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TRUCK-MOUNTED AREA-WIDE APPLICATION OF PYRIPROXYFEN TARGETING *AEDES AEGYPTI* AND *AEDES ALBOPICTUS* IN NORTHEAST FLORIDA[1]

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ABSTRACT. This study was conducted to determine the efficacy of truck-mounted ultra-low volume applications of pyriproxyfen against *Aedes aegypti* larvae in artificial water containers and wild adult *Ae. albopictus* populations in an urban setting. The study was conducted over a 3.5-month period (June–October 2012), during which 3 pyriproxyfen applications were conducted. Beginning 6 wk prior to the 1st pyriproxyfen spray, 10 Biogents-Sentinel® traps were used each week to survey the adult *Ae. albopictus* population at each experimental plot through the end of the study. The treatment and control plots contained specimen cups, each containing 10 laboratory-reared *Ae. aegypti* larvae, placed at 8, 15, and 23 m from the spray line. Emergence inhibition (EI) of 82% or greater was observed among *Ae. aegypti* larvae exposed to the 3 pyriproxyfen sprays. The EI of these same *Ae. aegypti* larvae at the 3 distances from the spray ranged from 84% to 92% and were not significantly different. Laboratory analysis of water samples taken from the larval cups independently confirmed the presence of pyriproxyfen. Similar levels of EI were achieved in *Ae. aegypti* and *Ae. albopictus* larvae when the measured field concentrations of pyriproxyfen were recreated in laboratory assays. Trap captures of wild adult *Ae. albopictus* were not markedly reduced following the 1st pyriproxyfen spray, perhaps due to heavy rainfall at the time and the lower rate of pyriproxyfen applied. Within 2 wk following Spray 2, however, *Ae. albopictus* collections from the treatment plot averaged approximately 50% of those from the control plot, and the reduction trend continued following Spray 3.

KEY WORDS Pyriproxyfen, area-wide treatment, larval control, *Aedes aegypti*, *Aedes albopictus*

INTRODUCTION

Aedes albopictus (Skuse) (Asian tiger mosquito) and *Ae. aegypti* (L.) (yellow fever mosquito) are important nuisance and disease vector species. These mosquitoes are peridomestic container breeders, daytime biters, anthropophilic, and capable of transmitting dengue and chikungunya viruses through infective bites (Sucharit and Surathin 1994, Mitchell 1995). These species are usually difficult to control, although there is extensive literature on dengue vector control methods and strategies (WHO 2012, Stoops et al. 2014). Mosquito control districts typically mount aggressive control campaigns against salt marsh, floodwater, and other rural-based mosquito pest species by implementing conventional mosquito abatement methods that focus on treating or eliminating larval habitats. These approaches are most efficient when larval sources are large and easily accessible; however, targeting *Ae. aegypti* and *Ae. albopictus* larvae is challenging because the larval habitats tend to be small and cryptic, plentiful, and located in residential areas where human–mosquito contact is common (Hawley 1988). The highly urban peridomestic nature of these species, where responsibility for control rests largely with private citizens, has hampered the control efforts of mosquito control districts/public health agencies.

Pyriproxyfen, a juvenile hormone mimic, functions as an insect growth regulator (IGR) preventing normal development of larvae into adults and is effective against a number of mosquito species, including *Ae. aegypti* and *Ae. albopictus* (Ali et al. 1995, Nayar et al. 2002, Sihuincha et al. 2005, Invest and Lucas 2008, Seng et al. 2008). Studies to date have been limited to either laboratory applications or direct container treatment of pyriproxyfen in the field to control larvae.

