Journal of Medical Entomology, 57(1), 2020, 164–172
doi: 10.1093/jme/tjz164
Advance Access Publication Date: 27 September 2019
Research

Effects of Carrion Relocation on the Succession of Newly Arriving Adult Necrophilous Insects

Angela Cruise,[1,2] Madhavi L. Kakumanu,[1] David W. Watson,[1] and Coby Schal[1,3,]

[1]Department of Entomology and Plant Pathology, North Carolina State University, Campus Box 7613, Raleigh, NC 27695-7613, [2]Present address: Cruise Consulting Group, LLC, Burlington, NC, and [3]Corresponding author, e-mail: coby@ncsu.edu

Subject Editor: Christopher Geden

Received 30 May 2019; Editorial decision 21 August 2019

Abstract

Ecological succession of necrophilous insects follows a predictable sequence, related to their differential attraction to changing odor profiles associated with carrion and colonizing insects. However, the dependency of insect arrival on the duration of the carrion's residency at a location has not been investigated. To assess the fidelity of necrophilous insects to carrion of specific decomposition ages, independent of its location, we monitored the decomposition of neonate pigs in one field and then simultaneously relocated carcasses of different decomposition ages to an ecologically similar but remote field. We examined the effects of decomposition age and relocation on the assembly of the necrophilous insect community, using a novel vented-chamber trap, which excluded all sensory cues except odors. Community composition differed over a 4-d decomposition period, showing that insects were differentially attracted to pigs of different decomposition ages. There was overall concordance between respective decomposition ages in the two fields, with similar relative abundances of taxa before and after transfer. Although different decomposition ages continued to attract different insects, differentiation of the necrophilous insect communities relative to the age of decomposition was less pronounced after transfer. The results of this study demonstrate that translocating a decomposing body to a new, but geographically and ecologically similar location continues the predicted insect succession, albeit with greater variance, based on olfactory cues alone. Several rare taxa were sampled only prior to relocation, including the first documentation of the invasive hairy maggot blow fly, *Chrysomya rufifacies* (Macquart) (Diptera: Calliphoridae), in central North Carolina.

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

After death, a series of physical and chemical changes occur within the body as it decomposes. Decomposition begins within minutes of death, as the cessation of essential metabolic functions triggers cellular changes (Clark et al. 1996, Carter et al. 2007, Statheropoulos et al. 2007). Decomposition first begins with autolysis, a period of cellular self-digestion. While the depletion of oxygen after death destroys cells, anaerobic microbes from the gut and respiratory system thrive in these conditions (Carter et al. 2007). The nutrient-rich cell contents released during autolysis fuel the process of putrefaction, the decomposition of tissue by microorganisms like bacteria, protozoans, and fungi (Vass et al. 2002).

Aiding and accelerating the process of decomposition are necrophilous insects primarily of the orders Diptera and Coleoptera (Haskell et al. 1996, Byrd and Castner 2000, Campobasso et al. 2001), without whose feeding activity the decomposition rate is significantly slowed (Simmons et al. 2010). Necrophilous insect taxa arrive to a decomposing body in a predictable sequence (Payne 1965, Goff 1993, Anderson and VanLaerhoven 1996, Sharanowski et al. 2008, Merritt and De Jong 2015). Several sensory cues contribute to the predictability of insect succession, but primary among these are visual stimuli and volatile organic compounds (VOCs) that are by-products of decomposition (Stensmyr et al. 2002, Urech et al. 2004, Kalinová et al. 2009, Von Hoermann et al. 2011, Paczkowski et al. 2012, Brodie et al. 2014). Nearly 80 compounds have been identified in headspace analysis of decomposing pig models (Dekeirsschieter et al. 2009). Phenolic molecules (skatole, indole), sulfur- and nitrogen-containing gases (dimethyl di- and trisulfides, hydrogen sulfide, ammonia), aliphatic and aromatic hydrocarbons (methane, toluene), esters (butanoic butyl ester, butanoic ethyl ester), ketones (2-nonanone, 2-butanone), alcohols (ethanol, butanol), amino acids (alanine, proline, methionine, GABA), and small carboxylic acids (oxalic acid, propionic acid) are just some of the products produced as carbohydrates, lipids, proteins, and nucleic acids are catabolized in the decomposition process (Gill-King 1996; Vass et al. 2002, 2004; Statheropoulos et al. 2005, 2007; Carter et al. 2007; Dekeirsschieter et al. 2009). Tissues decompose at different rates, are composed of organic macromolecules in different

ratios, and are colonized by different microbial assemblages, so the VOCs that emanate from a body vary over time (Gill-King 1996, Hoffman et al. 2009, Hyde et al. 2015).

