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SCIENTIFIC NOTE

IMPROVED SURVEILLANCE OF *AEDES TRISERIATUS* USING THE BG-PRO TRAP: IMPLICATIONS FOR SAMPLING HOST-SEEKING LA CROSSE VIRUS VECTORS

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ABSTRACT. La Crosse virus (LACV) is responsible for the majority of pediatric arboviral encephalitis in the United States. At present there are limited options for host-seeking surveillance for the primary vector (*Aedes triseriatus*) and, to a lesser extent, two invasive species (*Ae. albopictus* and *Ae. japonicus*) capable of transmitting LACV. We evaluated four host-seeking trap configurations (Centers for Disease Control and Prevention [CDC] Light trap, BG-Pro with BG lure, and BG-Sentinel 2 with and without BG lure) via two 4 × 4 Latin square field studies. Over the course of 128 trap-days, 436 mosquitoes were collected with the two most common species being *Aedes triseriatus* (n = 156, 35.8% of total) and *Ae. albopictus* (n = 182, 41.7% of total). The BG-Pro, on average, collected approximately 3 times more female *Ae. triseriatus* than the CDC light trap or the BG-Sentinel with BG lure. Similarly, the odds of collecting *Ae. triseriatus* with the BG-Pro trap were 3.02 times (95% CI: 1.96–4.67) than the CDC light trap; statistically greater than any other trap. There was no statistical difference in the odds of collecting *Ae. triseriatus* by the BG-Sentinel 2 (irrespective of lure presence) when compared to the CDC light trap as the reference. There was no difference in the odds of collecting *Ae. albopictus* using the BG-Sentinel 2 (OR: 4.62, 95% CI: 2.76–7.74) or the BG-Pro (3.06, 95% CI: 1.78–5.24) when compared to the CDC light trap as the reference. The limited collection of *Ae. japonicus* precluded any meaningful comparisons. Taken together, the BG-Pro trap should be considered for the surveillance or collection of the primary LACV vector, *Ae. triseriatus*.

KEY WORDS *Aedes albopictus*, *Ae. triseriatus*, BG-Pro, BG-Sentinel 2

La Crosse virus (LACV) is a leading cause of pediatric arboviral encephalitis in the U.S., particularly affecting children under 16 (Fagre et al. 2023). While LACV disease was initially recognized in Wisconsin, recent cases are concentrated in Ohio, North Carolina, West Virginia, and Tennessee (Day et al. 2023). In the southern Appalachian region, LACV exhibits persistent, localized clusters at county and zip code levels, with household-level risk suggesting the need for residential public health interventions (Byrd et al. 2018, Day et al. 2024).

The primary LACV vector is *Aedes triseriatus* (Say) with *Ae. albopictus* (Skuse) and *Ae. japonicus* (Theobald) as invasive secondary vectors (Westby et al. 2015). *Aedes triseriatus* is considered the main vector because of consistent findings of infected specimens near human cases, efficient laboratory transmission, transovarial transmission, and feeding behaviors that include humans and amplifying hosts (Pantuwatana et al. 1972, 1974: Watts et al. 1973, 1974). Vertical transmission within *Ae. triseriatus* populations likely facilitates overwintering and persistence of LACV. Because LACV disease risk is clearly associated with exposure to infected mosquitoes, effective methods to estimate biting rates, particularly for *Ae. triseriatus*, are necessary to better understand LACV exposure risk.

Host-seeking traps, baited with CO_2 or synthetic human odorants, capture mosquitoes actively seeking hosts and may serve as proxies for human exposure. However, standard traps like the Centers for Disease Control and Prevention (CDC) light trap (underrepresent diurnal container *Aedes* species. The BG-Sentinel 2 trap, designed to address this, effectively collects *Ae. albopictus* and *Ae. aegypti*, but its efficacy for *Ae. triseriatus* is less known (Maciel-de-Freitas et al. 2006, Camara et al. 2022). The BG-Pro a modified trap combining features of the CDC light trap and BG-Sentinel 2 is known to effectively trap *Ae. albopictus* (Degener et al. 2021). However, it has not been evaluated in the context of LACV vector surveillance, namely *Ae. triseriatus*, using a comparative approach in a LACV disease endemic area. This study aims to establish more effective methods for collecting host-seeking *Ae. triseriatus*, with secondary emphasis on invasive LACV vectors, by evaluating the BG-Sentinel 2 (with and without the BG human lure) and the BG-Pro (with the BG human lure), using the CDC light trap as a reference (Figs. 1a–d).

