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Variation in Baiting Intensity Among CO₂-Baited Traps Used to Collect Hematophagous Arthropods

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ABSTRACT. Hematophagous arthropods transmit the etiological agents of numerous diseases and as a result are frequently the targets of sampling to characterize vector and pathogen populations. Arguably, the most commonly used sampling approach involves traps baited with carbon dioxide. We report results of a laboratory study in which the performance of carbon dioxide-baited traps was evaluated using measures of baiting intensity, the amount of carbon dioxide released per unit time during trap deployment. We evaluated the effects of trap design, carbon dioxide source, and wind speed on baiting intensity and documented significant effects of these factors on the length of sampling (time to baiting intensity = 0), maximum baiting intensity, and variation in baiting intensity during experimental trials. Among the three dry ice-baited trap types evaluated, traps utilizing insulated beverage coolers as dry ice containers sampled for the longest period of time, had the lowest maximum but most consistent baiting intensity within trials and were least sensitive to effects of wind speed and dry ice form (block vs. pellet) on baiting intensity. Results of trials involving traps baited with carbon dioxide released from pressurized cylinders suggested that this trap type had performance comparable to dry ice-baited insulated cooler traps but at considerably higher cost.

Key Words: carbon dioxide trap, compressed gas, dry ice, mosquito sampling, tick sampling

Hematophagous arthropods play central roles in the transmission of numerous pathogens of wildlife, livestock, and humans. Diseases caused by mosquito- and tick-borne pathogens are particularly noteworthy because of their geographic ubiquity and the significant effects on the health of host populations (e.g., Bock et al. 2004, LaDeau et al. 2007, Randolph 2010, Bird and Nichol 2012, Bhatt et al. 2013, Kuehn 2013, World Health Organization 2014). Because of their ecological, agricultural, and public health significance, surveillance of mosquito and tick populations is regularly conducted to monitor vector populations and measure the prevalence of infection by pathogens. Sampling of mosquitoes as part of vector control and arboviral surveillance programs has a particularly long history (reviewed in Service 1993, Silver 2008). Various methods and associated equipment have been employed in these efforts, but arguably the most widely used and broadly effective approach involves traps baited with carbon dioxide (CO₂) (Sudia and Chamberlain 1962, Pfunter 1979, Rohe and Fall 1979). Gas released from CO₂-baited traps chemically mimics the exhalation of vertebrate hosts and thereby attracts female mosquitoes in search of a blood meal. Carbon dioxide-baited traps are also effective for the collection of ticks of some species (Garcia 1962, Wilson et al. 1972) as well as individuals of a variety of dipteran taxa (reviewed in Gibson and Torr 1999). Dry ice often serves as the carbon dioxide source in CO₂-baited traps (e.g., Newhouse et al. 1966), but carbon dioxide released from pressurized (compressed gas) cylinders (Hall-Mendelin et al. 2010, van den Hurk et al. 2012), produced by the burning of propane (Kline 2002), respired by live animals or humans (Service 1977), or generated through chemical reactions (Kline et al. 2006a) are also sometimes used.

Carbon dioxide-baited traps differ in their design and manner of deployment, and different trap types are generally compared according to their catch success (e.g., Reisen et al. 1999, 2002; Mboera et al. 2000; Kline et al. 2006b; Obenauer et al. 2010). Use of this metric to evaluate results of comparative field trials, although intuitive, can be

problematic because variation in catch success will reflect both intrinsic differences in trap performance as well as spatiotemporal variability in extrinsic factors such as the abundance of target arthropods and environmental conditions that can influence trap performance (e.g., wind speed). Disparities in catch success may thus reflect undetected or seemingly trivial differences in the location and timing of trap deployment rather than true performance differences among trap types. An alternative method of comparison involves direct quantification of a metric of trap performance, here termed baiting intensity, measured as the amount of carbon dioxide released by a trap per unit time during deployment (e.g., Cooperband and Carde 2006). Baiting intensity should on average be positively correlated with catch success and is an attractive complement to the latter because of its relative insensitivity to environmental biases. Further, quantification of baiting intensity in a controlled laboratory setting is much more feasible and allows the influence of environmental factors on trap performance to be controlled and measured. Associated insights can help to explain the extent to which variation in field-measured catch success is due to environmental effects and aid in the design of traps and trapping methods that are less sensitive to these impacts.

We conducted a laboratory study to evaluate the effects of trap design, carbon dioxide source, and wind speed on the baiting intensity of CO₂-baited traps used to sample hematophagous arthropods. Trap components associated with CO₂ delivery were deployed in purpose-built flumes and baiting intensity measured every 30 s during trials lasting up to 24 h. Raw baiting intensity data were used to quantify three performance measures: trial length (time to reach baiting intensity = 0 from the start of a trial), maximum baiting intensity (average baiting intensity during the first 15 min of a trial), and baiting consistency (variation in baiting intensity during a trial). Our study had two general objectives. First, for dry-ice baited CO₂ traps, we quantified the effects of dry ice form, trap design (specifically, the container used to hold dry

ice), and wind speed on the trap performance measures. Second, we evaluated traps baited with carbon dioxide released from pressurized cylinders and compared their performance and deployment cost with those of traps baited with dry ice.

Materials and Methods

Two rectangular flumes measuring 64 cm in width by 244 cm in length by 91 cm in height were constructed using 1.1-cm thick plywood (Fig. 1). Plywood panels were screwed together along their edges and the resulting hollow structure reinforced at either end with (8.9 in width by 3.8 cm in height) attached to the inner walls of the panels. All seams were then sealed with silicon caulking. The output end of each flume was left open, and the input end was fitted with a removable plywood cover joined to the flume with elastic cords and a rubber gasket. A hole 20 cm in diameter was cut into the center of this cover and a piece of metal HVAC (heating, ventilating, and air conditioning) ducting 150 cm in length was inserted into it. The ducting was secured to the cover with metal mounting brackets and the seam sealed with silicon caulking. A wooden support was used to hold the ducting in an orientation parallel to the long axis of the flume. A direct current fan 20 cm in diameter (model 30100393, Spal Automotive, Ankeny, IA) was mounted into the opposite end of the ducting with a hose clamp. The two flumes were positioned side-by-side, 28 cm apart and parallel to each other. A 15-cm section of flexible Mylar ducting was used to

connect the fan end of both flumes to a Y-shaped intake assembly constructed of metal HVAC ducting that terminated in a single vertical pipe 150 cm in length by 20 cm in diameter. A cardboard box was placed underneath the intake assembly for support. Seams in the intake assembly were sealed with duct tape. When operating, the fans (running at 13 volts) drew air through the intake assembly and then pushed it down the metal ducting and through the flumes. One of the flumes served as the test flume into which trap components associated with CO₂ delivery (dry ice containers for dry ice-baited traps, silicon outlet tubing for traps baited with CO₂ from compressed gas cylinders) were placed during trials. The other flume, designated the control flume, was used to measure ambient CO₂ concentrations during trials. The entire apparatus was located in a ~17,500 m³ storage warehouse at the headquarters of the National Ecological Observatory Network (NEON), Inc. The warehouse had regular air exchange with the external (outdoor) environment.

