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Research

Sampling

A Novel Passive Sampling Technique for Collecting Adult Necrophilous Insects Arriving at Neonate Pig Carcasses

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Abstract

Neonate pigs have been used as decomposition models in experimental forensic entomology studies. Their small size, however, poses challenges to traditional sampling methods of necrophilous insects, like the sweep net, the most commonly used sampling method in forensic entomology research and practice. Previous research experimentally demonstrated the potential for sticky traps as an effective sampling method for collecting necrophilous insects from neonate pigs. While sticky traps effectively sampled fly diversity from the pigs, they shared with the sweep net low sample diversity and abundance, particularly of necrophilous beetles. Motivated by chemosensory host-finding of necrophilous insects and the architecture of carrion-mimicking thermogenic flowers, we developed a 'vented-chamber' method and optimized its design experimentally. In this approach, a neonate pig was transiently enclosed in a chamber. The decomposition process thermally convected the natural decomposition odors in the headspace above the pig toward a pair of sticky traps. The vented-chamber method collected significantly more necrophilous flies, representing a greater diversity, than the sweep net. Nevertheless, this approach caught few beetles, and hand collections must be used as well to most effectively sample beetle diversity.

Key words: sampling, biodiversity, ecology & behavior, forensic entomology

Necrophilous insects from orders Diptera and Coleoptera are important indicators in forensic entomology, as their arrival patterns and growth rates can be used to calculate a post-mortem interval (PMI) (Goff 1993). In death investigations, the PMI is used to estimate a person's time of death, potentially corroborating or contradicting an alibi (Catts and Goff 1992). A dead body, whether carrion or human, is a rare and ephemeral resource in the ecological landscape (Carter et al. 2007, Yang et al. 2008). Therefore, necrophilous insects, including both specialists and generalists, have evolved specialized, fine-tuned chemosensory systems to detect and compete for such a transient resource (Dethier et al. 1963, Wells and Greenberg 1994). A central tenet of PMI determination is that necrophilous insects rapidly locate and colonize a carcass in a predictable ecological succession (Payne 1965, Keh 1985, Anderson and VanLaerhoven 1996, Grassberger and Frank 2004). Blow flies (Diptera: Calliphoridae) and flesh flies (Diptera: Sarcophagidae) are typical first responders that have been shown to arrive to a body within minutes of death unless any of the well-documented insect delaying factors like darkness or concealment exist (Payne 1965, Grassberger and Frank 2004, Pechal et al. 2014, Charabidze et al. 2015, Bonacci et al. 2016). Thus, the period of insect activity (PIA), which can be estimated using growth tables and local temperatures, is closely related to the PMI (Tomberlin et al. 2011).

Experimental research in forensic entomology requires the use of animal models. Neonate pigs have been used as decomposition models in a number of forensic studies, and their low cost and ease of acquisition enables greater replication and less reliance on pseudoreplication (Archer 2004, Zimmerman and Wallace 2008, Michaud et al. 2012). However, while carcass size has little effect on the successional pattern of necrophilous insects, it significantly influences the overall number of insects attracted to the carcass, challenging the effectiveness of typical sampling procedures (Kuusela and Hanski 1982, Hewadikaram and Goff 1991). The combination of sweep nets and hand-collections has been demonstrated experimentally to representatively sample the fauna colonizing a decomposing body (Byrd et al. 2010) and serves as the standard practice for crime scene investigators. This approach is significantly constrained, however, by small collections of insects, which is further exacerbated by the disturbance caused by the sweep net over a small carcass (Schoenly et al. 2007). Therefore, for active sampling methods like the sweep net to be effective, multiple, short, interrupted sampling events are required at each replicate body, which may limit the number of replicates that can be sampled concurrently. This is further aggravated if insect sampling is coupled with other intensive biological and environmental sampling.

Previous research on the suitability of sampling methods for ecological succession studies using neonate pigs showed that passive sticky traps effectively sampled arriving insects (Cruise et al. 2018b). Indeed, collecting necrophilous insects with sticky traps is not a new technique; such traps are frequently baited with natural or synthetic odors for blow fly monitoring or trapping purposes (Ashworth and Wall 1994, Hall et al. 2003). Unique to our previous work, however, was that the decomposing pig served as bait, with sticky traps mounted to an open wooden frame placed above it. The sticky traps sampled a diversity of flies, but they were relatively ineffective at sampling beetles, and it was clear that necrophilous insects were adept at orienting to the carcass while avoiding the traps. Thus, the efficacy of sticky traps was depressed by the spatial separation of olfactory cues from the decomposing pig and the position of the sticky traps.

In the present work, we sought to increase the efficacy of sticky traps by directing the decomposition odors toward the sticky trap and guiding the arriving insects to this attractive target. Our 'vented-chamber' method was inspired by two observations: 1) necrophilous insects quickly locate hosts primarily through orientation to decomposition odors (Ashworth and Wall 1994, LeBlanc 2008, Chaudhury et al. 2010, LeBlanc and Logan 2010, Paczkowski et al. 2012) and 2) carrion-mimicking plants attract necrophilous insect pollinators with a combination of odors, heat, and visual cues to guide the insect to the flower opening (Meeuse and Raskin 1988, Stensmyr et al. 2002, Seymour et al. 2003, Angioy et al. 2004).

