Field Efficacy of BG-Sentinel and Industry-Standard Traps for *Aedes albopictus* (Diptera: Culicidae) and West Nile Virus Surveillance

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J. Med. Entomol. 46(4): 919–925 (2009)

ABSTRACT Standard surveillance traps in North America for adult *Aedes albopictus* (Skuse) (Diptera: Culicidae), an invasive mosquito with public health implications, are currently ineffective. We compared the efficacy of the BG-Sentinel trap (BGS) with and without lures (BG-lure, octenol, and CO_2), the Centers for Disease Control and Prevention light trap (CDC) with and without lures, and the gravid trap (GT) for *Ae. albopictus* collection in two urban sites in New Jersey. The BGS with or without lures collected more *Ae. albopictus* compared with other trap configurations and was more specific for *Ae. albopictus*. In Camden County, the BGS with lures collected three times more *Ae. albopictus* than the CDC (with CO_2 only) and five times more than the GT. In Mercer County, BGS with lures collected the most mosquitoes, with 3 times more *Ae. albopictus* than the CDC with all lures and 50 times more than the GT. The BGS collected more male *Ae. albopictus* than other traps in both counties, providing further population monitoring. The GT and BGS provided a relative measure of the enzootic activity of West Nile virus in *Culex* spp. and the potential epidemic activity of WNV in *Ae. albopictus*. The BGS provides effective chemical and visual cues for host-seeking *Ae. albopictus* and should be used as a part of existing surveillance programs and new initiatives targeting this mosquito.

KEY WORDS arbovirus, Asian tiger mosquito, CDC light trap, gravid trap, mosquito surveillance

The first invasive population of the Asian tiger mosquito *Aedes albopictus* (Skuse) in the United States (Diptera: Culicidae) was reported from Houston, TX, in 1985 (Sprenger and Wuithiranyagool 1986). *Ae. albopictus* has now spread to an additional 36 states and is no longer a novel curiosity but a threat to public health (Enserink 2008). The specific role of *Ae. albopictus* in human-associated, arbovirus transmission in the United States remains elusive; however, Cache Valley virus, eastern equine encephalitis virus, Jamestown Canyon virus, La Crosse virus, and West Nile virus (WNV) have been isolated from field populations (Moore and Mitchell 1997, Gerhardt et al. 2001, Turell et al. 2005). *Ae. albopictus* distribution has expanded rapidly in New Jersey, and the mosquito has been associated with WNV in several foci (Farajollahi and Nelder 2009). The public health threat posed by *Ae. albopictus* has made it a top priority for control efforts; however, our ability to monitor accurately their populations and involvement in WNV ecology is hampered by ineffectual surveillance tools.

Surveillance of *Ae. albopictus* has been problematic because standard surveillance equipment such as the New Jersey light trap are inefficient surveillance methods for this species. Traps using light as the sole attractive component are considered ineffective at capturing *Ae. albopictus* because they target crepuscular or nocturnal mosquito species attracted to emitted light sources (Silver 2008), whereas *Ae. albopictus* is primarily a diurnal host-seeking mosquito (Robertson and Hu 1935). These light traps, usually deployed 1.5 m above the ground, do not target *Ae. albopictus* that typically host seek near the ground surface (Robertson and Hu 1935). The low efficacy of existing surveillance methods for *Ae. albopictus* has likely led to underestimations of population sizes and difficulty with assessing control methods.

The BG-Sentinel (BGS) trap was initially designed to collect and monitor populations of the yellow fever mosquito *Aedes aegypti* L. (Kröckel et al. 2006). Few studies have investigated the efficacy of BGS traps. A study conducted in suburban Virginia investigated the efficacy of the BGS trap for collecting *Ae. albopictus;* the BGS collected more *Ae. albopictus* than CDC and collapsible mosquito traps (CMT-20) (Meeraus et al.