A series of wind calibration trials was conducted to identify low, medium, and high wind speed levels for use in the experimental trials. Wind speed in the flumes could be manipulated by changing two flume-related settings: the distance from the input end of the flume where the CO₂ delivery components (e.g., dry ice container) were placed (wind speed reduced by moving traps away from the input end of the flume) and the aperture diameter of the vertical pipe of the flume intake assembly (wind speed reduced by partially covering the aperture

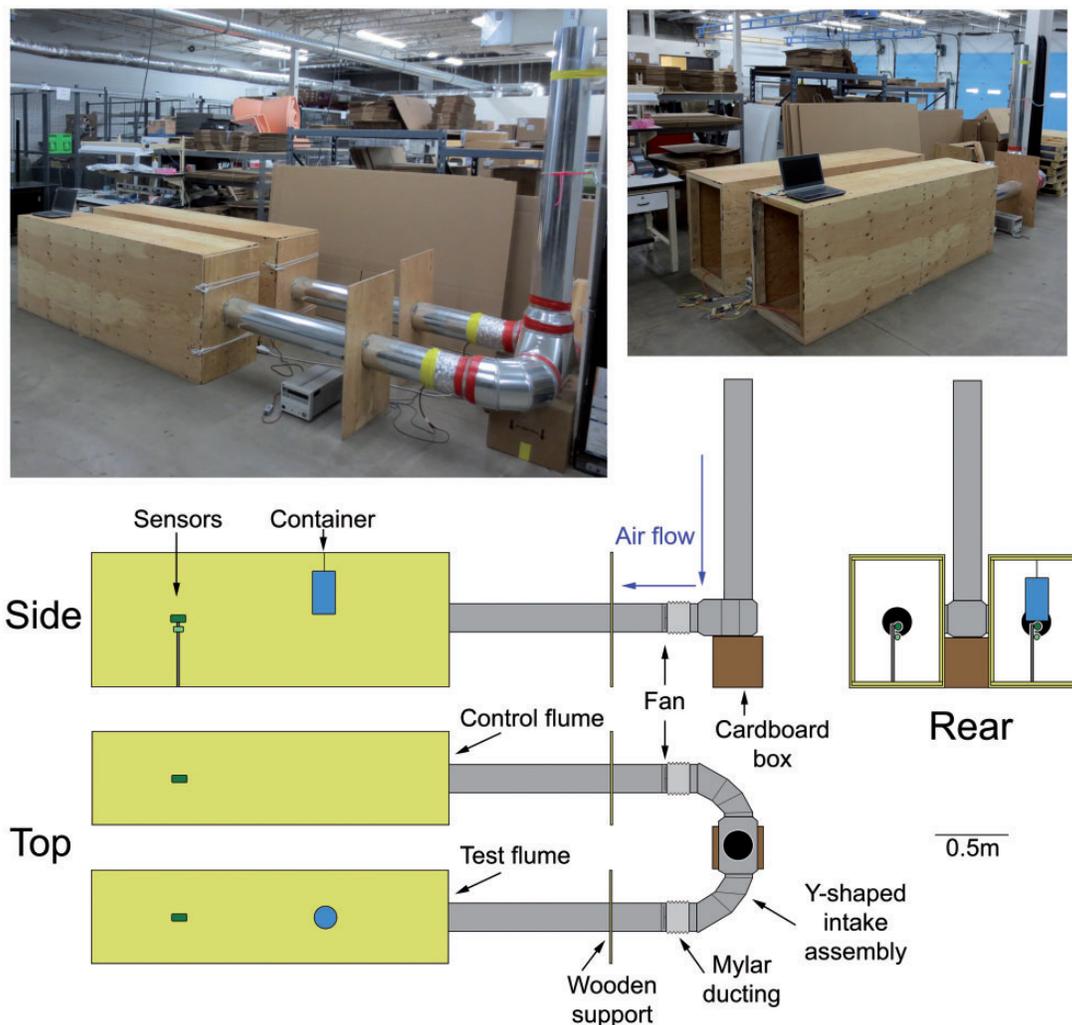


Fig. 1. Photographs and scale drawings of the flume assembly. Photographs show the pair of rectangular plywood flumes from the intake end (left) and output end (right). Scale drawings show flume assembly from side, top, and rear. Side and top views illustrate internal locations of fans, sensors (CO₂, temperature/RH), and container (insulated cooler is pictured).

with a flow restrictor plate). We varied these two settings and measured resulting impacts on wind speed by deploying an anemometer (Windobserver model 65, Gill Instruments, Lymington, UK) in place of the CO₂ delivery components in the test flume. Once the setting combination necessary to achieve each of the three desired wind speed levels was identified (distance of anemometer from input end of flume, aperture diameter of flume intake pipe), we ran 15 separate 24 h wind calibration trials (five replicates of each wind speed level, the level simulated in each trial randomly selected) to confirm that the target wind speed associated with each setting combination was consistently maintained in the test flume. In addition to collecting wind speed data, we deployed carbon dioxide and temperature/relative humidity (RH) sensors in the test and control flumes to confirm that these conditions did not differ between flumes. In each flume, a carbon dioxide sensor (model GMP343, Vaisala, Helsinki, Finland) and a temperature/RH sensor (Hobo model U23-001, Onset Corp., Pocasset, MA) were attached to a freestanding metal mount positioned 60 cm inside of the output end of the flume and at the same height that CO₂ delivery components would be placed during experimental trials. Carbon dioxide sensors were calibrated to ambient pressure and RH just prior to the start of each trial using data collected by NEON, Inc.'s collocated calibration and validation facility. Measurements of carbon dioxide concentration (hereafter designated as [CO₂]) were recorded every 30 s during trials, while temperature and RH measurements were recorded every 60 s.

Once wind speed levels had been established, we began experimental trials by comparing dry ice-baited traps. Collectively, these trials involved tests of every combination of two forms of dry ice, three container types, and the three wind speeds identified in the wind calibration trials. Containers were filled with 1,400 g of dry ice in the form of cylindrical pellets (typical dimensions 40 mm in length by 15 mm in diameter at purchase) or blocks (typical dimensions 40 cm in width by 40 cm in length by 5 cm in thickness at purchase). A band saw was used as needed to cut block ice into pieces of the largest size that would fit within each of the three containers. The first container consisted of a basket made of coarse wire mesh. This served as a container control and also simulated a sampling method (deployment of uncovered dry ice) often used to collect ticks (Grothaus et al. 1976, Norval et al. 1987, Petry et al. 2010, Carr et al. 2013). The second container was a padded shipping envelope (model 39261, Sealed Air Corporation, Elmwood Park, NJ) 26 cm in width by 34 cm in length with one corner cut off for CO₂ venting. Because of their wide retail availability, low cost, and easy of transport (light weight, compact), shipping envelopes are popular as dry ice containers used for mosquito sampling. The third container was a 1.9-liter cylindrical insulated beverage cooler (Legend model 00001755, Igloo Products Corp., Kay, TX) with a vent hole 13 mm in diameter drilled in the bottom. This type of container is standard equipment on many commercially available dry ice-baited mosquito traps (e.g., New Standard Miniature Light Trap models 1012 and 1.1, John W. Hock Company, Gainesville, FL) and has a capacity of 1,400 g of dry ice in pellet form. Insulated beverage coolers, or approximations thereof, have also been used in dry ice-baited sampling of ticks (e.g., Wilson et al. 1972, Kensing and Allan 2011).