Two 4 × 4 Latin square design studies were conducted in Jackson County, North Carolina, during summer 2023 (June 27–July 1 and August 25–29). Traps were placed in mixed hickory-oak forests near Western Carolina

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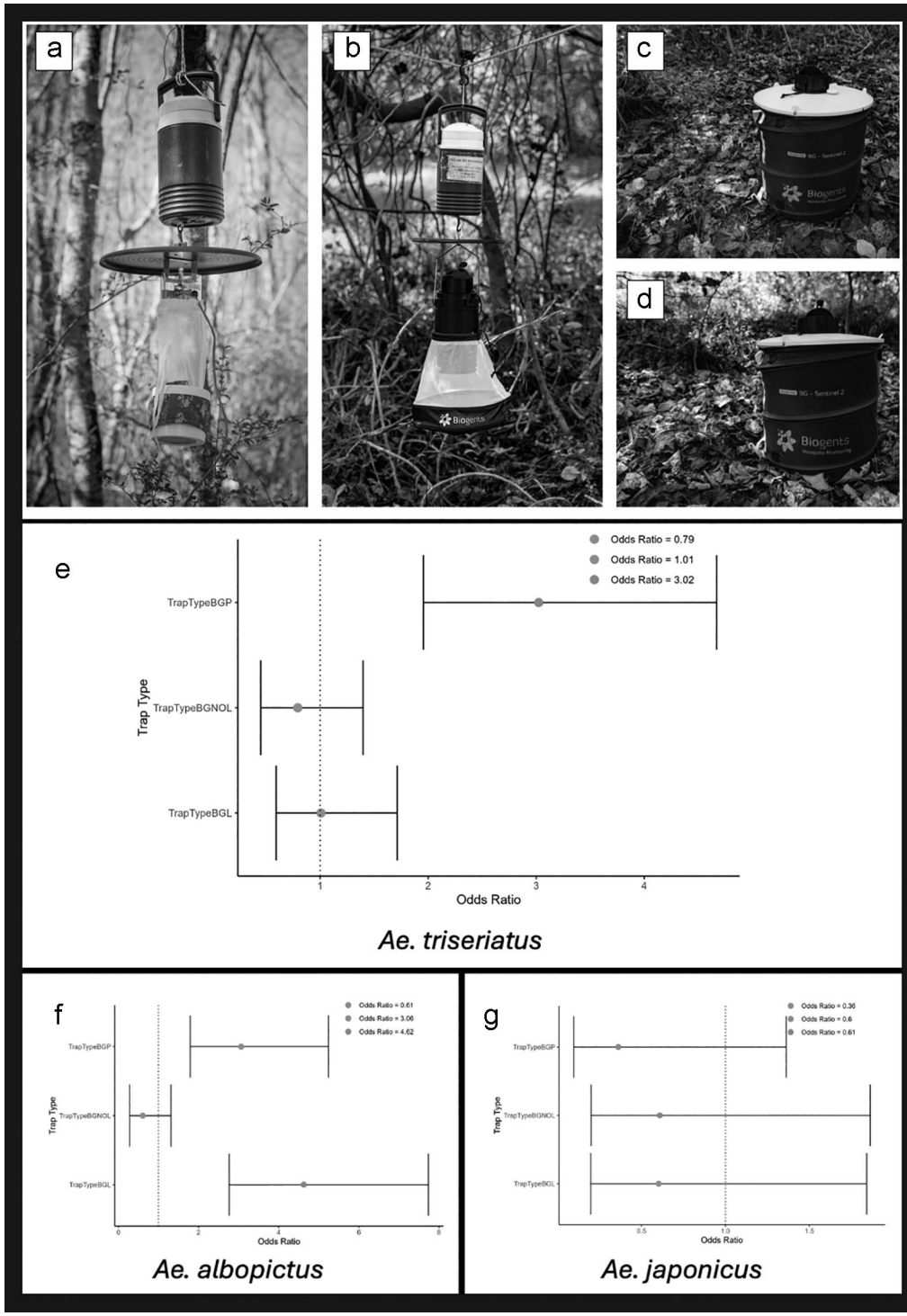

Fig. 1. Trap configuration and odds of collecting LACV vectors. (a) CO_2-baited CDC Light Trap, (b) CO_2-baited BG-Pro with BG-lure cartridge, (c) CO_2-baited (internal) BG-Sentinel 2 with BG-lure cartridge, (d) CO_2-baited (internal) BG-Sentinel 2 without BG-lure cartridge. Odds of trapping *Ae. triseriatus* (e), *Ae. albopictus* (f), and *Ae. japonicus* (g).

