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OPERATIONAL NOTE

FIELD COMPARISON OF CARBON DIOXIDE SOURCE WITH BIOGENTS SENTINEL-2 AND PRO TRAPS FOR ADULT *AEDES* MOSQUITO SURVEILLANCE

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ABSTRACT. The BG Sentinel-2 (BGS-2) and BG-Pro traps (BGS-2 configuration) were compared for their effectiveness to collect *Aedes* vectors and related nuisance mosquitoes in north central Florida during 2022. Traps were baited with either dry ice pellets, pressurized carbon dioxide (CO_2) gas, or the novel BG yeast-derived CO_2 generator. Additionally, each trap was fitted with the BG Sweetscent lure. Sixteen species were collected including *Aedes albopictus* and *Ae. aegypti,* which accounted for about 20% of the collections. The BGS-2 collected more mosquitoes compared to the BG-Pro, but the relative percent abundance of each species to total collection from each trap type was similar. Overall mosquito abundance was significantly greater in both trap types baited with dry ice compared with the other CO_2 sources. Significantly more *Ae. albopictus* were collected from BGS-2 traps baited with dry ice than all other CO_2 and trap configurations. Lastly, we did not observe any significant differences in *Ae. aegypti* abundance between trap type or CO_2 source.

KEY WORDS *Aedes albopictus*, *Aedes aegypti*, disease vector, sampling, carbon dioxide

The choice of field sampling method for female mosquitoes is focused on the host-seeking behavior of the target species, and this is especially true regarding disease vector surveillance. *Aedes aegypti* and *Aedes albopictus* are primary targets of many mosquito control and surveillance programs given their role as vectors for arboviruses such as chikungunya, dengue, yellow fever, and Zika (Kraemer et al., 2015). With the global distribution of these *Aedes* vectors the need for effective trapping methods to detect these species is crucial.

New traps and baits have developed in recent years to target the capture of *Aedes* mosquitoes. The BG Sentinel-1 (BGS-1) was introduced in 2004 (Biogents AG, Regensburg, Germany) and was widely adopted by the mosquito research community as the new standard to collect this vector (CDC 2018). Biogents later improved the durability of this trap and introduced the BG Sentinel-2 (BGS-2) in 2014. Most recently, Biogents developed a "3-in-one" modular trap (BG-Pro) that incorporated the design features of a CDC, Encephalitis Vector Surveillance (EVS), and BG Sentinel trap, the latter specifically to survey for *Aedes* mosquitoes. At the same time, Biogents also introduced a novel yeast-derived CO_2 generator as an alternative to conventional dry ice or pressurized CO_2 for use in their BG-Pro. We report here on the comparative effectiveness of the standard BGS-2 with the BG-Pro trap baited separately with either dry ice, pressurized CO_2 gas, or BG CO_2 generator as attractants to capture *Aedes* vectors and other mosquito species for surveillance purposes.

Evaluations were conducted in the city of Gainesville, FL at 4 locations: a cemetery (29.625970, −81.824110) and three residential properties (29.656070, −82.331630; 29.6568391, −82.3357427; 29.69920, −82.38085) from July through August 2022. All locations had a previous documented history of *Ae. albopictus* and *Ae. aegypti* populations (Y Jiang, pers comm). The cemetery and one of the residential properties were large enough for placement of two traps at least 100 m apart in each location and positioned so that they were not visible to one another, the remaining 2 properties consisted of 1 trap each (n=6 total sample locations). Each of the 4 properties were at least 5 km from one another.

BGS-2 and BG-Pro (the latter as a BGS-2 configuration, i.e., positioned on ground surface) were baited separately with either dry ice, pressurized CO_2, or the BG-CO_2 generator. In addition, a BG Sweetscent cartridge lure was inserted into each trap as suggested by the manufacturer. Carbon dioxide produced by dry ice consisted of 1.5 kg dry ice pellets in a 2.0 L Igloo container suspended above each trap with a tube inserted from the bottom of the container into the air intake of the trap. Pressurized carbon dioxide was delivered at 200 ml/min via a manufacturer preset regulator (Bioquip, Inc. El Rancho, CA). Yeast-derived CO_2 was delivered via a BG-CO_2 generator (75–125 ml/min) using Biogents proprietary yeast product per manufacturer's instructions (Biogents AG, Regensburg, Germany). The generator yeast product was replaced daily. Because the BGS-2 does not possess lights, the light string was removed from the BG-Pro sentinel set up. In addition, all traps were protected from rain with a 52-cm diameter translucent plastic lid affixed 29 cm above each trap (Degener et al. 2021).

The study followed a 6x6 Latin square design. Three rotational repetitions were performed among the six trap locations with initial random assignment of a specific trap and location at the start of each full 6-day rotation. Trap contents were collected daily at 24 h intervals. Female mosquitoes were identified to the species level using the taxonomic key of Darsie

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Table 1. Relative percentage of mosquito species (females) collected from BG Sentinel-2 and BG-Pro traps (sentinel configuration) Gainesville, FL 2022.

