Effects of Larval Habitat Substrate on Pyriproxyfen Efficacy Against *Aedes albopictus* (Diptera: Culicidae)

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ABSTRACT Pyriproxyfen, a juvenile hormone analog insect growth regulator (IGR), is a recommended insecticide for the control of container-inhabiting mosquitoes. The effects of eight container substrates (three plastics, wood, tire rubber, clay pot, concrete, and glass) were studied on reduction of bio-efficacy of pyriproxyfen for *Aedes albopictus* (Skuse) in the laboratory. Insect growth regulator-bioassay of third instars showed significant increase of LC_{50} with some substrates compared with control probably because of pyriproxyfen adsorption to the substrate. The LC_{50} for the control substrate (glass mug) was 0.029 μg/liter. In comparison to the control, LC_{50} increased 150.1 times with tire rubber (4.354 μg/liter) and 8–10 times with wood and high-density polyethylene plastic containers. Slight increases of LC_{50} were detected for clay pots, polypropylene, and polystyrene plastics (1.9–2.7 times). Pyriproxyfen efficacy with concrete substrate was higher than with glass containers, which might be caused by high pH. The pH of water with wood (5.6) and concrete (9.8) substrates differed from the other substrates tested (6.3–7.1). There was no pupal mortality at pH 5–11 without pyriproxyfen; however, interaction of pH (5–11) with pyriproxyfen (0.05 μg/liter) showed additive effects at both low and high pH values. Different substrates have variable impacts on pyriproxyfen efficacy, which might be associated with the failure of larval control strategies for the container mosquitoes under certain field conditions.

KEY WORDS IGR, larval habitat, larval control, adsorption, additive effect

Mosquitoes are highly diverged in morphology and behavior, yet are specific in their larval habitat selection. A well-defined group of mosquitoes that inhabit containers is characterized by the properties of its larval habitats. More than 30 mosquito species adapted to containers have been documented from 16 countries (Vezzani 2007). Many of the container-breeding mosquitoes, such as *Aedes aegypti* (L.), *Aedes albopictus* (Skuse), *Anopheles stephensi* (Liston), and *Culex pipiens* (L.), are important vectors of various dreadful human diseases (Nagpal et al. 2005, Vezzani 2007). Every year >1 million people die worldwide because of mosquito-borne diseases such as dengue, chikunguniya, and urban malaria (American Mosquito Control Association [AMCA] 2012).

Ae. albopictus is one of the most invasive species colonizing tropical to temperate areas on most continents. This species prefers to breed in small to medium-size containers located in shady and cryptic habitats (Hawley 1988). The substrates of larval habitats can vary geographically among human lifestyles. The Center for Disease Control and Prevention (CDC 2012) has provided detailed information regarding container mosquitoes and their breeding habitats in natural and artificial containers. Tires, battery boxes, waste containers, earthenware, paper cups, discarded metal drums, coconut shells, leaf axils, and glass bottles were key larval habitats of dengue vectors *Ae. aegypti* and *Ae. albopictus* in industrial, urban, semiurban, and rural areas of the seven states of northeastern India (Dutta and Mahanta 2006).

The management strategy of the World Health Organization (WHO) for container mosquitoes recommended environmental management and personnel protection. However, insecticide use is advised when the other methods fail to reduce mosquito populations (WHO 2009). Temephos, an organophosphate, and insect growth regulators (IGRs) such as methoprene, diflubenzuron, and pyriproxyfen were recommended as larvicides (WHO 2009). Larval control is considered to be appropriate for container mosquitoes because of their confined habitats. However, development of resistance in mosquitoes against organophosphates and other neurotoxic insecticides compelled the use of new insecticides with a different mode of action (Mulla 1995).

