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Truck-mounted area-wide applications of larvicides and adulticides for extended suppression of adult *Aedes albopictus*

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Abstract

BACKGROUND: Given the lack of vaccines for most vector-borne diseases, vector control is often the primary option for disease control. *Aedes albopictus* are difficult to control because the immatures primarily develop in containers ubiquitous in residential properties. Conventional adulticide campaigns often result in brief, rebounding population declines, so incorporating new techniques into an integrated pest management program is imperative. We performed combined area-wide applications of the larvicides *Bacillus thuringiensis* var. *israelensis* and pyriproxyfen with the adulticide sumithrin and prallethrin to achieve extended suppression of *Ae. albopictus* populations in Trenton, NJ, USA. We deployed bioassay cups to assess the spatial penetration and efficacy of the applications.

RESULTS: Inhibition of adult emergence was significantly higher in the treatment bioassay cups than in laboratory controls ($z = 4.65$, $P < 0.0001$) and field control bioassay cups ($z = 8.93$, $P < 0.0001$). We observed a lower trend in adult numbers following season-long combined application of pyriproxyfen and adulticide, with numbers of adult *Ae. albopictus* at the treatment site up to five times lower than at the control site.

CONCLUSION: Pyriproxyfen is a powerful mosquito larvicide and pupacide with low mammalian toxicity that shows promise for area-wide vehicle-mounted (either ground or airborne) applications.

Keywords: area-wide larviciding; *Bti*; pyriproxyfen; public health; New Jersey; USA

1 INTRODUCTION

Worldwide, *Aedes* (*Stegomyia*) *albopictus* (Skuse) is an important vector of dengue, chikungunya and Zika.[1–3] In the USA, *Ae. albopictus* has been the cause of several autochthonous dengue outbreaks since 2000: Hawaii (2001), Texas (2005) and southern Florida (2009–2011).[4–6] This mosquito species is now of concern because of the recent spread of the Zika virus on the American continent. *Aedes albopictus* is a container-inhabiting mosquito, strongly associated with humans, that lays diapausing eggs capable of surviving the winter in cold northern latitudes.[7] Historically, both worldwide and in the USA, most mosquito abatement efforts to control *Ae. albopictus* have focused on the drainage or removal of containers in yards (source reduction) to kill larvae and reduce immature production.[8] This approach requires a large workforce,[9,10] and cost-effective area-wide approaches such as truck-mounted or aerial application of adulticides. These techniques have demonstrated successful suppression of *Ae. albopictus* populations,[11,12] but the results tend to be transient[13] due to emergence from untreated containers or the reintroduction of containers by residents in urban environments.[9,14] The high cost and labor associated with removing or emptying containers (i.e. source reduction),[9,10,15] combined with the observed need for repeated treatments limit the viability of this method as an ongoing control strategy.

As an alternative to source reduction, containers can be treated with a residual larvicide. To reduce the labor costs associated with larviciding campaigns, the use of truck-mounted spray equipment to apply larvicides on an area-wide basis has been examined.[16] This has the added benefit of treating containers in residential properties to which mosquito control personnel have limited access.[17,18] Most studies have applied liquid formulations of *Bacillus thuringiensis* var. *israelensis* (*Bti*) de Barjac 1978 (Valent BioSciences Corp., Libertyville, IL, USA) using truck-mounted ultra-low volume (ULV) sprayers or mist blowers for the successful control of container-inhabiting *Aedes* species.[16,19,20] However, other pesticide formulations utilizing insect growth

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regulators such as pyriproxyfen and methoprene have also shown promise.[21,22]