Preliminary studies in northeast Florida by the US Navy Entomology Center of Excellence (Jacksonville, FL) and the Anastasia Mosquito Control District (AMCD; St. Johns County, FL) indicated that truck-mounted aerosol applications of pyriproxyfen from ultra-low volume (ULV) dispersal equipment might be effective as an area-wide control method of container-breeding *Aedes* mosquitoes. The current study was meant

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14. ABSTRACT

This study was conducted to determine the efficacy of truck-mounted ultra-low volume applications of pyriproxyfen against Aedes aegypti larvae in artificial water containers and wild adult Ae. albopictus populations in an urban setting. The study was conducted over a 3.5-month period (June???October 2012), during which 3 pyriproxyfen applications were conducted. Beginning 6 wk prior to the 1st pyriproxyfen spray, 10 Biogents-SentinelH traps were used each week to survey the adult Ae. albopictus population at each experimental plot through the end of the study. The treatment and control plots contained specimen cups each containing 10 laboratory-reared Ae. aegypti larvae, placed at 8, 15, and 23 m from the spray line. Emergence inhibition (EI) of 82% or greater was observed among Ae. aegypti larvae exposed to the 3 pyriproxyfen sprays. The EI of these same Ae. aegypti larvae at the 3 distances from the spray ranged from 84% to 92% and were not significantly different. Laboratory analysis of water samples taken from the larval cups independently confirmed the presence of pyriproxyfen. Similar levels of EI were achieved in Ae. aegypti and Ae. albopictus larvae when the measured field concentrations of pyriproxyfen were recreated in laboratory assays. Trap captures of wild adult Ae. albopictus were not markedly reduced following the 1st pyriproxyfen spray, perhaps due to heavy rainfall at the time and the lower rate of pyriproxyfen applied. Within 2 wk following Spray 2, however, Ae. albopictus collections from the treatment plot averaged approximately 50%of those from the control plot, and the reduction trend continued following Spray 3.

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to further evaluate the efficacy of pyriproxyfen delivered by truck-mounted ULV sprayer to control peridomestic, container-breeding mosquitoes using laboratory-reared sentinel *Ae. aegypti* larvae and wild adult populations of *Ae. aegypti* and *Ae. albopictus* in St. Augustine, FL.

MATERIALS AND METHODS

Pyriproxyfen and experimental use permit

Nyguard® IGR EC (10% pyriproxyfen), provided by MGK Chemical Co. (Minneapolis, MN), was used for all experiments. The current Nyguard label prohibits area-wide truck-mounted application. Therefore, an experimental use permit (EUP) was obtained (FL12-EUP-02) from the Florida Department of Agriculture and Consumer Services (effective July 5–December 31, 2012) that allowed off-label application for this study.

Study site and accompanying insecticide applications

Two residential areas in St. Augustine, FL, were selected for treatment and control plots. The area of each plot was 40 ha (100 acres) and consisted primarily of 50-year-old single-family homes located on 0.2-ha (0.5 acre) lots. Ten of these residential lots in each plot were identified as locations for placement of sentinel larvae.

Laboratory mosquitoes

Laboratory-reared *Ae. aegypti* (Orlando Strain, in colony since 1952) and *Ae. albopictus* (Gainesville Strain, in colony since 2009) were reared from eggs provided by the US Department of Agriculture–Agricultural Research Service Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, FL. Eggs were hatched by flooding egg papers, and the larvae were held in enamel trays with 1 liter of deionized water. Larvae were provided a diet of 30 mg of dried yeast and maintained in an incubator set at 26°C, 75% RH, and 16:8 light:dark h photoperiod.

