Research Article

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Effectiveness of autocidal gravid trapping and chemical control in altering abundance and age structure of *Aedes albopictus*

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Abstract

Background: ***Aedes albopictus*** **is a nuisance pest mosquito of public health importance commonly managed with adulticides and larvicides. We investigated whether adding Gravid** ***Aedes*** **Traps (GATs), Autocidal Gravid Ovitraps (AGOs) or In2Care traps would extend the effectiveness of chemical control methods in Wake County, North Carolina, USA, by combining barrier sprays and larval habitat management (LHM) with each trap type at suburban households. We compared these three treatment groups to untreated controls and to backyards treated only with barrier sprays and LHM. Once a week, for ten weeks, we collected adult mosquitoes at each house using lure-baited surveillance traps and dissected a portion of** ***Ae. albopictus*** **females to determine parity.**

Results: Barrier sprays and LHM alone or combined with any supplemental autocidal ovitrap significantly reduced female ***Ae. albopictus*** **through Week 3 post-treatment. GATs significantly extended chemical control effectiveness for the duration of the study. Compared to the untreated control, the AGO and GAT treatment groups had significant overall female** ***Ae. albopictus*** **reductions of 74% and 80.4%, respectively, with populations aging significantly slower at houses treated with AGOs.**

Conclusion: This household-level study, though limited in size, observed significant reductions in nuisance ***Ae. albopictus*** **when combining AGOs and GATs with chemical controls for an eight-week period. Delayed population aging in AGO-treated yards suggests that traps also could mitigate disease transmission risk. Future studies should test these control methods at the neighborhood level to evaluate large-scale effectiveness as well as assess the effect of autocidal ovitraps without chemical intervention.**

Supporting information may be found in the online version of this article.

Keywords: *Aedes albopictus*; autocidal gravid ovitraps; mosquito control; barrier spraying; residential ecosystems; integrated pest management

1 BACKGROUND

The tiger mosquito, *Aedes (Stegomyia) albopictus* (Skuse), is a widespread invasive, anthropophilic species originally from East Asia. Discovered in the United States in 1985 and present in North Carolina since at least 1987,[1–4] *Ae. albopictus* has been identified in nearly 44% of counties in the country from 1995 through 2016, and in almost every place examined in North Carolina.[5–7] An aggressive biter, *Ae. albopictus* is the primary nuisance mosquito for homeowners in suburban North Carolina and is collected in abundance around our study area.[6,8]

In addition to being a nuisance, *Ae. albopictus* is a public health threat, capable of transmitting dengue, chikungunya and Zika viruses and implicated in disease outbreaks in temperate climates.[9–11] Current understanding of arbovirus vectorial capacity suggests that long-lived females are critical to pathogen transmission; when a female mosquito lives long enough to ingest a pathogen through an initial blood meal, incubate that pathogen, and then feed on subsequent different hosts, the risk of disease transmission increases with each blood meal.[12,13] Therefore, skewing the age structure of a mosquito population towards younger females decreases vectorial capacity and can reduce the likelihood of epidemic spread of disease.[13] Control approaches that target ovipositing mosquitoes – which for

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anautogenous mosquitoes have already taken a blood meal and are therefore older and more likely to have been exposed to a pathogen – should skew population age structures toward younger mosquitoes.

The conventional approach to controlling mosquitoes – whether to manage disease risk, nuisance behaviors, or both – includes the use of chemical insecticides (i.e. sprays and larvicides) and larval habitat management (source reduction). These techniques aim to eliminate adults and immatures using insect neurotoxins (e.g. pyrethroids), insect growth regulators (e.g. pyriproxyfen) and breeding site removal. Larval habitat management can be a nontoxic, low-cost control method that minimizes nontarget effects, but the diverse array of containers that *Ae. albopictus* occupies makes control efforts difficult by source reduction alone. Chemical control is effective, and bifenthrin-treated foliage may suppress *Ae. albopictus* populations for two to six weeks.[14–17] Over time, however, the effect of barrier sprays may wear off due to new plant growth, direct sunlight, and exposure to high temperatures and heavy precipitation.[18,19] Mosquito populations inevitably rebound and require multiple treatment applications over the course of a season to maintain levels below a tolerance threshold. Increasing insecticidal use promotes resistance and can negatively impact nontarget species, whereas public perceptions of environmental chemical controls also grow increasingly negative.[20–22]

Some alternatives to environmentally distributed chemical control are available, including the Autocidal Gravid Ovitrap[23] (AGO) and the commercially available Gravid *Aedes* Trap[24] (GAT; Biogents AG, Regensburg, Germany). These passive traps function as attractive breeding sites that lure gravid females with an attractant but kill them with a sticky card or insecticide-treated netting before they can deposit their eggs.[23,24] Some field studies have seen reductions in *Ae. aegypti* populations when deploying AGOs in large numbers over a small area[25] and when combined with larval habitat management over a large area.[26–29] Few field studies tested GATs for control, but Johnson *et al.*[30] observed significant overall and week-to-week reductions in *Ae. albopictus* females at the neighborhood level where GAT density was highest. Otherwise, most studies involving GATs ran trap comparisons as surveillance tools. In one study comparing AGOs, GATs and CDC Gravid Traps, the deltamethrin-treated GATs collected slightly more *Ae. albopictus* than the AGOs, although the CDC Gravid Traps outperformed both.[31] When compared with BG-Sentinel traps, GATs treated with insecticidal sprays collected a similar number of, if slightly fewer, *Aedes* females and captured more gravid females.[32–34] GATs tested without insecticidal treatments – sticky cards or canola oil – also performed just as well as GATs treated with insecticides in capturing *Ae. aegypti* in the field.[35] Though seemingly useful for population monitoring, the lack of research on GATs for control, a trap specifically marketed for backyard use, highlights the need to investigate these traps and their applications beyond surveillance.

