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Short communication

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TrapTech R-Octenol Lure Does Not Improve the Capture Rates of *Aedes albopictus* (Diptera: Culicidae) and Other Container-Inhabiting Species in Biogents Sentinel Traps

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Abstract

Aedes albopictus (Skuse) and other container-inhabiting species have become important public health concerns due to the transmission of dengue, chikungunya, and Zika viruses. Effective surveillance is dependent on the ability to collect a sufficient number of mosquitoes for population monitoring and pathogen isolation. The Biogents Sentinel (BGS) trap supplied with a proprietary human skin lure has become the standard tool for container-inhabiting *Aedes* species collections worldwide. Recently, R-octenol, a single isomer of the well characterized mosquito attractant octenol, was shown to greatly improve the capture rate of some *Aedes* species when utilized with the Center for Disease Control and Prevention (CDC) light traps and Mosquito Magnet traps. This study evaluated the effectiveness of the TrapTech lure (TT lure), containing R-octenol, alone or in combination with the human skin lure in a BGS trap to capture *Ae. albopictus* and other species. BGS traps with human skin lures or a combination of the two lures collected approximately twice as many *Ae. albopictus* females compared to those with TT lures. Unlike previous studies, baiting BGS traps with TT lures did not result in increased diversity of mosquito species, or in higher numbers of other container-inhabiting *Aedes* species. Although human skin lures were clearly superior to TT R-octenol lures in BGS traps, R-octenol lures are more widely available and might still be used as an alternative lure, especially when *Ae. albopictus* populations are high.

Key words: mosquito surveillance, trap evaluation, BGS trap, olfactory kairomone, New Jersey

Aedes albopictus (Skuse), the Asian tiger mosquito, is a container-inhabiting species that thrives in artificial habitats found in peridomestic environments (Bonizzoni et al. 2013, Unlu et al. 2013). This diurnally active mosquito also negatively impacts human quality of life (Halasa et al. 2014) and is of great public health concern because of its ability to vector many arboviral diseases such as dengue, chikungunya, and Zika (Gratz 2004, de Lamballerie et al. 2008, Lambrechts et al. 2010, Paupy et al. 2010). The continuous expansion of *Ae. albopictus* and the increasing role that the species plays in emerging and re-emerging vector-borne diseases accentuates the need for improving surveillance of field populations (Meeraus et al. 2008, Unlu and Farajollahi 2012, Bonizzoni et al. 2013, Crepeau et al. 2013).

The Biogents Sentinel trap (BGS, Biogents AG, Regensburg, Germany; Krockel et al. 2006) has become the standard tool for surveillance of *Ae. albopictus* populations (Meeraus et al. 2008, Farajollahi et al. 2009, Obenauer et al. 2010). The primary olfactory attractant in the trap is the human skin lure or BG Lure (Biogents AG), a combination of fatty acids, ammonia, lactic acid, and other proprietary components, which are designed to mimic human odors. The human skin lure has been shown to significantly increase the capture rates of *Ae. albopictus* mosquitoes compared to unbaited traps (Farajollahi et al. 2009).

Other olfactory attractants used with BGS traps may also enhance capture rates and increase species diversity, while reducing cost. One of the most promising recently developed mosquito attractants is R-(-)-1-Octen-3-ol (hereafter R-octenol), which is a single isomer of the racemic mixture known as octenol (1-Octen-3-ol). A commercial R-octenol lure (MosquitoMagnet, Woodstream Corporation, Lititz, PA Kline et al. 2007, Lühken et al. 2014, Rochlin et al. 2016) is widely available, and an experimental lure (TrapTech, Bedoukian Research, Inc., Danbury, CT) has been shown to greatly increase collections of the Asian bush mosquito, *Aedes japonicus japonicus* (Theobald) and associated species in northeastern United States (Anderson et al. 2012, Rochlin et al. 2016). The TrapTech lure (TT lure) is a proprietary blend primarily consisting of R-octenol and ammonium bicarbonate (Anderson et al. 2012). While other studies have compared different traps with several attractants (Rochlin et al. 2016) or the addition of TT lures to standard CDC light traps (Anderson 2012), none of the studies have compared the efficacy of R-octenol lures to that of the human skin lures with BGS traps. Additionally, no studies have evaluated whether a combination of the human skin and R-octenol-containing lures may lead to increased numbers of mosquito species collected in BGS traps. This study evaluated the effectiveness of the TT lure, containing R-octenol, alone or in combination with the human skin lure in a BGS trap to capture *Ae. albopictus* and other species.