We conducted three replicate trials of each combination of dry ice form, container, and wind speed. Replicates were blocked in time such that one trial of each of the 18 combinations was performed before any particular combination was tested again. The order in which combinations were tested within blocks was randomized. Each trial began with a 1-h baseline period to allow flume intake fans to reach steady-state wind speed conditions and ambient [CO₂] to be measured. Measurements of [CO₂], temperature, and RH were made as described for the wind calibration trials with the exception that temperature and RH measurements were collected every 30 s. At the conclusion of the baseline period, a container filled with dry ice was hung in the test flume at the position (height, distance from input end of flume) corresponding to the wind speed being tested. Containers were located

between 1.0 m and 1.5 m upwind of the freestanding metal sensor mount in the test flume. The trial then ran for 24 h or until sublimation of the dry ice was complete, whichever occurred first. During trials, [CO₂] in the flumes was displayed in real time on a laptop connected to the CO₂ sensors, allowing this endpoint to be detected. Any dry ice remaining at the conclusion of the trial was weighed.

Once the experimental trials involving dry ice were completed, we conducted trials to evaluate traps baited with carbon dioxide released from pressurized cylinders. The cylinders used in these trials measured 44 cm in height by 13 cm in diameter and are widely available as refillable rentals from purveyors of welding and home brewing supplies. They are sometimes referred to as “five pounders” because they come filled with 2.3 kg of liquid CO₂. Cylinders were fitted with a pressure regulator to standardize the gas flow rate, which was selected such that cylinders would be empty after approximately 24 h (roughly equivalent to a rate of 800 ml/min). Carbon dioxide from a cylinder was introduced into the test flume via a length of silicon outlet tubing connected to the regulator, passed through a small hole drilled into the side of the test flume, and attached to a freestanding metal mount placed at the position (height, distance from input end of flume) corresponding to the wind speed being tested. As with the dry ice trials, carbon dioxide and temperature/RH sensors recorded data at 30 s increments. Each of these trials ran until the pressurized cylinder had been emptied (per sensor measurements of [CO₂] displayed on the aforementioned laptop).

For each experimental trial, measurements of [CO₂], temperature, and RH made in the test and control flumes were matched using sensor-generated timestamps. We calculated the baiting intensity ([CO₂] above ambient or amount of carbon dioxide released by the dry ice or pressurized cylinder in the test flume, in ppm) for each 30 s measurement increment of a trial by subtracting the [CO₂] measured in the control flume (ambient concentration) from that measured in the test flume. We used these time-series baiting intensity data to quantify three performance measures. First, we calculated the length of each trial (time to reach baiting intensity = 0 from the start of the trial) and used proportional hazards survival analyses to statistically assess the effects of container (dry ice trials only), ice form (dry ice trials only), and wind speed on trial length. In these analyses, wind speed was treated as a continuous variable and the mean value measured for each of the three speeds in the wind calibration trials was used. The distribution of trial length data did not deviate sufficiently from normality to warrant transformation, especially given that the survival analyses are relatively robust to deviations from normality. Second, we calculated the maximum baiting intensity of each trial (average of baiting intensity measurements recorded during the first 15 min of the trial) and used analyses based on a general linear model to evaluate the effects of container (dry ice trials only), dry ice form (dry ice trials only), and wind speed on maximum baiting intensity. Maximum baiting intensity data were log-transformed to meet assumptions of normality. Linear regression of trial length against log-transformed maximum baiting intensity data indicated that these two variables were not strongly colinear ($R^2_{\text{adj}} = 0.51$, $MS = 32.30$, $F_{1,53} = 55.86$, $P < 0.0001$) and could be analyzed separately. All the aforementioned analyses were performed using JMP v10.0 (SAS Institute 2012). Finally, we used a graphical integration approach to assess the consistency of baiting intensity during trials. For each trial, we used the measurements of baiting intensity collected at 30 s increments to create a raw baiting intensity curve (graphical trace of baiting intensity through time during a trial). We grouped the raw curves for the three replicates of each combination of ice form, container, and wind speed (dry ice trials) or wind speed (compressed gas trials) (e.g., Fig. 2A), smoothed each raw curve using a 25-point smoothing interval (e.g., Fig. 2B) and then averaged the three replicate smoothed curves at each 30 s measurement increment to generate a mean baiting intensity curve and associated standard deviation (e.g., Fig. 2C). We calculated the area under this mean curve as an estimate of the total amount of CO₂ released during the trial and determined the average time to reach three CO₂ release thresholds: 25%,

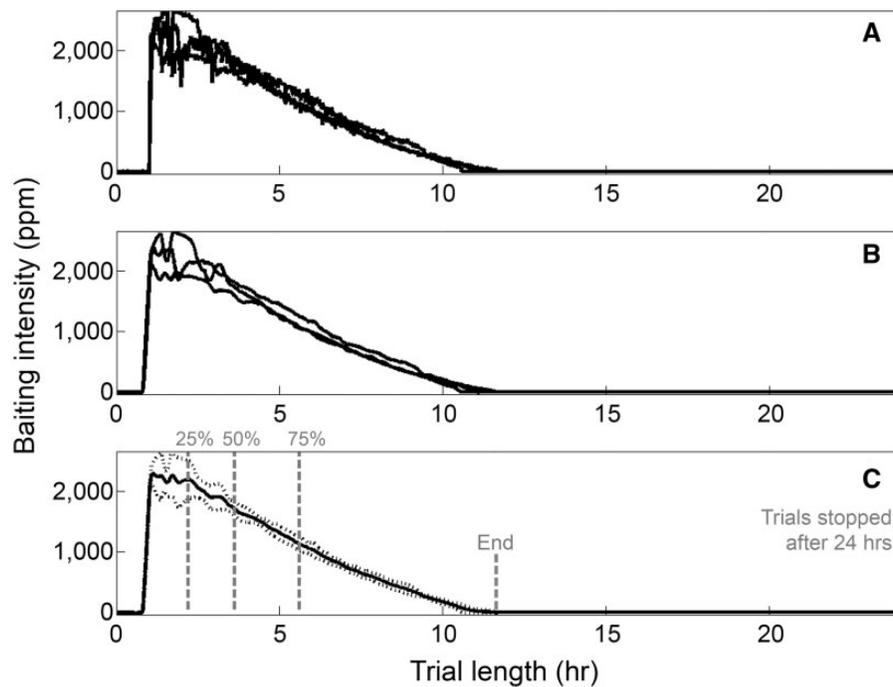


Fig. 2. Data processing sequence to evaluate consistency in baiting intensity during dry ice and compressed gas trials, illustrated with data from dry ice trials involving the combination of pellet ice deployed in a shipping envelope at medium wind speed. (A) Raw baiting intensity curves for each of the three trial replicates. Raw curves were generated using measurements of the concentration of carbon dioxide above ambient in the test flume recorded at 30 s increments during each trial. (B) Smoothed baiting intensity curves generated for each of the three raw curves by applying a 25-point smoothing interval. (C) Mean baiting intensity curve and associated standard deviation (dashed line) generated by averaging the three smoothed curves at each 30 s measurement increment, with vertical gray lines demarcating the 25%, 50%, and 75% CO₂ released thresholds.