Pyriproxyfen is a juvenile hormone mimic that suppresses embryogenesis, metamorphosis and adult emergence in insects.[23] Pyriproxyfen is virtually non-toxic to mammals and birds, is non-carcinogenic[24], and is recommended by the World Health Organization (WHO) for use as an insecticide in drinking water sources and containers, because it is safe to humans.[25] It exhibits poor adsorption into soil and movement into groundwater.[26] Although pyriproxyfen has a short half-life in soil, it adsorbs readily to suspended organic matter and can remain active for up to 2 months.[26] Despite this persistence, pyriproxyfen has a very low risk of resistance development because toxicity is maintained.[27] It exhibits excellent efficacy against a wide variety of mosquito species, especially container-inhabiting species such as *Aedes aegypti* L. and *Ae. albopictus*.[28–30] The extremely low median lethal concentration (LC_{50}) values[31] of pyriproxyfen against these species[32] suggests that a few ULV drops of pyriproxyfen within a container may deliver a lethal concentration. Additionally, because of its favorable environmental profile, low risk to vertebrates, efficacy and persistence, pyriproxyfen is an ideal candidate for truck-mounted area-wide larviciding efforts in urban and suburban settings.

Prior insecticide resistance testing showed that *Ae. albopictus* populations from New Jersey were susceptible to both pyriproxyfen and pyrethroid compounds.[33] Therefore, in this study, we tested the combination of area-wide treatment with insect growth regulator (pyriproxyfen) and *Bti* with a combination of pyrethroids against *Ae. albopictus* populations in the northeastern USA.

2 MATERIAL AND METHODS

2.1 Study area

In 2008, the Asian Tiger Mosquito Control Project was undertaken by Rutgers University, Mercer County Mosquito Control, Monmouth County Mosquito Extermination Commission, and the United States Department of Agriculture's Agricultural Research Service to develop strategies for effective area-wide control of *Ae. albopictus* for transference to other mosquito abatement and public health agencies. To explore the effectiveness of an integrated pest management approach for *Ae. albopictus* in Mercer County (NJ, USA), we identified three similar sites of ~1000 residential parcels each to compare treatment effects on mosquito populations utilizing the following guidelines: lot size, socio-economic status, and *Ae. albopictus* abundance. Trials were conducted in Mercer County in the vicinity of the city of Trenton (population ~ 83 000, area 21.1 km^2).[34] The treatment site has been described in detail previously.[9,34] The site on Cummings Avenue (40°21′N, 74°74′W), was 30 ha with 23 residential blocks, occasionally divided lengthwise by a drivable alley. The control site (S Clinton Avenue, 40°12′N, 74°43′W) was 62.4 ha consisting of 48 residential blocks in Hamilton Township.

During applications, bioassay cups were deployed each time in treatment and control sites. We placed bioassay cups (250 mL wide mouth polyethylene jars, Uline, Pleasant Prairie, WI, USA)[16] in 30 residential parcels in the Cummings site (treatment) and five parcels in the S. Clinton site (control). Within each selected parcel, dry bioassay cups were placed in front, alongside and in the backyard of each home. Bioassay cups were placed in the field 2 h prior to application and removed 1 h post application to allow time for droplets to settle into cups.[16] In all, 120 bioassay cups (90 treatment and 30 field controls) were deployed during an application in September.

2.2 Truck-mounted larviciding and adulticiding

The field trial was implemented in 2011, from 8 June to 27 September, covering the full active mosquito season in New Jersey. The timing of larvicide applications was based on a degree day model developed for *Ae. albopictus*.[16] We calculated total degree days for emergence based on cumulative temperature, indicating required mean daily temperature in degrees Celsius before larvae would emerge as adults.[35] However, because pyriproxifen was not labeled for area-wide applications in the USA, we had to apply for an Experimental Use Permit, which only became available in early August 2011(88144-EUP-1). To maximize use of the 2011 active mosquito season we treated the Cummings site on 8 and 15 June 2011 with *Bti* using an Ag-Mister LV-8 low-volume sprayer (Curtis Dyna-Fog, Westfield, IN, USA). Partial results of this study were published in Williams *et al.* showing a reduction efficacy of 72.0 ± 1.3% (based on bioassay cups).[16,36] Subsequently, in early August, which is peak season for *Ae. albopictus* in NJ, we tested pyriproxyfen combined with ULV adulticiding [Duet™ Adulticide: sumithrin, 5%, 44.94 g L^{-1} active ingredient (AI) and prallethrin 1%, 8.99 g L^{-1} AI with the synergist piperonyl butoxide 5%, 44.94 g L^{-1} AI; Clarke Mosquito Control, Roselle, IL, USA]. The combination of larviciding and adulticiding at half the label rate was followed by a second adulticiding also at half the label rate within 2–4 days, depending on weather conditions, using truck-mounted ULV sprays.[37] The purpose of this additional adulticiding campaign was to increase the efficacy of intervention efforts by targeting the adults that emerged because the larvae were already late fourth instar or pupae at the time of the intial larviciding event.[38,39] We repeated pyriproxifen larviciding and pyrethroid adulticiding on 16 September 2011 when the mean number of *Ae. albopictus* was five or more (males and females), which was a pre-set intervention threshold for this study.[40,41] This number was chosen because three bites have been reported as a nuisance threshold driving residents indoors, and an average of five bites per day from *Ae. albopictus* has been recorded as intolerable.[40,42]

