florida1. *Journal of the American Mosquito Control Association*, *30*(4), pp.291-297.

11. Dowling, M.A.C., 1953. Control of malaria in Mauritius Eradication of *Anopheles funestus* and *Aedes aegypti*. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *47*(3), pp.177-198.
12. Faraji, A. & I. Unlu (2016) The Eye of the Tiger, the Thrill of the Fight: Effective Larval and Adult Control Measures Against the Asian Tiger Mosquito, *Aedes albopictus* (Diptera: Culicidae), in North America. *J Med Entomol,* 53**,** 1029-47.
13. Gaugler, R., Suman, D. and Wang, Y., 2012. An autodissemination station for the transfer of an insect growth regulator to mosquito oviposition sites. *Medical and veterinary entomology*, *26*(1), pp.37-45.
14. GIS, Government Information Service, Prime Minister's Office, Mauritius. Fake news on Covid-19: Health Minister severely condemns anti-patriotic behaviour http://www.govmu.org/English/News/Pages/Fake-news-on-Covid-19-Health-Minister-severely-condemns-anti-patriotic-behaviour.aspx (2020)
15. Harris, C., Lwetoijera, D.W., Dongus, S., Matowo, N.S., Lorenz, L.M., Devine, G.J. and Majambere, S., 2013. Sterilising effects of pyriproxyfen on Anopheles arabiensis and its potential use in malaria control. *Parasites & vectors*, *6*(1), pp.144.
16. Hawley, W.A., 1988. The biology of *Aedes albopictus*. *Journal of the American Mosquito Control Association.* Suppl. 1, 1–39.
17. Health Statistics Unit, Ministry of Health and Wellness, Mauritius. Health statistics report 2018, Island of Mauritius and Island of Rodrigues http://health.govmu.org/English/Statistics/Health/Mauritius/Documents/HEALTH%20STATS%20REPORT%202018.pdf (2019)
18. Issack, M.I. *et al.* Reemergence of dengue in Mauritius. *Emerg. Infect. Dis.* **16**, 716 (2010).
19. Itoh, T., Kawada, H., Abe, A., Eshita, Y., Rongsriyam, Y. and Igarashi, A., 1994. Utilization of bloodfed females of Aedes aegypti as a vehicle for the transfer of the insect growth regulator pyriproxyfen to larval habitats. *Journal of the American Mosquito Control Association-Mosquito News*, *10*(3), pp.344-347.
20. Iyaloo, D.P. & Facknath, S. Optimization of *Aedes albopictus* rearing procedures: Preliminary steps towards large-scale rearing of the species within the laboratory in Mauritius. *J. Entomol. Zool. Stud*. **5**, 46 – 53 (2017).
21. Iyaloo, D.P., Elahee, K.B., Bheecarry, A. & Lees, R.S. Guidelines to site selection for population surveillance and mosquito control trials: A case study from Mauritius. *Acta Tropica*, **132**, 140 – 149 (2014).
22. Iyaloo, D.P., Facknath, S. & Bheecarry, A. Seasonal changes in the distribution and abundance of the Dengue vector, *Aedes albopictus* (Skuse) in two villages in Mauritius: Implications for a sterile release programme. *African Entomology*, **27**, 218-227 (2019).
23. Jaddoo J. Dengue Fever: alarming situation in Mauritius https://defimedia.info/dengue-fever-alarming-situation-mauritius (2019)
24. Juri, M.J.D., Zaidenberg, M. and Santana, M., 2013. The efficacy of a combined larvicide–adulticide in ultralow volume and fumigant canister formulations in controlling the dengue vector Aedes aegypti (Diptera: Culicidae) in Northwest of Argentina. *Parasitology research*, *112*(3), pp.1237-1246.
25. Maoz, D., T. Ward, M. Samuel, P. Muller, S. Runge-Ranzinger, J. Toledo, R. Boyce, R. Velayudhan & O. Horstick (2017) Community effectiveness of pyriproxyfen as a dengue vector control method: A systematic review. PLoS Negl Trop Dis, 11, e0005651.

