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Area-wide management of *Aedes albopictus*. Part 2: Gauging the efficacy of traditional integrated pest control measures against urban container mosquitoes†

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Abstract

BACKGROUND: ***Aedes* (*Stegomyia*) *albopictus* (Skuse) is an important disease vector and biting nuisance. During the 2009 active season, six ∼1000-parcel sites were studied, three in urban and three in suburban areas of New Jersey, United States, to examine the efficacy of standard integrated urban mosquito control strategies applied area wide. Active source reduction, larviciding, adulticiding and public education (source reduction through education) were implemented in one site in each county, an education-only approach was developed in a second site and a third site was used as an untreated experimental control. Populations were surveyed weekly with BG-Sentinel traps and ovitraps.**

RESULTS: A substantial reduction in *Ae. albopictus* populations was achieved in urban sites, but only modest reductions in suburban sites. Education alone achieved significant reductions in urban adult *Ae. albopictus*. Egg catches echoed adult catches only in suburban sites.

CONCLUSIONS: There are significant socioeconomic and climatic differences between urban and suburban sites that impact upon *Ae. albopictus* populations and the efficacy of the control methods tested. An integrated pest management approach can affect abundances, but labor-intensive, costly source reduction was not enough to maintain *Ae. albopictus* counts below a nuisance threshold. Nighttime adult population suppression using truck-mounted adulticides can be effective. Area-wide cost-effective strategies are necessary.

Keywords: integrated mosquito management; BG-Sentinel traps; ovitraps; door-to-door source reduction; education; larvicides; adulticides; New Jersey

1 INTRODUCTION

Since 1994, the USDA Agricultural Research Service (ARS) has been funding area-wide integrated pest management projects focusing specifically on invasive species.[1,2] Area-wide integrated pest management (AW-IPM) programs reflect the realization that pest movement requires a large-scale (area-wide) approach that needs to be balanced by the need to minimize the development of insecticide resistance as well as preserve local ecosystems and human health, the core drivers of IPM.[3] An AW-IPM program aims to reduce (manage) pest populations in a broad geographic area to levels that are below acceptable economic or health costs[4] and requires the development of combined strategies involving operational intervention, education and economic evaluation. Owing to the scale on which they have to be implemented, successful AW-IPM projects have been developed with extensive input from farmers, citizens or pest control professionals. Therefore, successful area-wide programs have benefited from experience and ideas generated by the customer-stakeholder base.[4]

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Aedes (*Stegomyia*) *albopictus* Skuse, the Asian tiger mosquito, is native to Asia,[5] but presently this aggressive human-biting species infests at least 28 countries outside its native range on all inhabitable continents.[5,6] *Aedes albopictus* is a container-inhabiting mosquito strongly associated with human habitats (especially outside its native range) and is capable of laying diapausing eggs that survive even the cold northern temperate latitudes in its native (e.g. northern Japan and China) and introduced (e.g. Europe and northeastern United States) ranges. The used tire trade is thought to have led to the recent spread of the species[7] and is still the most likely conduit for its long-distance spread, although the trade of small potted plants with water reservoirs, such as 'lucky bamboo', has also been implicated.[8,9] Detailed theoretical analyses indicate that the spread of *Ae. albopictus* may well continue into many more regions of the world.[5,6] Importantly, the epidemic of chikungunya fever occurring in Africa and the Indian Ocean Basin since 2004[10] was principally caused by *Ae. albopictus*, after mutations in the chikungunya virus (CHIKV) increased the vector competence of *Ae. albopictus* for this virus.[11,12] Although chikungunya fever has not spread broadly in the temperate zone, an epidemic in northern Italy in 2007 affected over 200 people,[13] and small numbers of locally transmitted CHIKV cases have been identified in southern France since 2010.[14] It is probable that the European expansion of CHIKV would not have been possible without the prior invasion of Europe by *Ae. albopictus*.[15]

Diseases caused by pathogens transmitted by vectors are not new to the United States or to Europe.[16,17] After the discovery in the late nineteenth century that mosquitoes could transmit disease-causing pathogens,[18] and also as residential communities expanded into coastal areas and prime mosquito habitat, organized mosquito control programs in many states were established to control brackish and salt-water species.[19] From its early beginnings, with few tools[20] but notable successes in Cuba, Panama, Greece and Italy,[21] mosquito control has become a multifaceted procedure with many techniques, inputs and concerns.[22] The application of modern AW-IPM programs to control *Ae. albopictus* is therefore a logical consequence of the expansion of its range, its status as a day-biting pest and its potential as a disease vector.

The goal of the area-wide project for the management of the Asian tiger mosquito (AW-ATM) is to examine the area-wide efficacy and sustainability of existing strategies for reducing the nuisance and threat to human health posed by *Ae. albopictus*. The project is based on detailed comparisons between previously defined groups of 1000 parcels (= single-home and surrounding yard) in urban and suburban settings in two counties of the state of New Jersey.[23] In each county, the abundances of *Ae. albopictus* in sites where specific control strategies were performed were compared with those in a no-intervention (untreated) site.

Several researchers have advocated that source reduction, which is the removal or destruction of containers that can hold water during larval development and pupation, is the best method for efficient control against *Ae. albopictus*.[24,25] Indeed, others have reported that source reduction campaigns achieved temporary suppression of immature *Ae. albopictus* in North Carolina[26] and more recently in Spain.[27] Therefore, source reduction is often the first strategy for control of *Ae. albopictus*, as well as of other peridomestic container mosquitoes. As containers in private residences are overwhelmingly the primary sources of *Ae. albopictus*,[28] education campaigns helping the public to identify and eliminate small water containers from their properties have become a basic component of organized mosquito control.[22] When containers cannot be removed or emptied regularly, larvicides are applied to prevent mosquito production. If adults become a serious nuisance, insecticides targeting the adults are applied. The current standard procedure is to apply this methodology locally, inside a yard or at most a housing block, as a reply to a request for service from citizens. The present objective was to test the efficacy of aggressive reduction by removal or treatment with larvicides of larval habitat and if necessary the use of adulticides. The efficacy of container reduction by the public, instigated by extensive education campaigns, was examined separately.

2 MATERIALS AND METHODS

2.1 Description of study sites

The present study focused on six predefined ∼1000-parcel sites (a parcel is the combination of a house and its surrounding yard), ranging in area from 1 to 2 km^2, with similar socioeconomic parameters, geography and *Ae. albopictus* abundance. For details of where the sites were located, how they were chosen and their similarity in socioeconomic terms, human population density and *Ae. albopictus* populations, please refer to the first paper in the series.[23] A group of three equivalent sites was established in Mercer County (centered at 40° 21′ N, 74° 74′ W) and another group of three in Monmouth County (40° 44′ N, 74° 17′ W), New Jersey.