Materials and Methods

The study was conducted in the City of Trenton, Mercer County, NJ, between July and mid-October (the peak time for *Ae. albopictus* in this area). Three highly urbanized sites were selected for the study. The first site (0.5 ha) was an automobile salvage yard (40° 14′06.1″ N, 74° 44′41.5″ W); the second site (1.2 ha) encompassed industrial and residential properties (40° 14′24.5″ N, 74° 44′27.9″ W), and the third site (4.0 ha) was located in an industrial section of the City (40° 13′58.0″ N, 74° 44′21.6″ W). The ubiquity of tires and other containers, along with over-grown vegetation, created numerous habitats for *Ae. albopictus* and other container-inhabiting mosquitoes.

The following lure combinations were used with duplicate BGS traps at each site (six traps total): 1) TT lure only, 2) BG lure only, and 3) TT lure and BG lure combined. The traps were placed ~50 m apart and operated for ~24-h periods for 17 and 13 trapping nights in 2013 and 2014, respectively. Each trap was labeled and always received the same lure. The traps with different lures were rotated for each trapping session using a random number generator to determine the first trap placement. The traps were rotated counter-clockwise for each subsequent trap week until the end of the study.

All statistical analyses were done using R v. 2.15.1 (R Development Core Team 2015) and the packages lme4 v. 1.0-4 for mixed effects models and vegan v. 2.0-10 for multispecies comparisons. A linear mixed-effects model with Poisson distribution was used to compare the number of *Ae. albopictus* mosquitoes (male and female) collected by each lure or lure combination. The numbers of duplicate traps at the same site were averaged and rounded to the nearest integer to avoid pseudoreplication (Hurlbert 1984). The model contained lures as a fixed effect, whereas site, year, and collection date nested within the year were random effect variables to account for spatial and temporal autocorrelation. The mixed-effects model accounted for random slopes and intersects, i.e., baseline or through time differences among the site–date–lure combinations. To check the model's assumptions, residual plots were visually inspected for any deviations from homoscedasticity or normality. Individual-level random effect was added to the model to estimate correct model parameters to address overdispersion in *Ae. albopictus* count data ($P < 0.05$).

For species composition and abundance comparisons among the three lure combinations, the ordination method with a Bray–Curtis distance measure was used (Beals 1984). The species counts were log (x+1) transformed to decrease the contribution of the most abundant species. Nonparametric analysis of variance (PERMANOVA) based on dissimilarities constrained by collection date was performed using the adonis function in the vegan package. *P*-values were corrected for multiple tests using the Holm–Bonferroni correction factor. If significant, the contributions of each individual species to the overall dissimilarity of mosquito species composition and abundance between two lure combinations were determined by similarity percentage (simper function).

Results and Discussion

BGS traps with BG lures collected approximately twice as many *Ae. albopictus* females (mean ± SE = 24.2 ± 2.65) compared to traps with TT lures (mean ± SE = 12.8 ± 1.68), $P = 0.002$ (Table 1; Fig. 1). Traps with BG lures alone also detected *Ae. albopictus* females more often compared to TT lures alone, 95% versus 75% of trapping occasions, respectively. Combining BG lures and TT lures did not result in significant changes in the *Ae. albopictus* collections compared to BG lures alone ($P = 0.491$). However, the difference between BG + TT lures combination versus TT lures alone was significant ($P = 0.013$). A similar trend was present in *Ae. albopictus* male collections (Table 1; Fig. 1): traps with BG lures collected significantly more males compared to those with TT lures ($P = 0.033$), but not with combined lures (BG + TT, $P = 0.85$). The difference between combined lures and TT lures alone was borderline not significant, at $P = 0.065$. Traps with TT lures alone detected *Ae. albopictus* males less often (62%), versus with BG lures alone (78%) and BG + TT (76%).

