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Short communication

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Evaluation of Lambda-Cyhalothrin and Pyriproxyfen Barrier Treatments for *Aedes albopictus* (Diptera: Culicidae) Management in Urbanized Areas of New Jersey

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Abstract

Mosquito control programs in the United States are still searching for best management practices to control the Asian tiger mosquito, *Aedes albopictus* (Skuse; Diptera: Culicidae). Most intervention methods for this species are either labor intensive (e.g., source reduction) or short-term (e.g., ultra-low-volume adulticiding). We investigated the effectiveness of barrier spray pesticide applications within urban and suburban residential yards in New Jersey as a control strategy using a before-after-control-impact (BACI) approach. Applications of Demand CSR pyrethroid (9.7% AI lambda-cyhalothrin) only or combined Demand CSR and Archer IGR insect growth regulator (1.3% AI pyriproxyfen) applications resulted in significant and similar decreases in adult mosquito abundance post-treatment ranging from 78 to 74% respectively, compared with the untreated control. Both insecticides exceeded the 70% reduction threshold considered as effective for *Ae. albopictus* control for 2 to 4 wk. However, applications of Archer IGR alone did not reduce adult mosquito abundance. The field study results were supported by laboratory no-choice bioassays using treated leaf foliage. Our study is the first data driven evidence of the residual efficacy of barrier pesticide applications in New Jersey with lambda-cyhalothrin that provided significant reductions in adult *Ae. albopictus* populations for an extended duration.

Key words: *Aedes albopictus*, barrier spray, lambda-cyhalothrin, pyriproxyfen

Since its establishment in New Jersey in 1995, *Aedes albopictus* (Skuse; Diptera: Culicidae) has become a major biting species driving most residential mosquito complaints and service requests in August and September (Unlu et al. 2016). In urban areas, this species' immature stages are found in artificial containers such as tires, buckets, bird baths, plant saucers, trash, and recycle bins (Richards et al. 2008, Unlu et al. 2013). Conventional mosquito control methods developed for salt marsh and floodwater mosquitoes are not effective for container inhabiting species (Rochlin et al. 2013). Novel techniques are needed to manage *Ae. albopictus* and *Aedes aegypti* (L.; Diptera: Culicidae) populations in the face of increasing risk of transmission of arboviruses including dengue, yellow fever, and Zika (Cardoso et al. 2015). Mosquito control programs in the United States are still searching for best management practices to control *Ae. albopictus*. Conventional intervention methods are either labor and time intensive, such as source reduction (YiBin et al. 2009, Fonseca et al. 2013), or achieve only short-term control using ultra-low volume (ULV) adulticiding (Farajollahi et al. 2012). A combination of several control methods is usually required to lower adult *Ae. albopictus* populations effectively (Abramides et al. 2011).

Barrier treatment is one of the tools that can be used to reduce adult mosquito abundance, and in some cases, may lower the risk of mosquito-borne pathogen transmission (Trout et al. 2007, Britch et al. 2009). Despite widespread use of barrier treatments by the private pest control industry (Trout et al. 2007), the number of peer-reviewed studies on the efficacy of this technique for *Ae. albopictus* control under field conditions is limited. Lambda-cyhalothrin and bifenthrin barrier treatments were effective against *Ae. albopictus* for periods ranging from 5 to 9 wk (Trout et al. 2007, Doyle et al. 2009, Li et al. 2010, Muzari et al. 2017). Other commonly used pyrethroids such as permethrin, deltamethrin, cyfluthrin, and tau-fluvalinate were effective under semi-field conditions (Cilek and Hallmon 2006, 2008).

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Area-wide larviciding with liquid formulations of insect growth regulators (IGRs) is another *Ae. albopictus* and *Ae. aegypti* control option under active investigation (Doud et al. 2014, Williams et al. 2014). Pioneering studies using ULV truck-mounted applications demonstrated significant reductions in container-inhabiting *Aedes* species populations with only pyriproxyfen (Doud et al. 2014) or in combination with an adulticide (Lucia et al. 2009). Moreover, the addition of pyriproxyfen increased the duration of suppression for 5 wk (Lucia et al. 2009).

