Evaluation of Novel Trapping Lures for Monitoring Exotic and Native Container-Inhabiting Aedes spp. (Diptera: Culicidae) Mosquitoes

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Abstract

Surveillance for diurnal container-inhabiting mosquitoes such as Aedes albopictus (Skuse), Aedes japonicus japonicus (Theobald), and Aedes triseriatus (Say) have routinely relied on the deployment of multiple trap types, including CO₂-baited light traps, gravid traps, oviposition traps, and BG-Sentinel. These trap configurations have met with varying degrees of effectiveness and in many instances likely under-sample these key mosquito vectors. Most recently, the BG-Sentinel trap used in conjunction with the human-scent lure has been largely accepted as the gold-standard for monitoring Ae. albopictus. However, its ability to attract other container-inhabiting Aedes species has not been fully evaluated. During 2018, we tested new scent lures, TrapTech Lure-A and Lure-H (Bedoukian Research, Inc.), using BG-Sentinel traps with CO₂ in two regions of Connecticut, Stamford and Hamden, against the BG-Lure. Pooled mosquitoes were additionally screened for arbovirus infection. A total of 47,734 mosquitoes representing 8 genera and 32 species were captured during the study, with the Stamford site deriving on average three times as many mosquitoes per trap, adjusting for sampling effort. Lure-A and Lure-H outperformed the BG-Lure in terms of total numbers, diversity evenness, and the proportion of both Ae. japonicus and Ae. triseriatus. There were no significant differences among lures in capturing Ae. albopictus, and in terms of species richness. Fifty-seven isolates of virus (West Nile, Jamestown Canyon, and La Crosse viruses) were obtained during the study, with no significant difference between trap-lure. We highlight both novel lures as effective attractants for use in mosquito surveillance=, which either outperform, or equal, BG-Lure.

Key words: surveillance, lure, arbovirus, mosquito, container-inhabiting

There are numerous examples of invasive species expanding their geographical distribution around the world; many associated with adverse consequences via habitat damage, species competition, or disease facilitation (Lowe et al. 2000; Crowl et al. 2008). Arthropod vectors of pathogens are no exception, shown by the recent emergence of the tick Haemaphysalis longicornis (Neumann) in the United States, invasive insect vectors of plant phytoplasmas, or mosquitoes including Aedes aegypti (Linnaeus) facilitating the spread of dengue, chikungunya, and Zika viruses in many parts of the world (Akiner et al. 2016; Queiroz et al. 2016; Beard et al. 2018). Several additional problematic container-inhabiting Aedes species (Diptera: Culicidae) have found their way into the United States. Since arriving in Texas by 1985, Aedes albopictus (Skuse) (the Asian tiger mosquito) is currently present in an estimated 40 states, and is of concern for public health and vector control, as a competent vector of 20 arboviruses (Paupy et al. 2009). Aedes japonicus japonicus (Theobald) (Asian bush or Asian rock pool mosquito) has also moved rapidly across the United States and is an invasive mosquito species from which La Crosse virus (LACV) (family: Peribunyaviridae) and West Nile virus (WNV) (family: Flaviviridae) have been isolated; laboratory competence for these and other arboviruses has also been demonstrated (Andreadis et al. 2001; Kaufman and Fonseca 2014). The native mosquito vector Aedes triseriatus (Say) (Eastern tree hole mosquito) is found in eastern United States and southern Canada and coexists with Ae. albopictus and Ae. japonicus in natural and artificial container-breeding habitats. It is the primary vector of LACV, a reportable encephalitic bunyavirus mainly affecting children, generally reported in the Midwestern and Appalachian states.

The State of Connecticut conducts mosquito surveillance from June to October at 91 sites statewide using CDC Light traps, CDC
Gravid traps, and BG-Sentinel traps. A 20-yr dataset produced by this monitoring has shown *Ae. triseriatus* to be widespread across Connecticut and that *Ae. j. japonicus* now occurs widely (Andreadis and Wolfe 2010). *Aedes albopictus* was first detected in the state in 2006, then from 2010 onwards, with geographical expansion and increase abundance in the southwest of the state, and some evidence of overwintering, at this northern limit of its range (Armstrong et al. 2017).