One of the sites in each county was subjected to multiple types of intervention aimed at controlling *Ae. albopictus*, including education, and this site will hereafter be referred to as the 'full intervention site'. In a second site, interventions related only to informing and engaging the public to remove water-filled containers from their yards that could become sources of *Ae. albopictus* were implemented. This site will be referred to as the 'education site'. A third site was left undisturbed except for weekly mosquito surveys performed on the same scale and intensity as in the other two sites, and acted as an experimental control 'untreated site'. As school districts comprise entire municipalities, and the desire was to investigate the effect of education campaigns on *Ae. albopictus* populations, these activities were designed so that they would impact only upon the elementary schools and citizens located within the full intervention and education sites.

Based on the 2008 study of these same sites,[23] in Mercer, South Olden was chosen as the full intervention site and Cummins as the education site, both located within the city (and township) of Trenton. To prevent 'information contamination' from influencing the ability to assess the impact of the education-only campaigns, the untreated site in Mercer County was assigned to South Clinton, located in the township of Hamilton, and therefore in a different school district to the other sites. In Monmouth County, the treatments were assigned randomly across the three study sites, as they were shown to have similar socioeconomic characteristics and equivalent populations of *Ae. albopictus* and were all located in different municipalities.[23] Thus, Cliffwood Beach became the full intervention site, Middletown became the education site and Union Beach became the untreated site.

The intervention phase focusing on source reduction was started on 27 April and 30 April 2009 in Mercer and Monmouth counties respectively, before any larvae or adult *Ae. albopictus* had been observed. Mosquito surveillance was started on 12 May 2009 in both counties and continued up to 24 and 12 November 2009 in Mercer and Monmouth, respectively, at which point the number of *Ae. albopictus* in any of the traps being used had been zero for at least 1 week.

2.2 Education

Public education consisted of elementary-school-based education and adult outreach. An in-depth account of the public education efforts developed in 2009 has been published;[29] please refer to it for specific information. Briefly, a 5 day elementary school curriculum was developed, geared towards 8–12-year-old children (3–5 elementary school grades). Each component of the curriculum adhered to New Jersey State Science Curriculum standards for that particular age group, and included information about mosquito life cycles, food chains, biology, problem-solving and classification. Each component consisted of a lecture, hands-on activity and an assignment. To educate adult residents over the spring and summer of 2009, the authors handed out four brochures in the full intervention and education sites. Materials also included links to their educational website (http://www.rci.rutgers.edu/~krisb/ATM/), where the public could find additional information on the Asian tiger mosquito, videos of mosquitoes and source reduction, maps tracking the distribution of *Ae. albopictus* (the Asian tiger mosquito), kids' pages with games and activities and a weekly blog.

2.3 BGS surveillance

As in 2008, in 2009 it was decided to monitor adult populations of *Ae. albopictus* with BG-Sentinel^{TM} traps (BGS traps; Biogents AG, Regensburg, Germany); however, because the objective this time was to compare sites to assess the effect of interventions, it was also decided to minimize variance by using fixed collection sites instead of sampling randomly across each site every week, as in 2008.[23] The fixed collection sites were chosen by overlaying a grid of specific distance intervals, creating a group of cells (Fig. 1). A 200 m grid was used for BGS trap placement in Mercer County, and a 300 m grid for Monmouth County, as the sites in Monmouth are much larger than those in Mercer.[23] The grids for determining trap placement were constructed using the Fishnet tool within ArcGIS Desktop 9.2 (Environmental Systems Research Institute, Redlands, CA). These distances were based on current knowledge of *Ae. albopictus* flight range[30,31] and the ability to deploy and collect all BGS traps in each county within 24 h. The 200 m grid resulted in 12 traps per full intervention site, 10 traps per education site and 15 traps per untreated site in Mercer County, while the 300 m grid resulted in 21 traps per full intervention site, 13 traps per education site and 23 traps per untreated site in Monmouth County (Fig. 1). Therefore, 94 BGS traps were deployed weekly during the 2009 mosquito season. The choice of fixed surveillance

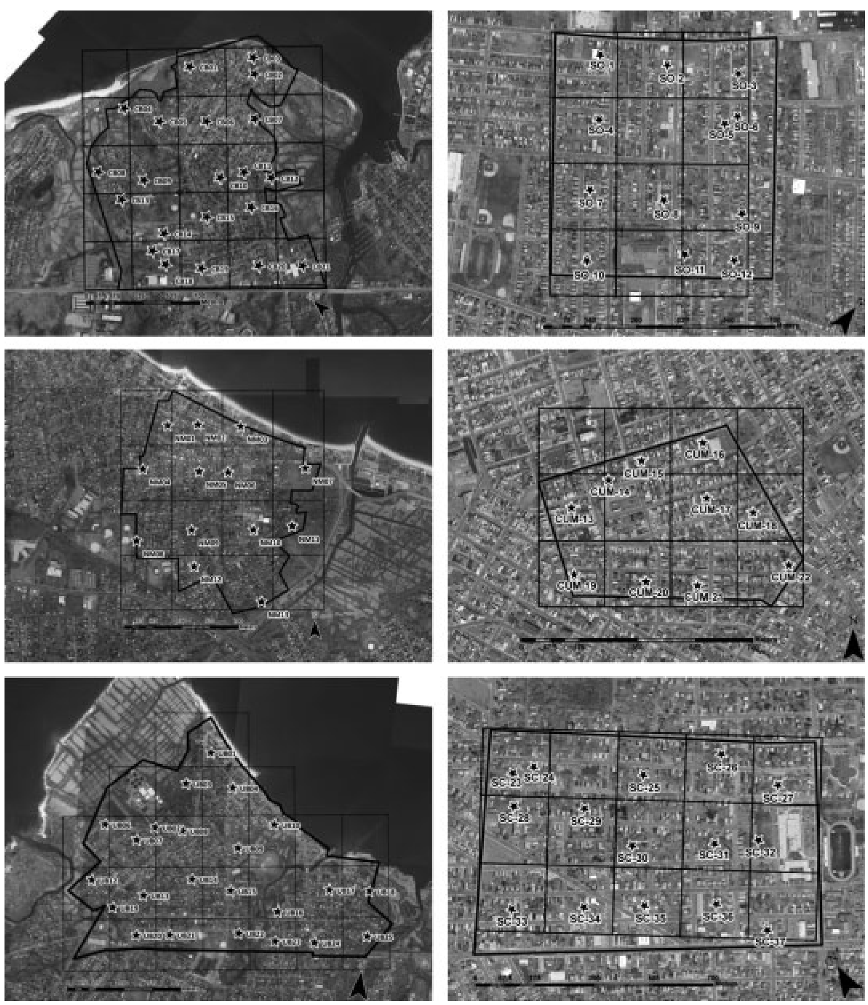

Figure 1. Aerial overview of experimental sites in Monmouth and Mercer counties. From top to bottom: full intervention, education and untreated sites. On left, Monmouth County; on right, Mercer County. The scale of Mercer County is half that of Monmouth County (1:5000 ft and 1:10 000 ft). The grid cell width in the Monmouth sites is 300 m, and in the Mercer sites 200 m.