Field evaluation

Three truck-mounted ULV applications (August 7, August 29, and September 22, 2012) of pyriproxyfen were performed on the treatment plot. Sprays were conducted between 8:00 and 10:00 a.m. using a Clarke Cougar® ULV truck-mounted sprayer (Clarke, Roselle, IL). The application rate for Spray 1 (August 7) was 164 ml/ha (2.25 oz/acre), and the application rate for both Spray 2 (August 29) and Spray 3 (September 25) was 329 ml/ha (4.5 oz/acre). These application rates translate to 21% of the maximum label rate (based on small-area applications) during Spray 1 and 42% for Sprays 2 and 3. These rates were established based on the total amount of Nyguard available to apply over 3 applications. A 91-m (300 ft) swath width was assumed, and the vehicle speed ranged from 5–13 km/h (3–8 mi/h). Thirty black 450-ml oviposition containers attached to wooden stakes were placed at 8, 15, and 23 m from the spray truck route at the 10 locations per plot before the application (i.e., $n = 10$ containers for each of the 3 distances). Each oviposition container held a sentinel cup, which consisted of a 125-ml specimen cup containing 100 ml of distilled water with 10 3rd to 4th instars of colony-reared *Ae. aegypti*. Thirty oviposition containers with sentinel cups with larvae were placed in a similar manner at the 10 locations in the untreated control area, located approximately 1 km away from the treatment plot. The oviposition containers were left in place throughout the study period to ensure that sentinel cups were placed in the same location for all sprays.

Following each of the 3 pyriproxyfen sprays, the 60 specimen cups containing colony-reared larvae were returned to the laboratory to monitor mortality and/or adult emergence. Any one cup was only exposed to a single spray. Cups were held at 25.5°C and 70% RH. BG-Sentinel® traps (Biogents AG, Regensburg, Germany) were used weekly at the 10 locations in the treatment plot and at the 10 locations in the control plot to monitor adult *Ae. aegypti* and *Ae. albopictus* populations prior to and following each application of pyriproxyfen.

Precipitation data were collected for the period June 27 to October 10, 2012, from the National Oceanic and Atmospheric Administration weather station (92814/SGJ) located at the local airport, approximately 5 km from the study site.

Pyriproxyfen concentration

Following the Spray 2 treatment, 10-ml samples were obtained from each of the 60 sentinel cups and submitted to Golden Pacific Laboratories (Fresno, CA) for pyriproxyfen analysis. Samples were frozen and stored in glass vials prior to analysis. Samples were thawed, vigorously mixed, and an aliquot was removed and combined with an equal volume of acetonitrile. The diluted samples were filtered through a 0.45-μm polytetrafluoroethylene syringe filter. Filtered samples were further diluted, if necessary, with 1:1 water:acetonitrile and analyzed by liquid chromatography–tandem mass spectrometry (LC-MS/MS) without further preparation. Analysis was conducted using reverse-phase high-performance liquid chromatography with detection by an AB Sciex (Framingham, MA) API 5000 mass spectrometer monitoring both primary and confirmatory ion pairs. Each set of samples contained a

negative control water sample, as well as positive control water samples fortified with pyriproxyfen at 0.002 ng/ml and at 1 ng/ml. Calibration standards prepared in 1:1 acetonitrile:water at concentrations ranging from 0.0005 ng/ml to 0.02 ng/ml were used to establish response versus concentration for each analytical set. Recoveries from fortified control samples were 92–106%. Five samples were submitted from sentinel cups from the control plot and were all negative for detectable pyriproxyfen residue.

Laboratory assay

The objective of this study was to compare the toxicity of pyriproxyfen on laboratory-reared 3rd- and 4th-stage *Ae. albopictus* larvae in addition to the *Ae. aegypti* larvae. However, there were not sufficient resources available to expose *Ae. albopictus* larvae in the field alongside the *Ae. aegypti* larvae, so a proxy study was conducted in the lab. Results from the LC-MS/MS analysis of Spray 2 at Golden Pacific Laboratories were used to calculate mean pyriproxyfen concentrations for 8-, 15-, and 23-m distances from the spray line. These mean concentrations were re-created in 125-ml specimen cups in the laboratory to compare emergence results for colony-reared *Ae. aegypti* and *Ae. albopictus* larvae in laboratory-mixed pyriproxyfen to emergence results for colony-reared *Ae. aegypti* larvae in field-collected pyriproxyfen. Ten cups of each species had concentrations that correlated to the mean pesticide exposure for 8, 15, and 23 m (0.01, 0.0055, and 0.0014 ppm pyriproxyfen, respectively) from Spray 2, for a total of 30 cups for each species. Ten cups of each species also served as controls with no pyriproxyfen added. Each cup contained 10 3rd to 4th instars of colony-reared *Ae. aegypti* or *Ae. albopictus* as appropriate. Emergence for each assay cup was monitored every 24 h until all larvae/pupae had died or emerged as adults.