26. McKemey, A. and Adey, R., Innovative Vector Control Consortium, 2018. Autodissemination of insecticides for Mosquito control. https://www.ivcc.com/wp-content/uploads/2019/10/Autodissemination-Report-Adey-and-McKemey-Sep-2018.pdf
27. Miyamoto J, Hirano M, Takimoto Y, Hatakoshi M (1993) Insect growth regulators for pest control, with emphasis on juvenile hormone analogs. Present status and future prospects In: Duke SO, Menn JJ, Plimmer JR, eds. Pest control with enhanced environmental safety (ACS Symposium Series 524). Washington, DC: American Chemical Society; p 144–168
28. MoHQL, MINISTRY OF HEALTH AND QUALITY OF LIFE, 2009. *Operational plan for the prevention and Control of Chikungunya and Dengue in the Republic of Mauritius* [online]. Mauritius, MoHQL. Available from: http://health.govmu.org/English/Documents/deng-act-plan.pdf [Accessed 20 November 2015]
29. MoT, Ministry of Tourism of Mauritius, 2019. Overview of tourism sector in Mauritius. http://tourism.govmu.org/English/Pages/Overview-of-Tourism-Sector-in-Mauritius.aspx#
30. Ocampo, C.B., Mina, N.J., Carabalí, M., Alexander, N. and Osorio, L., 2014. Reduction in dengue cases observed during mass control of Aedes (Stegomyia) in street catch basins in an endemic urban area in Colombia. *Acta tropica*, *132*, pp.15-22.
31. Ohba, S.Y., Ohashi, K., Pujiyati, E., Higa, Y., Kawada, H., Mito, N. and Takagi, M., 2013. The effect of pyriproxyfen as a "population growth regulator" against Aedes albopictus under semi-field conditions. *PLoS One*, *8*(7).
32. Ponlawat, A., Fansiri, T., Kurusarttra, S., Pongsiri, A., McCardle, P.W., Evans, B.P. and Richardson, J.H., 2013. Development and evaluation of a pyriproxyfen-treated device to control the dengue vector, *Aedes aegypti* (L.)(Diptera: Culicidae). *Southeast Asian J Trop Med Public Health*, 44 (2), 167-178.
33. Ramchurn, S.K., Moheeput, K. & S-Goorah, S. An analysis of a short-lived out-break of dengue fever in Mauritius. *Eurosurveillance*, **14**, 3 (2009).
34. Seccacini, E., Juan, L., Zerba, E. and Licastro, S., 2014. Aedes aegypti (Diptera: Culicidae): evaluation of natural long-lasting materials containing pyriproxyfen to improve control strategies. *Parasitology research*, *113*(9), pp.3355-3360.
35. Seng, C.M., Setha, T., Nealon, J., Socheat, D. and Nathan, M.B., 2008. Six months of Aedes aegypti control with a novel controlled-release formulation of pyriproxyfen in domestic water storage containers in Cambodia. *Southeast Asian J Trop Med Public Health*, *39*(5), pp.822-6.
36. Sihuincha, 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. *Journal of medical entomology*, *42*(4), 620-630.
37. 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. *Journal of medical entomology*, *40*(4), pp.455-462.
38. Snetselaar, J., Andriessen, R., Suer, R.A., Osinga, A.J., Knols, B.G. and Farenhorst, M., 2014. Development and evaluation of a novel contamination device that targets multiple life-stages of Aedes aegypti. *Parasites & vectors*, *7*(1), p.200.

39. Suman, D.S., Farajollahi, A., Healy, S., Williams, G.M., Wang, Y., Schoeler, G. and Gaugler, R., 2014. Point-source and area-wide field studies of pyriproxyfen autodissemination against urban container-inhabiting mosquitoes. *Acta tropica*, *135*, pp.96-103.
40. Tatarsky, A., Aboobakar, S., Cohen, J.M., Gopee, N., Bheecarry, A., Moonasar, D., Phillips, A.A., Kahn, J.G., Moonen, B., Smith D.L. and Sabot, O., 2011. Preventing the Reintroduction of Malaria in Mauritius: A Programmatic and Financial Assessment. *PLoS ONE*, 6 (9), e23832.
41. Vythilingam, I., Luz, B.M., Hanni, R., Beng, T.S. and Huat, T.C., 2005. Laboratory and field evaluation of the insect growth regulator pyriproxyfen (Sumilarv 0.5 G) against dengue vectors. *Journal of the American Mosquito Control Association*, *21*(3), 296-300.
42. WHO (2008) World Health Organization, 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. WHO, Geneva, Switzerland: (WHO/HSE/AMR/08.03/9)
43. Yapabandara, A.M.G.M. and Curtis, C.F., 2002. Laboratory and field comparisons of pyriproxyfen, polystyrene beads and other larvicidal methods against malaria vectors in Sri Lanka. *Acta Tropica*, 81(3), 211-223.

A. Bheecarry
Head, Vector Biology and Control Division
Ministry of Health and Wellness,
Route du Jardin, Curepipe,
Mauritius
(Tel: +230 6740019, 6740031; Fax: +230 6742564; email: ambicabheecarry@gmail.com)