Pyriproxyfen is a juvenile hormone analog that has a high efficacy against mosquitoes (LC_{50} = 0.011 μg/liter; LC_{90} = 0.376 μg/liter; Ali et al. 1995). Owing to its mode of action and safety to nontarget organisms, it is classified as unlikely hazardous category by the WHO (WHO 2009). The U.S. Environmental Protection Agency (EPA) has permitted experimental use of

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pyriproxyfen for *Ae. albopictus* in urban areas of Florida and New Jersey (EPA 2013). Pyriproxyfen was evaluated in large-sized containers under field or semifield conditions by several workers in tires, polyethylene plastic tubs (100 liters; Nayar et al. 2002), earthen pots (60 liters; Vythilingam et al. 2005), plastic containers (Takagi et al. 1995, Morales et al. 1997), glass containers, and tires (Andrighetti et al. 2008). In these studies, pyriproxyfen concentrations were inconsistent and produced variable results, making it difficult to compare efficacy.

Different substrates of adult mosquito resting habitats reduced residual efficacy of various adulticides variably, including organophosphates and synthetic pyrethroids, against different *Anopheles*, *Culex*, and *Aedes* species (Dorta et al. 1993, Etang et al. 2011). However, effects of larval habitat substrates on IGR larvicidal efficacy have not been reported.

Because pyriproxyfen tends to adsorb onto organic matter (Schaefer et al. 1988), we hypothesized that different types of container materials would have variable impact on the bio-availability and consequently the efficacy of pyriproxyfen. We tested our hypothesis on *Ae. albopictus* under laboratory conditions using eight substrates of larval habitats. Accordingly, we also tested the effect of the pH on pyriproxyfen efficacy, as pH can be altered by the substrates.

Materials and Methods

Mosquito Rearing. *Ae. albopictus* was collected and colonized from Mercer and Monmouth Counties, NJ, in 1995. Colonies of *Ae. albopictus* were maintained under laboratory conditions at 26 ± 2°C, 60 ± 5% relative humidity (RH), and a photoperiod of 16:8 (L:D) h. Adult mosquitoes were kept in aluminum screened cages (30 by 30 by 30 cm) where they were fed a 10% sucrose solution dispensed on cotton rolls (Richmond Braided Cotton Rolls, Charlotte, NC). Females (>2–3 d old) were fed on a guinea pig to obtain eggs. Eggs were collected on seed germination paper and stored at 26°C. When larvae were needed, eggs were hatched by flooding egg papers and the larvae held in enamel trays with 1 liter of deionized water and 30 mg/liter of dried yeast.

Larval Habitat Substrates. Eight substrates of the most common containers holding mosquitoes in this region were selected (Table 1). These substrates were cut into pieces with a similar total surface area of 174 cm^2 to fit into bioassay containers (beer glass mugs with 8.4 cm diameter, 16.2 cm depth, and 760 ml volume). The glass mugs were compared with Pyrex glass beakers for pyriproxyfen larval bioassay at doses ranging from 0.05 to 2.0 μg/liter. There were no significant differences between glass mugs and Pyrex beakers for pyriproxyfen efficacy.

Insecticide. NyGaurdIGR Concentrate emulsifiable formulation (MGK McLaughlin Gormley King Company, Minneapolis, MN), which contains 10% pyriproxyfen (4-phenoxyphenyl (*RS*)-2-(2-pyridyloxy) propyl ether) was used for all the experiments. Fresh stock solution (0.1% vol:vol) was made in tap water for each replicate study across time. Five serial dilutions were prepared in tap water ranging from 0.01 to 2.0 μg/liter from the same stock solution. For the tire substrate, concentrations were increased up to 10 μg/liter because of low pupal mortality within the range of 0.01–2.0 μg/liter.

Table 1. Description of the substrates of containers

Container name	Substrate materials
Cemetery vase	HDPE plastic
Plastic food container 1	PP plastic
Plastic food container 2	PS plastic
White pine wood	Soft wood
Clay pot	Fire clay
Glass mug (beer stein)	Glass
Tire pieces[a]	Styrene–butadiene copolymer rubber
Concrete plate[b]	Cement and stone pieces

[a] Tire rubber was cut from the sidewall of the used tires.
[b] Concrete plate was made from a commercially available cement and pebbles, air drying for 2 d, and hardening by submersion into water for 2–3 d.