Another form of autocidal control involves using female mosquitoes as vehicles for transporting pyriproxyfen (PPF) to breeding sites and reducing larval populations.[36–38] Recently the In2Care mosquito trap was developed to treat gravid females with a mixture of PPF and the fungal pathogen *Beauveria bassiana* (In2Care BV, Wageningen, Netherlands). The goal of this trap is to allow females to land on a treated netting, picking up the larvicide and the fungus. Driven by the propensity of females to distribute their eggs into different containers, the treated females visit cryptic breeding sites and deposit the PPF, with very small doses lethal to larvae and pupae.[36,37,39–41] Then, before taking another blood meal, the exposed females die from exposure to the fungus, eliminating them as disease vectors.[42] Although uncertainty exists with using *B. bassiana* as an effective adulticide in the field, laboratory and semi-field trials suggest that exposed females exhibit a decrease in vectorial capacity, with reduced feeding behaviors and increased mortality.[43,44] In laboratory and semi-field conditions the In2Care traps appear attractive to *Aedes*, with successful PPF dispersal and high larval mortality to nearby breeding sites, as well as high adult mortality from *B. bassiana*.[45,46] Another study demonstrated that field-deployed In2Care traps attracted *Ae. aegypti* and *Culex quinquefasciatus* and killed immatures,[47] and a recent large-scale In2Care intervention effort in Florida observed significant reductions in *Ae. aegypti* larvae and eggs, but not adults, compared to a separate area treated with source reduction, larvicides, and adulticides.[48] One study in New Jersey, USA, tested the effectiveness of PPF autodissemination stations (but not In2Care traps) on *Ae. albopictus* at the neighborhood level, but again adult populations were unaffected.[49] With so few studies evaluating these traps under field conditions, especially targeting *Ae. albopictus*, the true effectiveness, and limitation, of In2Care control on mosquito populations has yet to be explored.

Autocidal ovitraps may be most effective when deployed at high densities, as performed in many field studies, but limits in community participation, labor and cost might make exclusive trap use impractical for long-term control. Therefore, integrating chemical applications to initiate a population knockdown might encourage prolonged *Ae. albopictus* suppression when using autocidal ovitraps. To evaluate the ability of *Aedes*-targeted traps to improve the impact of residual barrier spraying and larval habitat management, we conducted an experiment in suburban backyard settings in Wake County, North Carolina (NC). We hypothesized that the addition of traps targeting ovipositing females improves the duration of effectiveness of traditional spraying. From this, we predicted the addition of these traps would reduce overall Ae. albopictus abundance over an eight-week period while also limiting disease risk by altering the population structure from older mosquitoes to younger mosquitoes due to the targeting of ovipositing females.

2 MATERIALS AND METHODS

2.1 Participant selection

We used recruitment fliers and emails to recruit households in Wake County, NC (35° 47′ 24″ N, 78° 39′ 0″ W), in May 2019. We posted the fliers in parks, civic centers and NC State University campus buildings, and sent emails to the students, staff and faculty of the Department of Entomology and Plant Pathology encouraging recipients to forward the emails to interested homeowners. We also emailed participants in a previous study.[17] Fliers and email text available from the corresponding author upon request. Eligible properties were single-family, detached houses in Wake County that had not used professional mosquito treatment since January 2019. Households that met the criteria and were willing to participate were selected on a first-come, first-served basis, and informed consent procedures were completed in person (NCSU IRB #16880, approved 25 April 2019). Houses selected for treatment received free professional barrier spraying (Sp) and larval habitat management (LHM) services (hereafter abbreviated as SpLHM), and houses not selected for treatment were offered a free treatment at the end of the study. Mosquito

Authority – Raleigh, Durham & Chapel Hill, NC (Morrisville, NC) provided the treatments according to industry standards.

2.2 Ethics

Informed consent was acquired before any research was conducted, either on the property or in assessing human subject responses (NCSU IRB # 16680, approved 25 April 2019). Mosquito treatments were donated by a third-party vendor (The Mosquito Authority, Hickory, NC, USA), but neither the authors nor the participants were solicited to advertise nor received any benefit from The Mosquito Authority outside of a single mosquito treatment.

2.3 Study design

2.3.1 Pre-treatment sampling and participant elimination

We enrolled 35 households into our study that met the entry criteria and sampled each house in a two-week pre-treatment sampling survey to assess mosquito productivity at each property. We sampled each house twice during this period, seven days apart, for 24 h each sampling day, from 10 June 2019 until 21 June 2019.