Table 1a. Total number of mosquitoes collected by trap in Jackson County, NC (2023); June and August combined.

Species	BG-Pro			BG-Sentinel (+ lure)			BG-Sentinel (− lure)			CDC light trap			Total
	M	F	Total	M	F	Total	M	F	Total	M	F	Total	
Aedes albopictus	5	53	58	11	80	91	2	10	12	3	18	21	182
Ae. canadensis	0	0	0	0	1	1	1	4	5	0	0	0	6
Ae. japonicus	0	3	3	0	5	5	0	5	5	1	8	9	22
Ae. triseriatus	0	81	81	0	27	27	0	21	21	1	26	27	156
Ae. spp. (unknown)	0	2	2	1	1	2	0	0	0	0	0	0	4
Ae. vexans	0	0	0	0	2	2	0	0	0	0	0	0	2
Anopheles crucians	0	0	0	0	0	0	0	1	1	0	1	1	2
An. punctipennis	0	1	1	0	10	10	0	11	11	0	8	8	30
An. sp.	0	0	0	0	0	0	0	0	0	0	0	1*	1
Coquillettidia perturbans	0	0	0	0	4	4	0	7	7	0	11	11	22
Culex pipiens	0	0	0	0	0	0	0	2	2	1	0	1	3
Cx. salinarius	0	0	0	0	1	1	0	0	0	0	0	0	1
Cx. spp. (unknown)	0	0	0	0	1	1	0	0	0	0	2	2	3
Toxorhnchites rutilus	0	2	2	0	0	0	0	0	0	0	0	0	2
Total:			147			144			64			81	Σ = 436

M, male; F, Female; *sex unknown.

University, at sites with known LACV vector presence. Each site had 4 trap locations, approximately 50 m apart. Four trap types were tested: CDC Light Trap, BG-Pro, BG-Sentinel 2 with human lure, and BG-Sentinel 2 without lure. All traps were CO_2-baited with dry ice. Trap placement was randomized and rotated daily for 4 days. Traps were deployed between 1400–1500 h and collected between 0900–1010 h to align with mosquito activity and minimize loss of CO_2 sublimation.

Collected mosquito samples were frozen at −20°C, then identified and sorted by species, sex, location, date, and trap type (Harrison et al. 2016). Specimens were stored at –20°C until LACV testing. Mosquito pools (groups of ≤50 mosquitoes of the same species, collection site, collection date, and sex) in 2 ml microcentrifuge tubes with stainless steel beads were shipped on dry ice and stored at −80°C until further processing. To process the pools, 1 ml of (DMEM) Dulbecco's Modified Eagle Medium (supplemented with 5% Fetal Bovine Serum, 1% Penicillin-Streptomycin, 1 mg/ml Amphotericin B, and 100 mg/l Gentamicin) was added to each tube. Using a tissue lyser, the samples were homogenized at speed 20 for 3 min and immediately centrifuged at 12,000 RPM for 10 min. To extract ribonucleic acid (RNA) and test pools by quantitative reverse transcription-polymerase chain reaction (qRT-PCR,) 200 µl of each centrifuged sample was extracted using the MagMax Viral/Pathogen Nucleic Acid Isolation kit (Thermo Fisher, Valencia, CA, USA) in accordance with the manufacturer's instructions. Molecular testing was performed in duplex via qRT-PCR, using the QuantiTect Virus +ROX Vial Kit (Qiagen, Redwood City, CA, USA). Forward, reverse, and probe primers were used in equal concentrations of 4 µM as dictated by the kit protocol. 5 µl of RNA from each sample was used. La Crosse virus primers are available upon request. The following cycling conditions were utilized: 50°C for 20 min, 95°C for 5 min; and 45 cycles of 95°C for 15 sec, 60°C for 45 sec. Virus isolation attempts were performed as follows. Mosquito pools were thawed from –80°C storage and centrifuged at 12,000 RPM for 10 min. 200 µl of each sample was filtered using 0.45 µm Multiscreen HTS) HA filter plates (MilliporeSigma, Burlington, MA, USA) with a Multiscreen HTS 96 format vacuum manifold (MilliporeSigma). Filtered samples were directly applied to 12 well monolayers of Vero cells. Plates were incubated at 37°C for 45 min, rocking every 15 min. Following adsorption, an additional 1 ml of complete DMEM was added to each well. Cells were monitored for cytopathic effects for 14 days. Samples demonstrating cytopathology were harvested, supplemented with 20% FBS, filtered with a 0.45 µm spin column (Corning, Corning, NY, USA), and stored at −80°C until subsequent genomic sequencing.