Effects of Container Materials on Larvicidal Efficacy of Pyriproxyfen. Container substrates were paired with control containers in triplicate. All eight substrates (Table 1) and serial dilution of pyriproxyfen as described above were used for larval bioassays. LC_{50} and LC_{90} of pyriproxyfen for different substrates were calculated. All experiments were repeated three times. Data from only two repeats for the concrete substrate were used because of high mortality in the controls of the third repeat study. A ratio of LC_{50} for pyriproxyfen between the glass standard and other substrates was calculated with the following formula:

$$LC_{50}\ \text{ratio} = LC_{50}\ \text{of substrate}/LC_{50}\ \text{of glass}. \quad [1]$$

Larval Bioassay. Glass mugs (760 ml) were filled with 200 ml tap water, and each substrate was submerged in water. Then, treatment mugs were treated with pyriproxyfen dilutions. Control containers were not treated. Twenty third instar *Ae. albopictus* were inoculated into each bioassay mug. Larvae were fed dried brewer's yeast (30 mg/liter) twice weekly until all the larvae metamorphosed into pupae or died. Evaporated water was replenished with tap water every other day. Observations were made for larval and pupal mortality, and adult emergence twice per week. Incomplete emergence of an adult was considered as a pupal death. Substrates were discarded after a single use. Pupal mortality was used to estimate LC_{50} and LC_{90} using probit analysis as described in Statistical analysis section.

Effects of pH on Pyriproxyfen Efficacy. The pH of control bioassay containers of different substrates was measured after 2 d of experiment setup. To assess the pH effect, a separate experiment was conducted with buffer solutions (Table 2) without substrates. The buffer solutions of different pH (4, 5, 6, 8, 10, 11, and 12) were prepared to cover the pH range of substrates used for the study (Table 2). Ionic strength of buffer solutions ranged from 0.025 to 0.1 molar. A safe pH range for *Ae. albopictus* larvae (5–11) was determined using larval bioassay of buffer solutions, and this range

Table 2. Chemical composition of various pH buffer solutions used for larval bioassay

pH	Chemicals	pH adjustment solution
4	0.1 M KH_2PO_4	0.1 M HCl
5	0.05 M KH_2PO_4	0.1 M NaOH
6	0.05 M KH_2PO_4	0.1 M NaOH
7	Tap water	0.1 M NaOH/HCl
8	0.05 M KH_2PO_4	0.1 M NaOH
10	0.025 M $NaHCO_3$	0.1 M NaOH
11	0.025 M $NaHCO_3$	0.1 M NaOH
12	0.05 M KCl	0.1 M NaOH

All the buffer solutions were prepared in 1-liter volume at 26 ± 1°C.

was used to assess additive effect of pyriproxyfen with pH.

Additive Effect Assay. The combined effect of pH and pyriproxyfen was assessed using a series of pH buffers (5, 6, 8, 10, and 11) with 0.05 μg/liter concentration of pyriproxyfen. pH buffer solutions were prepared as described in Table 2. The bioassay procedure was similar to the pyriproxyfen larval bioassay described previously. Untreated water was used as negative control; pyriproxyfen-treated water (0.05 μg/liter) was used as positive control; and pH in both controls was adjusted to neutral using HCl/NaOH solutions. The pupal mortality in the positive controls was used to correct mortality in the pH treatments using Abbott's formula (Abbott 1925). The negative control was excluded from the analysis, as there was no mortality in any developmental stage of mosquito.

Statistical Analysis. Efficacy of pyriproxyfen was expressed as percent pupal mortality and lethal concentrations (LC_{50} and LC_{90}). Treatment pupal mortality was corrected with control using Abbott's formula (Abbott 1925). If any control had >20% average mortality in any stage, data were discarded. The corrected pupal mortality and their respective pyriproxyfen concentration were subjected to probit analysis to estimate LC_{50} and LC_{90} using PSAW Statistics 18 software package (IBM Corporation, Armonk, NY). One-way analysis of variance (ANOVA) was used to compare the pH of different substrates and the effect of pH on pyriproxyfen efficacy. Fisher least significant difference (LSD) was used to differentiate the means at $P < 0.05$ significance level.