Notably, there are differences in the ability of each trap-type to reflect the abundance and distribution of species present in the environment adequately. Adult *Ae. triseriatus* are rarely detected in general mosquito surveillance with conventional traps, yet high volumes of eggs of this species are typically collected via oviposition trapping (Trexler et al. 1998), indicating that the species is vastly under-reflected in the majority of adult monitoring methods (T. G. Andreadis, unpublished observations). *Aedes albopictus* is not efficiently captured by the most commonly used mosquito traps, such as the CDC miniature light trap or CDC gravid trap; the BG-Sentinel (Biogents AG, Regensburg, Germany) is reported as being the gold-standard tool for detecting a presence of this species (Odenauer et al. 2010; Centers for Disease Control and Prevention 2018). BG-Sentinel traps use a combination of visual and olfactory cues (Centers for Disease Control and Prevention 2018). The efficiency of BG-Sentinel traps can be increased by baiting them with attractants (including use of carbon dioxide), with BG-Lure (Biogents, Germany) being the standard attractant employed with the trap. Given the acceptance of the BG-Sentinel for monitoring *Ae. albopictus* (Li et al. 2016; Ulu and Baker 2018), we employed this trap for testing the hypothesis that novel lures used with the BG-Sentinel trap could improve attraction of other diurnal, container-inhabiting *Aedes* species. This hypothesis was assessed in two regions of Connecticut, USA—one not yet known for reported presence of *Ae. albopictus*, and a second which, during state surveillance, had shown a regular prevalence of the species by July–August each year. Here we evaluate two new chemical lures (here called ‘Lure-A’ and ‘Lure-H’; Bedoukian Research, Inc., CT) for their effectiveness in both overall species diversity, and capture of target container-inhabiting *Aedes* spp. We show that both lures either outperform, or equal, BG-Lure, both in terms of species diversity attracted to the trap, and in the number of target species captured.

Materials and Methods

Lures

Ten sachets of two proprietary research lures were provided for experimental use by Bedoukian Research, Inc. (Danbury, CT) in 2018. Lure-A contained 250 mg of R-1-octen-3-ol and 1900 mg of ammonium bicarbonate in a 6.3 g volume, and was equivalent to the ‘TrapTech’ mosquito lure examined by Anderson et al. (2012). Lure-H contained 300 mg of R-1-octen-3-ol and 1900 mg of ammonium bicarbonate in an 11.6 g volume. The BG-Lure is a commercial lure available from Biogents. Figure 1 depicts the physical lures used in the study. Each trap was also baited with CO₂ by suspending a cooler with dry ice pellets over the trap.

Mosquito Collections

During July and August 2018, BG-Sentinel traps were deployed in the field for 12–15 d at two regions in Connecticut (1: Lockwood Farm, Hamden; 2: Cove Park, Stamford; Fig. 2). These sites consisted of mostly wooded habitat near urban settlements, and represent regions of the state where *Ae. albopictus* was both unknown (Hamden) and commonly detected (Stamford). At each site, 15 traps were arranged in five groups of three traps, each of the three traps in a group being baited with a different lure (Fig. 2). The lures tested included 1) BG-Lure, 2) Lure-A, 3) Lure-H, and their respective trap was placed approximately 50 ft from neighboring traps in the group (a distance believed far enough to distinguish lure aroma, but close enough to enable mosquitoes in the area to choose). Each trap (the whole trap and its respective lure) was rotated counter-clockwise every 2–3 d to control for side location bias. Traps were functioning continually in the field; with batteries changed daily and trap catch nets retrieved each morning. Contents of the catch nets were frozen and a cold-chain maintained from thereon. Female mosquitoes were sorted from other insect fauna, identified to species level (Andreadis et al. 2004) and pooled by date, species, lure-type, and trap location. The species diversity of each trap site-lure combination was recorded. Trapping effort was equal for each type of lure; therefore, efficacy of lure-use was calculated simply as the number of individuals of each species captured.

