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Residual Treatment of *Aedes aegypti* (Diptera: Culicidae) in Containers Using Pyriproxyfen Slow-Release Granules (Sumilarv 0.5G)

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ABSTRACT The residual efficacy of pyriproxyfen against *Aedes aegypti* (L.) was examined by treating 2-liter buckets with a range of rates of Sumilarv 0.5G (100, 10, 1, and 0.1 mg product/liter or nominal dose of 500, 50, 5, and 0.5 ppb active ingredient) under semifield conditions. Approximately every 2 wk, pupal emergence inhibition (EI) was measured by using Cairns colony *Ae. aegypti*. Pooled water samples from the five replicate buckets were analyzed for active pyriproxyfen by using ultra-high-pressure liquid chromatography with tandem mass spectrometry detection. A strong dose–response in EI was exhibited, with the 0.1 mg/liter giving ≈50% EI for only the initial week, whereas the 10 and 100 mg/liter doses produced EI > 90% for 8 and 40 wk, respectively. Measurable levels of active ingredient were detected in the 100, 10, and 1 mg/liter treatments, with measured starting concentrations of just 1–2–1.4% of the delivered (active ingredient) dose. Pyriproxyfen was detected in the 100 mg/liter treatment through the entire course of the trial (60 wk).

KEY WORDS *Aedes aegypti*, pyriproxyfen, Sumilarv, dose–response, persistence

In northern Australia, vector control officers require residual treatment of containers, as they cannot revisit properties at <1-mo intervals during large-scale dengue control programs. The current *Aedes albopictus* (Skuse) eradication program in the Torres Strait of Australia uses vector control tours at 6–8-wk intervals and requires residual treatment of containers. To date, larval control consists of treatment of containers with s-methoprene pellets or surface spray with residual synthetic pyrethroid such as bifenthrin. The synthetic pyrethroids can have nontarget effects and are vulnerable to degradation by ultraviolet light outdoors. High "megadoses" of Bti granules have also been used to provide residual control (Ritchie et al. 2010).

The insect growth regulator pyriproxyfen has excellent potential for the residual control of mosquitoes such as *Aedes aegypti* and *Ae. albopictus.* Pyriproxyfen works effectively at very low doses (Invest and Lucas 2008, Devine et al. 2009, Webb et al. 2012). In the field, Sihuincha et al. (2005) obtained >90% control of *Ae. aegypti* in water tanks for up to 5 mo by using doses of just 50–83 ppb. Vythilingham et al. (2005) obtained 4–5 mo residual control of *Ae. aegypti* in 50-liter earthen jars with even lower doses (10–20 ppb), and Chang et al. (2006) suppressed adult emergence from water storage jars for at least 6 mo by using a resin-based slow-release formulation of pyriproxyfen.

Use of gravid mosquitoes to "auto-disseminate" minute doses of pyriproxyfen has been developed as a strategy to control container-breeding mosquitoes (Itoh et al. 1994, Sihuincha et al. 2005, Devine et al. 2009). Devine et al. (2009) obtained control of *Ae. aegypti* by auto-dissemination to adjoining breeding sites of an estimated dose as small as 0.012 ppb active ingredient (AI). In North Queensland, *Aedes*-breeding sites are often small, holding <5 liters of water, and the granular formulation of pyriproxyfen (Sumilarv 0.5 G) offers potential not only for control of *Ae. aegypti* breeding but also auto-dissemination of active ingredient from these sentinel breeding sites to adjoining untreated sites. In this trial, we measured the efficacy of Sumilarv 0.5 G against *Ae. aegypti* in small containers in a semifield cage in Cairns, Queensland, over a 60-wk period.

Materials and Methods

Impact of Pyriproxyfen Against *Ae. aegypti* Under Semifield Conditions. *Ae. aegypti* larvae were exposed to four different doses of pyriproxyfen in 2-liter white plastic buckets under semifield conditions. Treatment doses of Sumilarv 0.5 G were 0.2, 0.02, 0.002, and 0.0002 g product/2-liter bucket (or 100, 10, 1, 0.1 mg product/liter) yielding nominal concentrations of 500, 50, 5, and 0.5 μg (AI)/liter (ppb) of pyriproxyfen, respectively. An untreated bucket served as a control. Five buckets (one from each treatment group and the control) were placed inside five plastic trays that were set inside a screened 5 by 7 by 4-m semifield cage to prevent oviposition from wild *Ae. aegypti* that might