Results and Discussion

Our results showed that different substrates reduced pyriproxyfen bio-efficacy against *Ae. albopictus* larvae; the highest reduction was observed with the tire substrate followed by high-density polyethylene (HDPE) plastic and white pine wood (Table 3). This result supports our hypothesis that adsorption of pyriproxyfen onto various substrates reduces the availability of the IGR to mosquito larvae. In natural conditions, pyriproxyfen residue concentration decreased in the water column by 50% within 24 h posttreatment of a pond and showed a tendency to adsorb onto organic materials present in polluted water that extended biological activity when applied at higher concentrations (Schaefer et al. 1988). This finding indicates that the adsorption phenomenon can be useful as a slow release mechanism of the insecticides. Our test concentration was lower (<5 μg/liter) than the recommended concentration of pyriproxyfen (10 μg/liter) for the treatment of container-dwelling mosquito larvae (WHO 2009). We did not investigate the further release of adsorbed pyriproxyfen from different substrates or persistence of its bioactivity.

Median lethal concentration (LC_{50}) of pyriproxyfen against *Ae. albopictus* in glass containers was 0.029 μg/liter (Table 3). This toxicity was consistent with bioassays conducted by Ali et al. (1995). We observed a 2–150 times increase in the LC_{50} of pyriproxyfen in containers made up of various substrates when compared with glass, except for concrete. Tire rubber showed the maximum ability to adsorb pyriproxyfen, which increased the LC_{50} and LC_{90} to 4.354 and 47.666 μg/liter, respectively. The LC_{90} of pyriproxyfen in tire rubber habitats exceeded by 4.7 times the WHO-recommended concentration to treat mosquito container habitats (WHO 2009), indicating ineffectiveness of pyriproxyfen at recommended concentrations in tires. Field trials of pyriproxyfen in tires with direct treatment using a backpack sprayer at the label rate (789 ml/ha of NyGaurd 10 EC) showed gradual reduction of pyriproxyfen activity against *Ae. albopictus* 2 wk posttreatment (D.S.S. et al., unpublished data). Another study also showed that pyriproxyfen (Sumilarv 0.5G) efficacy against *Ae. aegypti* was significantly reduced in tires compared with glass containers (Andrighetti et al. 2008). That failure may be attributed to

Table 3. Median lethal concentrations of pyriproxyfen against *Ae. albopictus* exposed in glass containers containing various substrates

Substrate	Pupal mortality (μg/liter)		Slope	Intercept	χ^2	df	*p*	LC_{50} ratio[a]
	LC_{50}	LC_{90}						
Glass container	0.029 (0.014–0.050)	0.587 (0.293–1.966)	0.985	1.509	1115.28	43	0.0001	—
HDPE plastic	0.295 (0.181–0.521)	1.301 (0.695–4.091)	1.991	1.504	2483.20	43	0.0001	10.18
PP plastic	0.058 (0.040–0.085)	0.390 (0.226–0.969)	1.553	1.916	670.50	31	0.0001	1.99
PS plastic	0.072 (0.048–0.108)	0.538 (0.295–1.542)	1.463	1.676	863.75	34	0.0001	2.48
White pine wood	0.233 (0.094–3.006)	6.369 (0.986–71523.43)	0.891	0.565	1881.673	33	0.0001	8.04
Tire	4.354 (2.328–10.786)	47.666 (16.343–940.750)	1.233	−0.788	1088.283	29	0.0001	150.14
Clay pot	0.070 (0.029–0.143)	1.437 (0.531–12.691)	0.975	1.128	2152.63	43	0.0001	2.74
Concrete	0.006 (0.0001–0.012)	0.032 (0.016–1.033)	1.736	3.883	318.430	12	0.0001	0.20