The initial activation, orientation, and landing behaviors of necrophilous insects rely heavily on odor cues (Spivak et al. 1991, Ashworth and Wall 1994, Brodie et al. 2014). As a body decomposes, microbes proliferate and metabolically produce dozens of volatile organic compounds (VOCs) (Vass et al. 2008, Davis et al. 2013, Crippen and Singh 2015). These compounds, particularly nitrogen-containing compounds (e.g., indole), short chain alcohols (e.g., nonanol), acids (e.g., butanoic acid), and sulfur-rich compounds (e.g., dimethyl trisulfide), are attractive to necrophilous insects, including both early responding flies and later arriving beetles (Stensmyr et al. 2002, Urech et al. 2004, Kalinova et al. 2009, Von Hoermann et al. 2011, Paczkowski et al. 2012, Brodie et al. 2014). Several of these VOCs have also been used to develop synthetic odor blends for blow fly monitoring and trapping purposes (Ashworth and Wall 1994, Urech et al. 2004, Aak and Knudsen 2011). Carrion-mimicking plants like the dead horse arum exploit this reliance on host odors in necrophilous insects by producing sulfur-rich volatiles that attract necrophilous insect pollinators (Kite 2000, Stensmyr et al. 2002). Such plants are often thermogenic, where heat facilitates odor emission and thermotactic orientation by pollinators, and they include visual cues which guide insects to the flowers (Meeuse and Raskin 1988, Seymour et al. 2003, Angioy et al. 2004).

Similarly, we developed a way to thermally convect natural decomposition odors to attract necrophilous insects and guide them to a sticky trap target. The vented-chamber design takes advantage of the decomposition process to thermally convect natural pig decomposition odors through a chimney system leading to a pair of sticky traps. This passive trap design maximized trap catch with minimal active sampling by researchers. We experimentally assessed the effects of trap design features on trap catch. Although we used a forensic model in this research, this trap is generally useful for studies examining ecological succession, host-finding cues, necrophilous insect behavior, or dipteran pest monitoring.

Materials and Methods

Site and Pig Placement

All experiments were performed at North Carolina State University's Lake Wheeler Road Field Lab in Raleigh, NC (35.728713, −78.666719) during June–August 2015 and June–July 2016.

Thirty-seven fully frozen stillborn pigs (*Sus scrofa domesticus*), each weighing roughly 1.5 kg, were acquired from the University's Swine Educational Unit and remained in a freezer until placement in the field for all experiments. The vented-chamber method required that the pig be temporarily placed within a chamber during sampling for necrophilous insects. To facilitate this, each pig was placed on a layer of soil on a standard 35 × 46 cm cafeteria tray. Ten minutes before pigs were placed in the field, soil from the site was piled atop the tray to a height of ~4 cm. A pig was placed on the soil-covered tray on the ground. This arrangement allowed us to move the decomposing pigs into the chamber for sampling and ensured that there was still a soil/body interface for maggot and beetle activity (Carter et al. 2007, Forbes and Dadour 2009).

Trap Design

The design of the vented-chamber trap was inspired by preliminary field observations, necrophilous insect host-finding mechanisms, and the pollination strategies of carrion-mimicking thermogenic flowers. The basic design, which served as our positive control, consisted of a watertight 41.2 quart (39 liters, 59.7 × 45 × 20.3 cm [length by width by height]) plastic tub (#10054386, The Container Store, Coppell, TX) with locking airtight lid and several PVC adapters (Fig. 1). A stillborn pig placed on a 4 cm layer of soil on a cafeteria tray was placed in the chamber, and natural pig decomposition odors served to attract adult flies and beetles to the trap. The chamber had three openings: one at the center of each of the small (45 cm) sides of the tub, which allowed ambient air to enter the chamber, and one offset on one side of the lid, which led to a PVC 'chimney' (Fig. 1). Within each of these holes was a 5 cm internal diameter PVC coupling fitting that allowed for different lengths of PVC to be inserted at each port for easy, non-permanent experimental manipulation. The PVC chimney focused the odors through a small opening, guiding arriving insects to the sticky traps. In the basic design, as used in Cruise et al. (2018a), the side opening near the pig's head was capped, forcing ambient air to enter the chamber through the opposite side and vent the headspace through the chimney. All experiments were conducted with back-to-back unscented sticky traps (Super Catchmaster, AP&G Co, Inc., Bayonne, NJ) attached with a binder clip and inserted into slits made on the chimney coupling, one facing west and the other east.

Sampling Protocol and Experimental Design

Before sampling, a pig on a cafeteria tray was placed into the tub with the pig's head orientated toward the capped end (Fig. 1). The lid was then secured in place, ensuring that the PVC chimney was closest to the capped side of the tub. The vented-chamber was allowed to passively sample arriving insects for 15 min. The traps were then removed from the chamber, covered with plastic cling wrap, and stored at −80°C until insect identification. The pig was removed from the chamber and placed on the ground for 15 min to minimize disruption of its natural colonization and also prevent excessive heating within the chamber which might stress the maggots (Rivers et al. 2010). For all experiments, sampling occurred between noon and 1800 hours, the optimal time for maximizing abundance and diversity of necrophilous insects (Cruise et al. 2018b).