We deployed one BG-Sentinel II trap (Biogents AG, Regensburg, Germany) for 24 h in the backyard of each house each sampling day. Traps were placed ≥1 m from property boundaries and near vegetation to provide some cover. We baited each trap with a BG lure (Biogents AG) and octenol lure (Flowtron Outdoor Products, Malden, MA, USA), and each trap was assigned a specific catch bag and 12 V battery associated with each house. We removed trap collections at the end of the sampling day, froze the contents at −20 °C, and then sorted and identified all mosquitoes to species. Up to 20 *Ae. albopictus* females from each trap were set aside for further processing. All other mosquitoes collected were recorded and stored.

At the end of the pre-treatment sampling period, we ranked each house based on the total number of *Ae. albopictus* females collected. We retained the 25 highest-producing houses for the study and eliminated the ten lowest-producing houses. This strategy was used to avoid houses with little to no *Ae. albopictus* presence, which would limit our ability to statistically detect control effectiveness.[17]

2.3.2 Post-treatment trap setup and maintenance

Before any treatment we randomly assigned each of the 25 households one of five treatments: untreated control, professional treatment (SpLHM) only, professional treatment and five Autocidal Gravid Ovitraps (AGOs) (constructed according to Mackay *et al*.[23] and Barrera *et al*.[26]), professional treatment and five Gravid *Aedes* Traps (GATs) (Biogents AG), and professional treatment and five In2Care traps (In2Care BV, Wageningen, the Netherlands) (Fig. 1). None of the pre-treatment counts from each group were significantly different (Table S1).

Trained technicians of a local mosquito control company (The Mosquito Authority – Raleigh, Durham & Chapel Hill, NC, USA) conducted professional treatment applications at houses designated for treatment conducted professional treatment applications at houses designated for treatment during the third week of the study (hereafter referred to as Week (W)1 of the treatment period) on Day (D)14 and D16. Professional treatment involved LHM and barrier spraying in the front, back and sides of the property. For LHM, all containers with standing water were emptied and removed if possible. Standing water that could not be removed and did not contain fish was treated with Zoecon Altocid Pro-G larvicide (1.5% (*S*)-methoprene; Wellmark International, Schaumburg, IL, USA). For barrier spraying, Bifen IT (7.9% bifenthrin; Control Solutions, Pasadena, TX, USA) was applied following manufacturer and regulatory guidelines, specifically targeting potential mosquito resting habitats and avoiding flowering and edible plants (e.g. fruiting trees and gardens). We deployed all control traps within 24 h of the spray treatment.

We assembled AGOs, GATs and In2Care traps according to manufacturer instructions and filled each trap with tap water (2 L). We formed grass hay bundles (10 g hay +50 g stone) wrapped into a tight ball with tulle and secured with a zip tie, which we submerged in the water of the AGO and GAT traps. Per manufacturer's instructions, we added the supplied odor pellets to the water of the In2Care traps instead of hay bundles to deter animal interference. A 51.4 × 15.7 cm Catchmaster™ glue board (AP&G Co., Inc., Bayonne, NJ, USA) was wrapped around the interior of the AGO entry port, which then was covered with mesh (with 2 x 2 cm openings) and secured with an elastic band. A 15 × 7.9 cm GAT sticky card (Biogents AG) was hung within the GAT trap. A gauze netting treated with In2Mix (74.03% pyriproxyfen and 10% *B. bassiana*; In2Care BV) was secured around a floating fence, which was placed on top of the water in the In2Care trap. We placed traps ≥1 m from property boundaries, near vegetation to provide some cover, and approximately equidistant from the other traps in the yard. Because yard size ranged widely among houses, we could not standardize trap placement and instead used the unique features of each backyard to deploy traps in shady, vegetated areas as an informed homeowner might. Trap locations remained unchanged for the entirety of the post-treatment sampling period.

We placed two BG-Sentinel II traps at each house, once a week, for 24 h, to measure the mosquito populations. BG lures were left unchanged from the pre-treatment sampling period, but new octenol lures were added to each trap at the start of the post-treatment sampling period, W1. The location of the first BG-Sentinel II trap was left unchanged from the pre-treatment sampling period. The second trap was assigned a new location in the yard and left unchanged for the remainder of the post-treatment sampling period. The post-treatment sampling period lasted from 24 June 2019 until 16 August 2019, with two concurrent mosquito collections (from two BG-Sentinel II traps) at each house once every seven days, totaling 16 collections per house.

We assessed AGO, GAT and In2Care traps for maintenance once per week when BG-Sentinel II traps were deployed at each house. We took note of any disturbed traps (e.g. traps that had tipped over or had parts missing) and refilled the traps with ≤2 L tap water as necessary. If an In2Care trap was disturbed and the gauze netting was wet, we removed the gauze and replaced it with a new one, as per the manufacturer's instructions.

