Effectiveness of autodissemination stations containing pyriproxyfen in reducing immature *Aedes albopictus* populations

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**Abstract**

**Background:** *Aedes albopictus*, the Asian tiger mosquito, is an aggressive, highly anthropophilic, day-biting mosquito with an expanding geographic range. Suppression of *Ae. albopictus* is difficult because of the abundance and prevalence of larval habitats within peridomestic environments, particularly cryptic habitats such as corrugated extension spouts, fence post openings, discarded food containers, etc. Because of the challenges of eliminating or treating larval habitats of this species, we tested an autodissemination concept to contaminate these habitats with the insect growth regulator pyriproxyfen.

**Methods:** Our study was conducted in the City of Trenton (Mercer County), New Jersey, USA (40°12′N, 74°44′W). We selected six hot spots, where five or more *Ae. albopictus* males or females were collected based on weekly trap surveillance. A trapping unit was a city block, approximately 0.8 ha (hot spot), where we deployed 26 to 28 autodissemination stations per treatment plot. To gauge efficacy, we deployed BGS traps, oviposition cups, and sentinel cups in treatment and control locations.

**Results:** We found a significant reduction in eggs (*P* < 0.0001) and larval populations (*P* < 0.0001) as a result of treatment. Pupal mortality, as determined through bioassays, was also significantly higher in the treatment sites (*P* < 0.0001).

**Conclusion:** Our results clearly show the potential and unique use of the autodissemination stations to control immature *Ae. albopictus* in urban areas. Penetration of larvicides with existing methods are difficult to reach cryptic habitats, but the autodissemination approach, which exploits the oviposition behavior of the target pest, can be integrated into intervention programs. New tools are urgently needed to curb the expansion and public health implications of *Ae. albopictus* and other container-inhabiting species.

**Keywords:** Asian tiger mosquito, Autodissemination station, Pyriproxyfen, Egg surveillance, Adult surveillance
such as buckets and tires are abundant, poses a challenge
to control this species [9–12]. *Aedes albopictus* also ex-
hbits skip oviposition, where the species may oviposit
eggs from the same batch in multiple containers [13, 14].
Most water-holding containers within the peridomestic
environment are suitable larval habitats for *Ae. albopictus*
[9, 15]. Previous studies have reported that corrugated
extension spouts routinely contain larvae and pupae of
*Ae. albopictus* in residential backyards of urban and sub-
arban areas of northeastern USA [16].

Suppressing populations of *Aedes albopictus* has been
traditionally more difficult than *Ae. aegypti*, in part
because of differences in their oviposition preferences
[17, 18]. *Aedes albopictus* prefer numerous smaller habi-
tats, which is much different than wetland mosquito
species that develop in habitats that are large and pre-
dictable [9]. We have also shown in previous studies [16]
that immature *Ae. albopictus* numbers were higher in
cryptic corrugated extension spouts (containers posi-
tioned on the ground horizontally) than open containers
(containers positioned on the ground vertically) during
the peak season (August). Because of the affinity of *Ae.
albopictus* for cryptic habitats, area-wide larviciding
using backpacks [19] or truck-mounted sprayers are not
optimal control measures due to the poor penetration of
pesticides into these habitats [16, 20]. Door-to-door
source reduction campaigns are also labor and time in-
tensive, often leading to ineffective control outcomes
[21–23]. Theautodissemination approach may be a
means of insecticide delivery into cryptic habitats that
are otherwise hard to treat by conventional methods.