Values in parenthesis for LC_{50} and LC_{90} column for respective substrates show upper and lower fiducial limits.
[a] LC_{50} ratio of pyriproxyfen for different substrates is calculated as LC_{50} of substrate/LC_{50} of glass container.

adsorption of pyriproxyfen by tires, consistent with our lab results. Tires have been found to be major breeding habitats for *Aedes* mosquitoes in urban, semiurban, and industrial areas, including *Ae. aegypti* and *Ae. albopictus* (Dutta and Mahanta 2006). Tires were found to be important larval habitats for 32 mosquito species in the eastern United States, including native *Aedes triseriatus* (Say) and invasive *Ae. albopictus* (Yee 2008).

Ae. albopictus prefers various tree holes for larval habitats in species such as maple (*Acer buergerianum*) and camphor (*Cinnamomum japonicum*) in Japan (Dieng et al. 2003) and bamboo stumps, and pineapple leaf axils in India (Dutta and Mahanta 2006). We found that wood showed higher adsorption of pyriproxyfen than did polypropylene (PP) and polystyrene (PS) plastics, clay, and glass substrates (Table 3). The LC_{90} (6.369 μg/liter) for pyriproxyfen in wood substrates was high but was less than in the tires. The susceptibility data (LC_{99} = 94.640 μg/liter; recalculated from probit analysis) in wooden substrates indicated the ineffectiveness of larvicidal application when compared with the WHO (2009) recommended concentration of pyriproxyfen (10 μg/liter).

Plastic-ware is a major part of the today's lifestyle, improving hygienic conditions via the "use and throw away" concept. However, it generates waste management problems at a large scale despite large recycling programs in both developed and developing countries. In 2010, the United States alone generated 31 million tons of plastic waste. Eight percent of this plastic waste was recycled (EPA 2012). Improper disposal and accumulation of plastic containers create larval mosquito habitats. *Ae. albopictus* has the ability to complete its life cycle within a little amount of water inside bottle caps (Bartlett–Healy et al. 2011). Pyriproxyfen efficacy in three types of plastic containers (HDPE, PP, and PS) was reduced by 2–10 times that in the glass containers (Table 3). Among these plastics, HDPE plastic adsorbed highest amount of pyriproxyfen, increasing LC_{50} to 0.295 μg/liter, compared with 0.029 μg/liter for glass. However, PP and PS plastics reduced pyriproxyfen concentration around 2–3 times compared with glass. The reduction of pyriproxyfen bioefficacy may have great impact on control measures of container mosquitoes such as *Ae. albopictus.*

Application of pyriproxyfen on clay pot substrate increased LC_{50} up to 2.7 times that in glass containers, indicating adsorption of active ingredient to the substrate. The reduction of pyriproxyfen efficacy in clay pot substrate was almost in the range of PP and PS. We tested the bio-efficacy of pyriproxyfen using fresh unused clay pots; however, pyriproxyfen adsorption may be greater in old clay pots that contain organic matter or algal growth. Earthen pots are important larval habitats in urban and semiurban communities in India, contributing 8.7–36.8% CI (container index) for *Ae. albopictus* and 9.4–47.5% CI for *Ae. aegypti* (Dutta and Mahanta 2006). Vythilingam et al. (2005) compared pyriproxyfen efficacy in 60-liter earthen jars with plastic tubs treated at 0.02 mg a.i./liter and found that pyriproxyfen efficacy was reduced in earthen jars earlier than plastic tubs. Long-term efficacy reported by Vythilingam et al. (2005) may be attributed to two factors: 1) a granular formulation that made pyriproxyfen available for longer duration than EC formulation because of slow-release mechanism and 2) the high concentration of pyriproxyfen (two times the WHO concentration; WHO 2009).