Data were analyzed using generalized linear mixed models (GLMMs) with a Poisson link function using the *lme4* R package (Bates et al. 2015). Zero-inflated data were considered structural zeros (Blasco-Moreno et al. 2019). Odds ratios for each trap type were calculated relative to the CO_2-baited CDC trap. In any instance where the total number of individuals for a species collected in a trap type was equal to 0, we added a pseudocount equal to 1 which allowed for model estimation. Overdispersion was assessed, and negative-binomial GLMMs were used to account for any unequal mean/variance relationship. Non-parametric bootstraps (n = 250) estimated parameter distributions. Males were excluded from analyses as the focus was host-seeking female mosquitoes.

A total of 436 mosquitoes were collected over 128 trap days (142 in June, 294 in August) (Table 1a). *Aedes albopictus* was the most common LACV vector (n = 182, 41.7%), followed by *Ae. triseriatus* (n = 156, 35.7%) and *Ae. japonicus* (n = 22, 5.0%). These 3 species comprised 82.6% of total collections. The BG-Pro trap collected the highest average number of female

Table 1b. Summary statistics for LACV Vectors.

Species	Mean (Median, Variance)			
	BG-Pro	BG-Sentinel (+ lure)	BG-Sentinel (− lure)	CDC light trap
Aedes albopictus	1.65 (0, 9.20)	2.50 (1, 16.2)	0.30 (0, 0.49)	0.56 (0, 1.84)
Ae. japonicus	0.09 (0, 0.15)	0.16 (0, 0.20)	0.16 (0, 0.34)	0.27 (0, 0.69)
Ae. triseriatus	2.53 (1.5, 10.45)	0.84 (0, 1.56)	0.68 (0, 2.16)	0.87 (0, 9.70)

Ae. triseriatus (2.53 per trap day), approximately three times more than the CDC light trap or BG-Sentinel 2 with lure (Table 1b). The odds of collecting *Ae. triseriatus* with the BG-Pro were 3.02 times higher (95% CI: 1.96–4.67) than with the CDC light trap (Fig. 1e). The BG-Sentinel 2 with lure collected the most female *Ae. albopictus* (2.5 per trap-day), about 1.5 times more than the BG-Pro and 4.46 times more than the CDC light trap (Table 1b). However, the odds of collecting *Ae. albopictus* with the BG-Sentinel 2 with lure (OR: 4.62, 95% CI: 2.76–7.74) were not significantly different from the BG-Pro (OR: 3.06, 95% CI: 1.78–5.24) (Fig. 1f). Only 22 *Ae. japonicus* were collected, limiting statistical analysis (Fig. 1g). No LACV was detected in any mosquito pools by qPCR or Vero cell culture.

The BG-Pro trap demonstrated superior field effectiveness in capturing *Ae. triseriatus* compared to standard surveillance traps, collecting approximately three times more specimens than the CDC light trap or BG-Sentinel 2 with lure. The observed odds ratio of 3.02 (95% CI: 1.96–4.67) underscores the utility of the BG-Pro to sample the primary vector. Although the BG-Sentinel 2 with lure captured more *Ae. albopictus*, the difference was not statistically significant compared to the BG-Pro.

Our study's strengths include a robust 4×4 Latin square design, controlling for site-specific and temporal variations, and the use of GLMMs with appropriate adjustments for zero-inflated data. However, limitations include the relatively small sample size ($n = 436$), particularly for *Ae. japonicus* ($n = 22$), and the absence of LACV-positive mosquitoes, which limits conclusions about trap efficacy in monitoring infected populations. Notably, in summer 2024, the BG-Pro successfully collected LACV-positive *Ae. triseriatus* at the residence of a LACV-infected child (Byrd, unpublished data).

These findings suggest that the BG-Pro is a valuable tool for surveillance of host-seeking LACV vectors, especially *Ae. triseriatus* and *Ae. albopictus*. A comprehensive LACV surveillance strategy should incorporate multiple trap types to effectively monitor all three container-inhabiting vectors (Grim et al. 2007, Tamini et al. 2021, Sither et al. 2023). Further studies are warranted to enhance capture rates for these species and to validate the BG-Pro's utility in detecting LACV-infected mosquitoes (Day and Trout Fryxell 2025).

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