locations was initiated by asking permission from residents located near the center of the grid and proceeding to neighboring parcels until a suitable location was found. Sampling was performed with BGS traps deployed continuously for 24 h in the chosen yards. Shaded areas were targeted for trap deployment because traps experimentally located in the shade collected significantly more *Ae. albopictus*.[32] Each week, traps were placed in the same location within the backyards. The BGS trap was used with the human BG-Lure (BioGents GmbH, Regensburg, Germany) because previous studies had shown this lure to be most effective for capturing *Ae. albopictus*.[33] Each BGS trap was also fitted with an iButton® Hygrochron (Maxim, Sunnyvale, CA) which recorded local temperature and humidity. Each iButton was inside a small mesh pouch suspended from the BGS crossbar and was set to record data every 60 s without rollover.

2.4 Ovitrap surveillance

To assess the usefulness, compared with BGS traps, of an inexpensive trap that has already been used extensively to survey and model *Ae. albopictus*,[34–36] oviposition cups (ovitraps) were deployed approximately 5 m away from each BGS trap (Fig. 1). The ovitraps were set and collected once a week. Dark-green plastic cemetery vases (400 mL capacity; Eaton Brothers Corp., Hamburg, NY) were staked into the ground to reduce the probability of being disturbed by lawn maintenance, wildlife or environmental conditions. The vases were filled with 300 mL of oak leaf infusion, and germination paper was placed to cover the inside surface. Two small holes were predrilled above the 300 mL water level to prevent the vases from being completely filled with water after a rain event, which would reduce the oviposition surface to zero. The ovitrap infusion was prepared by mixing 5 g of dry oak leaves per 8 L of tap water in large (>50 L) trashcans. Oak leaves were used because previous studies had reported that oak leaf infusions elicited oviposition responses from container-inhabiting *Aedes* mosquitoes.[37] To prepare the infusion, fallen white oak (*Quercus alba*) leaves were collected in a single site and used throughout the season in both counties. The oak leaf infusion fermented for 1 week before use, and any batch was in use for no more than 2 weeks, which meant that a new infusion batch was started every 2 weeks. On the first trapping day (time zero), an ovitrap was placed in a shaded area of the yard and remained in the same location for the duration of the mosquito season. Every week the germination papers were collected, cups were emptied and rinsed and new germination paper and oak leaf infusion were added. Broken and stolen cemetery vases were replaced as required. Egg papers were placed in labeled plastic bags to maintain humidity and limit egg desiccation and taken to the laboratory. There, the number of eggs was counted under a dissection microscope and recorded. Because other species of *Aedes* in New Jersey, such as *Ae. triseriatus* (Say), *Ae. atropalpus* (Coquillett) and *Ae. japonicus japonicus* (Theobald), will, like *Ae. albopictus*, oviposit in small water-filled containers, and because their eggs are not easy to identify by non-specialists, it was decided to hatch all eggs and rear the larvae to third instar for identification using the key in Farajollahi and Price.[38] In particular, eggs of *Ae. japonicus*, another recently introduced species,[39] are very similar to those of *Ae. albopictus*, and their larvae are commonly found in sympatry in New Jersey.[28] In the laboratory, positive egg papers were submersed completely and kept under water for 7 days. A quantity of 5 mg of ground rat chow per 500 mL of tap water was used as a hatching stimulus, and the containers were kept at 25 °C in an incubator.

2.5 Door-to-door source reduction and larvicide applications

Source reduction and larvicide applications were carried out monthly in the full intervention sites in both counties (Fig. 2). Between the last week of April and the end of October, crews inspected yards and attempted to remove, destroy or treat with larvicides or pupacides every existing or potential water container.

In Mercer County the full intervention site in South Olden was 48.6 ha and included 1251 parcels, while in Monmouth County the full intervention site in Cliffwood Beach was 156.1 ha with 1247 parcels. To allow progress to be tracked carefully, the full intervention sites were subdivided and assigned to different teams. Owing to the unique characteristics of the urban and suburban landscapes, different strategies were necessary in each county. In Mercer the full intervention site was divided into the 77 city blocks, while in Monmouth the site was divided into nine zones, with several residential blocks per zone and with easily identified streets as boundaries. Each residence in each full intervention site was informed about the nature of the control program, and source reduction and larviciding were implemented when possible and as needed. In Mercer, a Superintendent Labor Assistance Program (SLAP) through the Mercer County Correction Center was engaged on 18 May and 15 June 2009 to help clean the narrow alleys between backyards as well as some abandoned parcels.

When source reduction (removal or destruction of water-holding containers) was not feasible, either one or a combination of two larvicides was applied with different modes of action:[40] a slow-release pellet formulation of the insect growth regulator methoprene (Altosid® pellets; Wellmark International,

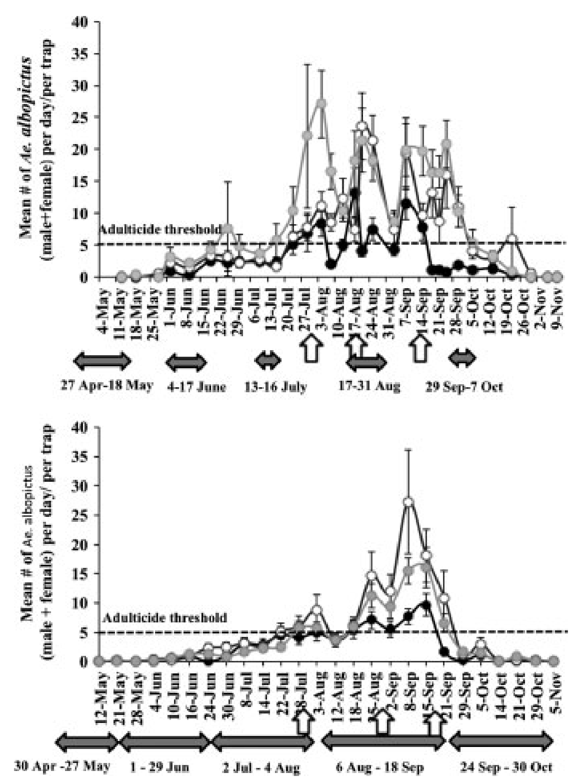

Figure 2. BGS adult collections and summary of main control interventions (except for education-only interventions). A, Mercer County; B, Monmouth County. Black symbols: full intervention site; grey symbols: education; white symbols: untreated. Longitudinal arrows represent the door-to-door interventions; vertical arrows indicate adulticide applications.