In addition, there are factors other than substrate texture that may be associated with mortality of larval mosquitoes. Organic and inorganic solutes can change physicochemical properties of habitat water that may become unsuitable for larvae (Clements 1992, Sota 1993). Our results showed that concrete increased pH (9.85 $\pm$ 0.28) significantly more than did the other substrates and control (6.47 $\pm$ 0.08). Wood decreased the pH significantly to 5.65 $\pm$ 0.14 (one-way ANOVA; df = 7, 64; F = 49.39; $P < 0.0001$; Fig. 1A). A slight increase in pH was observed in clay pot, PP- and PS plastics compared with the control. Concrete is composed of several inorganic compounds such as Ca, Mg, Si, Al, etc., which make hydroxides when reacting with water. In our study, concrete was freshly made, saturated with water for 2 wk and increased pH. We do not expect pH to increase as markedly in old concrete structures where the cement has set completely. However, pine wood releases various compounds that make acids such as tannic acid in the water, resulting in lower pH.

Adaptability to physicochemical properties varies among different mosquitoes. Larvae of *Ae. albopictus* performed better than *Aedes riversi* (Bohart & Ingram) in simulated tree hole water and when exposed to various tannic acid concentrations (Sota 1993). *Ae. albopictus* larvae grow comparatively fast when supplied with maple leaf litter (Dieng et al. 2003). There was no pupal mortality within a pH range from 5 to 11. However, high larval mortality was seen at pH below 5 and above 11 and none of the larvae reached to pupal stage at pH 4 and 12 without pyriproxyfen when exposed as third instar. This mortality may be because of the disruption of osmotic balance of *Ae. albopictus* larvae at high acidity and alkalinity. Other tree hole mosquito species such as *Anopheles plumbeus* (Stephens) and *Aedes geniculatus* (Olivier) may have advantages at lower pH, as they can survive in water with pH 4.4 (MacGregor 1921, Keilin 1932).

The combination of adverse pH conditions and pyriproxyfen showed additive effects for pupal mortality (Fig. 1B). We observed a threefold increase in pupal mortality when pH was <6 and up to a 5.5-fold increase of pupal mortality at pH 11 with 0.05 μg/liter of pyriproxyfen relative to that of control (one-way ANOVA; df = 5, 12; F = 2.08; $P < 0.138$; Fisher LSD = 18.466; Fig. 1B). It is interesting that we found higher efficacy of pyriproxyfen in the concrete substrates and LC_{50} for *Ae. albopictus* was reduced 4.8 times (0.006 μg/liter) in comparison with 0.029 μg/liter of control (Table 3). However, the efficacy in other concrete-made containers may vary with age and stability of the structures. Deer and Beard (2001) showed that water pH affected the chemical stability of various pesticides and that half-life varied