We reexamined all control traps at W5 to replace the reservoirs with 2 L fresh water and new hay bundles or odor pellets. GAT sticky cards were replaced. To fulfill the manufacturer's recommendation of replacing the In2Care gauze nettings after one month of use, we replaced all gauze nettings if they had not been changed already as a result of previous trap disturbances. We also replaced the octenol lures in the BG-Sentinel II traps to meet the manufacturer's recommendation of changing them after 1 month of use.

2.3.3 Mosquito processing

We removed BG-Sentinel II trap collections at the end of the sampling day, froze them at −20 °C, and then sorted and identified all mosquitoes. All mosquitoes collected were recorded by species and sex and stored at −20 °C, with up to 20 *Ae. albopictus* females from each trap, each sampling day, set aside for further

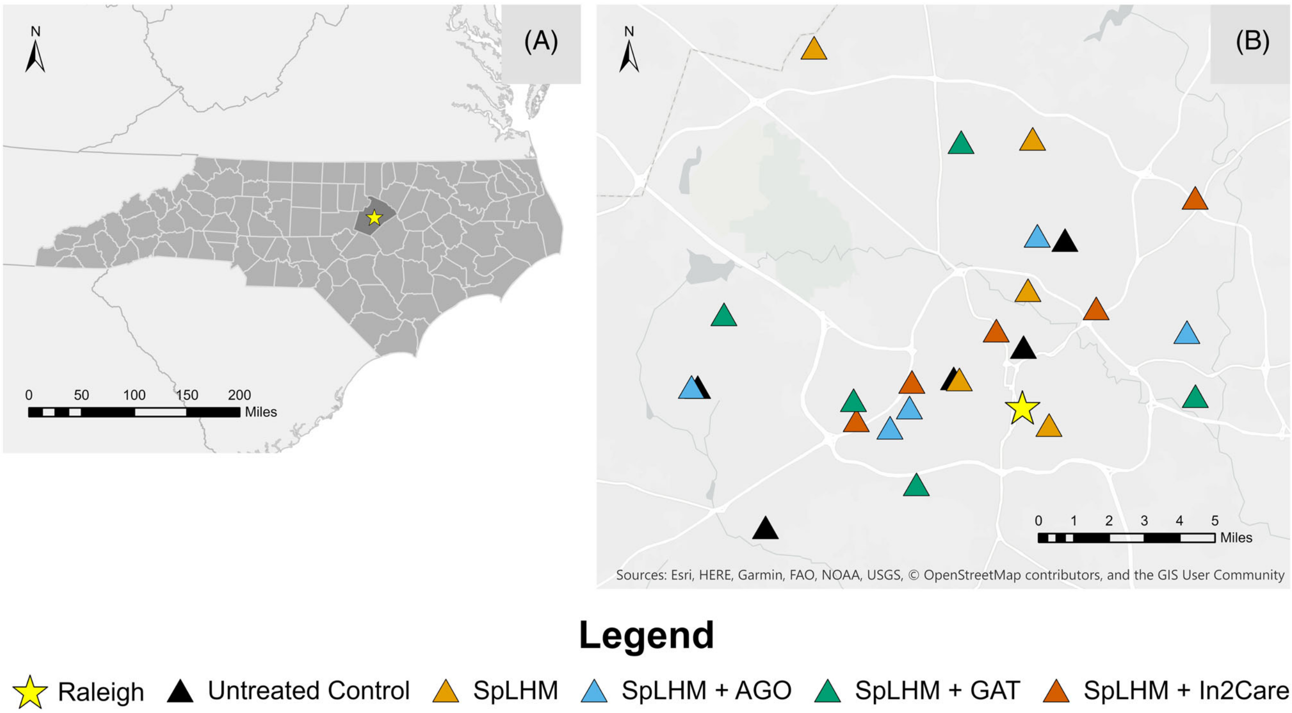

Figure 1. (A) Reference map of North Carolina, with Wake County in dark gray and Raleigh, NC, represented with a yellow star. (B) Map of experimental sites in Wake County in 2019. Household locations represented with triangles (black, untreated control; gold, SpLHM-only; blue, SpLHM + AGO; green, SpLHM + GAT; red, SpLHM + In2Care); yellow star, Raleigh, NC. The figure was produced in ArcGIS Pro 2.4.0 (ESRI) using the Light Gray World Base and Reference maps.

processing. We dissected at least five females per trap, if possible, to determine their parity status following the methodology of Spence Beaulieu *et al.*[50] Dissections occurred in a drop of distilled water on a glass microscope slide under a dissecting microscope. We extracted ovaries from the abdomen with forceps and placed them in a small drop of distilled water on a clean glass microscope slide to dry completely. We covered the dry, plated ovaries with clear nail polish (Revlon Super Lustrous Nail Enamel; Revlon, Inc., New York, NY) to determine parity classification later. We observed the preserved ovaries under a compound microscope to evaluate tracheation. We designated females as parous if they had loosely coiled tracheoles and nulliparous if they had tightly coiled tracheoles. If tracheoles appeared loosely and tightly coiled, we noted this, but considered them as likely being parous. We classified females identified with a blood meal or eggs in separate categories of blood-fed and gravid, respectively. Dissected ovaries too degraded to establish a parity status resulted in a female being classified as undetermined.