50%, and 75% of total CO₂ released. Graph-based integration calculations were performed using MATLAB (MATLAB and Statistics Toolbox Release 2012). Together with data on mean trial length, these data were used to evaluate the consistency of baiting intensity during trials.

We used prices (US dollars) charged by a local purveyor of welding supplies to compare deployment costs associated with CO₂ traps baited with dry ice and pressurized gas. Our calculations assumed that a sampling event would last for a continuous period lasting 24 h.

Results

Through the 15 wind calibration trials we identified low, medium, and high wind speed levels of 0.17 ± 0.031 m/s (mean \pm SD, $n = 14,296$ measurements from five replicate trials, 0.38 mph), 0.44 ± 0.053 m/s ($n = 13,926$, 0.98 mph), and 1.01 ± 0.072 m/s ($n = 14,228$, 2.3 mph), respectively. Based on the 95% confidence intervals, the test and control flumes did not differ significantly in terms of [CO₂] (470.38 ± 35.87 ppm vs. 408.59 ± 39.83 ppm, respectively, $n = 42,450$), temperature ($21.54 \pm 1.18^\circ\text{C}$ vs. $21.35 \pm 1.35^\circ\text{C}$, $n = 21,222$), or RH ($16.79 \pm 2.97\%$ vs. $17.18 \pm 2.98\%$, $n = 21,222$). Because of technical complications, temperature/RH sensors only recorded data during 11 of the 15 trials. We observed comparable results during the 1-h baseline period preceding the start of each experimental trial: the test and control flumes did not differ significantly in [CO₂] (472.35 ± 83.14 ppm vs. 472.35 ± 83.44 ppm), temperature ($23.03 \pm 0.66^\circ\text{C}$ vs. $22.98 \pm 0.63^\circ\text{C}$), or RH ($33.05 \pm 8.81\%$ vs. $33.84 \pm 8.75\%$).

Results of experimental trials involving dry ice demonstrated that, on average, trial length (i.e., time to reach baiting intensity = 0 from the start of the trial) was shortest for wire basket trials, intermediate for shipping envelope trials, and longest for insulated cooler trials (Fig. 3A). Across all combinations of ice form and wind speed, the mean trial lengths (\pm SD) for the three different containers were

7.03 ± 2.54 h, 12.69 ± 3.13 h, and 24 h, respectively. For wire basket and shipping envelope trials, trial length tended to be longer for block compared with pellet ice and at lower wind speeds. In analyzing these data using proportional hazards models with trial length as the response, we excluded data from trials involving insulated cooler traps because these trials all ran for and were stopped after 24 h (i.e., all data from this treatment were “censored” and there was no variation among the replicates). The three-way interaction term involving ice form, container, and wind speed in the full model was significant, so we parsed the dataset into trials involving wire basket or shipping envelope containers and analyzed these separately. For wire basket trials, both ice form (log rank $\chi^2 = 23.84$, $df = 1$, $P < 0.0001$) and wind speed (log rank $\chi^2 = 19.30$, $df = 1$, $P < 0.0001$) had significant effects on trial length. Trial length tended to be longer for block compared with pellet ice and at lower wind speeds. The interaction between these was not significant and was removed from the final model. For shipping envelope trials, ice form and wind speed interacted to determine trial length, such that wind speed reduced trial length for pellet ice (log rank $\chi^2 = 7.41$, $df = 1$, $P = 0.0065$) but not for block ice (log rank $\chi^2 = 0.35$, $df = 1$, $P = 0.85$).

Results of experimental trials involving dry ice further indicated that on average, maximum baiting intensity (i.e., average of baiting intensity measurements recorded during the first 15 min of the trial) was highest for wire basket trials, intermediate for shipping envelope trials, and lowest for insulated cooler trials (Fig. 4A). Across all combinations of ice form and wind speed, the mean maximum baiting intensities (\pm SD) for the three different containers were $3,379.43 \pm 1,435.28$ ppm, $1,209.16 \pm 721.40$ ppm, and 507.59 ± 414.16 ppm, respectively. Based on a general linear model with maximum baiting intensity as the response, we identified significant effects of container ($SS = 37.24$, $F_{2,53} = 118.26$, $P < 0.001$), wind speed ($SS = 15.21$, $F_{1,53} = 96.62$, $P < 0.0001$), and ice form ($SS = 2.20$, $F_{1,53} = 14.00$, $P = 0.0005$). Neither the three-way interaction term nor

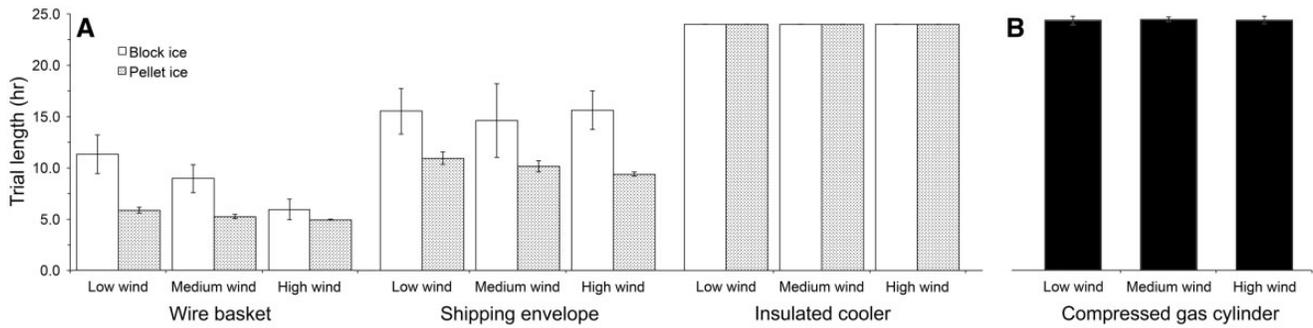


Fig. 3. Average and standard deviation of trial length for dry ice (A) and compressed gas (B) trials. Each bar represents the mean across the three replicates for a given combination of container, ice form, and wind speed (dry ice trials) or wind speed (compressed gas trials). Trial length is the time to reach baiting intensity = 0 from the start of a trial.