Viral Screening

Pools of mosquitoes were screened for evidence of arboviral infection using a Vero (African Green monkey) cell line (Armstrong and Andreadis 2006). Briefly, mosquitoes were homogenized in a 2 ml vial with 1 ml phosphate-buffered saline containing 0.5% gelatin, 30% rabbit serum, 1× antibiotic/antimycotic, and a copper BB pellet, using a mixer-mill set for 4 min 25 cycles/second as previously described (Andreadis et al. 2004). Samples were then centrifuged for 5 min at 7,000 rpm at 4°C. Hundred microliters of the supernatant were inoculated onto a confluent monolayer of Vero cells in 25-cm² culture flasks, allowed to absorb for 5 min on a plate rocker, then provided with 4 ml of minimum essential media supplemented with 10% fetal bovine serum, 1× antibiotic/antimycotic. Flasks were incubated at 37°C with 5% CO₂ and examined daily for cytopathic effect (CPE) for up to 7 d. Infected pools showing CPE were harvested and stored at −80°C. Virus isolated in cell culture was identified by molecular methods. RNA was extracted from viral isolates using a QIAamp Viral RNA mini kit (Qiagen, Germantown, MD), eluted in a final volume of 70 µl. A reverse transcription polymerase chain reaction (RT-PCR) was performed using a Titan One-Tube RT-PCR system (Roche Diagnostics, Indianapolis, IN) with generic orthobunyavirus primers (Dunn et al. 1994). Briefly, 5× Buffer, dNTPs (2.5 mM), DDT (100 mM), Titan enzyme mix, and 20 µM of each primer was used with 2 µl RNA template in a 25 µl reaction with the following cycling conditions: 1 cycle of 50°C for 30 min and 94°C for 2 min, followed by 35 cycles of 94°C for 15 s, 55°C for 30 s, and 68°C for 65 s, followed by 1 cycle of 68°C for 7 min. Amplification products of the appropriate size were purified using a QIAquick PCR purification kit (Qiagen) and commercially sequenced (Science Hill DNA Analysis Facility, Yale University, New Haven, CT). To identify WNV, a real-time RT-PCR was conducted according to Herman (2015) using the primer/probe set: WNV10533-F: AAGTTGAGTAGACGGTGCTG, WNV10534-R: AGACGGTCTCGAGGGCTTAC, WNV10560-P: 6-FAM-CTCAACCCGCCAGGACTGCGH, and the following cycling conditions: 1 cycle of 50°C for 30 min and 95°C for 10 min, followed by 30 cycles of 95°C for 15 s, and 60°C for 1 min.

Statistical Analysis

The number of mosquitoes captured by each lure was calculated as a percentage of the total number of that species. Percentages of predominant mosquito species among different trap-lures at a specific
region were compared by the χ² test with the Bonferroni correction for multiple comparisons.

To determine how the lures performed for key species, a one-way analysis of variance was used to assess whether the mean number of mosquitoes caught differed by Lure type. A multiple pairwise-comparison test was then used to examine the significance of any difference between pairs of lures. Since data did not tend to meet normality requirements, a Kruskal–Wallis $H$ test followed by a Pairwise Wilcox rank-sum test was used as a nonparametric multiple pairwise-comparison. All statistical tests were performed in R software (R Core Team 2015).

Species diversity was calculated using the Shannon Index:

$$H' = \sum_i - (P_i \times \ln (P_i))$$

where $P_i$ is the proportion of species $i$ captured

The index was normalized to a score between 0 & 1 using:

$$E (H) = \frac{H'}{\ln(S)}$$

where $S$ is species richness (number of unique species per lure or region) Higher values indicate more diversity while lower values indicate less diversity. This measure takes into account ‘evenness’ (i.e., an equally occurring distribution of different species, as opposed to one or two species dominating).

Results

Mosquito Attraction

A total of 47,734 mosquitoes, representing 32 species of 8 genera, were captured during the study (see Supp file S1 [online only]). For the Hamden site, the lures together caught 14,085 individuals of 26 different species in 6 genera during 15 d of sampling; at the Stamford site, the lures caught 33,649 mosquitoes of 27 species of 8 genera over 12 d. The most abundant species captured at each site are shown in Fig. 3. At Lockwood Farm, Hamden, Ae. j. japonicus (representing 32.2% of captures), Aedes vexans (Meigen) (28.5%), and Anopheles punctipennis (Say) (10.6%) were the most prevalent mosquito species detected; only six individual Ae. albopictus were detected from Hamden. In contrast, 3,340 Ae. albopictus (9.9% of site captures) were collected from coastal Stamford, CT, where the most prevalent species were Culex salinarius (Coquillett) (32.6% of captures), Ae. vexans (24.5%), and Ae. j. japonicus (13.9%).

All three lure types yielded Ae. albopictus at both regions of CT. The percentage of principal species captured by each lure, and the overall species richness and diversity detected by each lure are presented in Table 1. Both species diversity, and evenness or spread in abundance (standardized to a value between 0 and 1), were highest via use of Lure-A. Lure-A also provided the greater mosquito species richness; $n = 30$. 

![Novel lures used in the study](https://academic.oup.com/jme/advance-article-abstract/doi/10.1093/jme/tjz200/5686812/3)
*Culex pipiens* (Linnaeus) was the only species for which BG-Lure outperformed the novel lures ($\chi^2(2) = 10.64$, df = 2, $P = 0.005$; pairwise comparisons being significant between BG-Lure and Lure-A ($P = 0.048$) and between BG-Lure and Lure-H ($P = 0.005$); Lure-A and Lure-H were not significantly different ($P = 0.224$). There was no significant difference between the different lure types for

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**Fig. 2.** Positioning of traps at test sites in Connecticut.