2.3.4 *Statistical methods and analysis*

The number of female *Ae. albopictus* captured in each trap was modeled using a generalized linear mixed-effects model (GLMM), assuming a Poisson distribution with a log-link function, and fit using the LME4 package[51] using R.[52] Weeks since treatment and the treatment group, along with their interaction, were included as fixed-effects in the model, whereas the date of observation and trap position were included as a random effect to account for spatiotemporal variation in the population. In addition, we chose to include an observation-level random effect to account for overdispersion in the model.[53] We assumed a Poisson distribution with an observation-level random effect after confirming it accounted for overdispersion and visual comparison of diagnostic plots with those assuming a negative binomial distribution (fitted using the glmmTMB package[54]) (Figs S1 and S2). Comparisons between treatments were calculated as a relative reduction in female *Ae. albopictus*

$$\left(\%\text{Reduction} = \left(1 - \left(\frac{\text{Treatment}_t}{\text{Treatment}_{\text{Pre}}} \Big/ \frac{\text{Control}_t}{\text{Control}_{\text{Pre}}}\right)\right) * 100\%\right)$$

where subscripts denote the counts at time t and the pre-treatment counts, and control is either no treatment or SpLHM, depending on the comparison. All comparisons were done using estimated marginal means, calculated using R/EMMEANS.[55] Parity was modeled using a similar GLMM, assuming a binomial distribution with a logit-link function. Differences between treatments were estimated as the differences between estimated marginal trends using R/EMMEANS.[55]

3 RESULTS

We sampled *Ae. albopictus* for 470 trap days, 70 pre-treatment collections and 400 post-treatment collections. After the pre-treatment sampling phase, we dropped 10 houses at a threshold of less than nine *Ae. albopictus* females captured, with one house eliminated for being a neighbor to another participant.

3.1 Treatment effects: abundance

We collected a total of 4189 female *Ae albopictus* over the ten-week study period. We collected few mosquitoes in the first week of pre-treatment sampling (139 female *Ae. albopictus* in the first week *versus* 643 female

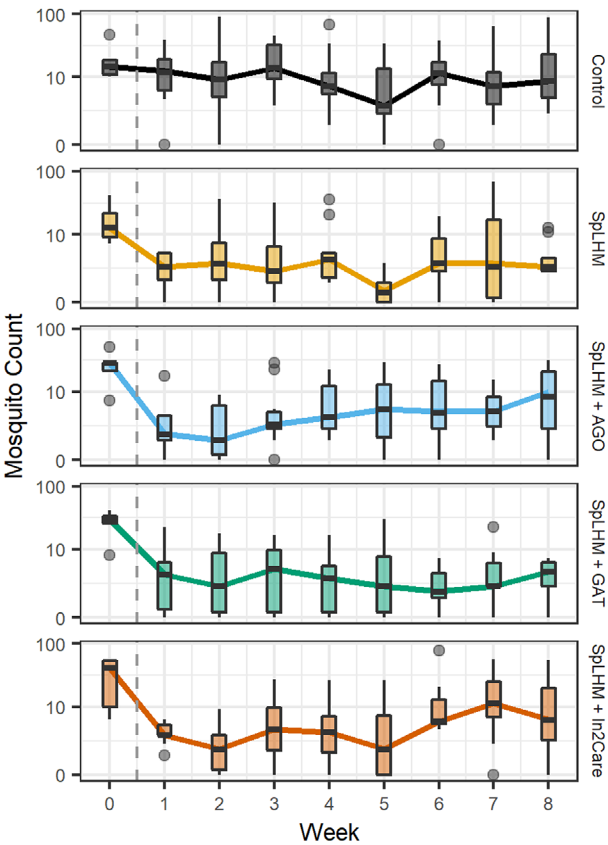

Figure 2. Weekly trap counts. Number of female *Aedes albopictus* recovered weekly from traps in each treatment group (black, untreated control; gold, SpLHM-only; blue, SpLHM + AGO; green, SpLHM + GAT; red, SpLHM + In2Care). Boxplot gives weekly median count and interquartile range for each treatment group, with outliers shown as points. Lines are included to illustrate the temporal trend of median trap counts. Week 0 denotes the pre-treatment measurement, and the vertical dashed line denotes the division between pre-treatment and post-treatment observations. Trap counts for the control group remained relatively stable throughout the study, whereas all other treatment groups saw a sharp decline following treatment. All treatments with the exception of SpLHM + GAT (green) saw trap counts recover to near pre-treatment levels by W8.

Ae. albopictus in W2) and therefore only used data from the second week of the pre-treatment period, hereafter referred to as W0, to compare to the post-treatment period. Figure 2 illustrates weekly trap count comparisons between the treatment groups. We also collected a total of 2297 male *Ae. albopictus* and 163 specimens of other species (116 females and 47 males), but we did not use these data in the final analysis.

Compared to the pre-treatment measurements we saw a 65.4% ($P = 0.0905$) mean reduction in *Ae. albopictus* females at houses treated only with SpLHM compared to untreated controls over the eight-week post-treatment period. Combining traps with the SpLHM application overall resulted in a 74.0% ($P = 0.0159$) mean reduction at AGO houses, a 80.4% ($P = 0.0020$) mean reduction at GAT houses, and a 65.5% ($P = 0.0812$) mean reduction at In2Care houses, compared to untreated controls (Table 1).