Autodissemination is a ‘pull’ (attraction and adhesion)
and ‘push’ (dispersal and transfer) technology which
allows mosquito control professionals to treat larval habi-
tats in a timely and economical fashion. Briefly, autodis-
semination is a pest management method in which
insects contaminated with an insecticide, transfer lethal
concentrations horizontally or vertically to other insects
via mating, oviposition, aggregation and other behaviors
[24–27]. The success of autodissemination stations de-
pends on three criteria: (i) attraction of mosquitoes to
the stations; (ii) transfer of chemicals to the mosquitoes;
and (iii) dissemination of chemicals to target habitats
[24]. Gaugler et al. [24] achieved all three conditions
with their 2012 design during semi-field trials and suc-
cessfully contaminated containers using mosquitoes as
the vehicles. A novel ovitrap was developed based on
pull and push strategy to control *Ae. aegypti* by Snetselar
et al. [28], using a combination of attractant media,
*Beauveria bassiana* (an entomopathogenic fungi), and
pyriproxfen (a biopesticide). Researchers achieved 100% larval mortality in the laboratory; however, the study did
not include any field trials. The most commonly used
chemical in autodissemination stations has been the
insect growth regulator (IGR) pyriproxyfen, which does
not cause immediate mortality or impair adult activity
[29], but is effective on immature mosquitoes at extraor-
dinarily low concentrations (LC50 in *Ae. albopictus*
0.012 ppb and *Ae. aegypti* is 0.023 ppb) [24, 30]. This
approach seems to be a feasible strategy based on *Ae.
albopictus* and *Ae. aegypti* skip-oviposition behavior,
facilitated by distributing eggs from a single gono-
thropic cycle [31]. Devine et al. [32] achieved 42–98% *Ae.
aegypti* adult emergence inhibition by using pyriproxyfen-treated stations in Peru [32]. Autodisse-
mination has also been tested by different groups in small
scale field experiments against two dengue vectors [10,
26, 32]. Recently, Abad-Franch et al. [33] evaluated the
efficacy of emulsifiable pyriproxyfen stations in a tropical
neighborhood by monitoring *Ae. aegypti*, *Ae. albopictus*
and *Culex* spp. populations. This approach resulted in a
10-fold decrease in adult mosquito emergence. However,
large scale autodissemination applications need further
investigation. A novel approach, “Auto-Dissemination
Augmented by Males” (ADAM), has proven that
pyriproxfen-dusted males were able to transmit lethal
amounts of insecticide to oviposition sites, resulting in
reduced adult populations of *Ae. albopictus* [26]. Most
studies have primarily shown reduction of immature
and adult populations caused by pupal mortality, and
reduced egg number of *Ae. aegypti* females exposed to
sub-lethal doses of pyriproxyfen [34, 35]. In the current
study, we evaluated the effects of deployed autodissemi-
nation stations in *Ae. albopictus* hot spots (localized
areas with high numbers of adults) through monitoring
of eggs, larvae, pupae and adult populations in a temper-
ate urban area, Trenton, New Jersey, USA.

**Methods**

**Study site description**

Our study was conducted in the City of Trenton
(Mercer County), New Jersey, USA (40°12′N, 74°44′W).
Trenton sites were urban residential neighborhoods:
South Olden (40°22′N, 74°73′W) was 48.6 ha consisting
of 24 city blocks, each containing a residential street on
all four sides and divided lengthwise by a drivable alley.
South Olden consisted of 1250 parcels (i.e. house with
surrounding yard), most often built as adjoining row
homes or duplexes [36]. Parcel sizes were approximately
200 m². We have continuous *Ae. albopictus* surveillance
data between 2008 and 2014 from this study site, dem-
onstrating high *Ae. albopictus* populations [9, 22, 37].

**Identification of *Ae. albopictus* hot spots**

We monitored adult populations of *Ae. albopictus* with
BG Sentinel™ traps (BGS traps; Biogents AG, Regens-
burg, Germany). A trapping site was a city block with a
BGS trap deployed in a parcel located in the center (or
as close as to the middle location as possible). A trapping site was classified as a hot spot when five or more *Ae. albopictus* males and females were collected in that individual trapping site based on weekly trapping surveillance [36]. This number was chosen because three bites have been reported as a common nuisance threshold driving residents indoors, and an average of five bites per day by *Ae. albopictus* has been recorded as intolerable [38–41]. For this study we used females and males for the nuisance threshold determination because *Ae. albopictus* is very focal within our study sites and male numbers are often followed by increasing female numbers in the traps [37]. We identified 6–10 hot spots based on 16 trapping sites between June 13 and July 23, 2014. Trapping sites that were identified as hot spots at least three times were selected for this study. To reduce the chances of control contamination, the site was split in two parts (treatment versus control) using the center most road as a dividing line (Fig. 1). Hot spots were often determined by the boundaries of a city block (Fig. 1), approximately 0.8 ha where we deployed 26 to 28 autodissemination stations per treatment site. Treatment sites are hereafter referred to as HA1 (hot spot autodissemination 1), HA2 and HA3, and control sites as HC1 (hot spot control 1), HC2 and HC3 (Fig. 1).