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2008). The BGS trap has also proved effective for the detection of new infestations of *Ae. albopictus* in the Torres Strait, Australia (Ritchie et al. 2006). Considering the BGS shows promise for *Ae. albopictus* surveillance, we aimed to verify its effectiveness when implemented in a mosquito control program using CDC and gravid traps (GTs) for mosquito surveillance. Furthermore, we assessed the BGS and accompanying traps for their ability to detect WNV in field-caught mosquitoes. We hypothesized that there would be a difference in the number of *Ae. albopictus* collected by BGS, CDC, and gravid traps as implemented according to manufacturer recommendations and under varying combinations of lures and CO_2 in two urban centers of New Jersey (Camden and Mercer Counties). Our study provides a more comprehensive assessment of *Ae. albopictus* surveillance techniques (i.e., comparing more trap and lure combinations over a longer period of the active season) and equips mosquito researchers and control operators with a more effective means of assessing population changes and the biology of this invasive mosquito.

Materials and Methods

Study Sites. Experiments and trapping were conducted from 6 August 2007 through 2 November 2007, using natural populations of mosquito species associated with two urban New Jersey counties. Sites were assigned based on locations supporting a ubiquity of habitats for immature *Ae. albopictus* such as human refuse. In Mercer County, an active automobile commercial site in Trenton (5,574 m^2) was chosen where discarded vehicle tires were abundant (40°14′ N, 74°44′ W). Mercer County, bordered to the south by metropolitan Philadelphia, PA, encompasses an area of 585 km^2, with a population of ≈270,000 (≈80,000 for Trenton). The center of the site contained a large, open field where discarded automobile tires and other artificial containers are exposed directly to the elements; the property perimeter was bordered by a 2-m-high chain link fence. Fencing and vegetation (*Quercus* spp.) along the property edge provided shade and resting areas for adult mosquitoes; *Ae. albopictus* adults were detected by visual inspection during collections. In Camden County, a residential yard within a suburban neighborhood in Cherry Hill (1,858 m^2) was chosen where various water holding containers were present (39°56′ N, 75°01′ W). Camden County, bordered to the north by Philadelphia, PA, encompasses an area >576 km^2 with a population of ≈517,000 (≈71,000 for Cherry Hill). Residential homes in the Cherry Hill site were comprised of single-family dwellings of similar age and size. The rear of the residence contained various artificial containers consisting of flowerpots, plastic tarps, and children's toy buckets. A 1.5-m-high chain link fence, interspersed with low-lying vegetation and a canopy dominated by deciduous trees (*Acer* and *Quercus* spp.), bordered the perimeter. Ornamental plants and shrubs, open grass areas, and low ground cover were also prevalent and provided shade for resting *Ae. albopictus* (detected during sampling events). Both locations have established populations of *Ae. albopictus*, as documented through County vector surveillance programs and service requests (i.e., requests for control efforts in response to *Ae. albopictus* populations and confirmed by operators; A.F., unpublished data).

Mosquito Collections. The BGS (BioGents, Regensburg, Germany) was designed to attract anthropophillic mosquito species such as *Ae. aegypti* and *Ae. albopictus* (Geier et al. 1999, Williams et al. 2006). The BGS attempts to mimic convection currents created by human body heat with a fan and mimics human odors through the BG lure (ammonia, caproic acid, and lactic acid) and an octenol lure (Meeraus et al. 2008). The contrasting black and white markings of the trap also provide visual cues that may be attractive to these mosquitoes (Kawada et al. 2007).

Traps were placed at least 10 m apart at permanent locations within each site and randomly rotated weekly, with at least one trap day per given week. Traps were placed on the periphery of each site, avoiding locations with direct exposure to the elements, between 0800 and 1000 hours EDST and collected between the same times after 24 h (i.e., a trap night). CDC miniature light traps (Clarke Mosquito Control, Roselle, IL) were placed at 1.5 m above ground; all other traps were placed on ground surface. Dry ice (2 kg) was placed within an insulated small bucket with ventilation holes and served as the source of CO_2 for designated traps. The BG and octenol lures were individually placed within a sealed plastic bag between trap nights to limit cross-contamination and to prevent the loss of lure ingredients. Octenol lures were replaced after 2 mo as suggested by the manufacturer, whereas the BG lure was not replaced because the manufacturer suggests that it will last for 4 mo.

