Journal of Medical Entomology, 2025, **00**(00), 1–7
https://doi.org/10.1093/jme/tjaf166
Research

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Vector Control, Pest Management, Resistance, Repellents

Does the fungus among us increase trap fidelity? Mycelium carbon dioxide generators for mosquito (Diptera: Culicidae) traps in two ecoregions of the United States

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Subject Editor: Lawrence Hribar

Mosquito control programs deploy mosquito traps for surveillance and targeting of vector mosquitoes. The primary attractant in these traps is generally carbon dioxide (CO_2), which acts as a powerful long-distance lure. Historically, dry ice and compressed gas have been accessible as lures, but they may exhibit logistical hardships (storage, safety, equipment, calibration, consistency, etc.). Additionally, the aforementioned standards can be inaccessible in remote or low-resource environments. Microbial lures have often been proposed to bridge this gap, but prior options, such as yeast, have proven inferior compared to traditional CO_2 lures. However, a mycelium-based CO_2 generator, the Exhale CO_2 Bag, is now commercially available. Initial studies suggested this product may be suitable for mosquito surveillance. To validate this, semi-field cage assays were conducted in Florida by releasing several species of vector mosquito into enclosures to be recollected by either Biogents or United States Centers for Disease Control and Prevention (CDC)-style traps paired with the Exhale, gas cylinders, dry ice, or unbaited. Dry ice and compressed gas performed equivalently, with Exhale collecting 25% to 50% fewer mosquitoes, and unbaited traps collected 10% to 30% fewer than Exhale. Additionally, field testing in Utah, with traps deployed across rural wetlands, industrial transition areas, and urban metropolitan areas, all yielded the same result: the Exhale mycelium and unbaited traps collected an average of 35 or fewer mosquitoes, as compared to regulated gas CO_2 traps collecting 1,000 to 3,000 mosquitoes in the same areas. Though some merits exist for a mycelium-based trap, the current data and recent literature fail to support this technology for host-seeking traps in existing mosquito surveillance programs.

Keywords: CO_2, surveillance, urban, rural, Aedes, Culex, host-seeking

Introduction

Mosquito surveillance networks provide essential data for enhancing quality of life and reducing the risk of mosquitoes and their associated pathogens (Connelly et al. 2020). Curated longitudinal information identifies changes in species, numbers, and local-level movement across habitats, all of which can be used for targeted treatments against larval and adult mosquitoes (Drakou et al. 2020, Aryaprema et al. 2023). Lures are an essential part of effective surveillance for hematophagous insects, with carbon dioxide (CO_2) being among the most significant enhancers of trap utility against mosquitoes since its discovery as an attractant (Reisen et al. 2000). Despite this, access to CO_2 generators for traps can be a limiting factor for programs globally. Dry ice is a common and simple means of adding CO_2 as a lure to a trap (Magnarelli 1975, Feldlaufer and Crans 1979, Reisen et al. 2000), while compressed gas cylinders can also be used to deliver a more regulated output (Reisen et al. 2000, Brandow et al. 2025).

There are instances where neither of these options are a reasonable solution, such as when operating in resource-poor environments or when technical maintenance and cost can be limiting (Steiger et al. 2016, Jerry et al. 2017). Baiting with CO_2 can become difficult when equipment has many moving parts, when cost becomes detrimental at a scale needed for routine monitoring, or cumbersome logistics from technical limitations such as storing dry ice safely for long-term use or keeping regulated systems calibrated (Brandow et al. 2025). These are rarely a fatal limitation when working in developed countries with significant infrastructure. However, even in highly developed regions such as the United States, there are contexts where it is valuable to simplify the tools when scaling up, for the risk of having a significant loss of data from equipment malfunction (Ritchie et al. 2007, Chen et al. 2011,