To evaluate treatment longevity, we defined the length of *Ae. albopictus* reduction as the last week during the study period for which the adult female population was significantly lower in a treatment group compared to the untreated control group. The SpLHM-only treatment group had fewer *Ae. albopictus* females for the first three weeks compared to the untreated control group, though only the reductions observed in W1 and W3 were significant. The SpLHM + AGO and SpLHM + In2Care treatment groups had significantly fewer *Ae. albopictus* females each of the first three weeks compared to the untreated control group. In the final five weeks of the study beyond W3, SpLHM-only resulted in 36.8–68.9% estimated reduction, SpLHM + AGO resulted in 54.8–66.1% estimated reduction, and SpLHM + In2Care resulted in 10.7–68.4% estimated reduction, but the high variability decreased our confidence in these estimates, and they were not significant at $P < 0.05$. Only the SpLHM + GAT treatment group significantly reduced *Ae. albopictus* abundance up to W8, with the exception of W5 and W7 (Table 1; Fig. 3).

Over the post-treatment period, compared to SpLHM-only treatments, we observed no significant evidence that the addition of traps had an effect on overall mean *Ae. albopictus* abundance (Table 2).

3.2 Treatment effects: parity

All treatment groups exhibited aging *Ae. albopictus* populations, with proportions of parous females increasing as the study progressed (Table 3). Only AGO-treated houses showed a significantly different trend compared with untreated controls; females aged slower in this treatment than in the other treatments ($P = 0.0143$).

Table 1. The percentage reduction in counts resulting from treatment, compared to the untreated control

		Week							
Treatment	Overall	1	2	3	4	5	6	7	8
SpLHM	65.4% (0.0905)	**80.2%* (0.0184)**	71.0% (0.094)	**86.6%* (0.00154)**	36.8% (0.788)	68.9% (0.222)	60.8% (0.281)	47.2% (0.607)	49.9% (0.536)
AGO + SpLHM	**74%* (0.0159)**	**84.4%* (0.00382)**	**91.4%* (<0.001)**	**84.7%* (0.00247)**	66.1% (0.156)	46.4% (0.615)	65.4% (0.164)	61.5% (0.251)	54.8% (0.391)
GAT + SpLHM	**80.4%* (0.00201)**	**80.4%* (0.0106)**	**81.7%* (0.00807)**	**85.3%* (0.0017)**	**80.4%* (0.0119)**	59.1% (0.322)	**88.4%* (<0.001)**	69.8% (0.0979)	**83.9%* (0.00392)**
In2Care + SpLHM	65.5% (0.0812)	**79.0%* (0.0172)**	**90.0%* (<0.001)**	**82.5%* (0.00498)**	68.4% (0.117)	52.3% (0.495)	39.4% (0.721)	10.7% (0.991)	38.0% (0.75)

Note: *P*-values, in parentheses, from contrasts between estimated marginal means between treatment groups. The marginal means were calculated based on best fit GLMM. *P*-values of <0.05 are in bold and noted with an asterisk (*).
SpLHM, barrier spray and larval habitat management; AGO, Autocidal Gravid Ovitraps; GAT, Gravid *Aedes* Traps.