Emergence inhibition

The effect of pyriproxyfen on the mosquito larvae/pupae is presented as emergence inhibition (EI). Total emergence was calculated as the sum of mosquitoes that emerged as adults; percent emergence was calculated as the ratio of total emergence to the number of larvae added; percent mortality was calculated as the ratio of total dead (= dead larvae + pupae) to the number of larvae added. For each mosquito species, the average percent emergence (APE) was calculated for the control and the 3 treatments by summing the individual percent emergences (PEs) over the 10 cups and dividing by 10:

$$\mathrm{APE} = \left(\mathrm{PE}_{\mathrm{cup1}} + \mathrm{PE}_{\mathrm{cup2}} + \cdots + \mathrm{PE}_{\mathrm{cup10}}\right)/10$$

Then, for each species, the APE for each treatment was divided by the APE for the species-specific control, and this ratio was subtracted from unity to obtain the EI for each treatment by species:

$$\mathrm{EI}(\mathrm{Species, Treatment}) = 1 - (\mathrm{APE}(\mathrm{Treatment})/\mathrm{APE}(\mathrm{Control}))$$

Finally, the corrected percent EI for each treatment was calculated as 1 minus the ratio of emergence for the given treatment to emergence of the control (Abbott 1925).

Statistical methodology

To assess EI significant differences among the 3 sprays, the 3 distances (8, 15, and 23 m from the spray line) for *Ae. aegypti*, between the 2 species (*Ae. aegypti* and *Ae. albopictus*) in the lab assay, and between the Spray 2 field assay and lab assay for *Ae. aegypti*, a series of nonparametric Kruskal–Wallis (K-W) hypothesis tests (Zar 1999) were conducted at the 95% confidence level ($\alpha = 0.05$): 1) 1-way analysis of field data: $N = 3$ sprays, $n = 3$ distances; 2) 1-way analysis of field data: $N = 3$ distances, $n = 2$ sprays (Sprays 2 and 3 only); 3) 2 1-way analyses of lab bioassay data: a) $N = 2$ species, $n = 3$ distances; b) $N = 3$ distances, $n = 2$ species. The K-W test was determined to be the most appropriate after a series of goodness-of-fit tests indicated that the data exhibit a nonnormal behavior.

To assess for significant differences in spray concentration of pyriproxyfen (ng/ml) among the 3 distances from the truck line (8, 15, and 23 m) in Spray 2, a 1-way nonparametric K-W hypothesis test (Zar 1999) was conducted at the 95% confidence level ($\alpha = 0.05$, $N = 3$, $n = 10$).

RESULTS

Emergence inhibition

The combined EI (averaged across all distance measurements) against *Ae. aegypti* larvae placed in sentinel cups to collect pyriproxyfen in the field for Sprays 1, 2, and 3 were 82%, 87%, and 87%, respectively (Table 1), and were not significantly different. The combined EI of *Ae. aegypti* that were in sentinel cups in the field for Sprays 2 and 3 for the 3 distances (8, 15, and 23 m) from the spray route were 92%, 85%, and 84%, respectively (Table 2), and not significantly different. Data were combined for Sprays 2 and 3 in Table 2 because the application rates were the same.

Pyriproxyfen concentrations at 8, 15, and 23 m following Spray 2

Samples obtained from the larval cups from Spray 2 were analyzed and actual pyriproxyfen

Table 1. Emergence inhibition (EI) of *Aedes aegypti* larvae/pupae exposed in sentinel cups to 3 truck-mounted ultra-low volume spray applications of pyriproxyfen. Sentinel cups were retrieved from the field immediately after each spray, and mortality and adult emergence were measured under laboratory conditions to derive EI.