The goal of our study was to assess the efficacy of a commonly used barrier treatment product containing lambda-cyhalothrin (Demand CS, Syngenta Crop Protection, Inc., Greensboro, NC) against *Ae. albopictus* adult populations in the northernmost part of this species range. Additional objectives were to evaluate of whether 1) the concurrent application of pyriproxyfen (Archer IGR, Syngenta Crop Protection) enhances the duration of lambda-cyhalothrin barrier treatment effect by preventing adult mosquito emergence, and 2) pyriproxyfen has potential as a stand-alone product for *Ae. albopictus* population suppression.

Material and Methods

Study Location, Design, and Mosquito Surveillance

This study was conducted in Mercer and Hudson counties, New Jersey, in primarily residential neighborhoods with single-family houses located on properties ranging in size from approximately 200–500 sq. meters. The study areas experience high *Ae. albopictus* populations (exceeding the threshold value of five adult mosquitoes per Biogents Sentinel [(BGS] trap per night) providing numerous opportunities for private pest control services (Unlu et al. 2016). For pesticide efficacy testing, a nested before-after-control-impact study design (Stewart-Oaten et al. 1986) was employed, whereby 20 residential properties were selected in each county and randomly allocated to either the treatment group (Demand CS, Archer IGR, or both) or the untreated control group for a total of five properties per group. The distance between the properties ranged from (mean [min–max]) approximately 0.2 km [0.04–0.5] in Hudson County to 2.6 km [0.1–6.6] in Mercer County.

For adult mosquito monitoring, one BGS trap baited with BG lure (Biogents AG, Regensburg, Germany) (Unlu et al. 2017b) was placed in a shaded area on each residential property (Crepeau et al. 2013). The traps remained in the same location each week to reduce variation from trap location (Crepeau et al. 2013). Adult mosquito sampling of 24-h duration was performed twice weekly starting 1 (Hudson) to 3 (Mercer) wk prior to treatments in late July to early August and continuing for 9 wk following the treatment until the end of September.

Pesticide Barrier Treatments and Cone Bioassays

During the first week of August, a single application was performed, treating residential properties with Demand CS (6.34 ml/liter), Archer IGR (7.40 ml/liter), or both according to the label. A backpack mist blower (model SR-450, Stihl Corp, Virginia Beach, VA) was used for all applications to treat vegetation below 3 m, leaf litter and resting habitats (e.g., under the porches and alcoves). For thick foliage, such as hedges and ivy, a hand tank with mist blower tip was inserted into the foliage to cover inner areas of dense vegetation (Trout et al. 2007). In the pyriproxyfen treatment, visible containers holding water, such as buckets and tires, were treated with 0.04 liter of Archer IGR at a rate of 1.2 liter/min.

For cone bioassays, we collected eight treated leaves (four from front and four from back of the properties) from plants in each residential property in the Demand CS, Demand CS + Archer IGR, and untreated control groups. Samples were collected 1, 2, 4, 6, and 9 wk post application. No leaf samples were collected from the Archer IGR treated group because no direct adult toxicity was expected. No-choice bioassays (WHO 2013) were conducted with insecticide-susceptible 2–5 d old nonblood fed *Ae. albopictus* from a colony established from eggs collected in Trenton and Keyport, New Jersey, in 2009. The eight leaves from each site were divided into four WHO cone bioassays. Leaves were positioned so that mosquitoes had access to both sides of the leaves. Using an aspirator, five female mosquitoes were introduced into each plastic cone with treated leaves and left for 3 min. Three replicates were performed for each sample resulting in 60 female mosquitoes (4 cones × 5 mosquitoes × 3 replicates) per treatment site per collection. After exposure, the mosquitoes were placed in 150-ml plastic cups, with sugar solution, and maintained in a climatic chamber at 26°C ± 2°C and 80% ± 10% RH. Knock-down measured at 1 h (60 min) post-treatment was used as a proxy for mortality.