Schaumburg, IL) and a monomolecular surface film (Agnique® MMF G; Cognis Corporation, Cincinnati, OH). Mosquito-larva-positive and larva-negative containers were both treated with Altosid® pellets. Pupa-positive containers were treated with an Agnique® MMF G and Altosid® pellet combination.[40] In addition to the larvicides used in Mercer County, for larval control in catch basins, Monmouth County employed Altosid® briquettes (Wellmark International, Schaumburg, IL) as well as Altosid® pellets, and in swimming pools Altosid® XR extended residual briquettes (Wellmark International, Schaumburg, IL). The active ingredient in both products is the insect growth regulator methoprene, and the residual effectiveness for pellets and briquettes is 30 and 150 days respectively. In Monmouth there were several low-lying overgrown areas with scattered containers and refuse which were treated with the methoprene granule Altosid® XRG (Wellmark International, Schaumburg, IL) or Altosid® pellets and VectorBac® WDG (Valent Biosciences, Libertyville, IL), dispensed with a backpack blower (Stihl SR 420; Stihl Corporation, Virginia Beach, VA). Altosid® XRG was also used in both counties to treat standing water in areas with amorphous surfaces such as tarps that contained many small pockets of water.

2.6 Adulticide applications

Adulticide applications were made to each of the full intervention sites when a threshold mean of ≥ 5 *Ae. albopictus* (male + female) adults was detected in weekly BGS surveillance (Figs 2A and B). This number was chosen on the basis of the fact that three bites have been reported as a common nuisance threshold driving residents indoors.[41,42] Ultralow-volume (ULV) applications of DUET™ dual-action adulticide (1% prallethrin, 5% sumithrin, 5% piperonyl butoxide; Clarke Mosquito Control, Roselle, IL) were performed using ground-based truck-mounted mist sprayers. All adulticide applications were conducted at a rate of 14.84 mL ha^{-1} (1.24 fl oz $acre^{-1}$; g/lb of active ingredient per ha/acre of 0.15/0.0008 prallethrin, 0.66/0.0036 sumithrin and 0.66/0.0036 piperonyl butoxide). Prallethrin is a relatively volatile pyrethroid with sublethal activity that causes excitation and may flush mosquitoes from cryptic habitats where they are more protected from ULV droplets.[43] The benign agitation component of prallethrin[43,44] makes DUET™ an appealing adulticide candidate for the present purposes because the required environmental conditions for ULV applications, such as a temperature inversion,[45] are better met at night when there is also less insecticide degradation by UV radiation[46] and a decreased risk of human exposure, but when diurnal mosquitoes such as *Ae. albopictus* may be less likely to be 'on the wing'. All adulticide applications were conducted using a single-nozzle Cougar® (Clarke Mosquito Control, Roselle, IL) cold aerosol fog generator. The unit was fitted with a SmartFlow (Clarke Mosquito Control, Roselle, IL) control that uses truck speed obtained from GPS connected to handheld computers running DataMaster. DataMaster 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 at a height of 0.79 to 0.89 m, bringing the spray nozzle to a height of 1.76 to 1.86 m, and the spray head was angled at 45° and pointing directly towards the back of the truck. The vehicle was driven at a speed between 2 and 20 mph.

The pesticide label for DUET states that, for ground-based applications, spray equipment must be adjusted so that the volume median diameter (VMD) is between 8 and 30 µm ($D_{v0.5} < 30$ µm). Calibration of the Cougar sprayers used in Mercer and Monmouth was accomplished using an AIMS hot-wire portable droplet counter (Model DC-III; KLD Laboratories, Huntington Station, NY) and both Hock (John W. Hock Company, Gainesville, FL) and Florida Latham Bonds[47] rotating impingers deployed within the full intervention site. The Mercer Cougar was determined to have a $D_{v0.5}$ of 15.2 µm, based on the AIMS machine, and an average $D_{v0.5}$ of 11.5 ± 0.3 µm and 14.9 ± 0.5 µm from the Hock and Florida Latham Bonds rotating impingers respectively. In Monmouth, two Cougars were used for each spray event. Monmouth Cougar A had a $D_{v0.5}$ of 16.39 µm and Cougar B had a $D_{v0.5}$ of 16.94 µm, based on the AIMS machine. Cougar A had an average $D_{v0.5}$ of 17.81 ± 0.7 µm and 13.51 ± 0.5 µm, and Cougar B had an average $D_{v0.5}$ of 20.01 ± 0.8 µm and 15.68 ± 0.6 µm from the Hock and Florida Latham Bonds impingers respectively.

During each ULV application, meteorological data were obtained from WeatherUnderground (wunderground®.com) or using a Vantage Pro2 (Davis Instruments, Hayward, CA) portable weather station set up within the treatment site 14 h prior to application and maintained until 8 h post-application.

2.7 Data analysis

Tests were conducted to establish whether the present activities in the full intervention and in the education sites led to a decline in the number of host-seeking or egg-laying females by comparing experimental and untreated sites using repeated-measures analyses across time (JMP 8; SAS Institute, Cary, NC). The dependent variable was either the total number of *Ae. albopictus* adults (males and females) or just the females caught in each BGS trap each week. The same repeated-measures analysis was performed on the number of total eggs per ovitrap, as well as on the number of eggs that were later identified as *Ae. albopictus* by examining the third- or fourth-instar larvae. Because the BGS traps cannot be deployed in the rain, it was not always possible to perform adult collections exactly 7 days apart. For this reason, and because the 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.

To calculate percentage control after application of adulticides, an algebraic variation of Henderson's method[48,49] was employed using the formula

$$\text{Percentage control} = 100 - \left[(T/U)\,100\right]$$

where T is the post-application mean divided by the preapplication mean in the treatment (full intervention) site, and U is the post-application mean divided by the preapplication mean in the control (untreated) site. Compared with Mulla's formula, another commonly used formula for calculating percentage control,[48,49] the Henderson formula provides conservative estimates of control efficiency, especially in the fall when the numbers of adults decrease naturally over time in both treated and untreated sites.

3 RESULTS

3.1 Education

As part of the adult education and outreach, approximately 24 000 brochures were distributed and 1125 elementary school students were taught. In addition to the curriculum, over 1500 mosquito magazines and 'build-a-mosquito' kits were provided to participating elementary schools. Teachers in 45 classes in the four study sites targeted for education received and provided the materials to their students in the spring of 2009. All 45 classes were allowed to keep the materials, including a digital microscope, in the hope that the curriculum would continue to be used in future years. Please refer to Bartlett-Healy and colleagues[29] for more detailed results. To reinforce the message of source reduction, 1500 take-home projects were distributed to the elementary schools during the last week of classes in 2009.