Spray[1]	EI ± SEM (%/100)
1	0.816 ± 0.068[2]
2	0.870 ± 0.021[2]
3	0.874 ± 0.046[2]

[1] Spray 1: 164 ml/ha; Sprays 2 and 3: 329 ml/ha.
[2] Nonparametric 1-way Kruskal–Wallis test ($\alpha = 0.05$): no significant difference ($n = 3$, df = 2, $\chi^2 = 0.2667$, $\chi^2_{crit} = 5.9910$, $P = 0.8771$).

concentrations at 8-, 15-, and 23-m distance from the application were 11.8, 5.5, and 1.4 ng/ml, respectively (Table 3), and were not significantly different.

Adult *Ae. albopictus* and *Ae. aegypti* sampling

Adult populations of *Ae. albopictus* and *Ae. aegypti* were sampled weekly at the control and treatment plots using BG-Sentinel traps; however, due to the low numbers of *Ae. aegypti* captured, only *Ae. albopictus* data are presented. Captures of *Ae. albopictus* on July 3 were the highest of the study at approximately 30–35 mosquitoes/trap from the 2 locations (Fig. 1). A reduction in the number of mosquitoes collected during the following 5 wk was observed culminating in a season low of approximately 3 mosquitoes/trap on August 9. One week following Spray 1, the numbers of mosquitoes captured from both plots rose to a mean of 18–23 mosquitoes/trap among the treatment and control plots, respectively. Two weeks following Spray 2 and through the remainder of the study, mosquitoes collected from the treatment plot were approximately half that of the control plot, both being relatively low (<5 mosquitoes/trap) from the 2 locations (Fig. 1). Precipitation peaked between July 31 and August 9 at 12 cm of accumulation (Fig. 1). A smaller rainfall peak of 7 cm occurred from August 22 to September 5.

Table 2. Emergence inhibition (EI) of *Aedes aegypti* larvae/pupae exposed in sentinel cups at 8, 15, and 23 m to truck-mounted ultra-low volume spray applications of pyriproxyfen, combined across Sprays 2 and 3. Sentinel cups were retrieved from the field immediately after each spray and mortality and adult emergence were measured under laboratory conditions to derive EI.

Distance from sprayer (m)[1]	EI ± SEM (%/100)
8	0.922 ± 0.032[2]
15	0.851 ± 0.024[2]
23	0.843 ± 0.049[2]

[1] $n = 20$ at 8 and 15 m; $n = 18$ at 23 m.
[2] Nonparametric 1-way Kruskal–Wallis test ($\alpha = 0.05$): no significant difference ($n = 2$, df = 2, $\chi^2 = 2.0000$, $\chi^2_{crit} = 5.9910$, $P = 0.3893$).

Table 3. Mean concentrations of pyriproxyfen sampled from sentinel larvae cups during Spray 2 at 8, 15, and 23 m from truck-mounted ultra-low volume sprayer. Sentinel cups were retrieved from the field immediately after the spray and maintained under laboratory conditions.

Distance from sprayer (m)	Concentration ± SEM (ng/ml)
8	11.80 ± 8.51[1]
15	5.49 ± 3.95[1]
23	1.40 ± 1.14[1]

[1] Nonparametric 1-way Kruskal–Wallis test ($\alpha = 0.05$): no significant difference ($n = 10$, df = 2, $\chi^2 = 0.0959$, $\chi^2_{crit} = 5.9910$, $P = 0.9534$).

Laboratory assay of EI for *Ae. aegypti* and *Ae. albopictus*

The 3 mean concentrations of pyriproxyfen from each of the 3 distances from the spray route determined in the Golden Pacific Laboratories analysis from Spray 2 were re-created in laboratory assay cups to compare emergence results to the field experiment data. There was no significant difference between the EI of *Ae. aegypti* exposed in the field during Spray 2 and the EI of *Ae. aegypti* exposed in the lab assay (data not shown). Furthermore, there was no significant difference between the EI among *Ae. aegypti* and *Ae. albopictus* exposed in the lab assay (Table 4).