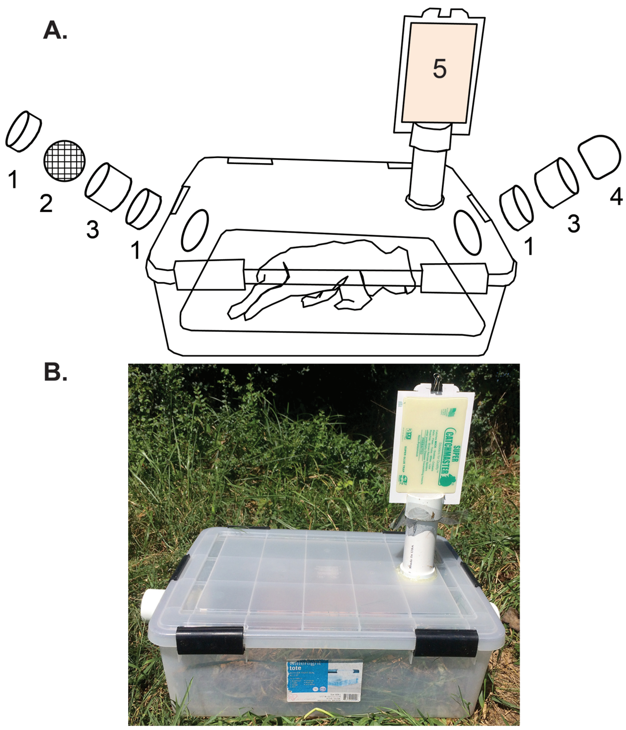

Fig. 1. The vented-chamber trap. (A) Schematic drawing with the left side of the chamber containing a mesh-covered opening, whereas the right side was capped. A pig was placed on a cafeteria tray within the chamber as illustrated, with its head closest to the capped side and chimney. (1) 5-cm PVC coupling, (2) 10 × 10 cm square of 17 × 13 mesh vinyl-coated polyester window screening, (3) 5-cm-diameter PVC coupling, (4) 5-cm-diameter PVC end cap, and (5) back-to-back unscented glue traps held together with a binder clip. (B) The assembled vented-chamber trap that served as the positive control for most experiments.

Experiment 1: Visual Versus Olfactory Cues

The sticky traps on the vented-chamber might serve as visual cues, and insects alighting on the traps might contribute to trap catch. Three frozen pigs were placed 25 m apart at the field edge in partial shade and allowed to decompose for 24 h. Each pig was then exposed to two treatments in a randomized order: a vented-chamber as positive control and a no-chamber experimental treatment (Fig. 2). For the latter, a metal ring stand adjacent to the pig held a PVC chimney with two back-to-back sticky traps. The chimney was set at the same height and position over the pig as in the vented-chamber control. Each of the two treatments was assayed for 15 min, with a 15-min rest period on the ground in between, during which the chamber and ring stand were removed. The three pigs were simultaneously sampled three times daily for two consecutive days. This experiment was performed in July 2015 and repeated with two additional pigs.

Experiment 2: Trap Size

Odors from the decomposing pig were directed toward either two back-to-back glue traps or an array of 12 back-to-back traps that formed a larger target (Fig. 3A schematic). The large trap consisted of two 41 × 41 cm white poster boards held together with binder clips, each covered with six glue traps. This large trap was supported between two 1-m-tall rebar rods and oriented North-South so traps faced East and West. A frozen stillborn pig was placed atop a soil-covered cafeteria tray and allowed to decompose for 48 h. The 2-d decomposing pig was then sampled for 15 min with the two-trap method, removed from the chamber to aerate on the ground for 15 min, and sampled again for 15 min with the 12-trap array. This experiment was repeated with four additional pigs in June 2015, for a total of five replicates, and the order in which the two trap types were performed was randomized across replicates. The overall numbers of necrophilous insects were compared, and their spatial distributions on the array of the 12 traps were assessed.

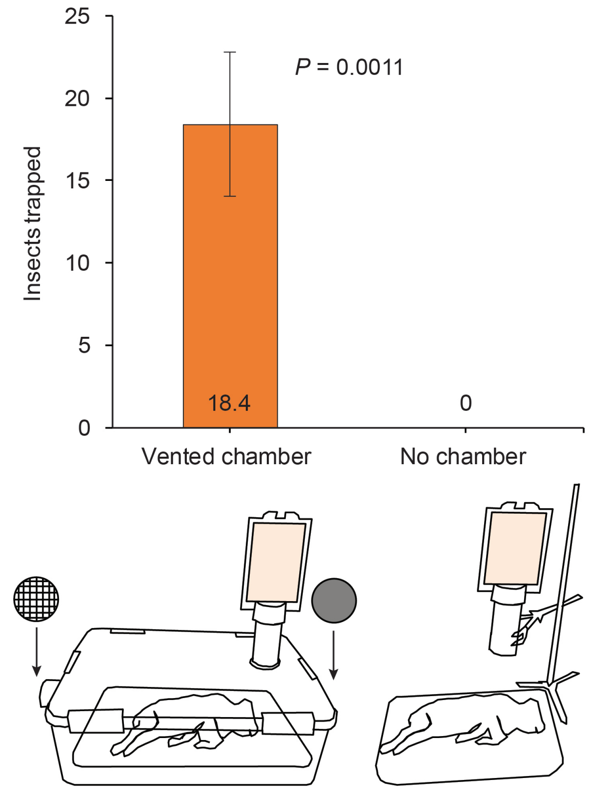

Fig. 2. Sticky traps do not constitute a visual cue for orientation or alightment of necrophilous insects. Mean number of insects (±SEM) trapped in the vented-chamber (shown within the bar) was significantly higher than in the no-chamber treatment, which failed to trap any insects (paired *t*-test: $T = 4.211$, df = 9, $P = 0.0011$).