Received: 20 August 2025. **Revised:** 3 October 2025. **Accepted:** 22 October 2025
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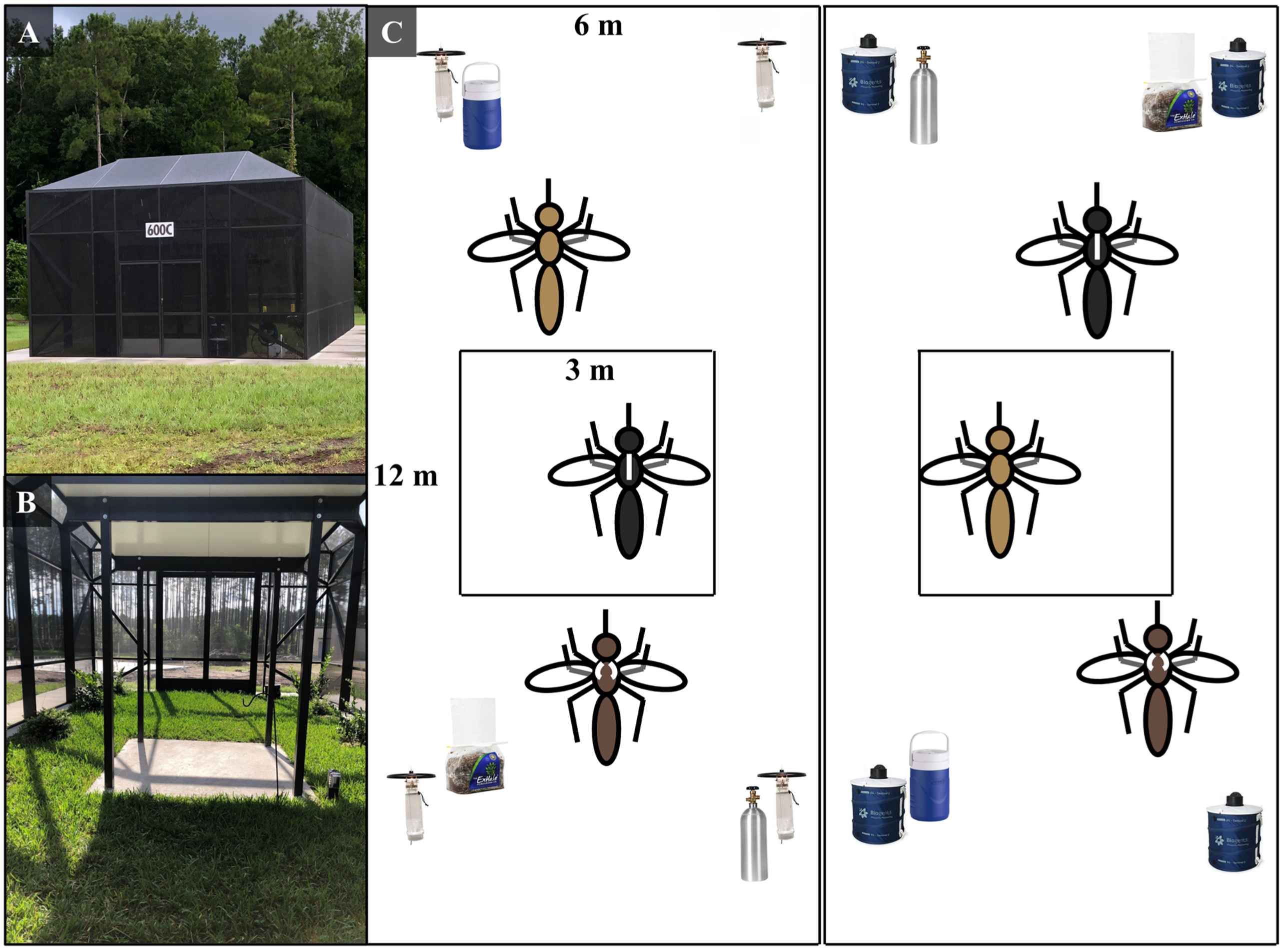

Fig. 1. A) Outdoor screened enclosure for semi-field bioassays; hosted at the Anastasia Mosquito Control District of St Johns County (Florida). B) Interior view of screened enclosure with planted grasses, shrubbery, and a 3 m × 3 m square patio with a shade roof interior to the screened enclosure. C) Dimensional layout of experiments using the 6 m × 12 m × 6 m interior space. Mosquitoes were acclimated and released in the central patio. Experimental lures were rotated among the far corners of cage when paired with either CDC-style miniature traps or Biogents Sentinel MkII traps.

Crepeau et al. 2013). This risk for any program leads to universal interest, and in some cases, necessity (Steiger et al. 2016, Jerry et al. 2017), for having a relatively stable and easy-to-work-with material that can be converted to CO_2 when needed.

Microbial lures have generally been the proposed solution for operational mosquito surveillance, with examples stemming from cheese fermentation (Peach et al. 2021), yeast (Steiger et al. 2016, Jerry et al. 2017), and now mycelial lures (Kim et al. 2025). In a resource-deprived context, any lure that may enhance capture is better than an unbaited trap. However, these alternative CO_2 generators may also experience problems, such as continuous rates of CO_2 output (Steiger et al. 2016) and reduced or negligible efficacy when compared to other lures (Murindahabi et al. 2022). For mycelium specifically, which produces CO_2 through its innate respiratory function, limited success has been demonstrated when using a commercial greenhouse supplement, Exhale CO_2 Bags (Kim et al. 2025). When mycelium-based CO_2 generators were used with BioGents Gravid *Aedes* Trap (BG-GAT) traps as a passive trap frame paired with Exhale bags, without infusion water, collections of peridomestic mosquitoes were significantly improved over standard usage (Kim et al. 2025). However, host-seeking traps for the same target species were only marginally improved over unbaited counterparts (Kim et al. 2025).