We used a truck-mounted Cougar® ULV machine (Clarke Mosquito Control) for space spraying applications of pyriproxyfen. The sprayer generates a cold aerosol using air pressure from a rotary lobe blower powered by a 10 HP gasoline engine. The spray droplet spectra were previously characterized at the Navy Entomology Center of Excellence (NECE, Jacksonville, FL, USA) in May 2010 with a 2D Phase Doppler Particle Analyzer® (TSI Inc., Shoreview, MN, USA). For this study, the transmitter and receiver were outfitted with 300 and 500 mm lenses, respectively, resulting in a measurable droplet size of 0.6–211 μm. Droplet size was measured at the horizontal center of the spray scanning the whole plume vertically in a continuous mode under a variety of flow rates and pressures with three replicated measurements recorded for each test. Mineral oil (BVA 13 ULV Oil, Adapco Inc., Sanford, FL, USA) was used in place of pyriproxyfen for the preliminary tests because the volume median diameter of the mineral oil droplets was found to be within ±3 μm of the pyriproxyfen droplets, regardless of the spray conditions. Final droplet characterizations were performed with pyriproxyfen. Given the efficient design of the spray nozzle, varying the flow rate and air pressure had little effect on the droplet spectra. There was no correlation between flow rate and volume median diameter (VMD, where 50% of the spray volume or mass is contained in droplets smaller than this value) with droplets ranging from 22.4 to 34.0 μm, but as expected, air pressure was

inversely correlated to droplet size ($R^2 = -0.99$).[37] Overall, the average VMD varied by ±10 μm over the range of air pressures tested (3.5 to 7 psi). Pyriproxyfen (NyGuard®, emulsifiable concentrate formulation; 10% AI, MGK Chemical Co., Minneapolis, MN, USA) was tested undiluted and diluted with water at ratios of 1:1 and 1:4. Dilution resulted in a bimodal droplet spectrum with many large and small droplets, but few median droplets. As a result, the VMD values for diluted NyGuard were not reflective of the actual spray plume. It is possible that the water and NyGuard were separating during application. The decision was therefore made to apply NyGuard undiluted for the present experiment.

A Cougar® cold aerosol ULV generator was used during all adulticide applications. DUET™ Dual-action Adulticide was used for adulticiding.[43] The Cougar was fitted with a SmartFlow controller (Clarke Mosquito Control) that registers truck speed obtained from a Global Positioning System (GPS) connected to hand-held computers running DataMaster (Elecdata, Jerome, ID, USA). This system allows for the speed of the vehicle to be used to ensure appropriate flow of insecticide, and accurate reporting and tracking of the amount of chemical used along with distance and area sprayed. The sprayers were mounted in the back of a pickup truck with the spray nozzle at a height of 1.76–1.86 m, and the spray head angled up at 45° and pointing directly out the back of the truck. The vehicle was driven at an average speed of 16.1 km h^{-1} and applications were conducted at a flow rate of 850 mL min^{-1} and an assumed swath width of 90 m for an application rate of 804 mL ha^{-1}. Applications were made to both streets and alleyways between the hours of 02:00 and 06:00 when human traffic was minimal and when meteorological conditions were appropriate for the requirements of a ULV applications[37] During each ULV application, meteorological data was obtained from WeatherUnderground (wunderground.com) and using a Vantage Pro2 (Davis Instruments, Hayward, CA, USA) portable weather station set up within the treatment site 14 h prior to application and maintained until 8 h post application. All pesticide applications were made by county mosquito control personnel under the authority of Title 26:9 of the New Jersey Administrative Code.