3.2 Source reduction and larvicide application

3.2.1 Mercer County

Door-to-door source reduction in the full intervention site was initiated at the end of April 2009 and, with the addition of larvicide and pupacide applications, was repeated during the months of May, June, July and August 2009 (Figs 2A and B). An average of 1185 parcels (94.7%) were inspected each month, with the highest inspection rate in June and the lowest in September–October (Table 1). Field crews identified 80 abandoned houses (6.4% of the houses in the full intervention site). Teams were unable to access yards mostly for three reasons: (1) dog present; (2) safety concerns associated with entering abandoned parcels; (3) resident refusal of inspection. Only six residents in the study site rejected any kind of mosquito surveillance and control measures on their property.

A total of 19 121 containers were either treated or removed from the full intervention site. This list included 533 tires and 5792 trash items (plastic bags, soda cans, etc.) that were removed (Table 1). An estimated 4.6 kg of methoprene and 183 g of monomolecular surface film were applied to unmovable containers (Table 1). Mercer County mosquito control personnel spent an average of 4 min to inspect a parcel (three inspectors per parcel), taking 3.5 days to inspect the 1250 accessible parcels in the site. Source reduction and larviciding efforts took over 1000 h to perform (Table 1), but the estimated time needed to inspect each parcel in the full intervention site did not include: (1) travel time; (2) time needed to acquire permission for property access; (3) time spent in public areas such as alleyways between parcels, some of the time by inmates in the SLAP (see above).

Early in the season, field crews had problems deciding which trash would produce *Ae. albopictus*. For example, discarded snack bags (ex: 170 g potato chip bag) were first excluded during trash collections because the numbers were overwhelmingly high and they seemed too small to sustain larval development to term. However, in July, numerous snack bags holding *Ae. albopictus* larvae were collected, and laboratory tests determined that even very small and shallow containers could remain wet long enough for adult production.[50] It was therefore decided to start removing most trash from the parcels. However, trash items were ubiquitous in many Trenton backyards, and often were hard to reach or detect.

3.2.2 Monmouth County

An average of 1047 parcels (85%) were inspected each month in the full intervention site, with the highest inspection rate in July and the lowest in April–May (Table 1). Teams were unable to access parcels mostly for two reasons: (1) locked garden gates not allowing access to the yard; (2) resident refusal of inspection and/or treatment. There were several residents in the study site who rejected any kind of mosquito surveillance and control measures on their property. In response, Monmouth began a 'Do Not Inspect List', which increased throughout the season (round 1 = 11 residents, 2 = 28, 3 = 29, 4 = 36, 5 = 44), although at its highest it was only 3% of the total parcels. Sanitation and treatment efforts took over 2000 h (Table 1). It took a team of two people 10 min to inspect a parcel. The estimation of the time needed to inspect each parcel in the full intervention site did not include travel time or time needed to acquire permission for property access. The three most common containers encountered on residential properties were buckets and lids, tarps and trash/recycling cans and lids. Use was made of an estimated 358.3 kg of Altosid® pellets, 27 Altosid® briquettes, three Altosid® XR briquettes, 129.9 kg of Altosid® XRG, 1.1 kg of VectoBac® WDG and 0.3 kg of Agnique® (Table 1). Three backpack applications of Altosid® pellets, Altosid XRG® and VectoBac® WDG were made to low-lying public properties containing large numbers of scattered containers and trash. The backpack applications accounted for the large amount of Altosid® pellets, Altosid® XRG and all of the VectoBac® WDG used during the July–August round of door-to-door source reduction and treatments.

3.3 Adulticide applications

Three ULV-DUET applications were performed in Mercer and three in Monmouth over the summer (Fig. 2).[51] The first application in the full intervention site in Monmouth was conducted between 17:00 and 00:00 h. This time was found to be unsuitable owing to human activity and traffic patterns within the communities. All

Table 1. Door-to-door activities in Mercer (MER) and Monmouth (MON). Inspection round statistics and pesticide usage

	Round									
	1		2		3		4		5	
	MER	MON	MER	MON	MER	MON	MER	MON	MER	MON
Begin date	27 Apr	30 Apr	4 Jun	1 Jun	13 Jul	2 Jul	17 Aug	6 Aug	29 Sep	24 Sep
End date	18 May	27 May	17 Jun	29 Jun	16 Jul	4 Aug	31 Aug	18 Sep	7 Oct	30 Oct
∼Employee hours	129	496	185	512	211	464	232	480	299	384
% Parcels inspected	80	69	86	91	80	96	66	91	88	84
% Inspected parcels with source reduction	33	17	9	3	23	3	57	1	37	7
Number of tires collected	158	21	100	14	76	4	100	2	77	0
% Inspected parcels treated: larvicide	74	35	60	34	49	29	51	23	68	18
Altosid® pellets (g)	774	54	604	174	179	35 437	450	103	2073	63
Altosid® XRG (g)	14	623	467	378	1843	11 644	235	195	69	154
Agnique® MMFG (g)	1	0	13	0	118	24	39	301	12	18

additional applications were conducted between 02:00 to 06:00 h. Pre- and post-trapping of *Ae. albopictus* showed a reduction in adult mosquitoes following all nighttime ULV applications,[51] but the populations started rebounding within less than 1 week, except after the September application (Fig. 2), which was performed after day-length had decreased below the observed threshold for egg diapause induction in New Jersey.[52] Spatial analysis of projected *Ae. albopictus* populations using ArcGis (ESRI, Redlands, CA) displayed population increases within the treatment site, and not on the periphery as would be expected if recolonization was occurring from outside the sites (Farajollahi A *et al*., unpublished data).

3.4 Impact of interventions

The first adult *Ae. albopictus* were collected on 19 and 21 May 2009 and the last on 6 and 29 October 2009 in the Mercer and Monmouth sites respectively (Fig. 2). A total of 7603 and 11 513 adult mosquitoes were collected in BGS traps in Mercer and Monmouth, respectively, during the 2009 mosquito season. *Aedes albopictus* was the most common mosquito species in Mercer, representing 73.8% (5611) of the collection, but in Monmouth, although still the most common mosquito species, it only represented 43% (4901 specimens) of the collection. Peak *Ae. albopictus* numbers were observed from July to September in Mercer County, especially in the untreated site, but much later in Monmouth (Fig. 2). iButton temperatures collected on the BGS traps revealed that mean daily air temperatures in the Monmouth sites were over 2 °C lower than in the Mercer sites, which were located within the City of Trenton.[32] The same general patterns were recovered for examining maximum and minimum daily temperatures. The spring and summer of 2009 were unusually rainy and relatively cold compared with the 2001–2010 means (http://climate.rutgers.edu/stateclim_v1/data/index.html).