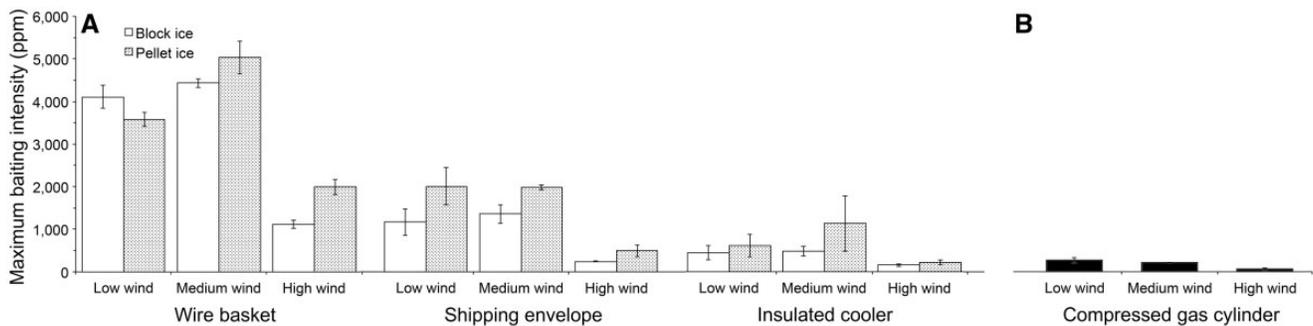


Fig. 4. Average and standard deviation of maximum baiting intensity for dry ice (A) and compressed gas (B) trials. Each bar represents the mean across the three replicates for a given combination of container, ice form, and wind speed (dry ice trials) or wind speed (compressed gas trials). Baiting intensity is the concentration of carbon dioxide above ambient. Maximum baiting intensity is the average of baiting intensity measurements (concentration of carbon dioxide above ambient) recorded during the first 15 min of a trial.

any of the two-way interaction terms were significant (final reduced model without the interactions: $R^2_{\text{adj}} = 0.87$, $MS = 13.67$, $F_{4,53} = 86.78$, $P < 0.0001$). Maximum baiting intensity tended to be higher for pellet compared with block ice. In a post-hoc test to further explore the effect of wind speed and whether it was nonlinear, we detected a significant quadratic effect of wind speed ($t = -2.13$, $P = 0.038$), reflecting the fact that the highest values of maximum baiting intensity were associated with trials involving medium wind speed, with lower values for low and high wind speeds, respectively ($R^2_{\text{adj}} = 0.28$, $MS = 9.53$, $F_{2,53} = 11.22$, $P < 0.0001$).

Visual examination of mean baiting intensity curves (i.e., average of the smoothed baiting intensity curves for each replicate trial of a given combination of ice form, container, and wind speed [dry ice trials] or wind speed [compressed gas trials]) (Fig. 5) and comparison of area-under-curve-derived measures of time to CO_2 release thresholds (Table 1) provided three main insights into the consistency of baiting intensity during experimental trials involving dry ice. First, baiting intensity declined most rapidly during wire basket trials, most slowly during insulated cooler trials, and at an intermediate rate during shipping envelope trials. For example, relative to wire basket, shipping envelope, and insulated cooler trials took 3.1 and 7.6 times longer to reach the 25% CO_2 release threshold, respectively (averaged across all ice form/wind speed combination), and 2.5 and 6.2 times longer to reach the 50% release threshold. Compared with shipping envelope trials, insulated cooler trials took 2.4 and 2.5 times longer to reach these two thresholds, respectively. When linear and two-parameter exponential models were fit to data on times to the CO_2 release thresholds for each container (across all ice form/wind speed combinations, and including trial length [time to baiting intensity = 0]), the exponential model improved the R^2 value of the linear regression most significantly for data from wire basket trials (0.70 vs. 0.83), to a lesser extent for data from shipping envelope trials (0.82 vs. 0.90), and least for data from insulated cooler trials

(0.96 vs. 0.97). Second, baiting intensity declined more rapidly for pellet ice compared with block ice. When averaged across all container/wind speed combinations, block ice took 1.5 times longer to reach the 50% release threshold than pellet ice. Finally, trials involving different wind speeds varied relatively little in times to CO_2 release thresholds. When averaged across all container/ice form combinations, trials involving low wind speed took 1.2 and 1.4 times longer to reach the 25% CO_2 release threshold compared with medium and high wind speed trials, respectively, and 1.1 and 1.2 times longer to reach the 75% threshold.

Data from experimental trials involving compressed gas indicated that the performance of traps baited with CO_2 from pressurized cylinders was most similar to that of dry ice-baited traps utilizing insulated coolers. This was true for both trial length (Fig. 3A and B) and maximum baiting intensity (Fig. 4A and B). Across all combinations of wind speed, the mean length of compressed gas trials (\pm SD) was 24.40 ± 0.31 h. Although the exact lengths of insulated cooler trials were not recorded, each lasted the allotted 24 h, and 16 of 18 trials had a very small quantity of a mix of ice and dry ice remaining when data collection was stopped (mean \pm SD = 93.4 ± 42.1 g). The average maximum baiting intensities of compressed gas and insulated cooler trials were also very similar: 180.38 ± 100.70 ppm versus 507.59 ± 414.16 ppm, respectively. Analyses using general linear models identified a significant effect of wind speed on the maximum baiting intensity of compressed gas trials ($SS = 3.96$, $F_{1,8} = 56.39$, $P < 0.0001$), but the wind speed by wind speed interaction was nonsignificant in the post-hoc analysis ($t = -1.62$, $P = 0.16$), indicating a linear, negative effect of wind speed on maximum baiting intensity. As illustrated in Figs. 3A and B and 4B, the average maximum baiting intensity measured in insulated cooler trials tended to be both higher and more variable compared with compressed gas trials. Relative to mean baiting intensity curves associated with wire basket and shipping