To reduce the immigration from outside the study sites, we conducted area-wide larviciding with a water-dispersible granular (WDG) formulation of *Bacillus thuringiensis* var. *israelensis* (Bti; VectoBac® WDG, Valent BioSciences Corp., Libertyville, IL, USA) prior to autodissemination station deployment. This was conducted within the barrier zone created around the sites, but excluded the experimental sites. We used a CSM2 Mist Sprayer (Buffalo Turbine, Springville, NY, USA) [20], for the Bti applications, and conducted the first application on 27 July between 01:00 and 06:00 h. Following the initial larviciding, ultra-low volume (ULV) adulticide applications using DUET™ Dual-action Adulticide (Clarke Mosquito Control, Roselle, IL, USA) were conducted each week around the treatment and control sites until 26 September. A Cougar® (Clarke Mosquito Control, Roselle, IL, USA) cold aerosol ULV generator was used during all adulticide applications. Truck-mounted adulticide applications were conducted at night using a single vehicle to drive buffer areas including all available roads and alleys to provide maximum coverage. Each application took about 2 h to complete and was conducted between 01:00 and 4:00 h [37].

**Autodissemination stations**

The autodissemination stations were modified from an earlier design [24] by introducing the mechanism of dual treatment [25]. The station consisted of a transfer chamber, unidirectional funnel, and an infusion reservoir.

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**Fig. 1** Map of the study locations, including trapping sites (n =1), and the locations of oviposition cups (n =5), sentinel cups (n =10), and autodissemination stations (26–28) in treatment and control sites, Trenton, New Jersey, 2014.
The transfer plate used by Wang et al. [25] was modified into a cartridge, which was inserted into the middle of the chamber body. The cartridge contains two formulation plates (top and bottom) and each plate (55 × 50 mm) contains an oil and powder band. The oil bands were coated with an oil formulation containing 20% pyriproxyfen a.i. (0.62 g/station) and the powder bands were coated with a powder formulation containing 60% pyriproxyfen a.i. (0.42 g/station). The oil formulation was coated by wicking the oil from a reservoir located on the bottom of the cartridge, which ensures availability of the oil formulation over the entire active season. The space for exiting gravid mosquitoes was 6 mm, which forced them to contact the oil formulation before picking up the powder. Gravid mosquitoes attracted by the infusion in the reservoir enter from the top of the unidirectional funnel to attempt to lay eggs. However, the mesh on top of the infusion reservoir prevents them from reaching the infusion. Fail to find a suitable oviposition site, the gravid mosquitoes are forced to exit the station from the gaps between formulation plates where they get contaminated with the oil formulation first and then pick up the powder before exiting the station. The formulation is then transferred into the containers where the contaminated mosquitoes seek oviposition. Additionally, the earlier station used oak leaf infusion [22], whereas here oak leaves were packed in mesh bags and placed into the reservoir water in an effort to extend attraction and duration. The tea-bag containing 7.5 g of shredded oak leaves and 15 g of oak wood chips was preloaded in the reservoir. The station was tested in a room assay (30 m³) using 10 oviposition cups filled with 250 ml of water by releasing 50 gravid females. The autodissemination station consistently achieved 100% pupal mortality within all oviposition cups. Oviposition cups were placed in the control and treatment sites on 28 July 2014 and autodissemination stations were deployed in the treatment sites only. Autodissemination stations were serviced weekly to ensure proper function, and field crews unblocked the opening of clogged stations which may have been caused by leaves or spider webs.

**Egg surveillance**

Black 360 ml oviposition cups (SpringStar, Inc., Woodinville, WA, USA] were zip-tied to a fence at ground level to reduce disturbance. The oviposition cups were filled with 300 ml of tap water, and seed germination paper was used to cover the inside surface acting as oviposition substrate. Two small holes were pre-drilled above the water line to prevent them from overflowing after a rain event. We deployed 30 oviposition cups (5 × 3 treatment sites, 5 × 3 control sites, Fig. 1). The oviposition papers were changed at 3 to 5 day intervals to coincide with adult trapping. Positive egg papers were subsampled (5 egg papers per week) and submersed in dechlorinated water in the laboratory. Because *Aedes japonicus japonicus* (Theobald) and *Aedes triseriatus* (Say) eggs other two mosquito species commonly found in containers in New Jersey [42], are very similar to *Ae. albopictus* eggs, larval food was provided until immatures reached third-instar stage and identified. Over 98% of the larvae were identified as *Ae. albopictus* [43].