Overall mosquito species composition and abundance collected with different lures were significantly different (PERMONOVA, $P < 0.001$). Pairwise comparisons indicated the difference between BG lures and a combination of both lures (BG + TT) was not significant ($P = 0.697$). However, the differences between BG or BG + TT combinations and TT lures alone were significant ($P < 0.001$, corrected significant alpha level at $P = 0.003$). Similarity percentage analysis indicated that the three mosquito species accounted for >90% of the significant dissimilarities (Table 1). BG lures and the BG + TT combination collected higher numbers of *Ae. albopictus* mosquitoes of both sexes (contributing ~75% of the observed dissimilarities) and *Culex pipiens* L. and *Culex restuans* Theobald mosquitoes (contributing ~10% of the observed dissimilarities). Traps with TT lures collected slightly higher numbers of *Ae. j. japonicus* mosquitoes (contributing ~8% of the observed dissimilarities). The contributions of the remainder of the species to the observed dissimilarities were minor (<8% combined).

Previous research has demonstrated that BGS traps were efficient for *Ae. albopictus* collections even if used without the addition any chemical attractant (Farajollahi et al. 2009). Unbaited BGS traps collected ~10 times more *Ae. albopictus* females than unbaited CDC light and gravid traps. Addition of CO_2 further increased the number of *Ae. albopictus* captured in baited BGS traps, with a corresponding increase in species diversity (Meeraus et al. 2008, Farajollahi et al. 2009). BGS traps with BG lures were very specific in collecting *Ae. albopictus*, and the diversity of other mosquito species was very low (Unlu and Farajollahi 2014). Our study suggests that the addition of TT lures did not increase or reduce the efficacy of BG lures to either *Ae. albopictus* or any other mosquito species. In this study, we expected that the increase in active compounds (BG + TT lure combination) naturally found in human emanations might improve the number of mosquitoes collected (Kline et al. 1990). Instead, including a few human odor blends was enough to attract mosquitoes without the need of all biologically active components found in human scent (Okumu et al. 2010). On the contrary, when Hoel et al (2007) combined lactic acid and octenol lures in the presence of CO_2, an increased the number of mosquitoes were collected including *Ae. albopictus* by using Mosquito Magnet Pro (Hoel et al. 2007).