Although the prior work with fungi has been a putative success, additional data in a broader context for mosquito surveillance are lacking. This is particularly important with the more widely used miniature CDC traps, or in surveillance environments not explicitly prioritizing invasive and container-inhabiting *Aedes* species. If mycelium-based CO_2 generators are more logistically feasible because of shelf stability, long-term storage, or simple usage, or effective enough to warrant cost analysis, then even well-developed surveillance programs could benefit from the shift in technology. We conducted a joint study between the Anastasia Mosquito Control District (AMCD) and the Salt Lake City Mosquito Abatement District (SLCMAD) in caged enclosures to assess efficacy of the Exhale CO_2 Bags for container-inhabiting mosquitoes. Additionally, we also conducted field studies near wetland habitats for floodwater mosquitoes using 3D-printed SL Traps (Bibbs et al. 2024), a variant of miniature CDC-style traps.

Materials and Methods

Semi-Field Evaluations

Semi-field assays were conducted at the AMCD, St Augustine, Florida, in 6 m × 12 m × 6 m screened enclosures (Fig. 1A). The screen enclosures are permanent outdoor fixtures constructed

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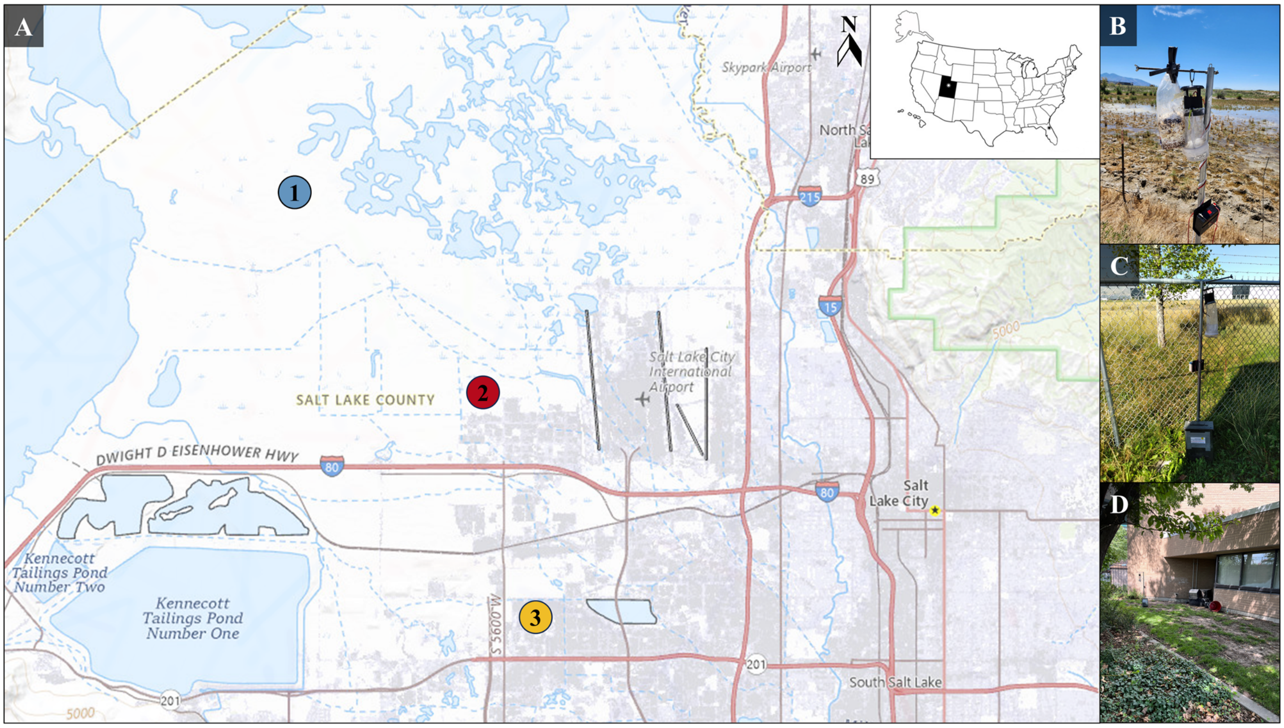