**Larval surveillance and pupal mortality**

Initially, 10 sentinel cups were placed at each hot spot and filled with 250 ml of dechlorinated tap water (Fig. 1). To improve stability during inclement weather or other disturbances, we developed an anchoring system using a similar black cup with a hole in the bottom where a 20 cm galvanized spike could be driven securely into the ground, creating a nest to hold the sentinel cups in place. The sentinel cups were sampled at 2 week intervals as fresh water are less preferred in comparison to

![Fig. 2 Autodissemination stations consisting of an infusion reservoir, transfer chamber, and a unidirectional funnel](image-url)
natural habitats [44]. Longer exposure provides the opportunity to deliver sufficient pyriproxyfen into cups through multiple visits of mosquitoes, which compensates for absorption of pyriproxyfen in sentinel cups. The original cups were left in the field for the entirety of the study. If a collected cup was below 250 ml or empty, additional dechlorinated water was added as needed, agitated in the cup, and poured into the new sample cup for bioassays. After sampling, the sentinel cups were replenished with 250 ml of water and returned to their corresponding locations. The control sites were always sampled first and placed in an enclosed container inside the cab of the vehicle, while the treatment samples were placed in the back of the vehicle upon completion. Gloves were changed for each sampling to prevent contamination. Each sentinel cup from the control and treatment hot spots were observed, and the number of larvae, pupae and pupal cases in each container was recorded. After counting immature mosquito populations, sentinel cups were filtered to remove debris, organic materials and immature mosquitoes. A 50 ml water sample was taken from the sentinel cups for pyriproxyfen residue analysis. The residue analysis of pyriproxyfen was carried out at Golden Pacific Laboratories (CA, USA) using a liquid-mass-mass spectrophotometry (LC-MS-MS) analysis as described by Wang et al. [25]. For the analysis, sample thawed, diluted 1:1 with acetonitrile and aliquot was passed through 0.45 µm filters. Primary and secondary ion pairs were monitored for the analysis using an AB Sciex API 5000 mass spectrophotometer. Each set of samples contained water blank and positive controls fortified at the limit of quantification and at a higher level to bracket expected sample concentrations. With the remaining 200 ml, we conducted larval bioassay by inoculating 20 third instars at 26 ± 1 °C and 16:8 L:D photoperiod. For colony mosquitoes, larval food (brewer’s yeast, 30 mg/l) was provided twice a week. Larval and pupal mortality and adult emergence were recorded to estimate efficacy. For negative control, three cups were set up using distilled water and 20 larvae per bioassay. Mosquito colony information and maintenance are described in detail elsewhere [25]. Briefly, larvae obtained for bioassays were from an Ae. albopictus colony established from the eggs of field populations collected in Mercer County, New Jersey, USA between 2008 and 2010 [25]. Restrained guinea pigs were used to blood-fed the females (Rutgers University Animal Use Protocol No. 86–129) and eggs were collected on seed germination paper and stored at 26 ± 1 °C. Third stage larvae were used in all bioassays.

**Adult surveillance**

We used BGS traps for adult mosquito sampling [37, 40], baited with BG lures (Biogents AG, Regensburg, Germany). Details of surveillance protocols are outlined elsewhere [22]; in short, trapping sites were chosen by overlaying a grid of 175 m intervals based on Ae. albopictus flight range [18] and available traps and personnel. The Fishnet tool within ArcGIS Desktop 9.2 (Environmental Systems Research Institute, Redlands, CA, USA) was used to determine potential trapping sites. The 175 m grid resulted in 16 traps within the study site (Fig. 1). Permission to place BGS traps was acquired from property owners. Traps were deployed in the field continuously for 24 h once per week during the mosquito season, and all mosquitoes were identified to species and gender [37]. Trapping started 14 May and ended 12 November when no mosquitoes were collected for 2 to 3 weeks.