DISCUSSION

Pyriproxyfen has been demonstrated to be an effective larvicide when applied on a small scale in laboratory or field settings (Nayar et al. 2002, Sihuincha et al. 2005, Invest and Lucas 2008, Seng et al. 2008). However, this is the first study to demonstrate the feasibility and efficacy of a truck-mounted ULV application of the chemical at the scale of a city neighborhood. The data presented in this study show that pyriproxyfen was successfully deposited into sentinel cups up to 23 m from the ULV spray, and show that the concentrations of deposited pyriproxyfen were sufficient to produce mean EI of at least 82% in sentinel colony-reared *Ae. aegypti* larvae. These findings provide evidence that area-wide application of pyriproxyfen could be effective against *Ae. aegypti* larvae.

The high variability of pyriproxyfen concentration among the sentinel larval cups at all distances suggests that, regardless of distance from the spray line, other environmental factors, such as wind turbulence and drift, may have affected the pyriproxyfen settling in larval containers. However, even the smallest mean pyriproxyfen concentration among the distances

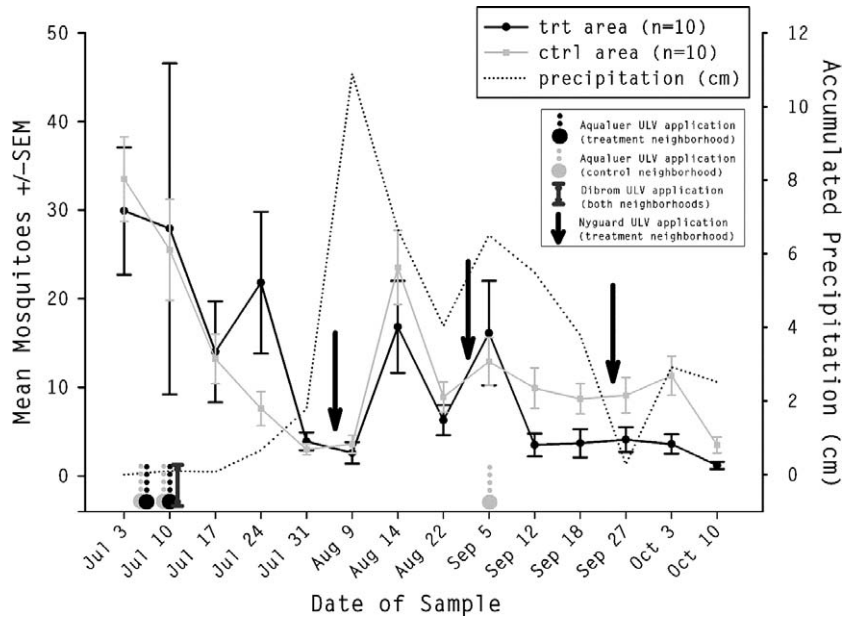

Fig. 1. Adult female *Aedes albopictus* captured with BG-Sentinel® traps and accumulated rainfall from June 27 to October 10, 2012. Arrows denote pyriproxyfen applications on August 7 (164 ml/ha), August 29 (329 ml/ha), and September 25 (329 ml/ha). ULV, ultra-low volume.

from the spray line was sufficient to produce substantial mortality in sentinel larvae. This finding is concordant with prior research on pyriproxyfen and its efficacy at low concentrations (Ali et al. 1995, Nayar et al. 2002, Sihuincha et al. 2005, Seng et al. 2008, Webb et al. 2012).