Experiment 3: Number of Chamber Openings

In this air flow experiment, we altered the number of side openings on the chamber. The use of PVC couplings and caps on the vented-chamber allowed for easy, non-permanent manipulation of air flow. Two fully frozen pigs were placed 25 m apart in a partially shaded location at the field edge in June 2015. Pigs were sampled for 15 min with each of three randomized treatments: one side open, both sides open, and both sides closed. All sampling began 24–48 h after pig placement in the field, and each pig received each treatment one time. This experiment was replicated with four additional pigs in July 2015.

Experiment 4: Chimney Orientation

In preliminary observations, we noticed that traps placed on a vertical chimney atop the chamber caught more insects than traps attached to a horizontal chimney on the chamber's side. Eight pigs were used in this experiment, with each pig exposed twice to each of four treatments. The design maintained the position of the trap either above the chamber or at its side, while varying the origin of the chimney between a position above the side vent hole or in line with it. These arrangements were expected to drive different thermal

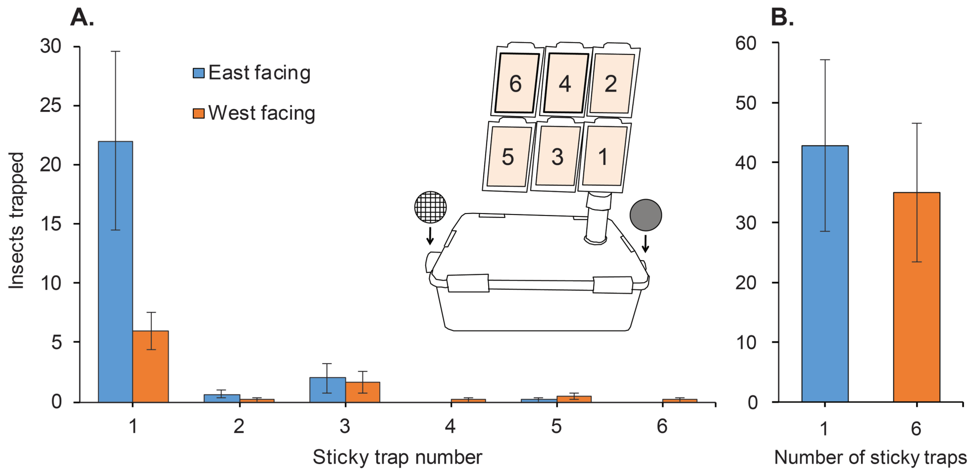

Fig. 3. Effect of trap size and orientation on mean number of insects (±SEM) trapped. The schematic illustrates six traps that were glued to each of two 41 × 41 cm pieces of poster board and attached by a binder clip to an equal sized piece of corrugated plastic. Each trap array included six East-facing traps and six West-facing traps. The schematic depicts the East-facing array. (A) Significantly more insects were trapped on the downwind East-facing side of the six-trap array (two-sided paired *t*-test, $T = 2.44$, df = 5, $P = 0.03$) and on trap #1 in the position just above the chimney (one-way ANOVA followed by Tukey's HSD test; East-facing: $F_{5,30} = 7.99$, $P < 0.001$; West-facing: $F_{5,30} = 9.45$, $P < 0.001$). (B) Comparison of a single trap (positive control) and the six trap array, combining insects trapped on both East- and West-facing traps (two-sided paired *t*-test, $T = 2.306$, df = 8, $P = 0.6830$).

convection forces within the vented-chamber. Treatment order was randomized for each pig, and sampling was performed for 15 min with a 15-min rest period on the ground between sampling periods. Pigs were spaced 25 m apart in partial shade in July 2015. Sampling began 48 h after pig placement in the field.

Experiment 5: Passive Versus Forced Venting of the Headspace

A 9.2 × 9.2 × 2.5 cm computer fan (Effizio, SilenX, Santa Fe Springs, CA) with an air flow of 42 ft^3/min (71 m^3/h) was used because of its low noise (15 dBA) and portability (12 VDC power source). The fan was attached to the 8 cm end of an 8-to-5 cm piece of PVC coupling with epoxy putty. This coupling was then joined to the 5 cm piece of PVC on the open mesh end of the chamber. Four frozen pigs were placed 25 m apart on soil covered cafeteria trays in the field in August 2015 and allowed to decompose for 48 h. The pigs were then sampled for 15 min with each of the four chimney orientations with the fan-modified vented-chamber. Chimney-trap orientations were randomized, and each orientation was repeated twice with each pig. As in all other experiments, the pigs on cafeteria trays were placed on the ground outside the vented-chamber for 15 min between successive 15 min sampling periods. This experiment was repeated with four additional pigs.

Experiment 6: Vented-Chamber Versus Sweep Net

Two frozen pigs were placed 50 m apart at the field edge in partial shade and allowed to decompose for 48 h. Two sampling treatments were performed twice on each pig: sweep net and the standard vented-chamber trap. As before, successive 15-min sampling periods were separated by a 15-min rest period with the pig placed on the ground. The order of the two treatments was randomized, and sampling was repeated the following day. This experiment was repeated once more with two pigs and a final time with a single pig in June and July 2016. Insects trapped by the sweep net were killed and stored in 70% ethanol.