In Mercer, repeated-measures analysis revealed that both the total number of *Ae. albopictus* and just the number of *Ae. albopictus* females caught in BGS traps were significantly lower in the full intervention site compared with the untreated site across the entire sampling season (both $P < 0.01$). The number of *Ae. albopictus* females was also significantly lower ($P = 0.046$) in the education site than in the untreated site, but only if the analysis was restricted to the 8 weeks between mid-July and mid-September, when *Ae. albopictus* was most abundant (Fig. 2). On average, across the entire season, the number of *Ae. albopictus* in the education site was 25% less than in the untreated site, and in the full intervention site it was 75% less than in the untreated site. Furthermore, in the full intervention site the nuisance threshold was surpassed only 6 times over the entire active season, while in the untreated site there were 17 occasions when BGS catch rates were above the threshold.

In Monmouth, the number of adult *Ae. albopictus* females caught in BGS traps was significantly lower in the full intervention site relative to the untreated site from late August to the end of

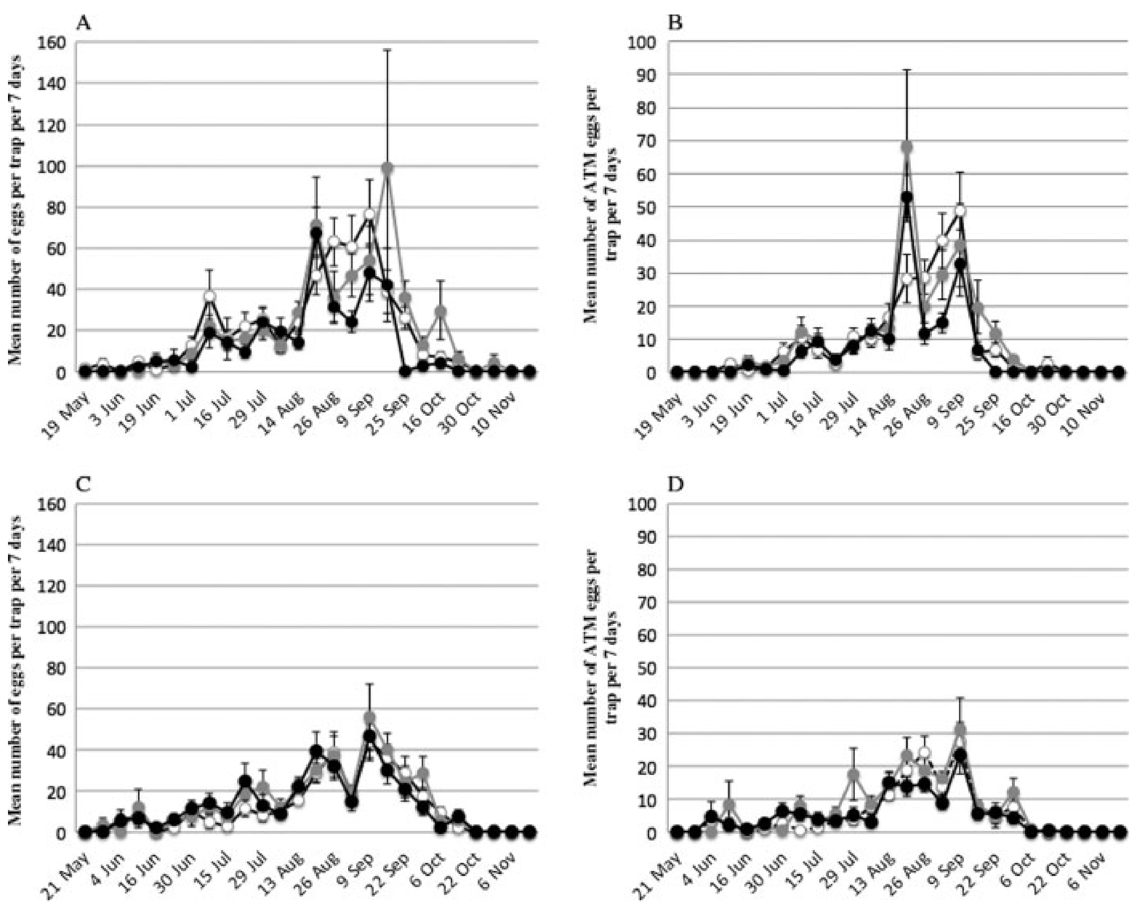

Figure 3. Mean egg collection per ovitrap, corrected so all counts are equivalent to 7 days of collection. Left panels: total number of eggs at each collection date; right panels: only eggs identified as *Ae. albopictus*. A and B, Mercer County; C and D, Monmouth County. Black circles: full intervention site; grey circles: education; white circles: untreated. *Ae. albopictus* represented 95.9 ± 0.006% (mean ± SE) of the total *Aedes* that hatched (see also Fig. 4), and therefore relative proportions of total eggs or just *Ae. albopictus* eggs across experimental sites were very similar.

September ($P = 0.04$), when *Ae. albopictus* abundance finally peaked across the Monmouth sites (Fig. 2). The number of *Ae. albopictus* adults or adult females caught in the education site was not significantly different from the number caught in the untreated site. On average, the number of *Ae. albopictus* in the full intervention site was only 25% less than in the untreated site, but 40% less in the peak months of August and September. In the full intervention site the nuisance threshold was surpassed only 5 times over the entire active season, while in the untreated site there were 8 occasions when BGS catch rates were above the threshold.

Although immediately after the ovitraps were deployed in mid-May a few eggs were collected in all three sites in Mercer and in the untreated site in Monmouth (Fig. 3), those that hatched (60%) were identified as *Ae. j. japonicus* (Fig. 4). The first eggs positively identified as *Ae. albopictus* were first collected in Monmouth County on 4 June, and in Mercer County on 11 June. After early June, hatch rates averaged 60–80% until early September, after which they steadily declined down to 10–20%. In late October, however, hatch rates increased again in Monmouth County, ranging from 30 to 100% driven by hatches of *Ae. japonicus* (Fig. 4). There was no significant difference in proportion of eggs that hatched as *Ae. albopictus* between experimental sites within each county, but Monmouth sites exhibited higher diversity of *Aedes* species (Fig. 4). Eggs of *Ae. japonicus* were mostly present in the spring collections, although they persisted through mid-July and reappeared in Monmouth in October. Eggs of *Ae. triseriatus* were almost completely absent from Mercer sites but occurred in small numbers from early June to late August in Monmouth sites (Fig. 3). A total of 17 444 *Aedes* eggs were counted from the egg papers in Mercer, from which 8030 larvae were identified as *Ae. albopictus*. In Monmouth, a total of 18 991 *Aedes* eggs were counted, and 8600 larvae were identified as *Ae. albopictus*, reflecting the larger number of traps deployed (37 versus 59 in Mercer and Monmouth respectively), not a higher average number of eggs per ovitrap (Fig. 3). Except for the collections in early spring and later after mid-September, all traps with more than ten eggs produced some larvae of *Ae. albopictus*, indicating that the moderate hatch rates may reflect the bet-edging behavior of this species and not catastrophic mortality of the eggs owing to the weather or mishandling. The mean number of *Aedes* eggs or *Ae. albopictus* eggs collected in each of the three sites in Mercer County was not significantly different, but there was a significant interaction between site and time ($P = 0.03$), reflecting an early spike in eggs in the full intervention and education sites (Fig. 3) and the fact that the number of eggs deposited in ovitraps in the full intervention site in Mercer declined to almost zero (mean 0.78 eggs ovitrap^{-1}) on 25 September and never recovered (Fig. 3), while in the education and untreated sites females continued depositing upwards of 10 eggs ovitrap^{-1} until the middle of October (Fig. 3). In Monmouth, ovitraps in the full intervention site collected a significantly lower number of eggs of *Ae. albopictus* than in the untreated or education sites ($P = 0.027$).