**Fig. 3.** Predominant species caught at Connecticut study regions (a) Hamden, (b) Stamford, showing number of mosquitoes.
capturing *Ae. albopictus* or *Ae. vexans* ($\chi^2(2) = 2.99$, df = 2, $P = 0.224$; and $\chi^2(2) = 0.081$, df = 2, $P = 0.961$, respectively). In contrast, all other mosquito species were better captured using the novel lures. Figure 4 displays the number of individuals caught by each trap lure type, for the most abundant mosquito species ($n > 1,000$). The 9,124 *Ae. japonicus* caught during the study (4,886 via Lure-H, 4,047 via Lure-A, and 281 via BG-Lure; average of 22.1 per night), showed a significant catch rate difference between the different lures ($\chi^2(2) = 169.93$, df = 2, $P < 0.001$); with pairwise comparisons showing differences between BG-Lure and Lure-A ($P < 0.001$); and between BG-Lure and Lure-H ($P < 0.001$); Lure-A and Lure-H were not significantly different ($P = 0.25$). On average, in the BG-Sentinel baited with CO2, 28.9 *Ae. japonicus* per night were collected per trap using Lure-A, 34.9 via Lure-H, and 2.0 per night with BG-lure. Similarly, for native vector, *Ae. triseriatus* (1,890 captured in total; 739 with Lure-H, 858 with Lure-A, and 293 with BG-Lure; average of 4.7 per night), lure performance differed ($\chi^2(2) = 44.37$, df = 2, $P = 0.001$; with a significant difference between BG-Lure and Lure-A ($P < 0.001$); and between BG-Lure and Lure-H ($P < 0.001$); Lure-A and Lure-H were not significantly different ($P = 0.4$). An average of 6.2 *Ae. triseriatus* were collected per trap per night using Lure-A; 5.3 with Lure-H, and 2.1 using BG-lure.

**Figure 4.** Relative contribution of lure-type in the detection of the predominant mosquito species (where over 1,000 detections of the species were recorded); asterisk indicates a significant difference between trap-type capture.

**Virus Prevalence**

From Stamford CT, the 33,649 captured mosquitoes were tested as 1,985 pools (grouped by species/date/lure). A total of 38 isolates of WNV were obtained, associated with 8 different species of mosquito (Table 2). The 14,085 mosquitoes captured at Hamden CT were tested as 1,609 pools producing 11 isolations of Jamestown Canyon virus, 4 of LACV, and 4 of WNV (Table 2).

Considering which lures had captured the virus-positive mosquitoes, at Hamden, 3 isolates were from BG-Lure baited traps, 8 each from Lure-A and Lure-H traps, at Stamford, 15 of the WNV isolates came via BG-lure and Lure-H each, and 8 from Lure-A. Table 3 indicates the different virus isolates made using each lure type, and the infection rates for each virus type. Each lure was tested in the field for a total of 140 trap nights (5 traps used over 28 nights). There was no significant relationship between the rates of virus detection and different lures.

**Discussion**

In this study, we show the efficacy of two new lure formulations for use in BG-Sentinel traps for mosquito surveillance. Our study
indicates that both Lure-A and Lure-H, in conjunction with CO₂, are effective attractants for the capture of container-inhabiting *Aedes* spp. in this region of the northeastern United States. We found that Lure-A and Lure-H were superior for collecting *Ae. japonicus* and *Ae. triseriatus* and performed as well as the current bait used within BG-Sentinel traps in capturing *Ae. albopictus*. Lure-A best reflected species diversity. Furthermore, both these novel lures demonstrated better or equal capture ability for nearly all other species than the BG human-scent lure, and thus, should receive strong consideration for use in BG-sentinel traps where diurnal container-inhabiting mosquitoes are the primary species of concern.

The detection of LACV in four mosquito pools from Hamden, CT is of notable significance. Historically, LACV has only rarely been detected in CT and the northeastern United States (Grimstad 1988, 2001). The species is a competent vector for WNV, Saint Louis encephalitis virus, Japanese encephalitis, LACV and dengue virus (Kaufman and Fonseca 2014). Seven of the 42 isolates of WNV obtained in the present study were from *Ae. japonicus* pools reaffirming its role as a potential ‘bridge’ vector.