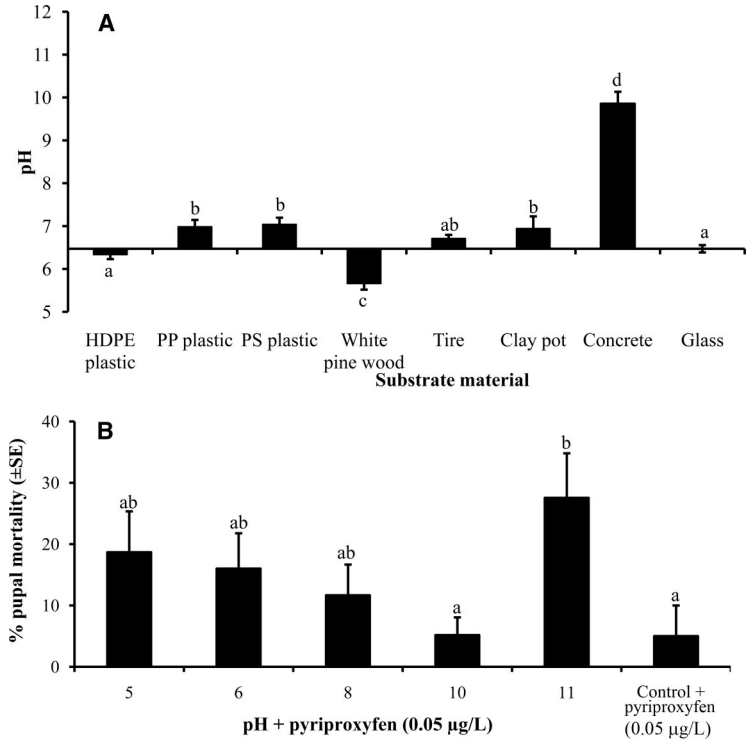

Fig. 1. The pH of water in bioassay using different substrates at 2-d postexposure (A) and the additive effect of pH on pupicidal efficacy of pyriproxyfen (0.05 μg/liter) (B) against *Ae. albopictus.* Scale bars designated with same letters are not significantly different ($P < 0.05$).

among the pesticides. Deer and Beard (2001) also found that low pH extended stability of insecticides. Stability of the active ingredient is important in IGR-bioassay experiments because IGRs bioassays are run for weeks rather than 24 or 48 h for many conventional bioassays.

In conclusion, different substrates reduced the bio-efficacy of pyriproxyfen against *Ae. albopictus* when exposed in the larval stages. Pyriproxyfen presumably adsorbed most strongly onto tire substrates, increasing LC_{50} up to 150 times compared with glass containers. IGR bio-efficacy was also significantly reduced in other container substrates (wood, plastics, and clay). Various substrates altered pH of water which affected pyriproxyfen efficacy. However, alteration of pH was not sufficient to overcome the reduction of pyriproxyfen efficacy caused by pyriproxyfen adsorption to the substrates. Despite pyriproxyfen adsorption to various substrates, the importance of this IGR cannot be underestimated, as it has high pupicidal efficacy at very low concentrations. To achieve maximum IGR efficacy and overcome differential adsorption of various substrates, this study suggests adjustment of the dose or concentration according to the targeted container substrate and to exploration of other formulations (e.g., slow-release, micro-encapsulation) to sustain bio-availability of pyriproxyfen in container habitats.

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References Cited

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265–267.

Ali, A., J. K. Nayar, and R. D. Xue. 1995. Comparative toxicity of selected larvicides and insect growth-regulators to a Florida laboratory population of *Aedes albopictus.* J. Am. Mosq. Control Assoc. 11: 72–76.

(AMCA) American Mosquito Control Association. 2012. Mosquito-borne diseases. (http://www.mosquito.org/mosquito-borne-diseases).

Andrighetti, M.T.M., F. Cerone, M. Rigueti, K. C. Galvani, and M.L.G. Macoris. 2008. Effect of pyriproxyfen in *Aedes aegypti* populations with different levels of susceptibility to the organophosphate temephos. Dengue Bull. 32: 186–198.

Bartlett–Healy, K., S. P. Healy, and G. C. Hamilton. 2011. A model to predict evaporation rates in habitats used by container-dwelling mosquitoes. J. Med. Entomol. 48: 712–716.

(CDC) Center for Disease Control and Prevention. 2012. Dengue home page. Mosquitoes' main aquatic habitats.

Dengue Branch 1324 Calle Cañada San Juan, Puerto Rico, 00920–3860. (http://www.cdc.gov/dengue/entomology Ecology/m_habitats.html).

Clements, A. N. 1992. The biology of mosquitoes, volume 1. Development, nutrition and reproduction. Chapman & Hall, New York, NY.

Deer, H. M., and R. Beard. 2001. Effect of water pH on chemical stability of pesticides. Utah State University, cooperative extension, UT. (http://extension.usu.edu/files/publications/factsheet/AG_Pesticides_14.pdf).

Dieng, H., M. Boots, Y. Tsuda, and M. Takagi. 2003. A laboratory oviposition study in *Aedes albopictus* (Diptera: Culicidae) with the reference to habitat size, leaf litter and their attractions. Med. Entomol. Zool. 54: 43–50.