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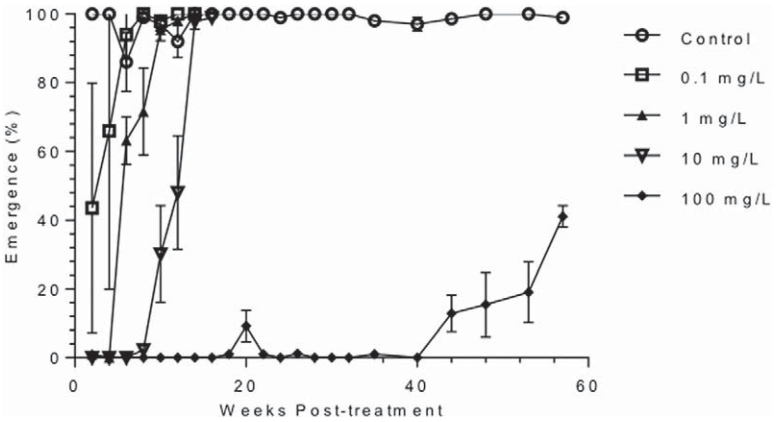

Fig. 1. Mean (+SE) pupal emergence of *Ae. aegypti* in 2-liter buckets treated with varying doses (in milligrams per liter) of Sumilarv 0.5 G. Trial runs from 11 April 2011 to 6 June 2012.

confound results, and to protect the buckets from direct sunlight that could degrade the active ingredient. The semifield cage was covered with 90% shade cloth awning that produced a total shade of 99%. However, rainfall could penetrate the shade cloth, and overflowing and water turnover occurred in the buckets but was not measured. Fortnightly, 20 third-instar *Ae. aegypti* (Cairns colony F3–10) were added to each bucket, with 0.5 g of Friskies cat food pellet (Nestle's Purina Petcare, Blayney, NSW, Australia) added for food when needed. Twice each week, pupae were collected by pipette and placed into 70-ml plastic jars containing tap water for emergence. For the highest dose, assessment periods were extended to 4 wk after 30-wk assessment. The trial was run for 60 wk or until control (emergence inhibition or EI) (The terms control and emergence inhibition may be used interchangeably) was <50% for a given treatment.

Meteorological data were obtained for the Cairns Airport site (10 km SE of the semifield cage) from the Australian Bureau of Meteorology. The buckets were exposed to heavy rainfall, with 2,596 ml of rainfall recorded at the Cairns Airport during the exposure period. The mean minimum and maximum temperature during the trial was 20.3 and 28.9°C, respectively.

Measurement of Pyriproxyfen in Treatment Buckets. Five 1-ml samples were extracted from each bucket weekly using a 1-ml pipette from each of the five buckets. The five samples for each treatment were pooled for subsequent analysis. Water samples were supplied to ACS Laboratories (Australia) (Kensington, Victoria) for analysis of pyriproxyfen content in August 2012. Each sample was diluted 1:1 with acetonitrile and then filtered through a 0.2-μm Teflon syringe filter into a 2-ml glass vial. Two microliters of the diluted sample was then injected onto a Waters BEH C18 column (1.7 μm, 50 by 2.1 mm) using a Waters Aquity UPLC-MS-MS (ultra-high-pressure liquid chromatography with tandem mass spectrometry detection). Pyriproxyfen was eluted with a mixture of acetonitrile and 5 mM trifluoroacetic acid (75:25) at 0.5 ml/min and quantified by external calibration. Limit of detection was 0.05 ppb.

Statistical Analysis. An analysis of variance (ANOVA) was used to compare mean adult emergence between the treatments. The lack of replication created by pooling the water samples prevented us from conducting a similar comparison on the pyriproxyfen detected in the buckets.

Results

Impact of Pyriproxyfen Against *Ae. aegypti* Under Semifield Conditions. A clear dose–response was noted in the efficacy of Sumilarv 0.5G against *Ae. aegypti.* The very lowest dose (0.1 mg/liter) provided ≈50% control for the initial week before rapidly tailing off. The 10-mg/liter dose gave good control for 8–10 wk. The highest dose (100 mg/liter) provided >95% control for 40 wk, with the exception of slightly higher emergence at 2 and 8 wk (88 and 94% EI, respectively). After 40 wk, control gradually declined to 60% at the conclusion of the trial. An ANOVA was conducted on mean EI for the initial 14 wk of treatment, after which only the high dose maintained any level of control (Fig. 1). There was a significant interaction between treatment group and weeks posttreatment ($F(24, 140) = 12.41; P < 0.0001$), as well as a significant effect of "treatment group" ($F(4, 140) = 269.1; P < 0.0001$) and a significant effect of "weeks posttreatment" ($F(6, 140) = 39.48; P < 0.0001$).