Jamestown Canyon virus (JCV) is an orthobunyavirus of the California serogroup causing febrile illness and neurologic disease in humans, and is transovarially transmitted in mosquitoes (Webster et al. 2017). The pathogen has been isolated from a variety of mosquito species of different genera (Andreadis et al. 2008). *Aedes canadensis* (Theobald) (one JCV isolate here) followed by a number of other woodland species including *An. punctipennis* have been incriminated as likely vectors of JCV in the Northeast (Andreadis et al. 2008). In this study, *An. punctipennis*, a species reported to feed readily on humans, yielded nine of the 11 isolates of JCV and it was more frequently captured using Lure-A and Lure-H than the conventional BG-Lure. It has been suggested that this species and *Anopheles quadrimaculatus* (Say) play a role in JCV transmission during the late summer when the majority of human cases occur in the northeastern and northcentral United States (Grimstad 1988, 2001).

Differences in the rates with which the lure type are associated with virus, could arise from either the total number of mosquitoes that the lure attracted, or the increased abundance of certain species associated as a vector for a virus. For example, increased isolates of LACV might be expected with a lure that attracts *Ae. triseriatus*; however, that was not the case here where BG-lure performs worse in capturing this vector, yet had an equal rate of LACV isolation as Lure-H. Future studies, involving more isolates of virus, for example during an outbreak, or over longer sampling periods, may reveal greater detail of the effect that lure has on capture of viral-infected mosquitoes.

Of the two study regions in Connecticut, Stamford is located on the coast, and the composition of attracted species reflected that location. The new lures collected greater numbers of two salt marsh inhabiting species that are widespread and abundant along the coast.
Atlantic coast of the United States, *Aedes sollicitans* (Walker) and *Culex salinarius* (Coquillett) than the BG lure. These species can be severe biting pests (Shone et al. 2006; Crans 2016) and have also been incriminated as bridge vectors of Eastern Equine Encephalitis virus (EEEV) and WNV (Crans 1977; Andreadis et al. 2004). Notably, two isolates of WNV were identified from *Ae. sollicitans*, which is infrequently collected in either CO2-baited CDC light or gravid traps and from which only a single isolate of WNV had been previously detected in nearly 20 yr of state-wide surveillance in CT (Andreadis and Armstrong, pers communication). Twelve isolates of WNV were obtained from *Cx. salinarius* consistent with its role as an efficient bridge vector (Andreadis et al. 2004,).

A key vector of WNV in the United States (Andreadis 2012), *Cx. pipiens* produced 13 isolates of this flavivirus during the study. Many more *Cx. pipiens* were collected at the coastal site than the inland site (1,016 mosquitoes at Stamford vs 76 at Hamden), and this was the only species for which conventional BG-Lure outperformed the two novel lures. This compared somewhat favorably for collection of *Cx. pipiens*; CDC gravid traps being the trap of choice for WNV detection in this peridomestic species.

It is generally acknowledged that *Ae. albopictus* is not efficiently collected by CDC light or gravid traps, and that BG-Sentinel or autocidal ovitraps are most effective (Farajollahi et al. 2009; Centers for Disease Control and Prevention 2018). BG-Sentinel traps use a combination of visual and olfactory cues. The olfactory cues, provided by careful consideration of lure choice, can promote more effective trapping of mosquito species. Unlu et al. (2016) used BG-Sentinel traps to show that R-octenol lures in combination with human-skin lure worked most effectively to trap *Ae. albopictus* females. Unlike the current study, they did not find the R-octenol and ammonium bicarbonate blended lures to result in increased diversity of mosquito species, however they did not bait traps with CO2 (Unlu et al. 2016). Anderson et al. (2012) found such a lure in combination with CO2, to be particularly effective for *Ae. j. japonicus*, using CDC light traps (Anderson et al. 2012). Some studies have tested alternative attractants, for example with BG-Sentinel traps, baits such as live mice have been used to effectively attract *Ae. albopictus* (Lacroix et al. 2009). A second lure manufactured for BG-Sentinel traps, BG-SweetScent (Biogens, Germany) however was not found to perform better than the BG-lure (Akatarovic et al. 2017).

In conclusion, we highlight two novel chemical lures that work effectively in BG-Sentinel traps to capture key *Aedes* spp. disease vectors. Since the performance of these lures generally surpasses that of the gold-standard BG-Lure in terms of diversity (Lure-A) and numbers caught, we suggest either of these products be considered as a replacement once commercially available, in order to deliver the more efficient mosquito monitoring.

**Supplementary Material**

Supplementary data are available at Journal of Medical Entomology online.

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**CDC Statement**

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**Author Contribution**

G.E., T.A., and P.A. conceived and designed the study; G.E. and A.D. conducted the field studies; R.B. and L.C. provided the lures; J.S. identified mosquito species; M.M. conducted viral screening; G.E. analyzed data and wrote the manuscript draft; T.A., P.A., and G.E. edited the final manuscript.

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