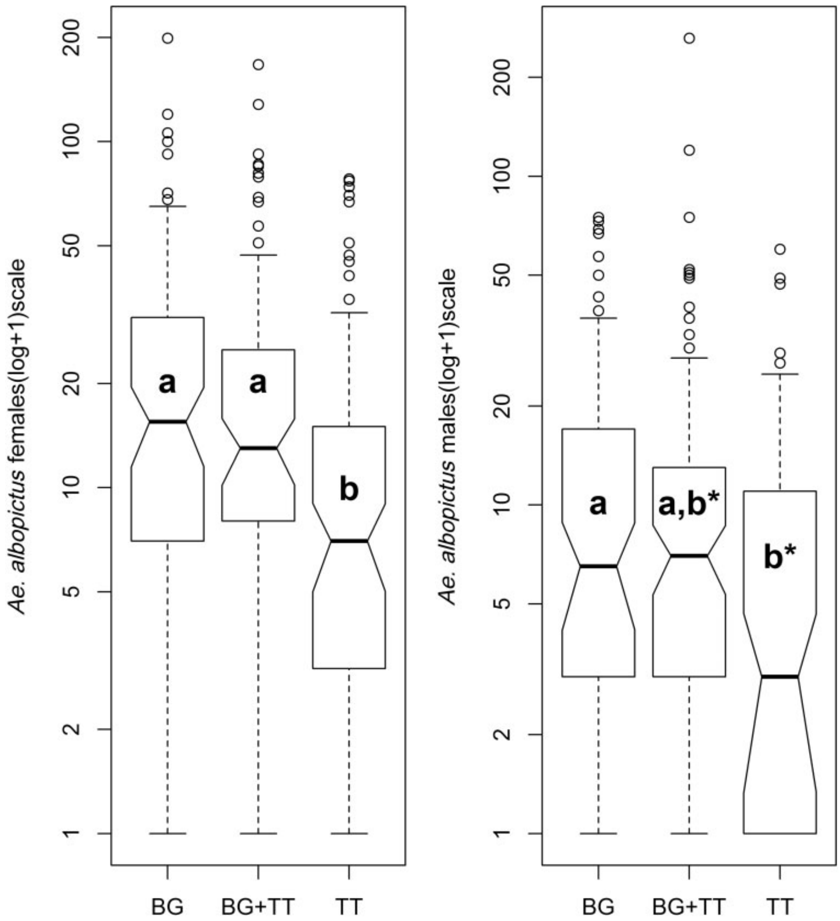

Fig 1. Female and male *Ae. albopictus* collections with three lure combinations in BGS traps. The line indicates the median, and the box shows the first and third quartile. The notches provide 95% CI for the median. The whiskers extend to 1.5 times the interquartile range from the box with the outliers indicated as open circles. Letters inside boxes indicate statistically significant differences in the mosquito collections by different lures in mixed-effects models.

Table 1. Mosquito species collected with three lure combinations in BGS traps

Species/Lure	BG lure[a]	Both lures[a]	TT lure[b]
Ae. albopictus F	24.7 ± 3.22[a] [0.95]	22.6 ± 3.05[a] [0.86]	12.7 ± 1.9[b] [0.75]
Ae. albopictus M	12.7 ± 1.83[a] [0.78]	14.8 ± 3.4[a,b] [0.76]	6.9 ± 1.15[b] [0.62]
Ae. atropalpus	1	1	nc
Ae. grossbecki	3	1	2
Ae. japonicus	0.3 ± 0.08 [0.21]	0.33 ± 0.07 [0.26]	0.42 ± 0.08 [0.17]
Ae. triseriatus	0.3 ± 0.1 [0.14]	0.18 ± 0.1 [0.11]	0.13 ± 0.04 [0.11]
Ae. vexans	8	7	4
An. punctipennis	7	4	4
An. quadrimaculatus	5	2	3
Cx. pipiens/restuans	1.12 ± 0.56 [0.26]	0.76 ± 0.28 [0.21]	0.42 ± 0.12 [0.14]
Cx. erraticus	2	4	nc
Cx. salinarius	1	nc	nc
Ps. ciliata	nc	1	nc
Ps. colombiae	1	1	1
Ps. ferox	4	5	6
Ps. howardi	2	2	nc
Tx. r. septentrionalis	1	nc	1

For common species, mean ± SE (proportion of trap nights collected) is shown. For uncommon species (i.e., collected on fewer than 10 trapping nights, usually as a single specimen each), integers show the number of detections. For *Ae. albopictus* female (F) and male (M) numbers, different letters indicate statistically significant differences among lure combinations. Different letters next to lures indicate statistically significant difference in mosquito species composition and abundance. The species which contributed the most to those differences are highlighted in gray.

nc—not collected.

Although the TT lure on its own was effective for attracting a higher diversity of mosquito species with other trap designs, our results for BGS traps are not in agreement with those studies (Anderson et al. 2012). There might be two reasons for these observations. One is that the BGS trap design is not effective at catching other mosquito species (Meeraus et al. 2008, Rochlin et al. 2016). Another possible reason is that the artificial "scent" lures in general, and BG lure in particular, appeared to work better in combination with CO_2 (Farajollahi et al. 2009, Obenauer et al. 2010). Anderson et al. (2012) reported an increase in the number of *Ae. j. japonicus* collected with TT lures alone, but an even larger increase was achieved after the addition of CO_2. Human skin and TT lure combinations with CO_2 and heat were very effective in collecting large numbers of *Ae. j. japonicus* and other low-flying *Aedes* species in Mosquito Magnets (Rochlin et al. 2016). Addition of CO_2 may lead to increased mosquito catch and diversity in BGS traps with TT lures. However, unlike CDC light traps or Mosquito Magnets, BGS traps are usually deployed without CO_2 for routine surveillance. The main reason for this is that the recommended way of supplying CO_2 is by a small cylinder (Obenauer et al. 2010), which is usually not available for field operations (Kline 2002). While not as effective as the BG lure, R-octenol lures (e.g., Mosquito Magnet R-octenol, very similar to TT lure) are widely available commercially and can be purchased at many large hardware stores. R-octenol lures may serve as an alternative when BG lures are not available, especially when *Ae. albopictus* populations are high.

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