Detection of Pyriproxyfen in Treated Water. Levels of pyriproxyfen declined steadily in the treated buckets (Table 1) and was dose dependent. After wk 2, pyriproxyfen was only detected in the highest dose (100 mg/liter). No active ingredient was detected in the smallest dose (0.1 mg/liter) at any time, and concentrations in water for the 1- and 10-mg/liter doses fell below the limit of detection after 2 wk and 1 wk posttreatment, respectively. Pyriproxyfen was detected for 60 wk for the highest treatment dose of 100 mg /liter, but levels in the bucket water rapidly declined to <1 ppb by 12 wk (Fig. 1).

Table 1. Pyriproxyfen (in ppb) detected in 2-liter buckets treated with Sumilarv 0.5 G and held under semifield conditions

Dose	100 mg/liter	10 mg/liter	1 mg/liter	0.1 mg/liter
AI ppb	500	2.5	0.25	0.025
Week posttreatment				
1	7.1	0.62	0.07	0
2	2.17	0.12	0	0
4	3.69	0	0	0
6	6.14	0	0	0
8	2.56	0	0	0
10	1.69	0	0	0
12	0.88	0	0	0
14	0.58	0		
16	0.09			
18	0.48			
20	0.5			
22	0.05			
24	0.1			
26	0.08			
28	0.05			
30	0.05			
32	0.19			
35	0.19			
40	0.13			
44	0.06			
60	0.06			

Discussion

These data indicate that Sumilarv granules can provide persistent control of *Ae. aegypti* under simulated field conditions. Both control and detection of (AI) in water samples were strongly dose dependent. The minimum dose, 0.1 mg/liter (or the equivalent of a single Sumilarv granule/bucket), even provided brief control for 2 wk, with increasing levels of control and residuality for the higher doses. The highest dose (100 mg/liter) provided >85% control for the entire study and >95% for all but two sample periods (88 and 94% at wk 2 and 8, respectively). High doses of Sumilarv could be used to provide very long residual control of *Aedes* in typical small breeding sites such as water storage containers, tires, sump pits, and other small water reservoirs that might serve as breeding sites. We acknowledge that the exposure of treated buckets was conducted under semifield conditions that may not reflect conditions in the field, particularly exposure to ultraviolet light, detritus, and rainfall. Additional studies of pyriproxyfen persistence should be conducted under a range of field conditions.

The persistence of active pyriproxyfen within treated buckets shows that active ingredient is slowly released into the water. The initial highest dose (100 mg/liter) was sufficient to create a concentration of 500 ppb of pyriproxyfen. However, we detected a concentration of only 7.5 ppb at wk 1, just 1.4% of the total (AI) dose. This was consistent for the lower doses too, with residual pyriproxyfen detected in water at wk 1 representing just 1.2 and 1.4% of the total doses applied for the 50 and 5 ppb treatments, respectively. Webb et al. (2011) reported initial levels of pyriproxyfen in water of ≈7 ppb, after application of a liquid formulation at a nominal rate of just 7 ppb, and this declined to <1 ppb within 5–7 d. In liquid form, the pyriproxyfen is freely available in solution initially, whereas the granular application releases only small amounts of pyriproxyfen over a longer period, making it more suitable for long-term residual control. A limitation of the study is that a single pooled water sample was used for analysis, effectively eliminating any replication.

Temporary control in breeding sites, even using very low doses for short periods, could be used to reduce wild populations of *Ae. aegypti* to enhance establishment of *Wolbachia* in open-field releases (Hancock et al. 2011, Hoffmann et al. 2011). Field workers have used bleach to provide short-term control of *Ae. aegypti* in breeding sites as part of a "crash and release" strategy (Jacups et al. 2013). However, the auto-dissemination of pyriproxyfen, either as a dust or dissolved in water, could be used to treat cryptic and inaccessible breeding sites such as septic tanks and roof gutters. Contaminated mosquitoes could be used to transfer effective doses of pyriproxyfen to such sites (Devine et al. 2009, Caputo et al. 2012, Gaugler et al. 2012). Devine et al. (2009) demonstrated that adult *Ae. aegypti* could be captured in traps where they pick up minute dust particles containing pyriproxyfen that are then disseminated to breeding sites by the mosquito. The dose data from Table 1 could also be used to estimate longevity of control based on the dose of pyriproxyfen and amount of (AI) in the water. This could be useful for estimating the timing of auto-dissemination treatment and release of *Wolbachia*-infected mosquitoes to avoid killing offspring of released mosquitoes.

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