Fig. 2. A) subsection of the service area for the Salt Lake City Mosquito Abatement District (Utah), with rural (1), industrial/transitional (2), and urban/peridomestic (3) mosquito habitats labeled for use during field trapping. B) An example of a commercial mycelium lure, the Exhale CO_2 Bag, affixed to a CDC-style miniature trap (3D-printed SL Trap) in rural wetlands. C) An example of a standard operation SL Trap in an industrial area with CO_2 dispensed at 300 ml/min from a pressurized gas cylinder. D) An example of an unbaited SL Trap in an urban sampling location. Surveillance data geomapping was compiled in VectorSurv and rendered in Tableau.

with aluminum frames and 20/20 coated fiberglass mesh. Interior space was planted with grass, harborage vegetation, and a 3 m × 3 m square patio with a shade roof (Fig. 1B). Experiments were conducted in two enclosures simultaneously, using one for miniature CDC-style traps without the light (Model 512, John W. Hock Company, Gainesville, FL) and one for Biogents Sentinel (BGS) traps (MkII Biogents Mosquito Trap Systems, Regensburg, Germany). Four traps were placed in each enclosure (Fig. 1C) one in each corner) and paired with one of the following lures: negative control (no CO_2 source); compressed gas CO_2 tank fitted with a regulator (Le Tkingok, Shenzhen, Guandong, China) and flowmeter calibrated to output 500 ml/min (JIAWANSHUN, Feng, Xing, China); 453 g dry ice loaded into a vented 3 L water container (Igloo, Katy Texas, United States); and the Exhale CO_2 Bag (20.32 cm × 12.7 cm × 35.56 cm, 1.81 kg, pre-activated version; Garden City Fungi, Missoula, MT, United States). The Exhale bag was allowed to prime for 2 weeks prior to inclusion in the study. Release rates for pressurized gas were based on a range of accepted operational norms that are common to mosquito surveillance (Reisen et al. 2000, Bibbs et al. 2024, Brandow et al. 2025).

Three species were tested in AMCD: *Aedes aegypti* (Orlando 1952), *Aedes albopictus* (Gainesville 2003), and *Culex quinquefasciatus* (Gainesville 1995 + Ocala 2003). All species were reared at AMCD insectaries under standard conditions: 26.6 ± 1.5 °C, 75 ± 10% RH, and 14L:10D photoperiod. Female mosquitoes aged 3 to 10 days, non-blood-fed, and sugar-starved for 12 to 16 h were used for each trial. A total of 250 female mosquitoes per species were simultaneously released into each enclosure (750 mosquitoes total) at the entrance point starting at approximately 5 PM and collected at approximately 8 AM the following day. After 15 h of study collection time, traps were collected and contents placed into a −20 °C freezer for a minimum of 1 h to kill all mosquitoes prior to counting. Trials were replicated 10 times throughout April and May of 2024, randomizing trap corner placement and treatment assignments to control for potential bias between cages, trap position, and environmental conditions. Average condition in the cages was 23.9 ± 1.7 °C, 84 ± 6% RH, as measured using a free-standing weather station (2000 series Watchdog, Spectrum Technologies Inc., Aurora, IL).

Field Evaluations

Field testing was conducted at the SLCMAD near freshwater wetland habitats on the outskirts of the Great Salt Lake, Utah. Field tests followed methods adapted from Harker et al. (2024). In brief, trap-poles were mounted on rebar with a minimum of 50 m of distance between any given trap location. The in-house manufactured SL Trap (Bibbs et al. 2024) was mounted on poles with corresponding lures and allowed a 24-h run cycle from 8 AM to 8 PM for each trap night of data. The positive control was regulated CO_2 dispensed at 300 ml/min via pressurized cylinders and no light (Bibbs et al. 2024, Harker et al. 2024). Negative controls were completely unbaited, with no lure or light. The mycelium comparison group was the Exhale CO_2 Bag (20.32 cm × 12.7 cm × 35.56 cm, 1.81 kg; pre-activated version; Garden City Fungi, Missoula, MT, United States) after allowing 2 weeks of growth at room temperature to become primed to readily express CO_2.