In Camden County, a duplicate of three trap types (six traps) were evaluated per manufacturer recommendations and trapped for 48 trap nights each: (1) BGS, (2) CDC, and (3) GT (BioQuip Products, Rancho Dominguez, CA) with grass infusion (GT). In Mercer County, five trap configurations (three traps each) were evaluated as follows and trapped for a total of 30 trap nights each: (1) BGS with BG and octenol lures plus CO_2 (BGS w/ lures and CO_2), (2) BGS with no lures or CO_2 (BGS w/ no lures or CO_2), (3) CDC with BG and octenol lures and CO_2 (CDC w/ BG-lure and CO_2), (4) CDC with no lures and no CO_2 (CDC w/ no lure or CO_2), and (5) GT with grass infusion (GT). Grass infusion was prepared following standard protocols (Scott et al. 2001).

WNV Detection. We assayed all mosquitoes collected from the various trap configurations for WNV. A cold chain was maintained by placing mosquitoes on dry ice for transport back to laboratory to prevent desiccation and virus inactivation. Mosquito pools were tested at the New Jersey Department of Health and Senior Services, Trenton, NJ, for WN viral RNA by a TaqMan (Applied Biosystems, Foster City, CA) reverse transcription-polymerase chain reaction (RT-PCR), using previously established procedures (Lanciotti et al. 2000, Farajollahi et al. 2005). Infection rates

Table 1. Female mosquitoes collected per trap day by trap type in Camden and Mercer Counties, NJ (6 Aug. to 2 Nov. 2007)

Species	Camden County[a]			Mercer County[a]				
	BGS	CDC	GT	$BGS_{lures + CO2}$	$BGS_{no\ lures}$	$CDC_{lures + CO2}$	$CDC_{no\ lures}$	GT
Ae. albopictus	23.63 ± 4.47	10.81 ± 2.07	3.69 ± 0.92	50.3 ± 8.25	6.46 ± 1.22	17.97 ± 2.57	0.50 ± 0.32	0.30 ± 0.07
Ae. atropalpus	0	0	0	0	0	0	0	0.02 ± 0.02
Ae. cinereus	0	0	0	0	0	0.03 ± 0.03	0.03 ± 0.03	0
Ae. japonicus	0.25 ± 0.11	1.31 ± 0.35	0.13 ± 0.06	0.81 ± 0.19	0.11 ± 0.06	0	0	0.10 ± 0.05
Ae. triseriatus	0.10 ± 0.05	0.35 ± 0.10	0	0.81 ± 0.22	0.04 ± 0.04	0.13 ± 0.07	0	0.03 ± 0.02
Ae. trivittatus	0	0.02 ± 0.02	0	0	0	0	0	0
Ae. vexans	0.35 ± 0.20	5.25 ± 1.16	0	0.66 ± 0.24	0	0.19 ± 0.08	0.03 ± 0.03	0
An. bradleyi/crucians	0	0.21 ± 0.21	0	0.06 ± 0.04	0	0	0	0
An. punctipennis	0.02 ± 0.02	0.69 ± 0.19	0	0.22 ± 0.11	0	0	0	0
An. quadrimaculatus	0.02 ± 0.02	0.33 ± 0.10	0.02 ± 0.02	0.16 ± 0.11	0	0	0	0
Cq. perturbans	0.04 ± 0.03	3.35 ± 0.05	0	0.03 ± 0.03	0	0	0	0
Culex spp.	0.33 ± 0.11	3.35 ± 0.62	20.92 ± 3.85	0.44 ± 0.13	0.29 ± 0.09	0.09 ± 0.05	0	7.08 ± 0.96
Cx. erraticus	0.04 ± 0.03	0.42 ± 0.03	0	0.06 ± 0.04	0	0	0.03 ± 0.03	0
Cx. territans	0	0.04 ± 0.03	0.02 ± 0.02	0	0	0	0	0
Cs. melanura	0	0.02 ± 0.02	0	0	0	0	0	0
Ps. columbiae	0	0.02 ± 0.02	0	0.03 ± 0.03	0	0	0	0
Ps. howardii	0	0	0	0.03 ± 0.03	0	0	0	0
Ur. sapphirina	0	0.04 ± 0.03	0	0	0	0.03 ± 0.03	0.22 ± 0.19	0
Total	9	15	5	12	4	6	5	5

[a] Two of each trap type (6 total) were used in Camden County and three of each trap (15 total) type were used in Mercer County.

were calculated using the Microsoft Office Excel plug-in PooledInfRate (Biggerstaff 2006), which allowed weekly calculation of a bias corrected maximum likelihood estimation (MLE).