Identifications

The plastic wrap was removed from sticky traps while the adhesive was still frozen. Traps were then thawed for 10–15 min, and insects were identified to the family or species-level in situ, without removing them from the traps. All traps were returned to the freezer for storage after identifications were complete. Insects trapped by the sweep net were removed from ethanol, allowed to dry, and identified. Insects were first classified as to whether or not they were forensically relevant. Forensically relevant insects are known to feed or breed on carrion and are useful to forensic investigations and PMI determination; literature documenting local fauna of interest was also consulted (Byrd and Castner 2000, Cammack et al. 2016). Forensically relevant insect taxa collected in these experiments were documented. For experiments 1–5, insects were counted without further classification. For experiment 6, insects were classified to family or species. Calliphorids were identified to species using Whitworth's taxonomic key (Whitworth 2006).

Analyses

Two-tailed paired *t*-tests were used to assess the effect of visual versus olfactory cues (experiment 1), insect trap size (experiment 2), and comparison of the vented-chamber versus sweep net (experiment 6) on the number of insects trapped ($\alpha = 0.05$). Differences in insect numbers trapped in each section of the large trap (experiment 2) were analyzed with one-way ANOVA followed by Tukey's honest significant difference (HSD) test (SAS 2012). To assess the effect of the number of openings on the number of insects trapped (experiment 3), a generalized linear model (GLM) was fit on insect count, with a Poisson error distribution and a logarithmic link function, using the statistical procedure GLIMMIX (SAS 2012, Zabala et al. 2014). All pigs were subjected to each experimental condition ($n = 6$ per experimental condition) and considered a random effect factor. Pairwise least squares means comparisons, with a Tukey's Kramer adjustment to control for type I error (reject null hypothesis of no effect when null hypothesis is true), were used to assess significant differences in the number of insects trapped by experimental condition ($\alpha = 0.05$) (Brodie et al. 2015).

Examination of the effects of trap orientation both with (experiment 5) and without (experiment 4) the addition of a fan was performed much like the analysis described for the number of sides open, except that both pig and a pig * orientation interaction were included as random effects in both analyses to better account for outliers present in these datasets. Means are reported ± SEM for all experiments.

Results and Discussion

The Vented-Chamber

We designed the vented-chamber (Fig. 1) in response to consistently small samples collected from neonate pig carcasses. The vented-chamber consisted of an airtight chamber into which a decomposing pig was placed during sampling times. Ports on the sides of the chamber allowed for manipulation of air flow, and a chimney at the top of the chamber opened to a set of sticky traps for insect collection. This design significantly increased the number of necrophilous insects trapped by directing the odors from the decomposing pig to a set of sticky traps.

Visual Versus Olfactory Cues

White-colored traps, like those used in the vented-chamber, are typically neither attractive nor repellent to various types of blow flies, but may increase fly alightment on the trap surface (Burg and Axtell 1984, Lee et al. 2013, Brodie et al. 2014). In experiment 1, insects did not use the sticky traps as visual cues and were adept at orienting to the decomposing pig. Whereas the standard vented-chamber trap captured 18.4 ± 4.37 insects, the no-chamber sticky trap did not trap any insects (Fig. 2; paired *t*-test: $T = 4.211$, df = 9, $P = 0.0011$). These results underscore the importance of the chamber and chimney in directing decomposition odors toward the trap.

Wall and Fisher (2001) also found higher trap catches when a white target was coupled with odor, but the white target itself was not attractive to flies. Other studies seeking to optimize traps for blow flies of veterinary significance have shown that certain colors like yellow, when added to an odor baited sticky trap, may increase trap catch (Lee 1937, Fukushi 1989, Wall et al. 1992, Wall and Smith 1996, Brodie et al. 2014). Interestingly, blow flies approaching an odor source responded to vertically-oriented visual cues (contrast) to initiate a landing response, but not to horizontally-oriented cues (Aak and Knudsen 2011). Further experiments altering trap color of the vented-chamber could help to better describe the roles of olfactory and visual cues at close range. For example, painting a black target on the vertically-mounted sticky trap may increase fly landing efficiency. Our focus, however, was to assess this method for olfaction-based ecological succession studies, and thus keeping the traps as neutral as possible was a priority.

Trap Size

On the large trap, significantly more insects were trapped on the sticky trap positioned just above the chimney than on any other trap position (East-facing: $F_{5,30} = 7.99$, $P < 0.001$; West-facing: $F_{5,30} = 9.45$, $P < 0.001$; Fig. 3A). In total, 85.1% of the insects were trapped directly above the PVC chimney (trap #1), indicating that insects used mainly olfactory cues and that the traps were rarely used as visual cues or resting sites. Significantly greater trap catch on the East-facing traps of the large trap (paired *t*-test; $T = 2.44$, df = 5, $P = 0.03$) was consistent with the predominance of winds from the West-Southwest during these assays in both 2015 and 2016 (Fig. 4). Olfactory navigation in flying insects (positive anemotaxis) involves upwind navigation, so the East-facing traps would be encountered first by newly arriving insects (Eisemann 1988).

In experiment 2, trap size did not affect the number of insects trapped. Although the square area of the large trap was six times larger than the standard small trap, both trapped similar mean numbers of insects (large trap: 35.0 ± 1.29 insects; standard trap: 42.8 ± 14.31 insects; paired *t*-test: $T = 2.306$, df = 4, $P = 0.6830$) (Fig. 3B). Moreover, the grouping of insects at the vicinity of the chimney opening was a clear indication of the importance of odors in attraction, as well as the success of the chimney itself as a way of directing necrophilous insects to a particular location for trapping. Thus, from a practical perspective, adding surface area with more sticky traps is unwarranted given the additional cost of the traps and effort to secure them.