Clusters for testing were rotated among three distinct areas in the Great Salt Lake area of Utah (Fig. 2A). Site 1 represented a strict rural mosquito population, far away from industrial

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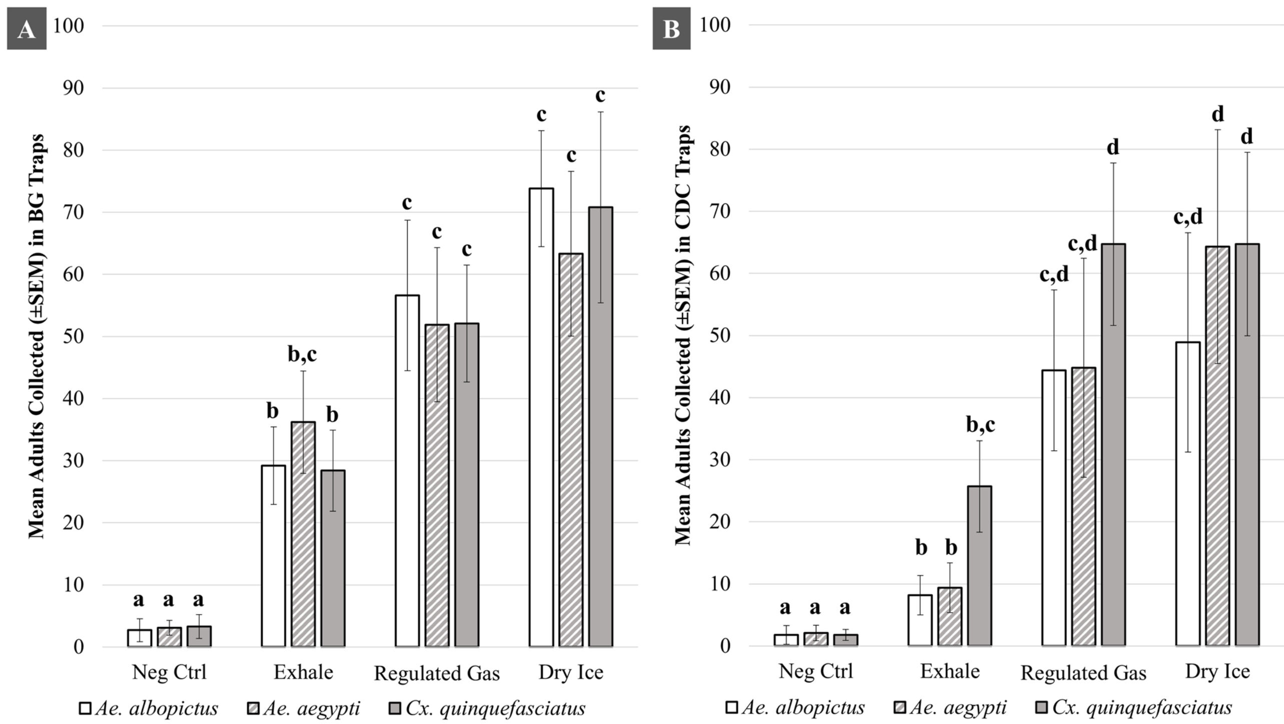

Fig. 3. Recapture efficiency when using CO_2 expressed from a gas cylinder at 500 ml/min, 453 g of dry ice loaded into a venting 3-L cooler, a commercial mycelium (the Exhale CO_2 Bag), or an unbaited negative control. Semi-field assays were conducted within outdoor, screened enclosures by simultaneous releases of 250 each of *Ae. albopictus*, *Ae. aegypti*, and *Cx. quinquefasciatus*, where they were exposed to abiotic conditions in north Florida. Tests were replicated 10 times and analyzed with two-way ANOVA for A) Biogents Sentinel MkII ($F_{39,119}$ = 16.6232, P < 0.0006) and B) miniature CDC traps ($F_{39,119}$ = 7.4609, P < 0.0005). Bar graphs are shown with I-bars as the standard error of the mean.