Statistical Analysis

The Henderson and Tilton correction, which accounts for natural population changes, was used to calculate the corrected treatment efficacy percentage for field treatment and laboratory testing (Henderson and Tilton 1955). To evaluate the effectiveness of the barrier pesticide applications on the field population of *Ae. albopictus*, a BACI design was employed (Stewart-Oaten et al. 1986). The full generalized linear mixed model (with negative binomial distribution because of overdispersion) contained treatment, before, and after time periods as the fixed effects, and week within individual locations as repeated random effects to account for potential autocorrelation. The overall treatment effect was considered significant if the interaction term treatment*before/after application was significant ($P < 0.05$) in the full model. Cone bioassay mortality data were analyzed, using the full mixed effects model with treatment as the fixed effect, and week within the nested term of sample, replicate and county as repeated random effects to account for potential autocorrelation and the differences in response among locations, replicates, and samples. *P*-values were obtained by likelihood ratio tests comparing the full model with and without the effect in question (Crawley 2012). To check the model's assumptions, residual plots were visually inspected for obvious deviations from homoscedasticity or normality. All statistical analyses were done using R v. 3.2.3 (Pinheiro et al. 2015) and the packages lme4 v. 1.1–10 (Bates et al. 2013) for mixed effects models (Bates et al. 2013).

Results and Discussion

In Mercer County, 3,236 mosquitoes (800 males and 2,436 females) were captured in 362 collections, whereas 2,956 mosquitoes (577 males and 2,541 females) in 263 collections were captured in Hudson County. Since the treatment effects did not differ between the two counties or between male and female mosquitoes (Table 1), all data were combined to increase statistical power for BACI analysis. *Ae. albopictus* populations were significantly reduced as much as 78% (mean ± SE 10.8 ± 1.5 to 4.6 ± 0.5) and 74% (16.9 ± 3.3 to 8.4 ± 1.1), respectively, from applications of either lambda-cyhalothrin or its combination with pyriproxyfen compared with controls (Fig. 1A). Although adult populations increased in properties treated

Table 1. Statistical analysis of interaction effects of treatment with other factors, and comparisons of treatment groups

	Coefficient	SE	Statistics[a]	*P*
Field trials				
Pre/Post × Treatment (BACI)			38.35	<0.001
Archer vs. Control	−0.15	0.34	−0.43	0.664
Demand vs. Control	−1.65	0.35	−4.71	<0.001
Archer+Demand vs. Control	−1.59	0.35	−4.57	<0.001
Demand vs. Archer+Demand	0.06	0.36	0.18	0.858
County × Treatment			2.07	0.559
Female/Male × Treatment			2.42	0.489
Laboratory cone assay				
Treatment			1201.80	<0.001
Demand vs. Control	5.51	0.5	10.99	<0.001
Archer+Demand vs. Control	5.44	0.5	10.86	<0.001
Demand vs. Archer+Demand	−0.06	−0.06	−1.14	0.256

Mixed effects models included time and place as random variables to account for differences among the sites or locations, and potential autocorrelation.
[a]Chi-square χ^2 for main and interaction factor effects (in bold), Z-values for Treatment levels.