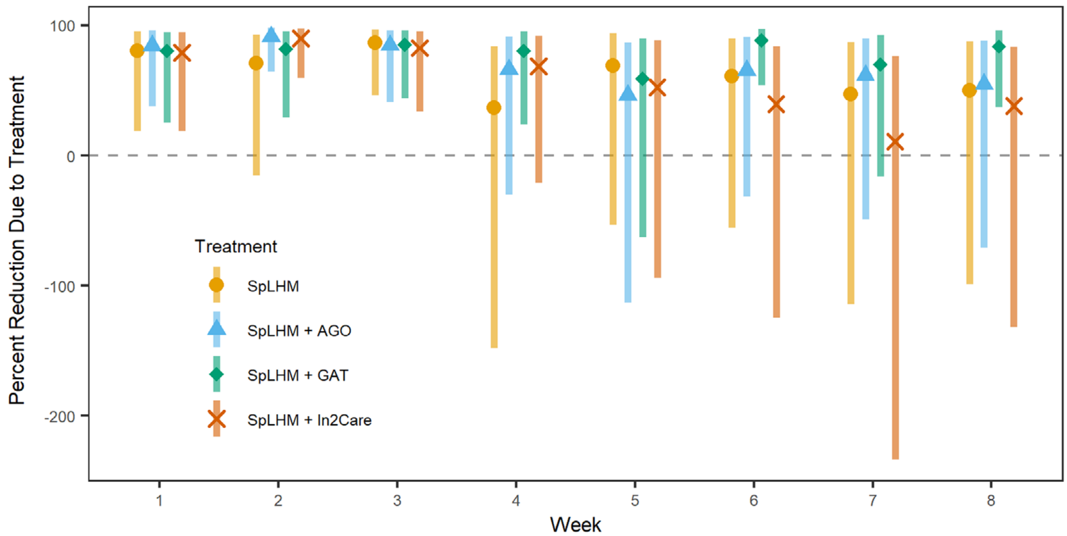

Figure 3. Percentage reduction due to treatment. Percentage reduction in the number of female *Ae. albopictus* each week compared to untreated controls for each treatment group that can be attributed to the treatment (gold circle, SpLHM-only; blue triangle, SpLHM + AGO; green diamond, SpLHM + GAT; red cross, SpLHM + In2Care). Mean estimates are given by points with 95% confidence intervals given by the vertical bars. Horizontal dashed line at 0 represents no effect of the treatment. Reduction is calculated as the proportional reduction in the treatment group compared to the control group (difference in differences). Following application of SpLHM, all treatment groups saw a significant effect of the treatment until W3, with the exception of SpLHM-only (gold circle) on W2. Following W3, only the group receiving SpLHM + GAT (green diamond) saw significant effects (W4, W6 and W8).

4 DISCUSSION

We found that all treatments (SpLHM-only and SpLHM plus traps) significantly reduced female *Ae. albopictus* abundance for the first three weeks post-treatment compared with untreated controls. These findings are consistent with the industry standard of approximately 21 days of mosquito control after a single SpLHM treatment application.[14] Indeed, the SpLHM-only treatment did not significantly reduce populations after W3. Likewise, after W3 reductions were not significant for the In2Care and AGO trap treatments. Most field studies using AGOs investigated large-scale mosquito control, with participant households receiving single or multiple traps to evenly cover a contiguous area, and have focused on *Ae. aegypti*, which is ecologically similar but not identical to *Ae. albopictus*.[25–29,56–59] These studies appeared successful at reducing local mosquito populations and disease transmission following these methods. Field In2Care studies are few, but some experiments involving pyriproxyfen autodissemination stations saw high juvenile mortality across a large treatment area, but no change in adult abundance.[38,49,60–64] These studies did not use *B. bassiana*, which may explain why adult populations were unaffected. We did not conduct any sampling to confirm PPF dissemination, but even if the In2Care traps successfully increased larval and pupal mortality, migrating adults may have contributed to the insignificant changes in adult abundance. However, even when Buckner *et al.*[48] deployed several hundred In2Care traps across 40 ha in Florida, they observed a 57% decrease in adult *Ae. aegypti* abundance, but these results were not significant. They also noted a high labor cost for maintaining the traps, a situation we observed in our own study. The greater maintenance needs of the In2Care traps relative to the AGOs and GATs suggests a potential drawback for practical large-scale control initiatives.

Even though we saw no significant adult reductions using AGOs and In2Care traps with SpLHM after W3, we did observe an effect with the combined SpLHM + GAT treatment, where W4, W6 and W8 saw significant estimated reductions of 80.4%, 88.4% and 83.9%, respectively. Few studies have evaluated GATs under field conditions, and those that have used the traps mainly for surveillance rather than for control.[32,33,35,65] We did not quantify GAT

Table 2. The percentage reduction in counts due to the trap, compared to SpLHM alone

		Week							
Treatment	Overall	1	2	3	4	5	6	7	8
AGO	25.0%	20.9%	70.2%	−14.1%	46.3%	−72.3%	11.7%	27.0%	9.69%
	(0.896)	(0.962)	(0.147)	(0.99)	(0.623)	(0.782)	(0.99)	(0.917)	(0.994)
GAT	43.4%	0.959%	37.0%	−10.1%	69.1%	−31.3%	70.4%	42.8%	67.9%
	(0.568)	(1.00)	(0.802)	(0.995)	(0.136)	(0.955)	(0.130)	(0.707)	(0.169)
In2Care	0.205%	−6.37%	65.6%	−30.7%	50.0%	−53.4%	−54.7%	−69.3%	−23.8%
	(1.00)	(0.998)	(0.209)	(0.940)	(0.532)	(0.872)	(0.806)	(0.715)	(0.963)

Note: *P*-values, in parenthesis, from contrasts between estimated marginal means between treatment groups. The marginal means were calculated based on best fit GLMM. *P*-values of <0.05 are in bold and noted with an asterisk (*).
AGO, Autocidal Gravid Ovitraps; GAT, Gravid *Aedes* Traps.

Table 3. Parity trends in each treatment group, compared to the untreated control

	Trend	Difference from control	Difference from SpLHM
Untreated control	−0.2811	—	—
SpLHM	−0.1316	0.1495	—
		(0.1437)	—
AGO + SpLHM	−0.0579	**0.2232***	0.0737
		(0.0143)	(0.733)
GAT + SpLHM	−0.1543	0.1268	−0.0964
		(0.256)	(0.9807)
In2Care + SpLHM	−0.2155	0.0656	−0.0839
		(0.731)	(0.5892)

Note: Negative values represent shifts towards parity, with more negative values representing a faster shift towards an older population. *P*-values in parenthesis. *P*-values less than 0.05 are in bold and noted with an asterisk (*).
SpLHM, barrier spray and larval habitat management; AGO, Autocidal Gravid Ovitraps; GAT, Gravid *Aedes* Traps.

collections in our study and used the traps only as a control tool. However, the possibility that these relatively inexpensive, low-maintenance traps could significantly reduce *Ae. albopictus* abundance when combined with SpLHM is a promising development for mosquito control and should be investigated further.