**Data analysis**

The effect of placement of autodissemination station in the treatment sites was investigated by comparing the number of eggs collected from oviposition cups at the treatment sites (HA 1-3) to the number of eggs collected from oviposition cups at the control sites (HC 1-3) using negative binomial regression (PROC GENMOD, SAS version 9.3; SAS Institute 2011), with treatment, week, and treatment*week as predictors, where treatment is an indicator variable (1 if site contained Autodissemination station, 0 otherwise). Since the number of days oviposition cups remained in the field varied, the natural log of the number of days a oviposition cup remained in the field was used as an offset. The number of larvae occurring in sentinel cups collected from the control and treatment sites were compared by negative binomial regression in SAS using the default log link. None of the sentinel cups contained larvae in the second week, therefore, data from this week were not included in the analysis. The model used treatment as a predictor for larval data showed signs of misfit, so instead the model was fitted using site, week, and site*week as predictors. Comparison of the mean number of larvae collected over the three treatment sites and three control sites was accomplished using a linear contrast. The mean pupal mortality from the laboratory bioassays using water samples collected from oviposition cups at the control sites (HC 1-3) using negative binomial regression using the default log link. The P-values between comparisons were adjusted using Holm’s test, which adjusts the calculation of probability in line with the number of comparisons made to avoid type I errors [45]. No offset was used since the same initial number of laboratory colony larvae (20) used to inoculate all samples. Negative binomial regression was utilized in lieu of logistic regression because negative binomial regression is better suited for data containing a large number of zero values.

Adult mosquito numbers were compared using piece-wise negative binomial regression in SAS [46]. Separate
slopes were fit for the before-treatment (12 June to 1 August) and after-treatment (5 August to 30 September) periods to examine whether the slope changed after placement of autodissemination stations in the treatment sites. We tested for the main effects of treatment, and of time in the before-treatment and time in the after-treatment periods, and for the interactions of treatment and time in both periods. These interactions were included to determine if the slopes differed between the treatment (HA1-3) and control (HC1-3) in either before or after-treatment periods. Time was treated as a continuous variable for the analysis of adult and egg data and as a categorical variable for the analysis of larval and pupal data.

Results

Egg populations

Between 11 August and 29 September 2014, a total of 4770 eggs were collected from the six study sites. Fewer eggs were collected from oviposition cups in the treatment sites compared to those in the control sites throughout the course of the study ($\chi^2 = 42.56; df = 1, P < 0.0001$; Fig. 3). The mean number of eggs collected in control sites were five times higher than the treatment sites, with $6.9 \pm 10.1$ (0–66) [mean ± standard deviation, (range)] per day per oviposition cup (Fig. 3). The mean number of eggs collected in treatment sites remained below two eggs per day per oviposition cup: $1.4 \pm 2.7$ (0–16).

Larval populations and pupal mortality

The model used to analyze larval data showed that the study sites were significant predictor of the mean number of larvae present in the sentinel cups ($\chi^2 = 36.40, df = 5, P < 0.0001$; Table 1). A linear contrast of the average number of larvae collected from HA1-3 (treatment sites) and from HC1-3 (control sites) showed a significantly lower mean number of larvae in the treatment sentinel cups ($Z = -5.23, P < 0.0001$; Table 1). Water samples collected from sentinel cups in the treatment sites resulted in higher pupal mortality of laboratory strain *Ae. albopictus* (12.4%) than did water samples from the control sites (0.58%) ($\chi^2 = 53.8, df = 1, P < 0.0001$). LC-MS-MS analysis detected 0.00400 to 0.0113 ppb pyriproxyfen residue in water samples collected from the treatment sites. We observed 70% pupal mortality at 0.00400 ppb, and 100% at 0.00478 ppb, while the highest concentration of 0.0113 ppb achieved 95% pupal mortality.

*Aedes albopictus* adult populations

A total of 623 adult mosquitoes were collected from treatment and control sites. The mean number of adults collected prior to autodissemination station deployment from control and treatment sites was $8.1 \pm 8.7$ (0–43) [mean ± SD, (range)] and $9.5 \pm 9.2$ (0–37) per day per BGS trap, respectively. Following the station deployment, the mean number of adults collected from control and treatment sites were $9.8 \pm 8.7$ (0–33) and $11.9 \pm 11.8$ (0–51) per day per BGS trap (Fig. 4). Neither interaction between treatment and time (before-and after-treatment) were significant, nor was the main treatment effect ($\chi^2 = 1.48, df = 1, P = 0.22$; Fig. 4).