Larval surveys performed during 2009 found that *Ae. albopictus* was very strongly and positively associated with small domestic water containers such as tires, buckets and flower pots.[28] Rain gutters in Mercer were sampled 167 times over the season, and *Ae. albopictus*, as well as *Ae. japonicus*, were found only once (Unlu *et al*., unpublished data). Also in Mercer, ten of the 68 catch basins located within the full intervention site were sampled monthly, from June to October, and *Ae. albopictus* was only recovered 3 times (once in July and twice in August). Nonetheless, as part of the standard Mercer County mosquito control program to control West Nile virus, an important urban arbovirus in the United States,[53] all catch basins were treated with a combination of VectoBac® 12 AS and VectoLex® WDG (Valent BioSciences, Libertyville, IL) twice a month. To assess the need to treat catch basins on a regular basis for *Ae. albopictus* control, on 9 July, 9% of the catch basins in the full intervention site in Cliffwood Beach in Monmouth County (30 out of 331) were surveyed, and only 13 were found to be wet. Of those, seven were just damp and had no mosquitoes. The remaining six were holding water and did have larvae present, but they were all identified as *Culex*. Several times during the AW-ATM door-to-door control efforts, catch basins were inspected as part of the property of adjacent homes. Overall, 58 catch basins noted to be holding water were treated throughout the season.

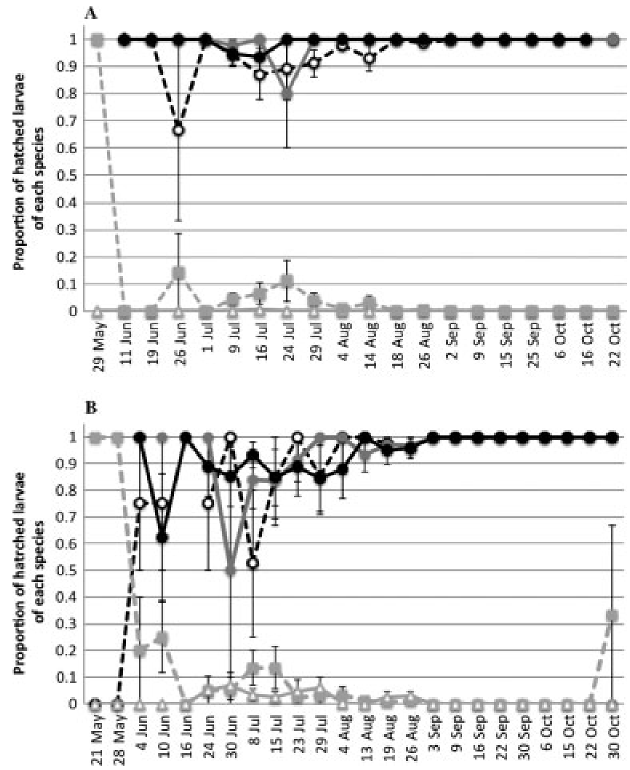

Figure 4. Proportion of eggs collected in ovitraps that were *Ae. albopictus* (separated across experimental sites) or other container *Aedes* (total proportion, proportions across species may not add up to 1) through the active season. The proportions of *Ae. albopictus*, *Ae. japonicus* and *Ae. triseriatus* were calculated only for egg papers that had eggs that hatched. No other *Aedes* were found. A, Mercer County; B, Monmouth County. Black circles and line: full intervention site; grey circles and line: education; white circles with dashed line: untreated; light-grey squares and dashed grey line: *Ae. japonicus*; white triangles and grey line: *Ae. triseriatus*.

4 DISCUSSION

Aedes albopictus is a difficult species to control because of the ubiquity and proximity to humans of its larval habitats. It is nearly impossible for an abatement district to eliminate all of these mosquito sources from people's yards, or even effectively to treat all of them with larvicides. To make matters worse, *Ae. albopictus* bites during the day when residents want to use outdoor spaces for various activities, generating many complaints to county and state mosquito control programs, and adult control using standard ultralow-volume applications applied at night have been thought to be ineffective because *Ae. albopictus* adults would not be on the wing during those times of the day.[54] Control strategies against *Ae. albopictus* have indeed been unsuccessful, except when new infestations are detected early while they are still restricted to relatively small areas and can quickly be controlled effectively.[55,56]

To assess the effectiveness of standard urban mosquito control strategies applied area wide, from April to October of 2009, monthly rounds of door-to-door mosquito inspection and control were carried out, incorporating education, source reduction and treatment of containers with insecticides, all standard strategies to control *Ae. albopictus*.[27] Independently, the effectiveness of education alone, aimed at reducing the number of mosquito-producing containers in private yards, was examined. BGS traps and ovitraps were compared as tools to assess mosquito control strategies, which also included non-standard nighttime truck-mounted applications of adulticides in the peak months of August and September.

The combination of source reduction by mosquito abatement personnel, source reduction by the public, larviciding and finally use of adulticides when the number of adults surpassed a predefined threshold significantly reduced the catches of *Ae. albopictus* in the traps (Fig. 2). Unexpectedly, greatest success was achieved in the inner-city urban sites within the city of Trenton, Mercer County (75% control compared with the untreated site). In contrast, the same procedures in suburban Monmouth County sites resulted in 40% reduction only during late August and September. In Mercer (Trenton), but not in Monmouth, the education site also experienced a significant reduction (25%) in the number of *Ae. albopictus*. These results occurred in spite of the clearly much greater visible amount of trash and abandoned properties providing suitable larval and adult habitat that appeared unresponsive to the door-to-door campaigns in the Mercer sites.