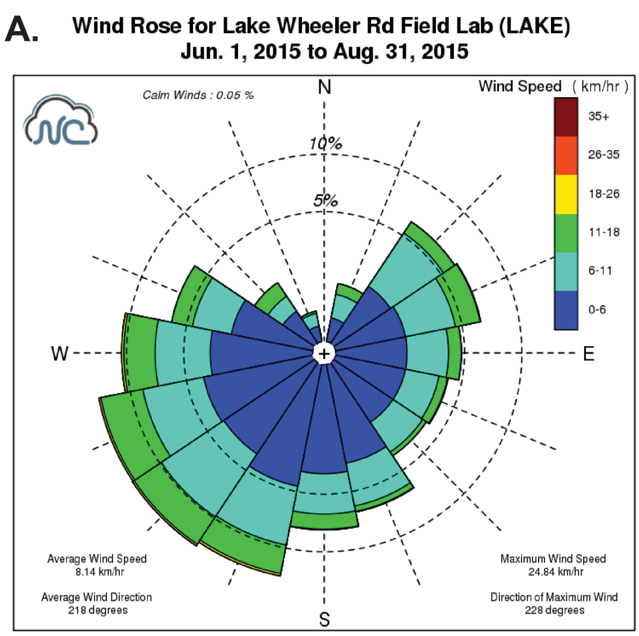

Fig. 4. Wind roses for (left) experiments 1–5 (June–August 2015) and (right) experiment 6 (June–July 2016). The predominant wind direction was from the West-Southwest. All data from the State Climate Office of North Carolina Lake Wheeler Rd. Field Station (http://climate.ncsu.edu/windrose.php?state=NC&station=LAKE).

Number of Chamber Openings

In experiment 3, presence of at least one opening to the outside air significantly increased the number of insects trapped by the vented-chamber (glmm: $F_{2,10} = 12.44$, $P = 0.0019$). Chambers with one or two openings trapped significantly more insects (22.9 ± 11.39 and 23.1 ± 11.49 insects, respectively) than chambers with both sides closed (14.1 ± 7.07 insects) (Tukey's HSD test, $P < 0.05$). These results indicate that decomposition odors diffuse through the chimney even when there is no air exchange within the chamber. However, the addition of a side opening in the chamber allowed outside air to enter the chamber and facilitated thermal convection of the decomposition odors.

Chimney Orientation

As predicted from our preliminary observations, chimney orientation in experiment 4 significantly affected the number of insects trapped (glmm: $F_{3,21} = 6.72$, $P = 0.0024$; Fig. 5A). Significantly more insects were trapped when odors emanated from the top openings (top = 16.8 ± 5.12 insects; top angled to the side = 16.5 ± 5.06 insects) than when odors emanated from the side (side = 5.8 ± 1.86 insects; side angled to top = 6.1 ± 2.09 insects).

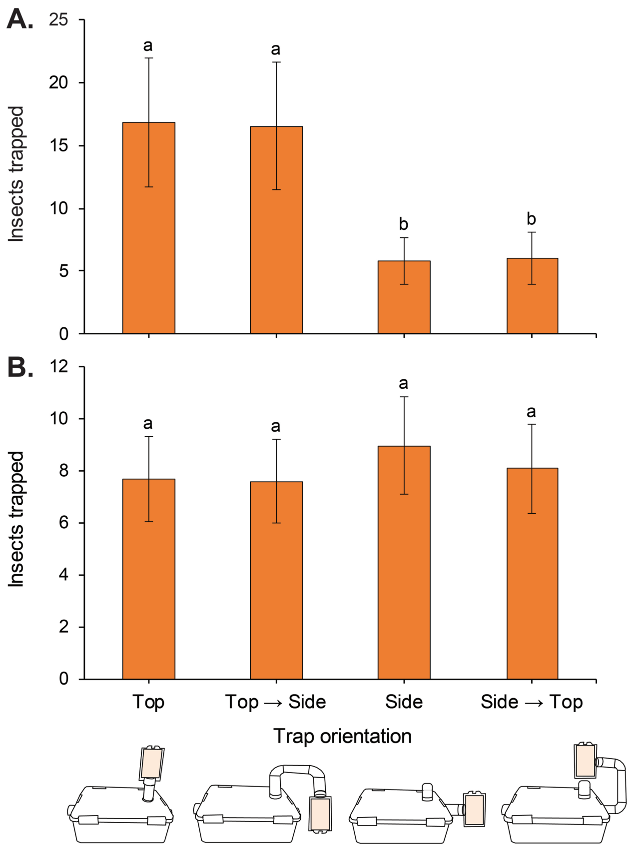

Fig. 5. Effect of chimney and trap orientation on the number of insects trapped by the vented-chamber. For all treatments, the port on the left side of the chamber was open and covered with window screening. Closed ports were capped with 5 cm PVC caps. The leftmost treatment ('top') served as the positive control. (A) Passive traps without fans. (B) A computer fan powered by a 12V battery pushed ambient air through the port on the left side of the chamber, which was covered with window screening. Bars show mean values ± SEM, and bars labeled with the same letter are not significantly different within experiment (glmm, A: $F_{3,21} = 6.72$, $P = 0.0024$, Tukey's HSD test $P < 0.05$; B: $F_{3,21} = 0.23$, $P = 0.8717$; Tukey's HSD test, $P < 0.05$).