Discussion

We assessed autodissemination as a means of reducing *Ae. albopictus* populations in hot spots, which was ultimately an attempt to manage this species area-wide. It has been shown that by targeting immature *Ae. albopictus* populations in hot spots, it is possible to achieve
Studies have demonstrated the efficacy of an autodissemination approach to reduce egg numbers in *Aedes* species in cages, rooms, and small scale field settings [10, 26, 32]. Our study targeted an area as large as 0.8 ha in an urban habitat and evaluated the impact of the stations on *Ae. albopictus* populations. We found egg and larval populations were reduced in autodissemination sites. These results were supported by higher pupal mortality during laboratory bioassays. There was no significant effect on the number of adult populations following autodissemination deployment; however, the overall trend in treatment and control sites were similar, indicating that adult numbers increased and decreased during repeated measurements of adult populations, mostly because our calculations were based on six traps (7 weeks). Even though we had long periods of follow-up with repeated measurements of adults using BGS traps to assess the effect of autodissemination station deployment, because of large variations in adult populations between sampling sites and across different sampling periods, the true effect on adults was not clear [47]. In addition, given the unique biology of container-inhabiting *Aedes* mosquitoes, we cannot discount recurring immigration into our sampling sites during the course of these studies. While the immigration of adults limited our ability to detect a decline in adult populations within the hot spots, these mosquitoes likely helped distribute pyriproxyfen to larval containers. This is supported by the detection of pyriproxyfen residue in treatment site sentinel cups, pupal mortality, and the presence of significantly less eggs and larvae compared to control site sentinel cups [33]. Although adult numbers were not significantly reduced, we recorded lower egg numbers from the treatment sites, which demonstrated that the stations were effectively contaminating adults and impacting their fecundity, leading to less egg collection from treatment sites. A previous study reported that pyriproxyfen contamination inhibits *Ae. albopictus* egg production [48]. In summary, adult movement makes it difficult to interpret the findings although we tried to reduce immigration by weekly ULV adulticiding events, however, measurements of all three immature stages support our main conclusion that autodissemination stations reduced *Ae. albopictus* populations.

### Table 1

| Site | No. cups | Larvae | Mean | 95% CI | Dead pupae | Mean | 95% CI |
|------|----------|--------|------|--------|------------|------|--------|
| HA1  | 36       | 0.1    | 0–4.0| 3.6    | 0–20.0     | 3.6  | 0–20.0 |
| HA2  | 39       | 3.9    | 0–25.0| 2.4    | 0–20.0     | 2.4  | 0–20.0 |
| HA3  | 39       | 0.4    | 0–4.0| 2.1    | 0–18.0     | 2.1  | 0–18.0 |
| LS mean (95% CI) | 0.5 | 0.3–0.9 | 2.2 | 0–0.9 |
| HC1  | 40       | 1.8    | 0–19.0| 0.1    | 0–2.0      | 0.1  | 0–2.0  |
| HC2  | 36       | 4.1    | 0–38.0| 0.2    | 0–2.0      | 0.2  | 0–2.0  |
| HC3  | 39       | 7.4    | 0–50.0| 0.1    | 0–2.0      | 0.1  | 0–2.0  |
| LS mean (95% CI) | 4.0 | 2.5–6.3 | 0.1 | 0.1–0.1 |

![Fig. 4 Least square means for treatment (red line) and control (blue line) sites with 95% confidence intervals and observed counts by site (treatment: red circles; control: blue circles) for total number of female and male adult *Ae. albopictus* collected in BGS traps. One BGS trap was deployed in each hot spot for 24 h once a week for 17 weeks. Autodissemination stations in the treatment sites on 28 July, 2014. Black line placed on 28 July indicates the oviposition cup, sentinel cup and autodissemination station deployment.](image-url)
in the sites. Our results on adult populations were inconclusive due to the low number of BGS traps deployed and immigration. The availability of additional adult data may still not capture the difference between treatment and control sites because adult immigration from adjacent sites was reduced but not eliminated by weekly adulticiding. Despite these limitations, the presence of less eggs and larvae could only be explained by sub-lethal exposure of the females to pyriproxfen. For future studies, we will increase the number of treatment and control sites along with the number of BGS traps per site to improve adult surveillance. Barrier treatments using backpack and hand sprayers on vegetation in the buffer areas, will be applied as an alternative to ULV adulticiding to further decrease immigration into the study sites.