Mosquito species	% BG Sentinel-2	% BG-Pro	% of overall collection
Aedes aegypti	5.0	9.6	6.2
Ae. albopictus	14.4	14.5	14.5
Ae. atlanticus	0.2	0.1	0.2
Ae. infirmatus	2.3	6.7	3.4
Ae. triseriatus	0.3	0.5	0.4
Anopheles crucians	0.3	0.3	0.3
An. quadrimaculatus	0.1	0.1	0.1
Culex erraticus	2.2	2.8	2.3
Cx. nigripalpus	4.7	4.6	4.6
Cx. quinquefasciatus	5.2	3.2	4.7
Culiseta melanura	0	0.1	0.04
Coquillettidia perturbans	0.9	0.4	0.8
Mansonia tittilans	59.8	43.8	55.9
Psorophora columbiae	0.1	0.1	0.1
Ps. ferox	3.9	9.0	5.2
Wyeomyia smithii	0.6	4.3	1.5
Total	100.0	100.0	100.0

and Morris (2003) and used in the reported data. Overall mosquito abundance data were log x+1 transformed prior to analyses. Initially, data were subjected to an ANOVA with a Tukey pair-wise mean comparison test (R Core Team 2023) was performed on the overall mosquito abundance data for each trap and CO_2 source, as well as *Ae. aegypti* and *Ae. albopictus* to determine differences ($P \leq 0.05$).

During the 8 wk study, 5,256 mosquitoes were collected; BGS-2 collected 3,952 and BG-Pro collected 1,294. Even though there was a difference in overall abundance for each trap type, both generally collected a relatively similar percentage of each species to total collection (Table 1). Sixteen species were collected from the BG-Pro and 15 in the BGS-2. *Culiseta melanura* (Coquillett) was absent from the BGS-2, presumably due to relatively low seasonal population and relative location in northern Florida at the time of sampling (Burkett-Cadena et al. 2015). The top three most abundant species collected from both traps were *Mansonia titillans* (Walker) (55.9%), *Ae. albopictus* (14.5%), and *Ae aegypti* (6.2%).

Overall, we found a significant difference in the number of mosquitoes collected between the BGS-2 and BG-Pro ($F = 23.24$, df $= 1, 2$, $P < .001$) (Table 2). The BGS-2 paired with dry ice or pressurized CO_2 collected the most mosquitoes on average (Table 2). The BGS-2 may have performed better as a result of the black outer surface of the BGS-2 trap being more attractive than the white outer covering of the BG-Pro. Bidlingmayer and Hem (1979) and Alonso San Alberto et al. (2022) have shown such preferences exist regarding visual contrast of mosquitoes to light and dark objects.

In our investigation we found that CO_2 source did significantly impact the number of mosquitoes collected ($F = 8.87$, d $= 1,2$, $P < 0.001$). Out of our CO_2 sources, dry ice baited traps collected more mosquitoes on average (Table 2). The BGS-2 baited with dry ice collected the most mosquitoes compared to all trap and CO_2 configurations (Table 2). The BG-Pro traps baited with pressurized CO_2 and the yeast generator performed similar to the BGS-2 with yeast (Table 2). The BG-Pro traps baited with the pressurized CO_2 collected the fewest mosquitoes (Table 2). Regardless of trap, dry ice outperformed pressurized CO_2 possibly related to an initially greater, but unregulated, release rate of CO_2 from the dry ice pellets.

When specifically examining *Aedes* collections we found that all trap and CO_2 configurations successfully captured *Ae. aegypti* and *Ae. albopictus*. We did see

Table 2. Comparison of mean mosquito abundance from BG Sentinel-2 and BG Pro (sentinel configuration) traps with associated CO_2 source within and between trap type and CO_2 sources Gainesville, Florida.

Trap type[a]	Carbon dioxide source	*Aedes albopictus*	*Aedes aegypti*	Overall mosquito abundance
BG Sentinel-2	Dry ice	19.4 ± 4.9Aa	3.9 ± 1.7Aa	142 ± 39.0Aa
BG Sentinel-2	CO_2 gas	7.7 ± 1.8Bb	4.2 ± 1.9Aa	41.1 ± 14.2Bb
BG Sentinel-2	BG-yeast generator	5.0 ± 1.5Bb	2.9 ± 1.7Aa	36.9 ± 10.2 BCb
BG-Pro	Dry ice	3.4 ± 1.0Ba	2.1 ± 0.9Aa	36.4 ± 11.1BCa
BG-Pro	CO_2 gas	2.8 ± 0.7Bb	1.9 ± 0.7Aa	11.7 ± 2.4Ca
BG-Pro	BG-yeast generator	4.2 ± 1.6Bb	2.9 ± 1.4Aa	23.4 ± 10.6BCa

[a] Capital letters in columns denote comparison between all 6 combinations (i.e., trap type and CO_2 sources). Means in columns with lower case letters compare mosquito abundance between CO_2 source within trap type ($P \leq 0.05$).

significant differences in the number of *Ae. albopictus* collected between trap type and CO_2 combinations ($F = 5.3$, $df = 1,2$, $P = 0.006$) but not for *Ae. aegypti* ($F = 0.3$, $df = 1,2$, $P = 0.7$). It is plausible that the low numbers of *Ae. aegypti* captured may reflect the general low historical occurrence of this species within the city of Gainesville during the sample period. Previous studies in other countries by Degener et al. (2021) found that BG-Pro traps placed in locations with high *Ae. aegypti* density can significantly outperform BGS-2 but at lower density both traps yield similar results for this vector.

From an operational deployment standpoint, it is well known that supplemental carbon dioxide increases mosquito catch in mosquito traps (Carestia and Horner 1968). However, the availability of CO_2 sources may vary by locality and may not always be accessible. We found that yeast-derived carbon dioxide from the BG-CO_2 generator may be a viable alternative for use in either trap as it captured similar mosquito species and did not deter or prevent the collection of *Aedes* vector species. Further studies are required to fully understand how trap and CO_2 configurations can impact the collections of *Aedes* mosquitoes. These insights could provide valuable information needed to target specific vectors for disease surveillance.

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