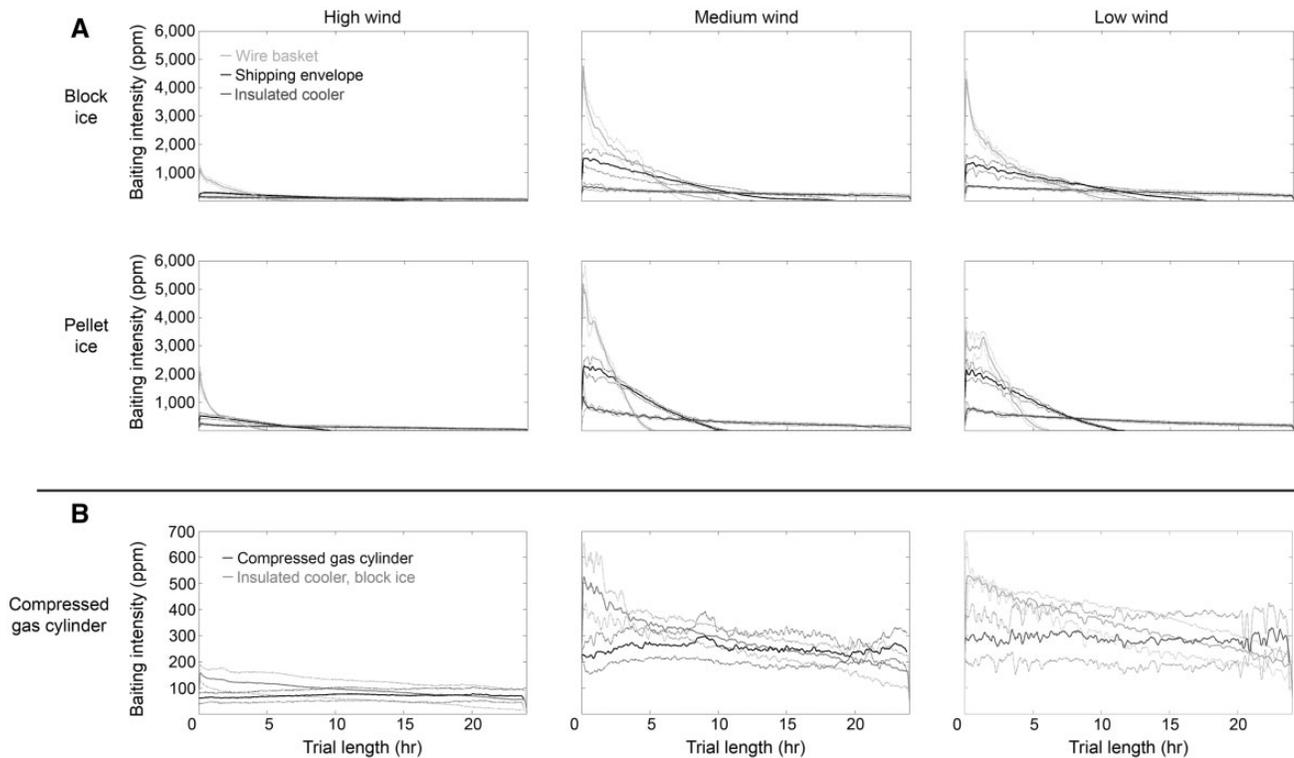


Fig. 5. Mean baiting intensity curves and associated standard deviations (dashed lines) for dry ice (A) and compressed gas (B) trials. Each plot illustrates a different combination of wind speed and CO₂ source. For comparison, plots in (B) also present data from dry ice trials involving insulated coolers filled with block ice. Note different y-axis scales in (A) and (B). Baiting intensity is the concentration of carbon dioxide above ambient.

Table 1. Average time required to reach each of three CO₂ release thresholds during dry ice and compressed gas trials for every tested combination of container, ice form, and wind speed (dry ice trials) or wind speed (compressed gas trials)

Container	Ice form	CO ₂ release threshold (% of total CO ₂ released)	Mean time to CO ₂ release threshold (h) ^a			
			Low wind speed	Medium wind speed	High wind speed	
Wire basket	Block ice	25	0.79	0.72	0.52	
		50	2.21	1.98	1.38	
		75	4.46	3.80	2.64	
	Pellet ice	25	0.69	0.46	0.23	
		50	1.48	1.16	0.80	
		75	2.49	2.10	1.83	
	Shipping envelope	Block ice	25	1.98	1.75	2.05
			50	4.50	3.99	4.64
			75	7.93	7.04	8.24
Pellet ice		25	1.26	1.20	1.18	
		50	2.84	2.63	2.65	
		75	4.96	4.61	4.60	
Insulated cooler		Block ice	25	4.18	3.91	4.21
			50	9.28	9.08	9.33
			75	15.49	15.48	15.57
	Pellet ice	25	3.39	2.61	3.78	
		50	7.97	6.74	8.56	
		75	14.19	13.08	14.41	
	Compressed gas cylinder	NA	25	5.84	5.93	6.42
			50	11.63	11.53	12.13
			75	17.78	17.60	17.94

^aDry ice trials ran for each value of mean time to CO₂ release threshold calculated as an average across three replicate trials.

Dry ice trials ran for 24 h or until sublimation of dry ice was complete, whichever came first. Compressed gas trials ran until pressurized cylinders had been emptied.

envelope trials, declines in baiting intensity during insulated cooler and compressed gas trials were minimal and occurred at an almost constant rate. When linear and two-parameter exponential models were fit to data on times to the CO₂ release thresholds (across all ice form/wind

speed combinations and including trial length [time to baiting intensity = 0]), the linear model was a better fit to the data than the exponential model based on their respective *R*² values (0.99 vs. 0.94, respectively).

Deployment cost calculations based on prices of \$2.56/kg for dry ice and \$17.56 per pressurized cylinder fill (obtained from General Air, Boulder, CO on February 1, 2015) indicated that a 24-h sampling event using a trap baited with compressed gas CO₂ would be almost five times more expensive than one using a dry ice-baited trap (\$3.58 per sampling event).

Discussion

Experimental trials involving dry ice documented three notable performance differences among the containers evaluated. First, there was considerable variation in trial length among containers. All insulated cooler trials lasted for 24 h, a duration that was on average 110% and 62% longer than that of wire basket (7.0 h) and shipping envelope trials (12.7 h), respectively (across all ice form/wind speed combinations). Second, maximum baiting intensity was inversely related to trial length. Insulated cooler trials were associated with the lowest mean maximum baiting intensity, approximately 500 ppm above ambient (across all ice form/wind speed combinations). In contrast, shipping envelope trials had a mean maximum baiting intensity of roughly 1,200 ppm above ambient (82% greater on average than insulated cooler trials), and wire basket trials had the highest mean maximum baiting intensity, nearly 3,400 ppm above ambient (149% greater on average than insulated cooler trials). Finally, consistency of baiting intensity during trials differed among containers. Fitting of linear and two-parameter exponential models to the estimates of time to CO₂ release thresholds indicated that while the decline in baiting intensity during trials exhibited an exponential decay signature for all three containers, the pattern was strongest (i.e., most exponential) for wire basket trials, intermediate for shipping envelope trials, and weakest (i.e., most linear) for insulated cooler trials.

Results of experimental dry ice trials additionally identified contributions of dry ice form and wind speed to variation in trap performance. On average, wire basket and shipping envelope trials had longer lengths but lower maximum baiting intensity when dry ice in block form was used. Among trials involving these two containers, those involving block ice lasted on average 43% longer than those involving pellet ice (across all container/wind speed combinations). In contrast, maximum baiting intensity for trials involving pellet ice was on average 19% higher than for those involving block ice. While wind speed and trial length tended to be negatively correlated, this relationship varied among containers and dry ice forms. For wire basket trials, the negative relationship between trial length and wind speed was significant for both block and pellet dry ice. For shipping envelope trials, the effect of wind on trial length was only significant for trials involving pellet ice. While not analyzed statistically, there was no apparent effect of wind speed on trial length for insulated cooler trials. For maximum baiting intensity, analytical results identified a nonlinear effect of wind speed, with the highest maximum baiting intensity realized at medium wind speed, intermediate values at low wind speed, and the lowest values at high wind speed.

The aforementioned results likely reflect the relationship between dry ice surface area and sublimation rate, and the modifying effects of air movement on this. At a given wind speed, the higher surface area of pellet ice should result in more rapid sublimation compared with block ice, producing both a higher maximum baiting intensity and shorter trial length. In general, increases in the speed of air moving over dry ice should increase the sublimation rate as the cold, high [CO₂] boundary layer surrounding the ice is progressively stripped away by warmer, lower [CO₂] air. This relationship should be asymptotic for a given set of temperature/RH conditions as the dry ice reaches its maximum sublimation rate without any boundary layer buffering. In contrast, a more parabolic relationship was observed in our dry ice trials. This result probably reflects the fact that as wind speed increases, rates of dry ice sublimation and dissemination of produced CO₂ from the flume both increase. Under low wind speed conditions, there may have been

insufficient air movement to maximize dry ice sublimation (e.g., through boundary layer removal and heating). Under high wind speed conditions, the rate of dry ice sublimation was greatest, but the rate of airflow was likely so high that produced CO₂ was rapidly diluted by ambient air being forced into the flume. These high wind speeds would additionally make it difficult for CO₂ to build up within the flumes. Medium wind speed conditions appear to have represented the balance of these two effects, resulting in a combination of more rapid sublimation of dry ice relative to low wind speed trials but reduced degree of CO₂ export/dilution relative to high wind speed trials. Finally, containers varied in the degree of physical and thermal protection they provided to the ice. Ice deployed in a wire basket was directly exposed to wind and experienced the full effect of the aforementioned boundary layer removal and heating. By comparison, shipping envelopes provided a physical barrier to wind but little if any thermal protection. Analysis of data on trial length showed that the negative relationship with wind speed was significant for both dry ice forms in wire basket trials but only for higher surface area pellet ice in shipping envelope trials. This result suggests a reduced effect of wind on dry ice sublimation rates in shipping envelope trials. Finally, insulated coolers provided the highest degree of both physical and thermal insulation. Qualitative evaluation of data for insulated cooler trials suggested no effect of ice form or wind speed on trial length.