Scott et al. (2013) conducted a parallel study in these same plots assessing pyriproxyfen deposition and effect. They exposed *Ae. albopictus* larvae to containers that had been placed in the treatment plot prior to the pyriproxyfen applications (direct exposure). Additionally, they sampled vegetation in the study area following the pyriproxyfen applications, which was washed and the rinsates collected into containers to which *Ae. albopictus* were added (indirect exposure). The EI of *Ae. albopictus* from both direct and indirect exposures averaged from 77–100% for each of the 3 pyriproxyfen applications (Scott et al. 2013). These results provide an independent measure of the efficacy of the truck-mounted ULV pyriproxyfen applications and are consistent with our results.

Since the larvae subjected to the pyriproxyfen sprays (*Ae. aegypti*) and those tracked as adults (*Ae. albopictus*) were similar, but distinct species, a laboratory assay was conducted to compare the response of larvae of both species to concentrations of pyriproxyfen derived from an analysis of field-collected pyriproxyfen concentrations from Spray 2 (Table 3). The results from this laboratory assay were compared with the results of the field assay from Spray 2 and no significant difference in EI was observed between the 2 species in the laboratory assay or between the laboratory and field assay for *Ae. aegypti* (Table 4). These findings suggest that a truck-mounted application of pyriproxyfen could be

Table 4. Laboratory assay of emergence inhibition (EI) of *Aedes aegypti* and *Ae. albopictus* larvae/pupae equivalent to the 3 mean pyriproxyfen concentrations at the 3 sampled distances (8, 15, 23 m) from sprayer during Spray 2.

	EI (%/100)			
Species/concentration	11.8 ng/ml	5.5 ng/ml	1.4 ng/ml	Mean ± SEM
Aedes aegypti	1.000	0.979	0.814	0.9313[1] ± 0.059[1]
Ae. albopictus	1.000	1.000	0.939	0.9797[1] ± 0.020[1]
Mean ± SEM	1.000 ± 0.000[1]	0.990[1] ± 0.010[1]	0.877 ± 0.062[1]	

[1] Nonparametric 1-way Kruskal–Wallis tests ($\alpha = 0.05$): Among 3 distances ($N = 3$, $n = 2$): no significant difference (df = 2, $\chi^2 = 4.1935$, $\chi^2_{crit} = 5.9910$, $P = 0.1337$); between 2 species ($N = 2$, $n = 3$): no significant difference (df = 1, $\chi^2 = 0.4839$, $\chi^2_{crit} = 3.8410$, $P = 0.4917$).

just as effective against *Ae. albopictus* larvae in the field as was observed against sentinel *Ae. aegypti* larvae.

The results indicate that the ULV sprays were effective at delivering a lethal dose of pyriproxyfen into the 125-ml sentinel larvae cups. What is unknown is how well pyriproxyfen would have been delivered beyond 23 m and into cryptic peridomestic or natural containers with wild populations of *Ae. aegypti* and *Ae. albopictus* larvae. For an indication of the impact of the spray treatments on wild populations, data of adult *Ae. albopictus* trapping conducted during the study using BG Sentinel traps will be considered.

Prior to this study, Tropical Storm Debby (June 24–26, 2012) deposited 21 cm of rainfall on the St. Augustine area. This weather event correlated with a seasonal population peak of *Ae. aegypti* and *Ae. albopictus* in the region and contributed to a significant mosquito problem for the AMCD in late June–early July. In response, a number of adulticide applications were made in the city's residential areas, which included the study treatment and control plots. Aqualuer® 20-20 (permethrin, PBO; All Pro® Vector Group, Bloomington, MN) was applied twice with a truck-mounted ULV sprayer in early July to both the treatment plots at the label rate of 7.8 g AI/ha (0.007oz/acre) on July 6 and 10, 2012, and to the control plot on July 5, July 9, and September 5, 2012. Additionally, an aerial application of naled (Dibrom® Concentrate; AMVAC, Los Angeles, CA) was applied to both plots on July 11, 2012, at the rate of 42 g AI/ha (0.6 oz/acre). The pyriproxyfen applications, which occurred in August and September, must be analyzed in the context of the greater mosquito control efforts and distinctive weather events preceding the study.