Several studies have identified decomposition VOCs that serve as attractant cues for necrophilous insects. Some compounds, such as dimethyl trisulfide, that co-occur in decomposing cadavers (Vass et al. 2004, Statheropoulos et al. 2007, Frederickx et al. 2012a) and in carrion-mimicking plants like the dead horse arum (Stensmyr et al. 2002), attract *Calliphora vicina* Robineau-Desvoidy (Diptera: Calliphoridae), *Lucilia caesar* L. (Diptera: Calliphoridae), *Lucilia sericata* Meigen (Diptera: Calliphoridae), and other calliphorids and even coleopterans, and are considered to be important cues in colonization (of cadavers) and pollination (carrion-mimicking plants) (Stensmyr et al. 2002, Kalinová et al. 2009).

Many factors are known to influence, delay, or alter the expected successional pattern of insects. Among them are geography (Hwang and Turner 2005, Battán Horenstein et al. 2010, Tomberlin et al. 2011a, Pechal and Benbow 2015), accessibility/concealment (Haskell et al. 1996, Campobasso et al. 2001, Pechal et al. 2014, Charabidze et al. 2015), seasonality (de Carvalho and Linhares 2001, Archer 2004, Sharanowski et al. 2008), interspecific competition (Brundage et al. 2014), carrion type (Watson and Carlton 2003, 2005; Zeariya et al. 2015), and level of sun exposure (Sharanowski et al. 2008, e Castro et al. 2011). The time course of the differential responses of necrophilous insects can alter the successional pattern, yet it is poorly characterized. A central question is: How is succession affected when a body undergoes decomposition in one place and is then transported to another location? In one scenario, insects would respond to specific compounds and blends of compounds that correspond to their preferred decomposition stage. Under this model, insects arrive sequentially and respond to a set of compounds independently of what transpired before these compounds were produced. Thus, the successional pattern would be expected to continue unchanged when the body is translocated to a new, ecologically similar site because new responders of the same taxa would be recruited to the carcass. Alternatively, it is possible that persistence of the carcass in one location might serve as a 'staging' arena even for late arrivers which might assemble in the area and be primed in the vicinity of the resource in 'anticipation' of the proper olfactory cues. If the carcass is relocated to a new site, the assembly of the successional colonizers would then need to start anew.

We sought to elucidate the mechanisms contributing to the predictable succession of necrophilous insects visiting carrion by examining the effect of carrion relocation on insect succession, while excluding all sensory cues related to the body except odors. Our approach was to characterize ecological succession on decomposing neonate pigs in one location and then simultaneously move them to a new, ecologically similar location. By using pigs at four different ages within the decomposition process, we were able to assess the differential attraction and community organization of insects to each decomposition age in two locations—where succession commenced in the first location, and changes in succession as it continued in the second location. It is important to note that this approach is substantially different from large-scale postmortem transportation of decomposing bodies, such as from an urban to rural area or across geographic regions, where locality-specific differences in insect fauna may assist in criminal cases (Haskell et al. 1996), although distribution inconsistencies are well-documented (Charabidze et al. 2017). Instead, in this study, we assessed post-relocation community assembly within the same microgeographic area, where the same necrophilous taxa are expected. Key to this approach was to exclude all sensory modalities except olfaction, and we accomplished this with a novel vented-chamber sampling method that effectively uncoupled thermally convected odors emanating from a decomposing pig carcass from all other close-range cues (Cruise et al. 2018a,b). Results from this approach provide insight into necrophilous insect attraction mechanisms and behavior, areas of needed research for these insects (Tomberlin et al. 2011a,b; Charabidze et al. 2017).