Statistical Analyses. Because female *Ae. albopictus* and *Culex* species (combined in this study: *Culex pipiens* L. and *Culex restuans* Theobald) were more abundant in the traps than other species, they were the only groups analyzed. A negative binomial distribution model was fitted for each species from each site, a model shown to be a robust analysis especially with respect to count data sets (Cunningham and Lindenmayer 2005, Martin et al. 2005, Sileshi 2006). Parameters were estimated with a log-link function, compared with Wald's statistic, and contrasts performed as follow-up tests for significant main effects using PROC GENMOD in SAS 9.1 (SAS Institute 2004). Differences between means were considered significantly different at a family error rate of $P < 0.05$.

Results

Twenty species of mosquitoes were collected using all trap types from both counties; 15 species were collected from Camden and 15 species from Mercer counties (Table 1). *Ae. albopictus*, *Culex* spp., and *Aedes vexans* (Meigen) were the most commonly collected mosquitoes (highest to lowest mean number females per day) using all trap configurations in Camden and Mercer Counties (Table 1). In Mercer County, the BGS collected more vector and human-biting mosquitoes such as *Aedes japonicus* (Theobald), *Aedes triseriatus* (Say), *Ae. vexans*, and *Anopheles punctipennis* (Say) than other trap configurations. In Camden, the most abundant mosquito collected by BGS and CDC was *Ae. albopictus; Culex* spp. was the most common in GT (Table 1). Trap type was a significant variable for the mean number of *Ae. albopictus* (Wald's statistic $\chi^2_2 = 45.26$, $P = <0.0001$) and *Culex* spp. collected (Wald's statistic $\chi^2_2 = 168.74$, $P = <0.0001$). Contrasts showed that BGS with lures trapped more *Ae. albopictus* females than the other traps, followed by CDC with CO_2 and GT (Fig. 1a). Alternatively, GT collected more *Culex* spp. females, followed by the CDC with CO_2 and BGS with lures (Fig. 1b).

In Mercer County, *Ae. albopictus* was the most commonly collected mosquito for all BGS and CDC configurations; *Culex* spp. was the most common in GT (Table 1). For *Ae. albopictus,* trap type was a significant variable (Wald's statistic $\chi^2_4 = 362.52$, $P = <0.0001$), indicating that the mean number of *Ae. albopictus* females caught in the traps were significantly different. Trap type was also a significant variable for *Culex* spp. (Wald's statistic $\chi^2_4 = 153.44$, $P = <0.0001$). Contrasts showed that BGS with lures trapped more *Ae. albopictus* females than any other traps, followed by CDC with lures, BGS without lures, CDC without lures, and GT (Fig. 2a). Contrasts for *Culex* spp. showed that GT collected more females than other traps (Fig. 2b).

The BGS was the most specific trap for collecting *Ae. albopictus* in both counties. In Camden County, *Ae. albopictus* comprised 95.3% of all mosquitoes collected by BGS with lures, followed by CDC with CO_2 (45.6%) and GT (14.0%). In Mercer County, *Ae. albopictus* comprised 92.9% of all mosquitoes collected by BGS with lures: BGS with no lures (93.8%), CDC with lures (96.4%), CDC with no lures (61.5%), and GT (3.6%).

In Camden County, males comprised 25.2% (n = 1,771) of all *Ae. albopictus* collected, 11.0% (544) for CDC with lures, and 3.92% (216) for GT. In Mercer County, males comprised 53.3% (2,571) of the *Ae. albopictus* catch for the BGS with lures, 6.16% (268) for BGS with no lures, 19.5% (680) for CDC with lures, 0.16% (16) for CDC with no lures, and 0.31% (34) for GT.