Based on monitoring of the adult *Ae. albopictus* population, control efforts carried out by AMCD in early July were successful in substantially reducing the number of the adult mosquitoes, as indicated by trapping results (Fig. 1). The 1st pyriproxyfen application occurred following this population reduction, which could be considered a method to sustain adult suppression through the use of an IGR following adulticide applications.

A control delay can be expected from the date of pyriproxyfen application until an effect would be observed in the adult *Ae. albopictus* population, due to a reduction in the number of larvae developing into adults. However, an increased number of adult *Ae. albopictus* were collected from both control and treatment plots approximately 1 wk following Spray 1. At this time, the *Ae. albopictus* population was on the rebound and may have been beyond the capabilities of the lower concentration of pyriproxyfen in Spray 1 (164 ml/ha) compared to the higher concentrations used in Sprays 2 and 3 (329 ml/ha). Spray 1 was used to refine the protocol to include establishing parameters that most effectively measure impact. Sprays 2 and 3 are most comparable and involved highest application rate.

Rainfall precipitation was recorded during the study. The greatest accumulated rainfall (12 cm) occurred from July 31 to August 9, which included the 1st application period (Fig. 1). The effect of precipitation on pyriproxyfen efficacy is unknown. This considerable accumulation of rain may have flooded the various containers and rinsed resting locations utilized by *Aedes* species and diluted the pyriproxyfen available to affect larvae. This could explain the apparent lack of efficacy on adult populations despite the EI achieved with low pyriproxyfen concentrations in the laboratory-reared *Ae. aegypti* larvae placed out prior to Spray 1 and collected up before the rains (Table 1). Precipitation was recorded following Sprays 2 and 3; however, the accumulated levels were less than half of those following Spray 1.

When considering the potential impact of Sprays 2 and 3, the largest difference in the number of adults collected with BG Sentinel traps from control and treatment plots, i.e., the strongest indication of the efficacy of the pyriproxyfen application against wild populations of *Ae. albopictus*, occurred approximately 2 wk following Spray 2 (Fig. 1). This is consistent with what might be expected if pyriproxyfen reduced the numbers of adults emerging in that area. Furthermore, an area-wide Aqualuer 20-20 application was conducted by AMCD on September 9, 2012, to the control plot, while no adulticide application was made to the treatment plot. This treatment may have reduced the number of adult *Ae. albopictus* collected from the control plot. Therefore, it may be that there would have been an even greater difference in the number of adults captured between the 2 plots had efforts to reduce the adults in the control plot by AMCD not been carried out. From September 12 to October 3, the number of adult *Ae. albopictus* collected from the treatment plot was approximately half of those from the control. No marked increase or decrease of adult *Ae. albopictus* was observed during either Week 1 or 2 following Spray 3; however, by the final collection in October the overall *Ae. albopictus* population numbers appeared to be declining for the season.

Results obtained during this study indicate urban area-wide pyriproxyfen application via truck-mounted ULV equipment is efficacious to an EI of $\geq$82% out to at least 23 m from the sprayer against colony-reared *Ae. aegypti* larvae and, by inference, *Ae. albopictus* larvae. Presently, the labeling of pyriproxyfen (Nyguard) does not provide for area-wide treatment against mosquito larvae, but the positive results from this study indicate that more field trials could be carried out to accumulate evidence to update this labeling. Future area-wide trials could: improve timing

to isolate effects of pyriproxyfen from effects of adulticiding, for instance by conducting trials when container-inhabiting mosquito populations are lower and local mosquito control measures are less likely to occur, or by conducting trials in urban areas that lack mosquito control; investigate nontarget effects of pyriproxyfen; improve estimates of effects on local adult populations with more intensive trapping in fewer locations; include a long-term field method that leaves sentinel cups in place in the field with colony or locally acquired larvae to look at the stability of the treatment under field conditions; or include long-term tracking of pyriproxyfen concentrations and larval survival in nonexperimental containers that are organic to the neighborhoods used in the study at various distances from the spray line.

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