2.3 Larval bioassays

To assess the efficacy of pyriproxyfen application we added second to third instars from an insecticide-susceptible reference laboratory colony of *Ae. albopictus*[33] to the bioassay cups shortly after they were collected from the field. The colony was fed on restrained guinea pigs (Rutgers University Animal Use Protocol #86-129) and after hatching, larvae were reared at densities of 500–1000 per pan (22–25 °C, photoperiod of 12:12 h L/D). Larval food (brewer's yeast, 30 mg L^{-1}) was provided twice a week. Between 15 and 17 third instars were added to each cup and the larvae were kept at 26 ± 1 °C and 16:8 h L/D in vertical incubators in a laboratory used exclusively for these studies; fish food was added to the bioassay cups every 2 days. Larval and pupal mortality, as well as adult emergence were recorded to estimate efficacy. Because of miscommunication among personnel, no field controls were available for the August bioassays, only laboratory controls created by adding water and larvae to cups that were never in the field. For the September tests, field control and experimental cups were available. Half of the field controls were kept in the same incubator as the treatment cups. To assess possible contamination across cups during testing (which took over 30 days), the other half of the field control cups were kept at the same temperature and humidity conditions and otherwise handled equivalently but in an incubator in a different building. Additionally, for the September bioassay, we created a set of laboratory control cups kept among the field controls. Tests continued until the last larva or pupa died or adults emerged from every cup.

2.4 Adult surveillance

Surveillance was conducted using Biogents Sentinel™ (BGS) traps (Biogents AG, Regensburg, Germany).[17,44] Trapping locations were selected by laying a 175 m grid over the study sites using the Fishnet tool within ArcGIS Desktop 9.2 (ESRI™, Redlands, CA, USA).[34] These distances were based on the available traps and personnel within the county and on current knowledge of *Ae. albopictus* flight range.[45] The 175 m fishnets resulted in 11 traps within the treatment site (Cummings) and 24 traps in the control site (S. Clinton).

We selected the trapping locations by asking permission from residents located near the center of each Fishnet grid. If we could not obtain approval from a pre-selected resident, we sought permission from the next closest resident. Sampling was performed once a week for 24 h, placing traps in the shaded areas of backyards (near vegetation) for each parcel selected. The same trapping location was used weekly from 30 April to 25 October 2011.

2.5 Egg surveillance

We deployed oviposition cups in each BGS trapping location at the treatment and control sites during the field trials. Oviposition cups (400 mL dark green plastic cemetery vases; Eaton Brothers Corp., Hamburg, NY, USA) were placed at least 5 m from the BGS traps and away from productive larval habitats (i.e. tires). The protocol for egg surveillance has been described elsewhere,[9] but briefly, ovitraps were filled with 300 mL of oak leaf infusion and seed germination paper was used to cover the inside surface. We prepared the infusion by mixing 5 g of dry white oak (*Quercus alba*) leaves per 8 L of tap water in large (> 50 L) trashcans.[9] On the first trapping day (time zero), an ovitrap was placed in a shaded area of the yard and left in the same location for the duration of the mosquito season. When the traps were serviced, germination papers were collected, and cups were emptied and rinsed. Germination papers were changed every 6–9 days, synchronized with BGS trapping events. The number of eggs was counted in the laboratory and a subsample was frozen in TE buffer (10 mM Tris and 0.1 mM EDTA). When confirmation of species identification was needed we used a modified Real-time quantitative PCR-based rapid assay[46] optimized for New Jersey container species.