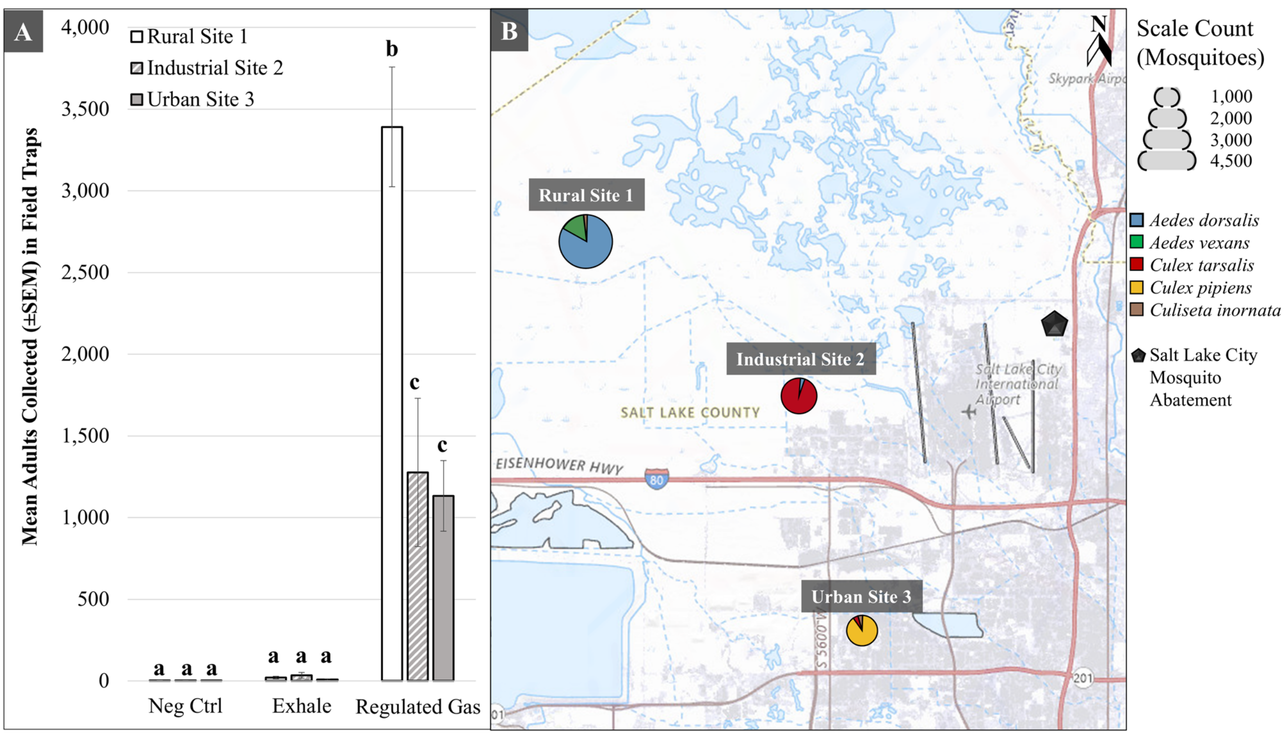

Fig. 4. A) Collection efficiency when using CO_2 expressed from a gas cylinder at 300 ml/min, a commercial mycelium, the Exhale CO_2 Bag, or an unbaited negative control when using a CDC-style miniature trap (3D-printed SL Trap). Field trapping was replicated 8 times per site, with only data from the standard CO_2 lure found to be significant across rural, industrial, or urban ($F_{2,4}$ = 12.393, P < 0.0001) field sites. Bar graph is shown with I-bars as the standard error of the mean. B) Species composition using mean collection data at each site. Collections tended to be nearly monotypic, with *Ae. dorsalis*, *Cx. tarsalis*, and *Cx. pipiens* being dominant at rural, industrial, and urban locations, respectively. This tendency was the same regardless of the lure used. Surveillance data geomapping was compiled in VectorSurv and rendered in Tableau.

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centers and in the middle of managed wetlands (Fig. 2B); Site 2 represented an industrial–rural transition zone (Fig. 2C), where human development was extensive but natural swales and ditches still intermixed with the available habitat; and Site 3 represented an urban/suburban mosquito habitat within peridomestic human environments (Fig. 2D). Traps were rotated among the intra-site locations at each replicate, with 9 replicates per urban, industrial, and rural site, conducted throughout June (25.3 ± 3.2 °C, 39 ± 20% RH), July (27.5 ± 2.1 °C, 29 ± 23% RH), and August (25.5 ± 5.4 °C, 41 ± 15% RH) of 2024 with environmental conditions noted through local weather services. Adult mosquitoes were anesthetized at −80 °C and then counted using a hybrid camera and microscopy system (Kesavaraju and Dickson 2012, Faraji et al. 2025). Species identifications were rendered using published dichotomous keys (Darsie and Ward 2016).

Data Analysis

Semi-field assays in walk-in flight cages were analyzed using a mixed effects two-way ANOVA whereby the first variable (lure type) was fixed but the second variable (# collected of given mosquito species) was randomized to observe the between and within subject variances, respectively, while treating species collected as a repeated measure. Bonferroni corrections were applied during main effects analysis to account for possible non-independence related to lure types being tested in the same enclosures. Because of significant non-normal skew in data, field data were analyzed by site with the non-parametric Aligned Rank Transform test using site and lure type as the main variables. Post-hoc analysis was performed with full factorial ANOVA and ranked comparisons to separate main effects from interactions. All statistical analyses were conducted using R v. 4.2.0 (R Core Team 2022).