One of the strengths of the experimental design for this study was to investigate the effect of our treatment on each life stage of the mosquito. In addition to monitoring adult populations, we collected larvae and water from sentinel cups for bioassays. The water was used to challenge laboratory colonies which allowed us to determine the effects of pyriproxfen on pupal stages. We also used residue data to confirm whether or not pyriproxfen is disseminating in the field. Correlation between the mortality in field collected cups and the residue analysis of samples is challenging as the amount of organic content varies in field collected samples. The organic content degrades and adsorbs pyriproxyfen, which makes it harder to extract from the water phasic substrates using organic solvents. Most of the time, this process resulted in loss of pyriproxyfen and that produced variations in the results [49]. Whereas in the larval bioassay, larvae are continuously exposed to the same water for 2–3 weeks, and organic contents degrade over time, which provides regular supply as similar to slow release granule formulations of insecticides.

The autodissemination approach presented here shows promise to be a new tool for the control of a mosquito responsible for reducing quality of life and initiating disease outbreaks. *Aedes albopictus* is difficult to control and integrated abatement methods must be used to suppress the populations [15, 17, 19, 22, 37, 50]. Autodissemination station deployments can be incorporated with existing integrated mosquito management programs to increase the effectiveness while reducing time, cost, and effort spent for methods such as door-to-door source reduction. Target containers (plant pot saucers, recycle bins, used tires, etc.) are usually located within private residential parcels which are sometimes [51] inaccessible to mosquito control personnel. Even if inspectors can gain access to the parcels and treat all containers with larvicides, new ones frequently appear [13]. This approach is labor- and cost-intensive, while the present study indicates that this exceptionally low risk and relatively inexpensive ($ 4.5 to $ 100 per kg) active ingredient, pyriproxyfen, offers promise. Pyriproxyfen is certainly deserving of further investigations, with the possible outcome of a modest label modification.

Another challenge with *Ae. albopictus* is its use of cryptic habitats for larval development. Unlu et al. [37] reported a dominant representation of *Ae. albopictus* in corrugated extension spouts [16]. Because container-inhabiting *Aedes* species prefer cryptic larval habitats, which are difficult to reach by traditional mosquito control techniques [9, 52], the autodissemination approach may be a uniquely useful tool because it exploits female oviposition behavior to deliver a toxicant to these habitats [53]. Container-inhabiting *Aedes* species also exhibit skip-oviposition and oviposit in a wide number and variety of artificial containers within peridomestic habitats [13, 53]. The autodissemination approach provides a unique opportunity for vector control operations to utilize skip-oviposition to their advantage globally.

**Conclusions**

Despite ongoing advances in the biology and ecology of *Ae. albopictus* and other container-inhabiting mosquito species, effective vector control remains as the primary option for the protection of human health and comfort. *Aedes albopictus* is also poised to expand its range in the next few decades; primarily because of warmer winter temperatures [54]. The lack of effective area-wide control measures will present a major challenge to mosquito control agencies tasked with the protection of public health. Better planning and integration of new control products and methods, such as the autodissemination approach, will be the key for successful intervention campaigns.

**Additional file**

**Additional file 1:** Data generated and analysed in this study. (XLSX 23 kb)

**Abbreviations**

BGS traps: BG sentinel traps; Bti: Bacillus thuringiensis var. israelensis; CHIKV: Chikungunya virus; LC-MS-MS: Liquid-mass-mass spectrophotometry; ULV: Ultra-low volume; ZIKV: Zika virus

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**Availability of data and materials**

All the datasets analyzed during this study are included within the article and in the Additional file 1.
Authors’ contributions
RG, DS, YW, AF and IU conceived the study and coordinated its implementation. RG, DS, YW, AF and IU participated in the experimental design. Statistical analysis performed by KK and IU. DS, YW and IU performed the experiments. IU and KK drafted the manuscript which was critically revised by RG, DS, YW and AF. All authors read and approved the final version of the manuscript.

Competing interests
The authors declare that they have no competing interests.

Consent for publication
Not applicable.

Ethics approval and consent to participate
All studies were conducted within the jurisdictions of the authors’ respective governance domain by professional mosquito control personnel. All entomological surveys and collections made on private lands or in private residences were conducted after acquisition of oral or written consent from residents. No specific permits were required for the mosquito collections. These studies did not involve endangered or protected species.

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