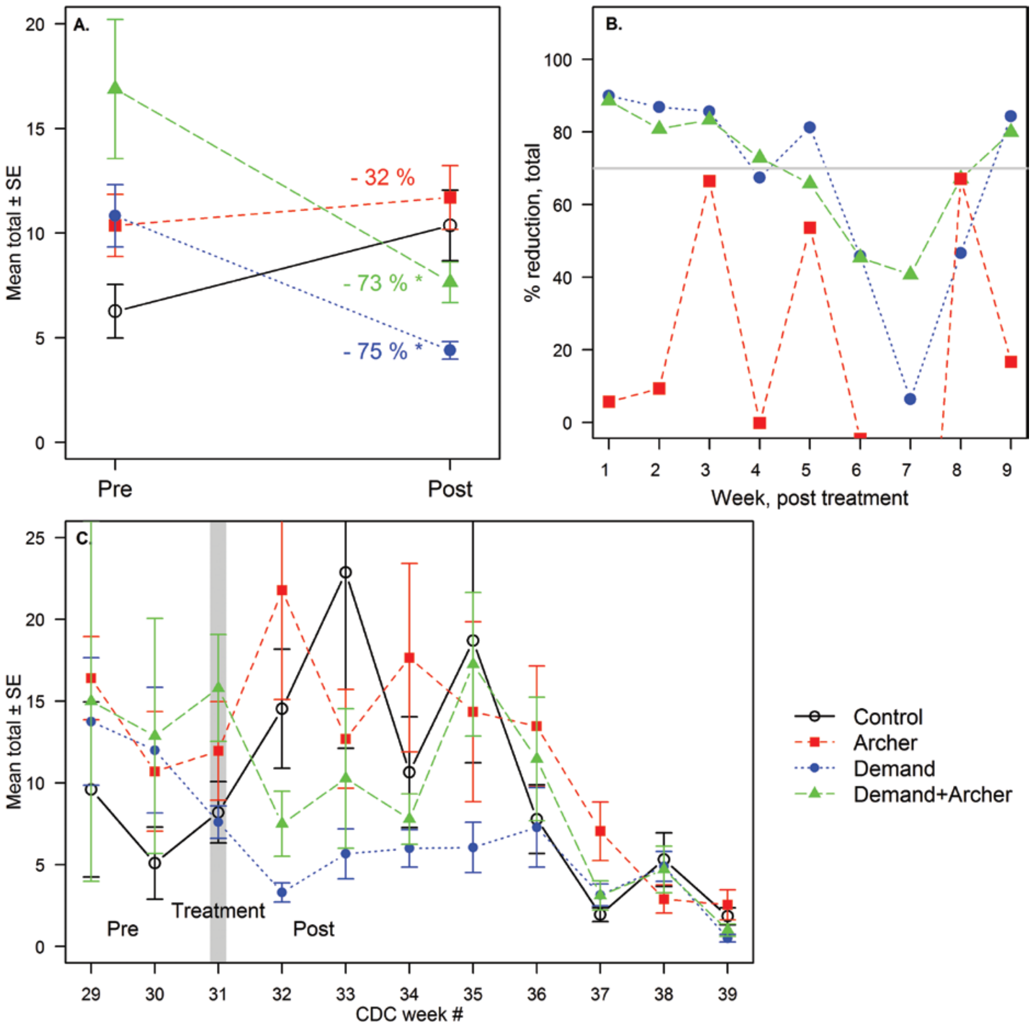

Fig. 1. Field barrier treatment trials. (A) Mean number of *Ae. albopictus* mosquitoes collected per trap pre and post treatment (BACI plot) showing significant effect when treated line intersects with the control line. These statistically significant differences in the overall percent change compared to the control group with Henderson-Tilton's correction are indicated with an asterisk (*). Henderson-Tilton's correction accounts for fluctuations in the control group and thus can show the overall reduction in the treatment population even if it increased post-treatment, but at a lesser degree than the control population. (B) Percent reduction in the total number of *Ae. albopictus* mosquitoes collected per BGS trap for each week post treatment. Each group was compared to the control group with Henderson-Tilton's correction. The grey line represents 70% reduction threshold considered as effective. (C) Mean number of *Ae. albopictus* mosquitoes collected per trap per CDC week. Treatment week (CDC week = 31, grey), and pre/post treatment periods are indicated.

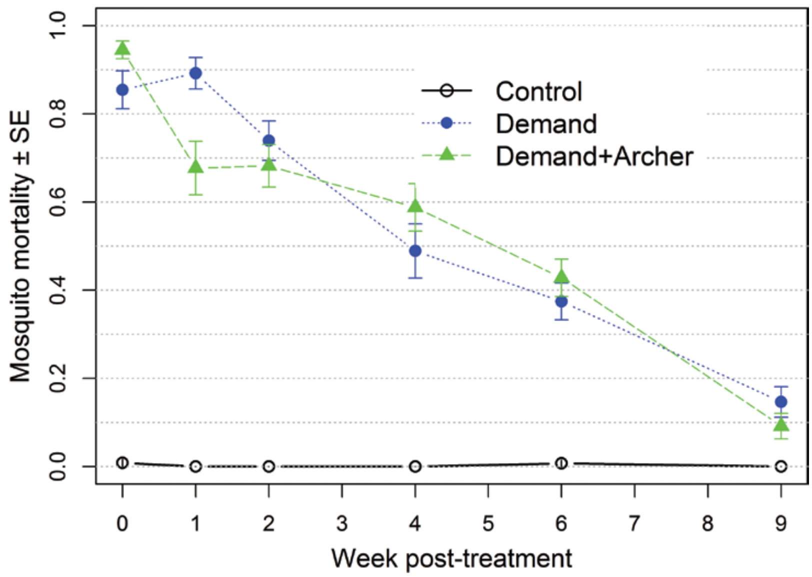

Fig. 2. Mean *Ae. albopictus* mosquito mortality in WHO adult cone bioassay starting 2 d post-treatment (week 0).

with pyriproxyfen only, this increase was smaller relative to the control populations (Fig. 1A, Table 1).