Materials and Methods

Experimental Animals

Stillborn pigs were acquired from North Carolina State University's Swine Educational Unit. A total of 20 pigs (*Sus scrofa domesticus*), each weighing roughly 1.5 kg, were used in this experiment. Pigs were placed in a freezer immediately after birth and remained fully frozen until they were placed in the field. This prevented early decomposition and ensured that all pigs were the same temperature at the start of the experiment. Frozen pigs (–12°C) reached ambient temperature in ~4.5 h in full sunlight (A.M.C., personal observations).

Study Site

This experiment was conducted during September and October 2015 in two open fields at North Carolina State University's Lake Wheeler Road Field Lab in Raleigh, NC. One field (35.730605, –78.673048) was designated the *decomposition field* (i.e., field 1), where pigs were placed and sampled daily. Pigs were positioned in this field at the edge of a tree line with their heads oriented eastward. This ensured that they experienced full daytime sun. The other field (35.731424, –78.667759) was designated the *transfer field* (i.e., field 2) into which all pigs were transferred and sampled on the fifth day of the experiment, henceforth referred to as the 'transfer day' or 'day of transfer'. Care was taken to select a transfer field that was as similar to the decomposition field as possible in terms of overall sunlight and vegetative characteristics. The two fields were approximately 426 m apart and were separated by two geographical barriers: a stream (waterway) and a dense row of trees. Directionality of pig placement was conserved when pigs were moved to the transfer field.

The average temperature during the September dates of the experiment (20.9 ± 0.03°C, N = 10,080 min-by-min readings, SEM) was almost double the average temperature during the October dates (11.6 ± 0.06°C, N = 11,520 min-by-min readings, SEM) (Fig. 1). During the September dates of the experiment, between 1.3 and 18 mm of rain fell each day, either before or after sampling (State Climate Office of North Carolina). There was no precipitation during the October dates.

General Field Methods

Frozen neonate pigs were added sequentially to the decomposition field over a 4-d period. In September 2015, three pigs were added to the decomposition field each day for 4 d (12 pigs total). At the time of placement, three shallow holes were dug 25 m apart in the decomposition field, and the displaced soil was piled ~4 cm high atop a standard plastic cafeteria tray (35 cm × 45 cm). Each of the three pigs was placed on a soil-covered tray. This allowed us to move each pig into the vented-chamber (described below) during sampling sessions, while preserving the insect-rich soil-body interface. Pigs were

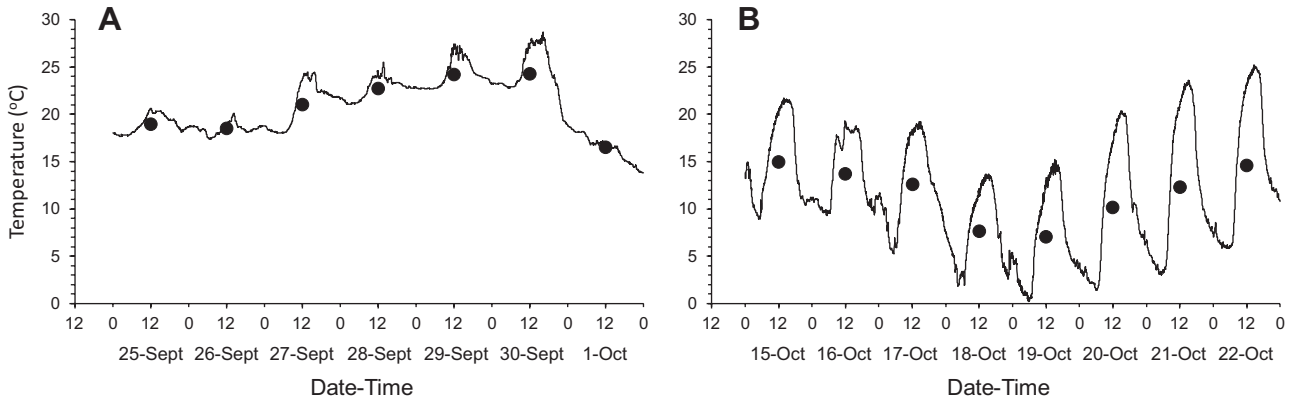

Fig. 1. Minute and daily average ambient temperature (°C) near the field research site during the experiment. The symbols at 12 (noon) indicate the average daily temperature. Temperature data were acquired from the State Climate Office of North Carolina's Lake Wheeler Road Field Lab weather station. The average temperature during the September experiment (A) was 20.9 ± 0.032°C (N = 10,080 min-by-min readings, SEM). Temperatures during the October experiment (B) were much cooler, averaging only 11.6 ± 0.057°C (N = 11,520 min-by-min readings, SEM).