We would expect barrier spraying and larval habitat management to successfully reduce populations for approximately three weeks, but GAT-treated yards observed persistently significant reductions in the following weeks after the effect of spray likely ceased. Autocidal ovitraps are designed to remove gravid females from the population, which would help slow the population rebound. However, we could not account for populations migrating from untreated neighbor yards, which may explain the insignificant reductions in the other treatment groups beyond W3. Hollingsworth *et al.*[17] demonstrated a spillover effect of treatment between neighbors, but only for SpLHM, and not beyond 25 days. After the effect of barrier spraying wears off, certain traps may not be able to handle the burden of rebounding populations from within or nearby, suggesting that a larger, neighborhood-scale level of treatment may improve trap effectiveness, as has been noted previously in a similar setting in Maryland, USA.[30] The GATs, however, were successful despite these potential obstacles, and although we could not account for possible underlying causes affecting the observed reductions (e.g. neighbors treating yards, shifting population dynamics, BG placement and yard layout), the results offer a compelling argument for continued field evaluation in combination with chemical controls.

Yards with the SpLHM + GAT treatments observed not only significant week-to-week reductions in *Ae. albopictus* females, but also significant overall reductions compared to the untreated controls. GAT-treated households saw an estimated 80.4% reduction over the eight-week study period. Although SpLHM + AGO treatments did not offer significant reductions after W3, the overall reductions were significant at 74%, suggesting that even if AGOs could not prolong SpLHM effectiveness, they still had a meaningful impact on the existing *Ae. albopictus* population. By contrast, SpLHM-only houses and SpLHM + In2Care houses did not observe significant overall or week-to-week reductions compared to untreated controls over the entire eight-week observation period. Limitations with SpLHM-only would be expected considering the typical 21-day treatment duration, but the lack of evidence for In2Cares to prolong SpLHM or reduce overall *Ae. albopictus* populations within this study design suggests the need to further explore options for using these traps as effective control tools. Compared to SpLHM-only houses, the addition of traps had no effect on overall *Ae. albopictus* female abundance. Even so, AGOs and GATs were predicted to offer 25% and 43.4% additional control, respectively. In2Care traps were estimated to offer no additional control.

In the control yards and treated yards female *Ae. albopictus* populations aged as the study progressed, with higher proportions of parous (i.e. older) females present at the end of the study than in the beginning. These findings would coincide with seasonal dynamics typically observed in *Ae. albopictus*, with greater numbers of young, nulliparous females present early in the season as opposed to later in the season. The one exception in our study was the AGO treatment, which saw the female populations in these yards aging more slowly compared with controls. Ball and Ritchie[66] discovered a bias in the BGS1 traps with respect to *Ae. aegypti* physiological state with significantly fewer nulliparous females captured than most of the gravid, blood-fed and parous female groups. Although our BG-Sentinel II traps may have been biased against collecting some nulliparous *Ae. albopictus*, the data are nevertheless consistent with expected seasonal observations. Importantly, based on recent evidence that a second blood meal significantly increases disease transmission from infected *Ae. aegypti* and *Ae. albopictus*,[67] using AGOs to remove older females could greatly impact the public health threat these species pose.

One limitation in our study design included small sample sizes ($n = 5$ per treatment), which hindered our ability to make strong inferences about the treatment effects. Conducting the study early in the mosquito season (June to August) presented additional problems, as population sizes were likely lower and our inferences on treatment effectiveness may have changed had we performed the experiment later in the summer, when *Ae. albopictus* populations peak.[6,68] Although our effort to improve statistical power and avoid the underlying heterogeneity in *Ae. albopictus* populations by eliminating low mosquito abundance houses helped, a study with increased replication during peak mosquito season may be able to see more subtle effects of control. The observation that control effectiveness of ≤68% was not statistically significant demonstrates the variability in these field data, and the need for increased sample sizes.

5 CONCLUSIONS

Mosquito control techniques must constantly evolve to balance the needs of public health and pest management with the growing risks of insecticide resistance in target species and negative impacts to nontarget species. Combining relatively inexpensive, low-maintenance Autocidal Gravid Ovitraps with barrier sprays and larval habitat management may be one solution for prolonged control with fewer environmental consequences. Not only can AGOs with SpLHM affect disease transmission risk with the removal of older, gravid *Ae. albopictus* females, but AGOs and

GATs offer relief from nuisance pests with overall and persistent reductions in *Ae. albopictus* females when combined with SpLHM. To the best of our knowledge, this is the first field study comparing AGOs, GATs and In2Care traps and their effectiveness at extending adulticidal and larvicidal backyard applications for control against *Ae. albopictus*. Further research exploring the effect of these traps on a large scale when *Ae. albopictus* populations are at their peak and without the combination of SpLHM will offer a better understanding of the practical capabilities of these traps for long-term control.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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