Lambda-cyhalothrin only and in combination with pyriproxyfen exceeded the 70% post treatment reduction threshold considered as effective for *Ae. albopictus* control (Farajollahi et al. 2012). This reduction persisted for 3 to 4 wk (Fig. 1B and C) during New Jersey's peak *Ae. albopictus* activity in August until obscured by the natural seasonal decline of populations in late September. Bolstering the field results, lambda-cyhalothrin only or in combination with pyriproxyfen resulted in significant mortality in adult mosquitoes in laboratory cone bioassays (Table 1). At the 70% effective treatment threshold, this effect persisted for 2 to 4 wk (Fig. 2) in agreement with the field data. The duration of the treatment effect was shorter than reported in the literature for lambda-cyhalothrin, i.e., approximately 4 wk at 70–80% reduction versus 5–9 wk at over 90% reduction (Trout et al. 2007, Li et al. 2010). Possible explanations for this discrepancy include different types of vegetation present affecting efficacy and duration of barrier treatments (Doyle et al. 2009), growth of new and therefore untreated foliage (Cilek and Hallmon 2006), or the timing of the barrier treatment application closer to the seasonal *Ae. albopictus* population decline in our study.

As an IGR, pyriproxyfen does not cause adult mortality or impair their mobility (Kawada et al. 1993). However, this juvenile hormone mimic is lethal to immature mosquito stages at extraordinarily low concentrations (LC_{50} in *Ae. albopictus* 0.012 ppb and *Ae. aegypti* is 0.023 ppb) (Gaugler et al. 2012). Area-wide ULV applications of pyriproxyfen were effective at inhibiting adult emergence of *Ae. albopictus* and *Ae. aegypti* mosquitoes. Pyriproxyfen acted synergistically with an adulticide to achieve over 90% reduction for up to 5 wk (Lucia et al. 2009) and over 80% when used as a stand-alone product (Doud et al. 2014). Moreover, this effect resulted in a 50% reduction in adult *Ae. albopictus* populations in pyriproxyfen treated areas (Doud et al. 2014). Although application techniques were different, we did not observe any significant treatment effect of pyriproxyfen or its combination with an adulticide in our study, likely due to the differences in the scale, frequency, and technique. The scale of area-wide applications ranged from 36 to 40 ha as opposed to 0.02–0.05 ha in this study, which was perhaps insufficiently large to entirely cover the larval habitat serving as the adult population source. A single application of pyriproxyfen, as in our study, was not effective, requiring 1–2 additional treatments to reduce adult *Ae. albopictus* abundance (Doud et al. 2014). ULV applications with smaller droplet sizes, providing greater drift, compared to a hand-held sprayer likely penetrates better into the *Ae. albopictus* cryptic larval habitat. The success of pyriproxyfen depends on the dissemination of chemicals to target habitats with a loss of efficacy if a large proportion of containers remain untreated (Unlu et al. 2017a). Despite these limitations, incorporating pyriproxyfen with container-inhabiting *Aedes* mosquito control in residential areas should be further investigated at different spatial scales, frequencies, and equipment to identify optimal conditions for its use. We did not measure the direct impact of pyriproxyfen on immature mosquitoes because larval mortality is of little consequence, unless it results in a measurable reduction in adult mosquitoes that defines success or failure of barrier treatments.

Farajollahi et al. (2012) showed that nighttime adulticiding reduced *Ae. albopictus* adult populations for 7–10 d. Because control achieved by adulticiding is transient, weekly treatments are required for continued suppression. The benefit of barrier treatments is their efficacy duration, which was about twice that of a conventional ULV application.

Our study was conducted during the adult *Ae. albopictus* population peak time (July to September) which is followed by a precipitous seasonal decline starting in mid-September (Unlu et al. 2011). Despite the limitations, we achieved 4 wk of control during the period when *Ae. albopictus* is most difficult to suppress. Control of *Ae. albopictus* is challenging, but increasing evidence on the efficacy of barrier treatments is encouraging and may be incorporated as part of an integrated mosquito management program (Wheeler et al. 2009, Muzari et al. 2014). More research is needed to demonstrate whether barrier treatments can also reduce the risk of mosquito-borne diseases as claimed by some private pest control operators without adequate support by scientific studies.

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