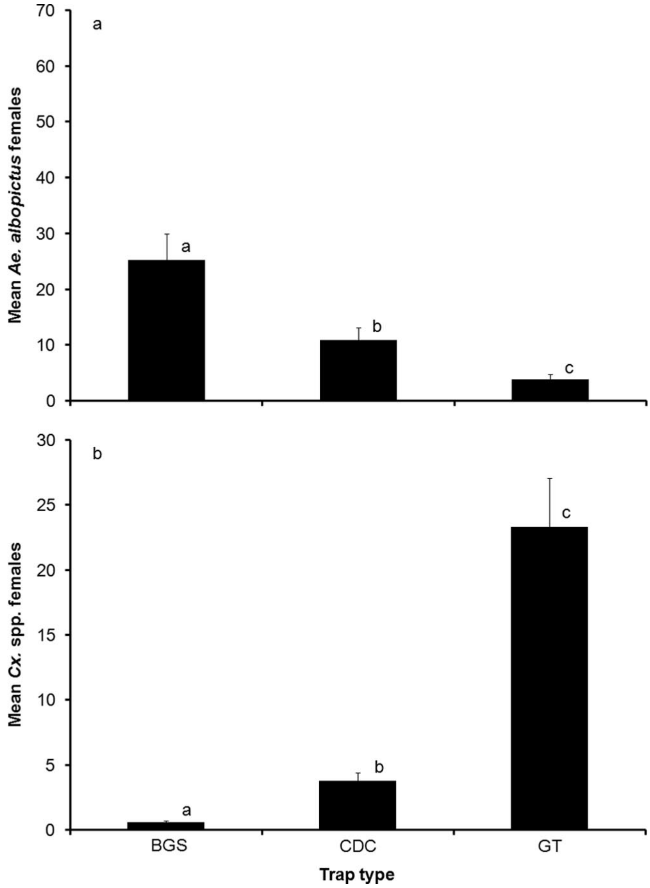

Fig. 1. Mean numbers of female *Aedes albopictus* (a) and *Culex* spp. (b) caught in traps from Camden County, NJ (6 Aug. to 2 Nov. 2007). Means with different letters are significantly different from each other at a family error rate of $P < 0.05$.

West Nile viral RNA was detected in 22 mosquito pools (total pools = 732) in both counties from three species (Table 2). In Camden County, WNV was detected from 11 pools of *Cx. pipiens* (MLE 13.6) and from 1 pool of *Cx. restuans* (MLE 10.5) using GTs; 2 pools of *Cx. pipiens* collected by CDC (MLE 13.5) were WNV positive. In Mercer County, WNV was detected from seven pools of *Cx. pipiens* (MLE 17.1) using GTs and from one pool of *Ae. albopictus* (MLE 5.6) using BGS with no lures. Overall, *Cx. pipiens* exhibited the highest MLE values for WNV in both counties using all trap types, with MLEs of 13.3 and 16.1 in Camden and Mercer Counties, respectively. A single pool of *Ae. albopictus*, collected from a BGS with no lures (MLE 5.6), was positive for WNV in Mercer County.

Discussion

The BGS trap efficiently collected *Ae. albopictus* females and was more specific for collecting this species compared with CDC and GT in urban sites of New Jersey. Similar efficacy for *Ae. albopictus* collection was reported in Virginia using the BGS (Meeraus et al. 2008); however, trap configurations differed in the two studies. In Virginia, the BGS (with BG-lure and CO_2) collected six times more *Ae. albopictus* than CDC with CO_2 alone compared with our study in which the BGS with both lures collected three times more than the CDC with both lures or the CDC with CO_2 alone. The BGS with lures provided the highest collections of *Ae. albopictus* in our study; however, BGS traps without lures still collected more *Ae. albopictus* than other traps. In New Jersey, *Ae. albopictus* comprised 95.3 (Camden) and 92.9% (Mercer) of all mosquitoes collected in the BGS with lures. This specificity was greater than the 73% reported in the Virginia study. Although chemoreception plays an integral part in the host-seeking behavior of *Ae. albopictus*, our increased capture without lures compared with other traps indicated that visual cues play an important role as well. Laboratory studies testing attraction of *Ae. albopictus* to the BGS have indicated that CO_2 and octenol, in conjunction with visual cues, are important variables in collecting host-seeking females (Kawada et al. 2007).