Results

During semi-field cage assays in Florida, lure type used was significant to the collection totals for either the BG traps (Fig. 3A; $F_{39,119} = 16.6232$, $P < 0.0006$) or miniature CDC traps (Fig. 3B; $F_{39,119} = 7.4609$, $P < 0.0005$). For BG traps, neither the species of mosquito tested ($P < 0.915$) nor the interactions between mosquito species were significant ($P = 0.9374$). For CDC-style traps, *Cx. quinquefasciatus* was collected to a more significant degree than either *Aedes* spp. tested ($F_{2,72} = 4.046$, $P < 0.0216$), while the interaction between species was not ($P = 0.5021$). In the case of either trap type, collections of all species from low to high trended as negative control < Exhale mycelium < gas cylinder CO_2 = dry ice (Fig. 3). Though statistically significant, the collections of *Ae. albopictus* and *Ae. aegypti* in CDC traps using the Exhale mycelium averaged fewer than 10 mosquitoes. With *Cx. quinquefasciatus* in CDC traps, or for all species in the BG traps, the Exhale mycelium was 3 to 10 times greater than the unbaited collection numbers, but was still only half the collection numbers as of either CO_2 from gas cylinders or dry ice. Given the statistically insignificant difference between CO_2 from gas cylinders and the dry ice, only the pressurized cylinders were used in the field in Utah to reduce complexity.

Collections of wild mosquitoes in the field resembled the trends of the semi-field assays, but with 10 to 35 times greater mosquito totals (Fig. 4A). The standard CO_2 lure at 300 ml/min was the only lure to collect significantly more than an unbaited trap, regardless of trapping in rural, industrial, or urban ($F_{2,4} = 12.393$, $P < 0.0001$) field sites. The mosquito collections by CO_2 lures varied significantly by site ($F_{2,63} = 12.4494$, $P < 0.0002$), with rural > industrial = urban, while unbaited traps and the Exhale lure were consistently equivalent to each other regardless of site. Although wild populations may be mixed, the field sites tended to be biased toward one or two common species (Fig. 4B). At rural site 1, over 75% of collections comprised *Aedes dorsalis* (Meigen) with some presence of *Aedes vexans* (Meigen) and *Culiseta inornata* (Williston). The industrial and urban transitional areas were dominated by *Culex tarsalis* Coquillett with intermittent *Ae. dorsalis* collections. The urban and peridomestic areas were primarily *Culex pipiens* L., with occasional collections of *Cx. tarsalis* and *Cx. inornata*. Collections in a given night ranged as high as 4,982 (rural), 4,066 (industrial), and 2,238 (urban) mosquitoes in a single trap night of collection. Collection diversity was nearly monotypic, regardless of the lure used, with respective site diversity (rural = 3, industrial = 2, urban = 3) conserved between Exhale and CO_2. Unbaited traps typically collected no mosquitoes, but when they did, they caught the same number of species in single-digit totals.

Discussion

The mycelium-based CO_2 generators were not a competitively effective lure when paired with commonly used host-seeking traps. The BG traps were more effective than CDC-style traps when paired with the Exhale mycelium. However, the percentage-wise change in collections between more abiotic CO_2 generators and an unbaited trap showed the Exhale CO_2 Bag to be consistently lower than conventional lures when used with the BG trap or a CDC-style trap. The finding that *Cx. quinquefasfasciatus* were collected more in the CDC-style trap than the tested *Aedes* spp. is consistent with the frequent difficulties of invasive, container-inhabiting *Aedes* (Steiger et al. 2016, Kim et al. 2025). The disparity in collections by lure type was exacerbated in the field, where the Exhale mycelium did not statistically improve collections over an unbaited trap, regardless of site-based effects. This could be due to many factors, such as poor spatial reach of the lure in an open environment and/or stronger competing CO_2 signals from adjacent areas that eclipsed the emissions from the mycelium. The CO_2 lure having increased collections in the rural site is indicative of the increased general mosquito abundance in that site type.