Although larval control measures were relatively successful, they required a dedication of resources that was only possible thanks to a federally funded project. Further, it was still necessary to apply adulticides several times during the summer to bring the number of adults below a threshold of five adult *Ae. albopictus* per 24 h of BGS trap collection. Even in Monmouth, where the *Ae. albopictus* population growth across all three sites (full intervention, education-only and untreated sites) was very slow, probably on account of low spring and early summer temperatures,[57] the threshold for adult treatment was exceeded 3 times. Encouragingly, predawn ultralow-volume applications of DUET (prallethrin and *d*-phenothrin) were effective.[51] Of note, in the weeks after the third application of the adulticide in Mercer, in mid-September, the number of adult *Ae. albopictus* did not rebound, and the number of eggs in the ovitraps decreased and stayed at close to zero until the end of the surveillance period, even while significant numbers of eggs were still being deposited in ovitraps in the education and untreated sites during October. This result indicates that the area-wide adulticide applications effectively removed adult female *Ae. albopictus* across the full intervention site. The present results support the conclusion that this species exhibits low autonomous dispersal,[30] and indicate that *Ae. albopictus* populations can remain very local even across highly homogeneous urban environments.

The mean number of *Ae. albopictus* eggs in ovitraps was not significantly different across all sites in Mercer County and therefore did not accurately measure the impact of control interventions. In contrast, it was found that in Monmouth lower adult catches did correlate with significantly lower numbers of eggs in the ovitraps, a result that agrees with other studies.[27,34] It is hypothesized that this difference between urban and suburban sites is the result of the types of oviposition site available. In the Trenton sites in Mercer County, in contrast to the overall cleaner suburban properties in Monmouth, there is an obvious high availability of above-ground containers in the many abandoned or poorly maintained properties. Further, because of the ongoing threat of West Nile virus, all catch basins in Trenton have been routinely treated with larvicides for the last 6 years. As a result, *Ae. albopictus* in the Mercer sites may exploit predominantly above-ground containers, not unlike the ovitraps deployed in the present study, leading to high numbers of egg catches. As *Ae. albopictus* females will skip oviposit in response to the presence of conspecific eggs, they may saturate the ovitraps, which are pristine at the beginning of each collection period, before moving onto other containers. As a result, smaller numbers of adult *Ae. albopictus* in the full intervention or the education sites will not be reflected in the number of eggs in the ovitraps.

In contrast, in the Monmouth sites, females may routinely exploit a larger variety of containers. Catch basins and other water-retaining structures underground may be sources of *Ae. albopictus* because these are not routinely treated there. Also, the lower-density suburban surroundings in Monmouth County include larger uninhabited areas that could contain hidden larval sources. Although *Ae. albopictus* is not considered to be a salt marsh or brackish water mosquito, it can survive in salinities up to 3 ppm,[58] which is higher than the salinity of many water-holding containers in the high marsh (located above the mean high water). Indeed, egg catches were consistently much lower across the Monmouth sites (Fig. 3), even when numbers of adults were similar in Mercer and Monmouth (Fig. 2 after mid-August). Because ovitraps may be less attractive in suburban sites, smaller numbers of adult females following effective interventions result in a perceivable decline in eggs in the ovitraps. Recent larval surveys in Japan and Italy[34,59] in suburban areas underscore the importance of catch basins as sources of *Ae. albopictus*, although in Japan only a small proportion (12%) were positive for *Ae. albopictus*.

The BGS traps were more sensitive than ovitraps to the presence of low numbers of *Ae. albopictus*. Early in the season it was possible to capture adult *Ae. albopictus* in BGS traps weeks before the first *Ae. albopictus* eggs were detected, indicating that it was possible to collect specimens from the first spring generation emerging from overwintering eggs. On the one hand this result supports other accounts of the unique attractiveness of BGS traps to *Ae. albopictus*[33,60] and their usefulness,[61–63] even considering their high costs and restrictive deployment,[64] and on the other hand ovitraps provide a different kind of data that can be very useful for operational decisions. For example, the significant and simultaneous increase in the number of eggs in ovitraps both in the full intervention and in the education sites relative to the untreated site in Mercer in mid-August (Figs 3A and B) may have been a direct consequence of the door-to-door deployment of educational brochures and information in those two sites, but not in the untreated site. Egg hatch rates also provide information on the onset of production of diapausing eggs.

The education campaign led to a significant reduction in adult *Ae. albopictus* females in Trenton, Mercer County, but not in Monmouth. It is interesting that the present results indicate that the Trenton community responded more energetically to an outreach program, considering that the high school graduation rate in that community is 31.5% compared with 85.3% in

Monmouth.[23] The results show that outreach education by mosquito control programs can impact upon the number of nuisance adult *Ae. albopictus*, although further studies have shown even stronger results when the community is actively involved in the education process rather than passively receiving information (Bartlett-Healy K *et al*., unpublished data). Efforts failed to document a reduction in the number of larval sites corresponding to the decrease in number of adults,[29] suggesting that the quality (in terms of productivity) as well as the quantity of larval sites is important.

Adulticiding activities in September after the onset of egg diapause in mid-August[65] caused long-lasting reductions in the adult population, while earlier in the season adulticiding resulted in only a temporary reduction. Late-season adulticiding as a strategy to reduce the population may become even more important if climate change results in an extension of warm temperatures in the fall, as *Ae. albopictus* populations track closely ambient temperature.[5] Temperature indeed appears to be a strong driver of the growth trajectories of *Ae. albopictus* in New Jersey sites. In 2008, populations of *Ae. albopictus* had been very similar in both counties and across all six sites chosen,[23] but during the spring and early summer of 2009 the Trenton sites were 2 °C warmer than the Monmouth County sites, possibly as a result of an urban heat island effect,[66] and *Ae. albopictus* populations were significantly depressed in Monmouth Country compared with Trenton in Mercer County.

Results of this study have several practical implications for operational control of *Ae. albopictus*. Firstly, interventions in early spring and summer may be particularly effective because they could mimic the natural control of populations caused by low temperatures, as observed in 2009 in the Monmouth sites. Secondly, late-season adulticiding can provide longer relief from biting mosquitoes than adulticide treatments earlier in the season. Thirdly, careful source reduction by mosquito abatement personnel, combined with efforts to educate the public to clean up sites, can result in a significant decrease in the numbers of adult *Ae. albopictus*. The present experimental efforts to apply source reduction in 1 year were difficult and seemed unsustainably intensive; however, like all procedures in mosquito abatement, source reduction would become more efficient over time. These methods are relatively expensive, but used effectively they have the greatest likelihood of convincing the public that they are worth the cost. Customization of programs to each community is probably necessary for success. For example, understanding the principal types of productive container and the specific socioeconomic characteristics of the locality would help target efforts. It is the authors' hope that integration of the techniques presented here can bring some relief to communities that suffer from this pest and that are threatened by the possibility of outbreaks of disease.

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