Male *Ae. albopictus* comprised a significant proportion of the total BGS catch. Males reportedly swarm at human baits, presumably anticipating female arrival and creating a mating arena (Gubler and Bhattacharya

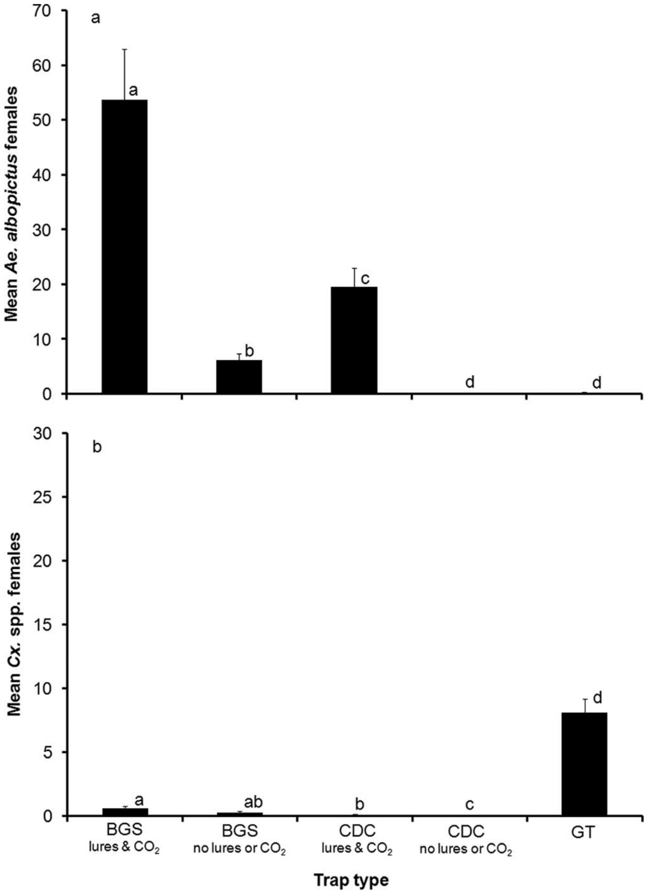

Fig. 2. Mean numbers of female *Aedes albopictus* (a) and *Culex* spp. (b) caught in traps from Mercer County, NJ (6 Aug. to 2 Nov. 2007). Means with different letters are significantly different from each other at a family error rate of $P < 0.05$.

1972). Male *Ae. albopictus* are potentially exploiting components of the lures or the CO_2 to locate a host in anticipation of intercepting potential female mates. The male proportion of the catch in the BGS can also be beneficial when attempting to predict female population levels, given that males will exhibit protandry and emerge 24–48 h before the females (Clements 2000). Such a predictive ability can aid mosquito operators, allowing control of populations before females emerge; however, this predictive ability is untested and requires further study. The biology underlying the attraction of male *Ae. albopictus* to human baits and baited surveillance traps, in this and similar studies, deserves further study.

The BGS and GT provide a relative risk measure for WNV activity: the GT assesses enzootic activity in *Culex* spp. and the BGS assesses the potential epidemic activity in *Ae. albopictus. Ae. albopictus* poses a risk as a WNV bridge vector because it will feed on birds and mammals (Savage et al. 1993; Sardelis et al. 2001, 2002; Turell et al. 2005). The BGS with lures and CO_2 provides an improved surveillance tool for *Ae. albopictus* and can augment enzootic and epidemic surveillance programs for WNV. The majority of WNV-positive pools detected in our study were collected from GTs (86%); this finding was not surprising because these traps target ovipositing enzootic vectors of WNV in the northeastern United States (Andreadis et al. 2004). Conversely, the WNV-positive pool of *Ae. albopictus* during our study was collected with BGS (with no lures or CO_2). We also collected two positive pools from host-seeking *Cx. pipiens* mosquitoes from CDC traps, underscoring that vector surveillance programs should deploy a variety of traps for data collection if possible and as resources allow.