2.6 Data analysis

The overall proportion of adults that emerged in the laboratory control, field control and treatment cups were compared using beta-binomial regression with a logit link (PROC FMM, SAS version 9.4 for Windows, SAS Institute Inc, Cary, NC, USA). Beta-binomial regression was used to account for overdispersion in the binomial model because of the clustering of observations in the bioassay cups.[47] All pairwise comparisons were performed using Holm's method to control for multiplicity.[48] Statistical analyses were performed only on the results of the September biosassay due to the lack of field controls for the August bioassays.

Time spent as larvae or pupae in laboratory controls, field controls and treatment cups was compared using Cox regression (PROC PHREG, SAS version 9.4 for Windows).[49,50] Correlation among the bioassay cups was accounted for using the robust sandwich covariance matrix estimate.[51] Ties were handled using the

exact method.[52] Time spent as immature mosquitoes was not significantly different for field control 1 and field control 2 ($\chi^2 = 0.092$, df = 1, $P = 0.76$), so results for these controls were combined. All pairwise comparisons were considered and Holm's method was employed to control for multiplicity.

The effect of pyriproxyfen combined with Duet™ applications during the 2011 season on *Ae. albopictus* adults and eggs in the treatment site was investigated by comparing the numbers of adults and eggs collected at the treatment sites with the numbers of adults and eggs collected from the control site using generalized linear model (PROC GENMOD, SAS version 9.4 for Windows; SAS Institute 1985),[49] with treatment, week and treatment*week as predictors. Overdispersion was detected in the Poisson models for adults (deviance/df = 11.18) so the models were refit using the negative binomial distribution (deviance/df = 1.1). The model regressed the number of adults by treatment (treatment versus control sites) and blocked by week resulted in several large Pearson standardized residuals, indicating that these data did not fit the model. Once we removed outliers, the model fit better to the data set (likelihood ratio test $P = 0.12$). Overdispersion was detected in the Poisson models for eggs (deviance/df = 56.54), so the models were refit using the negative binomial distribution (eggs; deviance/df = 1.02). Because the number of days oviposition cups remained in the field varied for each sampling (egg papers were left in the field between adult surveillance events), the number of eggs was divided by the number of days they were deployed and the final number was multiplied by 7, to obtain the number of eggs per 7 days.[9]

3 RESULTS

We surveyed the bioassay cups for up to 43 days until all larvae or pupae had died or adults had eclosed. We observed a smooth decline in numbers of larvae over several weeks without evidence of early high mortality associated with possible depositions of active pyrethroids from the adulticide applications. The proportions of adults that emerged from the field control cups interspersed among the treatment cups and those kept in a separate laboratory were not statistically different ($z = 0.19$, $P = 0.85$) and so were combined. We observed that 98.7% of the *Ae. albopictus* larvae emerged as adults from the laboratory controls, followed by 76.6% emergence from the field control; these values were statistically different ($z = 2.26$, $P = 0.02$) indicating possible cross-contamination during retrieval and transportation from the field but not in the laboratory.[53] Adult emergence from laboratory control bioassay cups ($z = 4.65$, $P < 0.0001$) and field control cups ($z = 8.93$, $P < 0.0001$) was significantly higher than from treatment cups (Table 1, Fig. 1). Overall, there was an 81% reduction in emergence from treatment cups compared with aggregate control cups.

BGS traps collected 6900 adult *Ae. albopictus*; 1567 (22.7%) from treatment sites and 5333 (77.3%) from control sites. The main treatment (pyriproxyfen and pyrethroid) effect was significant ($\chi^2 = 14.74$, df = 1; $P = 0.0001$) (Fig. 2) throughout the mosquito season, but interactions between treatment and week were not significant ($\chi^2 = 2.7$, df = 1; $P = 0.1$). A total of 26 945 *Ae. albopictus* eggs were collected; 9025 (33.4%) from treatment sites and 17 920 (66.6%) from control sites. Neither interaction between treatment and week was significant, nor was the main treatment effect ($\chi^2 = 0.27$: df = 1; $P = 0.60$) (Fig. 3). We observed a lower trend in adult numbers following the last pyriproxyfen and adulticide application (16 September 2011), with adult numbers up to five times lower than in the control site (Fig. 2).