The Exhale mycelium product, as used in this study, contained 1,814.4 g of mulch. It is generally accepted that wood contains an average of 50% releasable carbon from dry mass (Doraisami et al. 2022). This results in 907.2 g of hypothetically releasable carbon. With the molecular density of carbon at 12.011 g/mol, and of CO_2 at 44.009 g/mol, there is a maximum potential for 3,324 g of CO_2 to be available from each Exhale Bag. If we rationalized emissions across 30 days to expiry, we can expect 39.26 ml/min of CO_2 at maximum. Whereas host-seeking traps need to use much larger CO_2 signatures, reaching 300 to 800 ml/min to simulate one or more large animal hosts (Reisen et al. 2000). The product is marketed at a 60-day lifespan, which would half the emissions rate, assuming all the considerations are reasonably linear. This low emission is theoretical, but, if true, is not currently realistic to use in the field for host-seeking mosquito traps.

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Despite this, Kim et al. (2025) found meaningful improvement in collections in both cage assays and field settings in Florida when they paired the Exhale mycelium with a BG gravid *Aedes* trap. Their use of the BG-GAT was passive, omitting the infusion water. Consequently, one could interpret the authors' use to be akin to a passive host-seeking trap, or to have used the mycelium as a surrogate for the infusion water. In either case, results were statistically significant over unbaited equivalents. Ordinarily, host-seeking traps require 8 to 20 times greater output than what the mycelium could theoretically release in its current form. The passive nature of the BG-GAT is reminiscent of yeast-based live-collection traps that have found success in other regions (Steiger et al. 2016). However, the low CO_2 emission could potentially provide another angle to supplement surveillance.

Although neither Kim et al. (2025) nor this study directly tested gravid traps in conventional usage with organically enriched water (Reiter 1983), the low levels of CO_2 generation from the Exhale bags may be analogous to CO_2 output from bacterial decay processes in typical infusion water (D'Jesús et al. 2006) or natural pond habitats (Girard et al. 2024). The mycelium could enhance gravid trap efficacy with a low level of CO_2 expression, below a host cue, to stimulate mosquito interest in the water. After all, confirmation of oviposition sites seems to be verified through a menagerie of cues by gravid mosquitoes (Bentley and Day 1989, Rejmánková et al. 2005).

Unfortunately, as a strictly host-seeking mosquito lure, a mycelium-based CO_2 generator seems to encounter the same pitfalls as other microbial lures for mosquito surveillance to gauge biting pressure. Other microbial CO_2 generators have required a bulky amount of starter material (Steiger et al. 2016) and may still result in very low actual mosquito numbers in traps (Murindahabi et al. 2022) for reasons that are not always clear. Even for this specific mycelium option, the Exhale bags, the findings by Kim et al. (2025) were the first to show modest, insignificant improvement in captures with BG traps and low, even if significantly improved, captures in BG-GATs. When taken at the counted values, the Exhale lures performed the same in this current study; mosquitoes were collected in the dozens. In contrast, standard practices with abiotic CO_2 generators collect thousands of mosquitoes.

A case can be made that the absolute number of mosquitoes is less important than maintaining metrics on population trends and species diversity (Bibbs et al. 2024, Harker et al. 2024), but programs must decide on an individual basis whether the collections achievable with the Exhale lure will suit their goals, such as trends in abundance, diversity checks, or pathogen detection. Certainly, some specific utility still exists for using Exhale bags with gravid traps (Kim et al. 2025). Furthermore, mycelium lures are comparable to yeast lures and can be used in low-resource programs or remote environments that simply cannot deploy compressed gas or dry ice. Future work should focus on engineering mycelium-based lures with higher CO_2 output and exploring pairing strategies with gravid traps. However, based on the current data and how it reconciles with other literature, the Exhale CO_2 Bags are currently not a replacement for conventional CO_2 sources in host-seeking traps within areas with access to adequate infrastructure.

Acknowledgements

The Exhale CO_2 Bags for this study were provided gratis by Target Specialty Products. Surveillance data geomapping was compiled in VectorSurv and rendered in Tableau.

Author Contributions

David Christian Furness (Methodology [equal], Validation [equal]), Maggie Liu (Investigation [equal], Methodology [equal], Validation [equal]), Kai Blore (Investigation [equal], Methodology [equal], Validation [equal]), Nathaniel M. Byers (Conceptualization [equal], Project administration [equal], Supervision [equal], Writing—review & editing [equal]), Whitney A. Qualls (Conceptualization [equal], Investigation [equal], Methodology [equal], Project administration [equal], Supervision [equal], Validation [equal], Writing—original draft [equal]), Ary Faraji (Funding acquisition [equal], Project administration [equal], Resources [equal], Supervision [equal], Writing—review & editing [equal]), and Christopher S. Bibbs (Data curation [equal], Formal analysis [equal], Methodology [equal], Project administration [equal], Supervision [equal], Writing—original draft [equal], Writing—review & editing [equal])

Funding

None declared.

Conflicts of Interest

None declared.

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