These results are likely attributed to decomposition VOCs thermally convecting from the pig. Heat is released during the decomposition process, and it is expected to be vented through a chimney, especially when cooler fresh air is allowed to displace the escaping air (Janaway et al. 2009). Heat serves not only to transport VOCs, but heat itself is a known cue for blow flies at close range, contributing to its effect in the vented-chamber trap (Angioy et al. 2004).

Passive Versus Forced Venting of the Headspace

Experiment 5 investigated whether the addition of a fan would overcome the deficiencies of some treatments in experiment 4 by annulling the effects of differential passive thermal convection. Indeed, the forced discharge of the headspace with a fan equalized the insect counts from all chimney orientations (glmm: $F_{3,21} = 0.23$, $P = 0.8717$; Fig. 5B). However, the fan added cost and maintenance but did not significantly increase trap catches relative to our standard design. These results support our evidence that the decomposition odors play a pivotal function in host location by necrophilous insects, and the location of the access to the body (i.e., sticky trap position) is of minor significance as long as odors efficiently emanate from that position.

Vented-Chamber Versus Sweep Net

In paired comparisons of the passive vented-chamber and active sweep net approaches (experiment 6), the former method captured significantly more insects than the sweep net (34.1 ± 9.74 insects vs. 7.1 ± 1.79 insects; paired *t*-test, $T = 3.237$, df = 23, $P = 0.0018$) (Fig. 6). Both methods sampled seven common taxa, including most blow flies, flesh flies, and the house fly (Table 1). Although flesh flies (Sarcophagidae) were trapped in larger abundance as well as in greater percentage of sweep net samples, all other flies were trapped in larger numbers and in more samples in the vented-chamber method. Only the vented-chamber method trapped the blow fly *Lucilia cuprina* Meigen (Diptera: Calliphoridae), and only the sweep net method trapped a single histerid beetle.

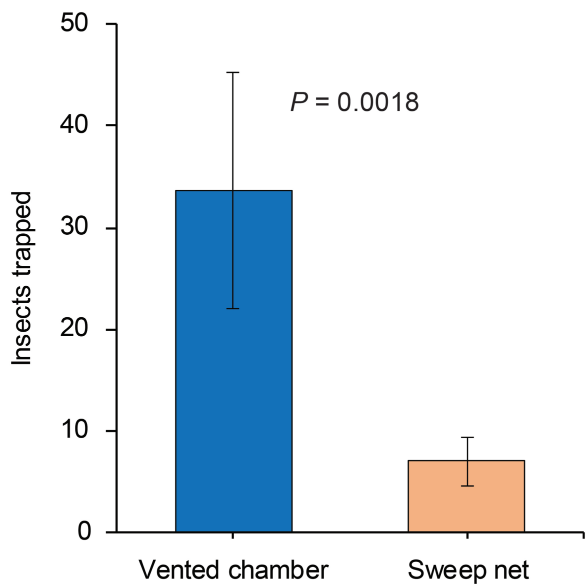

Fig. 6. Comparison of insects trapped with the vented-chamber trap and sweep net (paired *t*-test, $T = 3.237$, df = 23, $P = 0.0018$).

Table 1. Forensically significant insects sampled by the vented-chamber and sweep net methods

Order	Family	Genus and species	Vented chamber trap				Sweep net			
			Number trapped (mean ± SEM)	% positive[a] (n = 24)	Min, Max[b]	Total	Number trapped (mean ± SEM)	% positive[a] (n = 24)	Min, Max[b]	Total
Diptera	Calliphoridae	*Lucilia illustris* (Meigen 1826)	4.92 ± 1.17	62.5	1, 17	119	0.71 ± 0.18	50.0	1, 3	17
		Lucilia coeruleiviridis (Macquart 1855)	2.88 ± 0.99	50.0	1, 20	69	0.92 ± 0.32	41.7	1. 6	22
		Lucilia sericata (Meigen 1826)	1.46 ± 0.43	45.8	1, 6	35	0.29 ± 0.13	20.8	1, 2	7
		Lucilia cuprina (Wiedemann 1830)	0.17 ± 0.10	12.5	1, 2	4	0	0	0, 0	0
		Phormia regina (Meigen 1826)	22.29 ± 8.09	87.5	1, 159	535	3.50 ± 1.13	66.7	1, 22	84
		Cochliomyia macellaria (Fabricius 1775)	0.71 ± 0.32	29.2	1, 6	17	0.21 ± 0.10	16.7	1, 2	5
	Sarcophagidae	Sarcophagidae spp.	0.38 ± 0.17	20.8	1, 2	17	0.96 ± 0.28	54.2	1, 6	23
	Muscidae	*Musca domestica* (Linnaeus 1758)	0.88 ± 0.38	37.5	1, 8	21	0.42 ± 0.19	25.0	1, 4	10
Coleoptera	Histeridae	Histeridae spp.	0	0	0, 0	0	0.04 ± 0.04	4.2	1, 1	1

If no species is listed, taxonomic identifications ended at the family level.

[a]% positive represents the percentage of traps that caught at least one insect of the specified taxa.

[b]Range of trap catch (minimum, maximum) from traps with at least one insect.

Advantages and Disadvantages of the Vented-Chamber

An ideal sampling method should be easy to implement, unbiased, and cost-effective, while allowing the researcher to maximize sampled diversity and overall numbers of organisms. We systematically modified the vented-chamber to maximize trap catch. Results of all design modifications indicated that the prototype design was the most effective for trapping the largest number of insects. These modifications, however, were useful to understand which features of the trap design contributed most to its efficacy.