Table 1. Odds of adult emergence in laboratory control, field control and pyriproxfen cups

	Laboratory control		Pyriproxyfen
	5	30	80
No. of larvae per cup (range)	15–17	15–19	15–19
Total no. larvae tested	80	467	1264
Total no. adults emerged	79	358	252
Odds ratio (95% CI)	131.50 (16.80, 1029.26)	12.28 (7.08, 21.30)	Reference
	10.71, (1.37, 83.81)	Reference	—
Hazard ratio (95% CI)	11.31 (7.37, 17.35)	6.10 (4.26, 8.73)	Reference
	1.85 (1.39, 2.47)	Reference	—

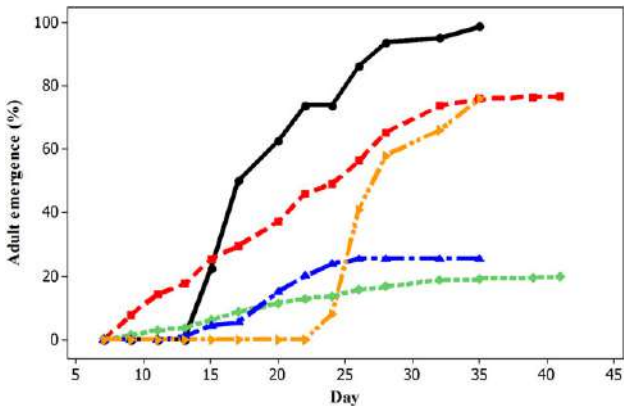

Figure 1. Survival curves of mosquito larvae placed in bioassay cups deployed at the treatment and control sites. Emergence was monitored daily for 35 days for August bioassays and 43 days for September bioassays. Orange line, August control; blue line, August treatment; black line, September laboratory control; red line, September field control; green line, September treatment representing adult emergence from bioassay cups.

4 DISCUSSION

One of the greatest challenges in managing container-inhabiting *Aedes* mosquitoes is to control the immature stage that quickly replenishes the adult populations following adult control. To address this limitation, we tested truck-mounted area-wide applications of *Bti* and pyriproxyfen in combination with an area-wide application of adulticides. Williams *et al.* previously demonstrated the success of area-wide *Bti* application by achieving > 90% larval mortality in post-application bioassays;[16] however, they did not examine the effects on adult populations. Here, we showed that truck-mounted area-wide applications of pyriproxyfen also successfully delivered significant amounts of larvicide to bioassay cups in residential properties. Although adulticiding alone