Results of compressed gas trials showed that the performance of traps baiting with CO₂ released from pressurized cylinders was most similar to that of dry ice-baited traps utilizing insulated coolers. When compared with wire basket and shipping envelope trials, those involving both compressed gas and insulated coolers had the longest length, the lowest maximum baiting intensities, and exhibited declines in baiting intensity during trials at low and relatively constant (i.e., linear) rates. In terms of these characteristics, insulated cooler and compressed gas trials differed minimally. The length of insulated cooler trials did not appear to be sensitive to effects of wind speed, although this relationship could not be statistically assessed for reasons explained previously. Similarly, the length of compressed gas trials did not vary by wind speed since the rate of production of CO₂ in these trials was determined by the cylinder's flow rate regulator and was thus unrelated to airflow. Maximum baiting intensity was low for both compressed gas and insulated cooler trials but tended to be slightly higher and more variable for the latter. Changes in baiting intensity during insulated cooler trials were better characterized by a pattern of exponential rather than linear decay, although the improvement in model fit was small. This result is not surprising given the aforementioned sublimation dynamics of dry ice. In contrast, the flow rate regulator used in compressed gas trials resulted in more consistent rates of CO₂ release. Changes in baiting intensity during compressed gas trials were minimal and better described by a linear than an exponential decay model. Post-hoc analyses revealed significant effects of wind speed on maximum baiting intensity for both trap types, but the effect was nonlinear for insulated cooler trials and linear for compressed gas trials. These results are consistent with the mechanisms described above: for insulated cooler trials, wind speed affected rates of both CO₂ production (via dry ice sublimation) and dilution/export from the flume, while in compressed gas trials, only CO₂ dilution/export was affected since regulator-controlled CO₂ release rates were held constant. In contrast to these performance similarities, our cost estimates indicated that a 24-h sampling event using a trap baited with compressed gas CO₂ would be five times as expensive as one using a dry ice-baited insulated cooler trap.

To date, much of the research into the use of olfactory cues by arthropods (including hematophagous taxa) to orient in and navigate through the environment (e.g., to find mates or hosts) can be divided into two categories. The first involves studies of the formation, structure, and physical dynamics of odor plumes. Chemical odors are transported away from their source by diffusion and advection on air currents, forming a plume. Each plume will develop a large-scale

structure that can be characterized by its length, general shape, and the average concentration of odor molecules within it (Murlis et al. 1992). In general, a high concentration of odor molecules at the source will result in longer plumes (Gillies 1980). The physical structure and heterogeneity of the environment will influence the patterns of air flow (e.g., wind speed and direction) that largely determine the shape of plumes (Brady et al. 1989). For example, in open habitats (e.g., grasslands), air currents can move relatively unobstructed through the environment, so plume structure will tend to be more linear and mixing will be greater (Murlis et al. 1992). In contrast, plumes generated in more closed habitats (e.g., woodlands, forests) will be carried on relatively lower speed air currents moving in a nonlinear manner as they flow around physical structures (e.g., shrubs and trees or features of the urban/built environment). The degree of mixing may be lower within these plumes, and they may develop a strongly nonlinear structure as they “meander” through the environment (Murlis et al. 1992). While the average concentration of odor molecules within a plume will decrease with distance from the odor source, the small-scale structure of plumes will generally be characterized by an intermittent signature in which odor molecules are concentrated within pulses that vary in their strength and duration (Murlis and Jones 1981). This heterogeneous structure is generated by turbulence within the plume and will result even if odor molecules are released continuously and at a fixed rate from their source (Murlis and Jones 1981). In general, smaller odor sources will result in higher intermittency and a greater degree of concentration heterogeneity within plumes (Murlis et al. 1992).

The second category of research investigating the role of olfactory cues in arthropod orientation and navigation includes studies that examine how organisms perceive and respond to different chemical cues. A good deal of this work focuses on the response of female mosquitoes to carbon dioxide plumes (reviewed in Gillies 1980, Mboera and Takken 1997, Takken and Knols 1999). Female mosquitoes can detect changes in $[\text{CO}_2]$ of as little as 50 ppm (Grant and O’Connell 1996), and results of CO_2 -based sampling of mosquitoes generally show that addition of CO_2 bait to traps using other attractants (e.g., light), and increases in the concentration/release rate of CO_2 in CO_2 -baited traps, enhance catch success (both numbers and diversity of mosquitoes) (e.g., Rudolfs 1922, Newhouse et al. 1966, Carestia and Savage 1967, Mboera et al. 2000). In mosquitoes of many species, CO_2 has been shown to act as both an activator (e.g., inducing takeoff and sustained flight) and an attractant that allows female mosquitoes to orient upwind as they fly along plumes toward CO_2 sources (hosts or traps) (Gillies 1980, Eiras and Jepson 1991, Geier et al. 1999). Results of multiple studies suggest that activation and attraction behaviors may be elicited solely or primarily when the CO_2 plumes are turbulent and intermittent in concentration structure and that these behaviors may be suppressed under continuous or homogeneous CO_2 stimulation (Gillies 1980, Geier et al. 1999, Dekker et al. 2001). There is additional evidence that responses to CO_2 , particularly the concentrations that elicit activation and attraction behaviors, vary among individuals of different mosquito species and stages of development or nutrition within particular species. For example, because species of mosquitoes vary in their host preferences, the types and concentrations of odors that initiate and sustain host seeking vary among species (Brady et al. 1989, Eiras and Jepson 1991, Takken 1991). Mosquitoes that prefer smaller vertebrate hosts such as birds may respond to CO_2 at relatively low concentrations, while species that feed on larger hosts with greater pulmonary capacity may only be activated at higher CO_2 concentrations. Reeves (1953) conducted CO_2 sampling using three CO_2 release rates (25 ml/min \approx chicken, 250 ml/min \approx human, 2,500 ml/min \approx horse or cow) and found that collection success associated with different mosquito species was consistent with their host preference(s). Further, individual mosquitoes should vary in their sensitivity to CO_2 relative to their need to locate hosts. Indeed, the sensitivity of CO_2 -detecting neurons in mosquitoes can vary with sex, age, nutritional status, and gonotrophic condition in females (Bowen 1991, Kline et al. 2006b). Also, CO_2 is only one of a suite of olfactory

cues that can influence mosquito behavior. Effects of CO_2 on mosquitoes are often modified by the presence and concentration of other chemicals that may be emitted from hosts (Takken 1991).