Dorta, D. M., V. Vasuki, and A. Rajavel. 1993. Evaluation of organophosphorus and synthetic pyrethroid insecticides against six vector mosquito species. Rev. Saude Publica 27: 391–397.

Dutta, P., and J. Mahanta. 2006. Potential vectors of dengue and the profile of dengue in the north–eastern region of India: an epidemiological perspective. Dengue Bull. 30: 234–242.

(EPA) U.S. Environmental Protection Agency. 2012. Wastes–resource conservation-common waste and materials. (http://www.epa.gov/osw/conserve/materials/plastics.htm).

(EPA) U.S. Environmental Protection Agency. 2013. Pesticides: registration review–pyriproxyfen. (http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2011-0677-0002).

Etang, J., P. Nwane, J. A. Mbida, M. Piameu, B. Manga, D. Souop, and P. Awono–Ambene. 2011. Variations of insecticide residual bio-efficacy on different types of walls: results from a community-based trial in south Cameroon. Malar. J. 10: 333.

Hawley, W. A. 1988. The biology of *Aedes albopictus*. J. Am. Mosq. Control Assoc. (Suppl). 4: 2–39.

Keilin, D. 1932. On the water reservoir of a horse chestnut tree. Parasitology 24: 280–282.

MacGregor, M. E. 1921. The influence of the hydrogen-ion concentration in the development of mosquito larvae (preliminary communication). Parasitology 13: 348–351.

Morales, R. E., Y. Tsuda, and M. Takagi. 1997. A field experiment of the emergence inhibition of a juvenile hormone mimic, pyriproxyfen, against *Aedes albopictus* in Nagasaki, Japan. Trop. Med. 39: 89–93.

Mulla, M. S. 1995. The future of insect growth regulators in vector control. J. Am. Mosq. Control Assoc. 11: 269–273.

Nagpal, B. N., A. Shrivastava, R. Saxena, M. A. Ansari, A. P. Dash, and S. C. Das. 2005. Pictorial identification key for Indian Anopheline. Malaria Research Centre, (ICMR), 20, Madhuban, Delhi, India.

Nayar, J. K., A. Ali, and M. Zaim. 2002. Effectiveness and residual activity comparison of granular formulation of insect growth regulator pyriproxyfen and S-methoperene against Florida mosquitoes in laboratory and outdoor conditions. J. Am. Mosq. Control Assoc. 3: 196–201.

Schaefer, C. H., T. Miura, E. F. Dupras, F. S. Mulligan, and W. H. Wilder. 1988. Efficacy, nontarget effects, and chemical persistence of S-31183, a promising mosquito (Diptera, Culicidae) control agent. J. Econ. Entomol. 81: 1648–1655.

Sota, T. 1993. Performance of *Aedes albopictus* and *A. riversi* larvae (Diptera: Culicidae) in waters that contain tannic acid and decaying leaves: is the tree hole species better adapted to tree hole water? Ann. Entomol. Soc. Am. 86: 450–457.

Takagi, M., Y. Tsuda, and Y. Wada. 1995. Evaluation of effective period of a juvenile hormone mimic, pyriproxyfen, against *Aedes albopictus:* preliminary experiments in the laboratory and the field. Trop. Med. 37: 87–91.

Vezzani, D. 2007. Review: artificial container-breeding mosquitoes and cemeteries: a perfect match. Trop. Med. Intl. Health 12: 299–313.

Vythilingam, I., B. M. Luz, R. Hanni, T. S. Beng, and T. C. Huat. 2005. Laboratory and field evaluation of the insect growth regulator pyriproxyfen (Sumilarv 0.05G) against dengue vectors. J. Am. Mosq. Control Assoc. 21: 296–300.

(WHO) World Health Organization. 2009. Dengue guidelines for diagnosis, treatment prevention and control, New edition 2009. WHO, Geneva, Switzerland. (WHO/HTM/NTD/DEN/2009.1).

Yee, D. A. 2008. Tires as habitats for mosquitoes: a review of studies within the eastern United States. J. Med. Entomol. 45: 581–593.

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