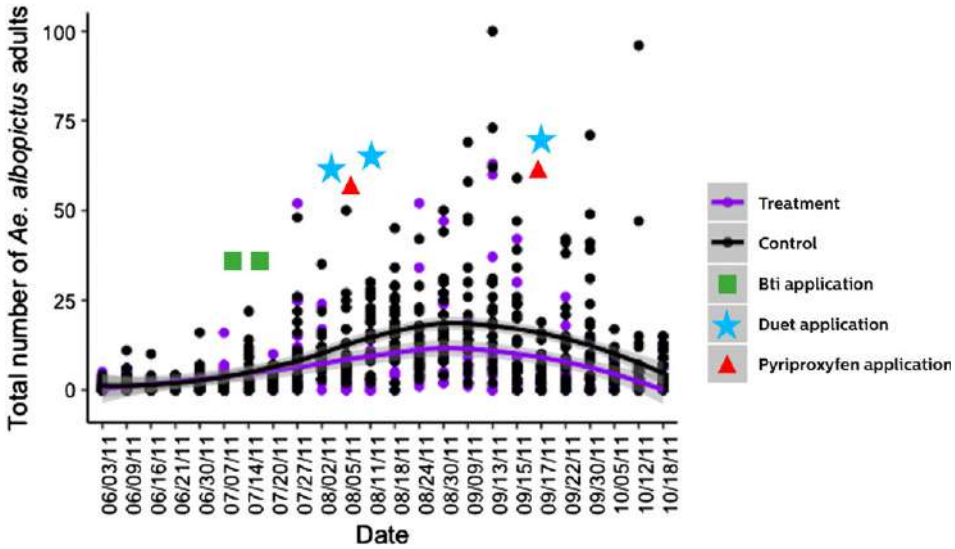

Figure 2. Observed counts by site polynomial regression lines (treatment = purple line and purple circles and control = black line and black circles) for total number of female and male adult *Aedes albopictus* collected in BGS traps. Eleven and 24 BGS traps were deployed at the treatment and control sites once a week for 24 weeks.

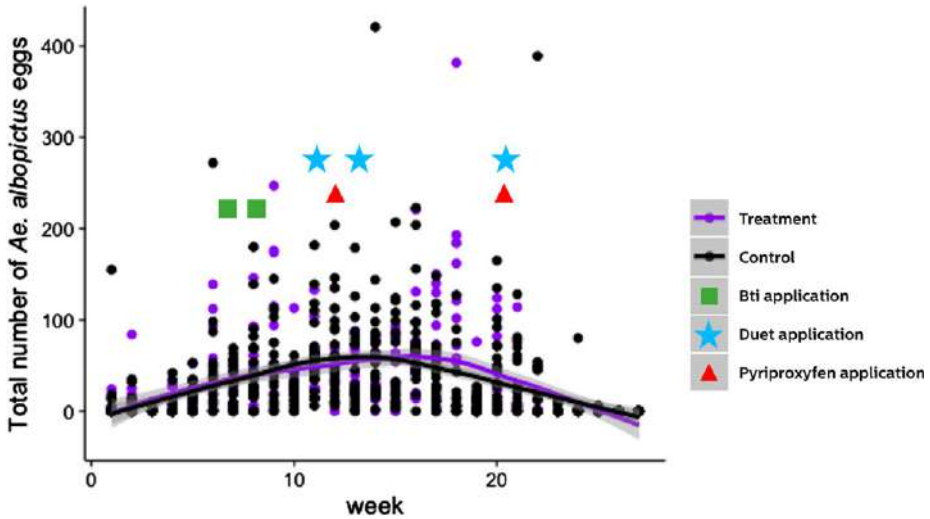

Figure 3. Observed counts by site with polynomial regression lines (treatment = purple line and purple circles and control = black line andblack circles) for total number *Aedes albopictus* eggs collected in oviposition cups. Eleven and 24 oviposition cups were deployed every 7–9 days at the treatment and control sites for a total of 24 weeks.

has only short-lived effects,[37] we found that a combination of adulticiding and larviciding ultimately reduced the numbers of *Ae. albopictus* adults in the treatment site during the entire mosquito season, demonstrating extended control with a small number of area-wide applications.

The need to obtain an Experimental Use Permit prior to the experiment complicated our ability to examine the direct effect of area-wide pyriproxyfen applications. Uncertain if we would obtain the permit and trying to prevent populations of *Ae. albopictus* from growing unchecked, we used *Bti* as a larvicide in the treatment site that later was treated with pyriproxifen. This means that we cannot easily separate the effects of each larvicide, particularly because early in the season when the populations of *Ae. albopictus* are still recovering from the winter and numbers of adults still low, it is difficult to observe significant effects. Therefore, the more dramatic effects in adult numbers were observed following the pyriproxyfen application in August, the peak season. A similar pattern was observed by Doud *et al.* that reported their second and third applications of pyriproxyfen achieved more reduction in adult populations.[21] Although bigger effects during peak may explain these results, it is also possible that accumulation of pyriproxyfen due to long residual effects could result in greater mortality after repeated applications. There are still questions to be addressed, but our results indicate that a combination of larviciding and adulticiding can be an effective method for area-wide management of *Ae. albopictus* populations over a season, which is supported by other studies.[16,37]

Although no resistance to pyriproxyfen was detected in New Jersey populations, significant but low resistance to pyriproxyfen was found in Florida[33] and a recent study showed that pyriproxyfen is metabolized by P450s (detoxification enzymes) associated with pyrethroid resistance in *An. gambiae*.[54] If this insecticide is to be used in future in the USA, constant monitoring of the insecticide resistance status of *Aedes* populations is recommended.