Responses of ticks to CO_2 have also been investigated, albeit generally to a lesser extent than in mosquitoes. Individuals of a variety of species have been collected using CO_2 -baited traps, and CO_2 receptors have been identified in both hard and soft ticks (reviewed in Wilson et al. 1972, Semtner and Hair 1975, Allan 2010). Sauer et al. (1974) showed that attraction intensity of *Amblyomma americanum* L. increased positively with $[\text{CO}_2]$ through a range from 2,000 ppm to 8,000 ppm and posited that while ticks may be stimulated by lower concentrations of CO_2 , consistent, positive chemotaxis only occurs at levels above 1,000 ppm. In contrast, work by Perritt et al. (1993) on adult *A. americanum* and *Dermacentor variabilis* (Say) provided evidence of responsiveness to CO_2 at levels as low as 9 ppm above ambient. Perhaps reconciling this apparent contradiction, results of a wind tunnel study by Steullet and Guerin (1992) documented effects of both inhibitory and excitatory CO_2 sensors in *Amblyomma variegatum* Fabricius, with the former capable of detecting changes in $[\text{CO}_2]$ of as little as 100 ppm around ambient concentration and the latter most sensitive to changes in $[\text{CO}_2]$ above 1,000 ppm. The authors suggested that these sensors might operate in concert to guide ticks to hosts, with inhibitory sensors detecting small changes in ambient CO_2 that indicate the presence of a host nearby and excitatory sensors guiding ticks toward these host. Indeed, they documented both activating and attracting effects of CO_2 on adult ticks, with response elicitation peaking at concentrations of roughly 1,500 ppm and 4,500 ppm, respectively. Finally, a laboratory experiment involving *Amblyomma hebraeum* Kock demonstrated that the effects of CO_2 on tick responsiveness were greatest during periods of the life-cycle associated with host seeking (Anderson et al. 1998).

Informed by what is known about the structure of odor plumes and how they are likely used by hematophagous arthropods to locate hosts, our results suggest a number of conclusions about the use of the CO_2 -baited traps evaluated in this study. Our documentation of a tradeoff between maximum baiting intensity and trial length was not surprising. Given this relationship, traps with higher maximum baiting intensity should on average produce longer odor plumes with higher average $[\text{CO}_2]$. This may enable sampling over a broader geographic area but for a relatively short period of time. If the desired area to be sampled is comparatively small (e.g., habitat patch or confined indoor space), a lower sampling intensity may be more appropriate, both because this could allow for longer periods of sampling and because extremely high sampling intensity may have a negative effect on catch success (e.g., carbon dioxide may have a repulsive or immobilizing effect at very high concentrations). At least two considerations arise related to sampling duration. First, collection success will reflect both the capacity of arthropods to detect CO_2 bait and their ability to travel to its source during the time that a trap is deployed. The latter constraint may be relatively unimportant for mosquitoes and other flying arthropods but is likely to be significant for less mobile taxa such as ticks. While mark/recapture studies of ticks sampled with CO_2 traps have documented collection of individuals at a distances of up to 21 m from traps (Wilson et al. 1972), the typical sampling radius seems to be within 3–5 m of a trap (e.g., Kensinger and Allan 2011). The collection of greater numbers of ticks of more mobile life stages (adults > nymphs > larvae) provides further evidence of the effects of arthropod mobility on sampling success (Koch and McNew 1982). This phenomenon may be most problematic for traps that sample with high intensity (e.g., blocks of raw dry ice commonly used to sample ticks) as arthropods activated at and attracted from long distances may not have adequate time or physiological capacity to reach traps before dry ice sublimation and associated sampling is complete. Second, sampling decisions related to duration could be informed by the length of the daily activity period of the target taxa and the portion of this period that is selected to be sampling. Sampling at higher intensity will be

possible if the desired duration of sampling is relatively short. These tradeoffs make clear that there will rarely be an optimal, one-size-fits-all approach to CO₂ sampling. One possible solution might be a mixed strategy involving a single trap with paired high and low intensity CO₂ sources, the former providing a short, concentrated initial burst of bait to initiate activation over broad areas, the latter maintaining a longer, lower intensity baiting signal to ensure extended attraction.

A second conclusion relates the value and paucity of both species-specific information on CO₂ sensitivity and spatial information about plume concentration structure. In instances where sampling is targeting specific arthropod species or closely related taxa, knowledge of the [CO₂] thresholds necessary to achieve activation and attraction (e.g., Eiras and Jepson 1991) would be valuable. When paired with predications about CO₂ plume concentration structure within a desired sampling radius, this information could be extremely useful in determining minimum and optimal CO₂ baiting intensities at the source (trap location). In general, [CO₂] within baiting plumes will decay with increasing distance from the source, with degree of decay positively related to wind speed and degree of turbulence/mixing. The exact baiting intensity realized at a given distance from a trap will be exceedingly difficult to estimate precisely due to complex and idiosyncratic effects of air movement, environmental structure, and interactions between the two. Nevertheless, it may be feasible to empirically characterize odor plume structure in the proximity of a CO₂ trap (e.g., within 5–10 m, Cooperband and Carde 2006) and use this information to approximate the relationship between baiting intensity and distance from a trap. This relationship could then be used to identify a CO₂ release rate or concentration from a trap that roughly achieves a target baiting intensity at a designated distance from the source. In addition to allowing sampling to be tailored to the CO₂ sensitivity of target arthropod taxa, such information would permit more accurate estimates of the area sampled by a given trap targeting these taxa. Our study has effectively achieved this for a distance of ~1.25 m for the trap types tested. Larger and longer flumes could be used to characterize plume structure and measure baiting intensity decay over larger distances from traps.

Our final conclusion pertains to variation in CO₂ release rates and consistency of baiting intensity during sampling events. Comparisons of carbon dioxide traps baited with compressed gas versus dry ice frequently focus on logistic considerations. For example, while dry ice is relatively less bulky than pressurized cylinders and may be more readily obtained in remote areas (Service 1993), it can be difficult and hazardous to handle, transport, and store (Singh et al. 2013). In contrast, while pressurized cylinders can be extremely dangerous if they rupture and require expensive and relatively delicate regulator accessories to operate, their ability to dispense CO₂ at a constant and easily controlled rate is frequently cited as a significant advantage over dry ice (Kline et al. 2006a). Our results indicate that, at least for the combination of 24-h sampling duration and vent hole bore size used in our insulated cooler traps, roughly the same consistency in baiting intensity can be achieved with dry ice-baited trap for a fraction of the cost. It is true that a cylinder flow regulator should easily allow higher CO₂ release rates to be set and uniformly maintained, but insulated coolers with larger diameter vent holes could in theory be used to achieve the same results. While the argument that uniform CO₂ release rates enhance comparability among sampling events seemingly points to a significant advantage of traps baited with compressed gas, our results indicate that for all three dry ice-baited trap types evaluated, baiting intensity curves exhibited a reasonable degree of consistency in structure among replicate trials at a given wind speed. Finally, even when relatively constant baiting intensity can be achieved at the source, it seems highly unlikely that such consistency in [CO₂] exists or can be maintained in the associated plume at further distances from the source in the field given effects of air movement and environmental structure. In light of these points, our results suggest that when logistic challenges of procuring, storing, and/or transporting dry ice can be overcome, dry ice-baited CO₂ traps,

particularly those using insulated coolers, should be strongly considered for use as they offer performance very similar to traps baited with compressed gas at a fraction of the price.

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