The vented-chamber is an effective method for trapping a relatively large number of necrophilous insects, particularly flies, as they arrive to the carcass. Its advantages are low cost, portability, and the ability to trap large and diverse insect communities. Active sampling requires a 1:1 ratio of researcher to sampling target with constant hands-on input. Conversely, multiple passive traps can be deployed in the field, enabling concurrent sampling from multiple carcasses, with minimal input from the researcher. The vented-chamber trap also eliminates sampler bias, which is almost unavoidable when active sampling methods are used in field research. It significantly increased trap catch over previous work with sticky traps alone, and our results demonstrated that without the chamber, the sticky traps were completely ineffective. Thus, a key feature of this chamber that contributes to its effectiveness is the directed venting of decomposition odors onto a sticky trap.

Nevertheless, a major shortcoming of the vented-chamber trap is that it, like most sampling methods, failed to trap necrophilous beetles. We frequently observed that necrophilous beetles flew at low altitude to an area near an exposed pig and then walked to the resource; some beetles hovered just centimeters above the carcass and then dropped onto it. This would explain why beetles were not found on the elevated traps. A modification of the vented-chamber could place the sticky traps in contact with the ground, possibly in combination with a fan to broadcast the decomposition odors close to the ground. It is possible that even these modifications may not trap beetles because they might be deterred from stepping onto the sticky surface. We postulate, however, that this modified, fan-assisted vented-chamber approach could focus beetles into a small area on the ground either above a pitfall trap or from which they could be hand collected. This is an acceptable approach, as a combination of active and passive methods has been shown to most effectively sample necrophilous beetle diversity (Zanetti et al. 2016, Cruise et al. 2018b). In forensic entomology practice and succession research, the sweep net is almost always coupled with hand-collections (Anderson and VanLaerhoven 1996, Haskell et al. 2000, Carvalho et al. 2004, Amendt et al. 2007, Byrd et al. 2010, Benbow et al. 2013). The drawback of incorporating hand-collections is that it reintroduces sampler bias into the system, although only for beetle sampling; fly sampling remains unbiased with the vented-chamber.

An existing passive trap that samples both flies and beetles is Schoenly's Demographic Bait Trap (Schoenly 1981). This trap features plywood sides in a dodecahedral arrangement surrounding a small mammal (rat, mouse, etc.) bait. Integrated pitfall traps, emigrating funnels, and a canopy leading to an elevated central collection chamber are additional features of the trap. A tripod and halyard are positioned above the trap to assist with trap lifting during sample collection. As in our vented-chamber, the soil/body interface is preserved (Schoenly 1981). This trap, with some modifications, was demonstrated to be more efficient than short traditional collections consisting of a sweep net and hand collection (Ordonez et al. 2008). While this trap may be a good option for some researchers, we found it impractical for our field studies due to its complex construction. Because we timed our field studies during periods of little precipitation, the clay soil at our field site was very dry and firm, limiting our ability to integrate belowground pitfall traps. Previous attempts to dig pitfall traps were unsuccessful and/or time-consuming, so the vented-chamber was designed without such passive beetle sampling. Additionally, the design and materials used to construct the Schoenly trap would have limited our ability to quickly and inexpensively create multiple traps for concurrent replications. e Castro et al. (2009) discussed the complexity of building one of Schoenly's traps and proposed their own modifications, including removal of the tripod and an increase in size for piglet carcasses; these modifications did not make the trap more suitable for our field studies; however, because the same issues with size, weight, and pitfall trap integration persisted. The modified trap also remained difficult to move, which presented problems for using this method in future studies in different parts of the field site. Perhaps most significantly, however, was the extended sampling interval of the Schoenly trap. We wanted sampling to be as short as possible to avoid oversampling the carcass and potentially interfering with the decomposition process or on-carcass insect interactions.

Our findings have implications for forensic entomology, as well as other investigations seeking to elucidate necrophilous insect host-finding cues or maximize necrophilous fly trap catches for generating inventories, monitoring or control purposes (e.g., neuroethology, veterinary entomology). First, by incorporating knowledge about odor, thermal, and visual cues used by necrophilous insects in navigation to carcasses, we developed a passive trap that maximized trap catches from small decomposing bodies. This simple, inexpensive design was a substantial improvement over the open frame sticky trap design used in our previous work (Cruise et al. 2018b). Additionally, and perhaps more importantly, the vented-chamber trapped significantly more insects than the sweep net. This novel sampling method could be readily adapted by forensic investigators to rapidly establish reference collections of local fauna using neonate pigs or other carcasses. There are obvious constraints scaling this method to larger bodies. We suspect, however, that solid (non-porous) tent-like devices with open bottoms, similar to insect emergence traps, could be used instead of chambers, with the collection jar of the emergence trap replaced by sticky traps. A porous (mesh construction) emergence trap failed to trap insects (Cruise et al. 2018b), likely because odors diffused in all directions. Therefore, a non-porous trap would be needed to focus the odors toward the sticky trap. Notably, larger collections of necrophilous insects throughout the carcasses' decomposition will provide greater resolution of the differential attraction of males, unmated females, and mated females to the carcass.

A second implication is the simple observation that more necrophilous insects are attracted to an odor source when it is passively or actively vented. Thus, a decomposing corpse within a 'chamber' with only one opening (e.g., a bag) may emit substantially less decomposition odors (and attract fewer insects) than when at least two openings are present (e.g., rolled up carpet). The latter would allow for faster air exchange and odor emission due to convective processes.

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