Interestingly, we did not find a significant difference in eggs collected from the treatment and control sites. Focks discussed the problems with egg data using oviposition cups, emphasizing the effect of skip oviposition behavior and competing containers.[55] Although oviposition cups are suitable for determining the presence or absence of dengue vectors (*Ae. aegypti* and *Ae. albopictus*), they are not reliable for adult population estimation alone. Therefore, egg data should be collected in tandem with adult data.[9] Based on our experience in urban New Jersey, the numbers of eggs in oviposition cups do not correlate with the numbers of females, especially during dry summers.[9] By contrast, Suter *et al.* showed 2.26 times higher egg density in their control site compared with the intervention site,[56] and their findings are in agreement with similar studies conducted in Italy indicating a positive correlation between treatment and egg numbers.[57,58] While Suter *et al.* attributed the lack of correlation between adult and egg data of dengue vectors to the choice of statistical analyses, following Suter *et al.* we used a negative binomial model working directly from the actual count data and still did not find a significant correlation.[56] Our conclusion is that due to conflicting results between using eggs and adult populations for surveillance of invasive *Aedes* mosquitoes, researchers should be cautious while considering either or both of these methods.

Aedes albopictus management is a challenge for mosquito abatement programs in the USA because current control methods are developed for floodwater and saltmarsh mosquitoes.[59,60] *Aedes albopictus* can be described as a backyard mosquito with numerous, highly dispersed larval habitats, most of which are artificial containers (buckets, tires, plant saucers, etc).[13,18] This underlies the difficulty in eliminating larval habitats because many of the target containers are located within private residential properties that are often inaccessible to mosquito control personnel. Even after permission is obtained in some areas, each container must be treated or removed, and despite complete removal, new containers frequently reappear.[14] Even if source reduction is effective alone or combined with other control methods, it is time and labor intensive, and thus also expensive.[9,13] The need for area-wide applications of larvicide is obvious to help control invasive *Aedes* mosquitoes.

Lam *et al.* treated vegetation with *Bti* using backpack mist blowers and truck-mounted ULV equipment in Singapore.[61] Human landing rates showed significantly lower adult abundance in treatment areas compared with control sites following *Bti* applications.[61] In St. Augustine, Florida, researchers demonstrated that they delivered pyriproxyfen to bioassay cups using a truck-mounted ULV sprayer and achieved a 50% reduction in adult numbers in treatment sites.[21] Although, as explained, we cannot specifically attribute the season-long reduction in adult numbers to pyriproxifen alone or the early season *Bti* alone, we found that larvicide applications in tandem with adulticide applications when adult catches crossed a threshold can be effective. Our results agree with previous studies on combined control methods. Chebabi *et al.* used source reduction, larviciding (*Bti* and diflubenzuron) and adulticiding (alfacipermetrin) to control *Ae. albopictus* populations in Catalonia, Spain, and showed a significant reduction of the number of eggs recorded in the treated areas.[11]

Because of regulatory constraints and the minor economic market that mosquito control represents, few new active ingredients are being developed. An alternative is to repurpose existing active ingredients from other industries, of which pyriproxyfen may be a prime candidate. Pyriproxyfen is already used against a wide range of agricultural and structural pests.[62,63] However, pyriproxyfen is not labeled as a mosquito larvicide for use with vehicle-mounted (either ground or airborne) application equipment.[21] Our truck-mounted studies required a label exemption (experimental use permit) granted by the U.S. Environmental Protection Agency. This study indicates that this exceptionally low-risk and highly effective active ingredient deserves further investigations as a tool against invasive *Aedes* and other mosquitoes.

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