The BGS provides effective surveillance for other important vector species in urban habitats. Rose et al. (2006) reported that the trap was effective for collecting *Cx. pipiens;* however, the GTs collected significantly more *Culex* spp. than the BGS and CDC in our study. The collections of *Ae. japonicus, Ae. triseriatus, Ae. vexans,* and *An. punctipennis* by BGS in Mercer County are noteworthy to surveillance programs targeting these species. Although highly specific for *Ae. albopictus,* programs using the BGS trap can enhance surveillance of other mosquito species without jeopardizing existing goals. The simultaneous use of the three trap types can provide effective surveillance for epidemic WNV vectors (i.e., *Ae. albopictus* using BGS), enzootic vectors (i.e., *Culex* spp. using GT), and

Table 2. Mosquito species (adult females) assayed for West Nile virus in Mercer and Camden Counties, NJ (6 Aug. to 2 Nov. 2007)

Species	Total pools/no. individuals	Camden County infection rates[a,b]				Mercer County infection rates[a,b]					
		BGS	CDC	GT	Total	$BGS_{lures + CO_2}$	$BGS_{no\ lures}$	$CDC_{lures + CO_2}$	$CDC_{no\ lures}$	GT	Total
Ae. albopictus	233/4,303	0	0	0	0	0	1 [5.6 (0.3–27.6)][b]	0	0	0	1 [0.4 (0.1–2.0)]
Cx. pipiens	166/1,687	0	2 [13.5 (2.4–44.2)]	11 [13.6 (7.1–24.2)]	13 [13.3 (7.4–22.6)]	0	0	0	0	7 [17.1 (7.5–34.1)]	7 [16.1 (7.1–32.1)]
Cx. restuans	60/136	0	0	1 [10.5 (0.6–50.9)]	1 [9.6 (0.5–46.6)]	0	0	0	0	0	0
Total	732/6,815	0	2 [1.7 (0.3–5.6)]	12 [10.8 (5.8–18.8)]	14 [4.0 (2.3–6.5)]	0	1 [5.3 (0.3–25.9)]	0	0	7 [14.8 (6.5–29.6)]	8 [2.7 (1.3–5.0)]

Infection rates were calculated per 1,000 female mosquitoes using a bias-corrected max likelihood estimate (MLE).
[a] Two of each trap type (6 total) were used in Camden County and three of each trap (15 total) type were used in Mercer County.
[b] Number of positive pools [MLE (95% CI)].

mosquito diversity (i.e., other host-seeking species using CDC).

In urban New Jersey, the BGS trap has improved the ability of mosquito control operations to monitor and react to *Ae. albopictus.* The increase in catch with the BGS can provide much needed information on the involvement of *Ae. albopictus* in WNV ecology and accurate determination of population densities in response to resident complaints. In 2006, 37 travelers returning from India and La Réunion to the northeast United States were infected with chikungunya virus, exemplifying the continued threat of exotic pathogens entering and establishing in the United States (Lanciotti et al. 2006). Considering that U.S. populations of *Ae. albopictus* are efficient laboratory vectors of chikungunya virus and *Ae. albopictus* was the principle vector in a chikungunya outbreak in Italy, an effective means of surveillance is crucial for protecting U.S. public health (Turell et al. 2001, Bonilauri et al. 2008). Where the Asian tiger mosquito is established in the United States, the BGS trap should be an integral part of any existing or planned surveillance program, and where *Ae. albopictus* has not become established, the BGS trap can provide an early warning system for detecting incipient invasion.

Acknowledgments

We thank A. Jamieson and J. Nunemaker for field assistance in Camden County and E. Williges for field and laboratory assistance in Mercer County. We also thank L. McCuiston for species identification and A. Raghavendran for statistical advice. This is New Jersey Agricultural Experiment Station Publication D-08-08292-04-09 supported by state funds with partial support from the New Jersey State Mosquito Control Commission.

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Received 29 October 2008; accepted 5 April 2009.