Table 1. Placement and sampling schedule for all pigs

Experiment day	Pig ID placed in decomposition field	Pig ID sampled in decomposition field (age)	Pig ID sampled in transfer field (age)
September 2015			
1	a, b, c	None	None
2	d, e, f	a, b, c (day 1)	None
3	g, h, i	a, b, c (day 2)	None
		d, e, f (day 1)	None
4	j, k, l	a, b, c (day 3)	None
		d, e, f (day 2)	None
		g, h, i (day 1)	None
5 (transfer)	None	a, b, c (day 4)	a, b, c (day 4)
		d, e, f (day 3)	d, e, f (day 3)
		g, h, i (day 2)	g, h, i (day 2)
		j, k, l (day 1)	j, k, l (day 1)
October 2015			
1	m, n	None	None
2	o, p	m, n (day 1)	None
3	q, r	m, n (day 2)	None
		o, p (day 1)	None
4	s, t	m, n (day 3)	None
		o, p (day 2)	None
		q, r (day 1)	None
5 (transfer)	None	m, n (day 4)	m, n (day 4)
		o, p (day 3)	o, p (day 3)
		q, r (day 2)	q, r (day 2)
		s, t (day 1)	s, t (day 1)

Each pig was assigned a unique number. The decomposition age of each set of pigs (in days) is indicated in parentheses.

first sampled 24 h (decomposition age 1) after they had been placed in the field, and then daily for three more days. On the fifth day of the experiment, no new pigs were added to the field and the pigs (decomposing for 1–4 d) were sampled in the decomposition field and then relocated to the transfer field. Pigs were sampled in the transfer field beginning 15 min after placement. The experiment was repeated in October 2015, when two rather than three pigs were added each day to the decomposition field (eight pigs total). Thus, in total the combined experiments represented 20 pigs. Scheduling of pig placement, sampling, and transfer is shown in Table 1. When not sampled, the carcasses were protected from scavengers within enclosures constructed of poultry netting that allowed for normal insect colonization.

Sampling Procedure

Pigs were simultaneously sampled twice daily between 1400 and 1900 h, beginning 24 h after their placement in the decomposition field. Previous work demonstrated that repeated sampling during this afternoon interval accounts for all observed diversity in our research fields (Cruise et al. 2018c). Insect sampling was achieved through the passive vented-chamber method, which thermally convected decomposition odors to a pair of sticky traps, as outlined in Cruise et al. (2018a) and Cruise et al. (2018b) (Fig. 2). At each sampling event, each pig on a cafeteria tray was placed in the chamber and an airtight lid placed on top. Back-to-back unscented glue traps (Super Catchmaster, AP&G, Bayonne, NJ) were attached to the chimney atop the chamber and allowed to collect insects for 15 min. A 15-min rest period was inserted between successive sampling intervals. During this time, the pig and tray were removed from the chamber and placed on the ground, allowing decomposition and colonization to occur freely. Sticky traps were wrapped in cling plastic wrap and stored in the freezer.

To sample beetles, hand-collections were performed on the pig both before and after its placement in the vented-chamber, as some beetles that remained hidden during the initial sampling moved to the surface from within or under the body during the cafeteria tray movement. Beetles were stored in 70% ethanol.

Translocation

On the transfer day, pigs were sampled for two 15-min sampling periods in the decomposition field, as previously outlined. At the end of the second sampling period, all pigs were again removed from the vented-chamber and allowed a 15-min rest period on the ground. Pigs were then enclosed in the chamber and all open ports closed with PVC end caps. This prevented cross-contamination while transporting pigs to the transfer field. All visible adult insects were removed from and around the body before its enclosure in the vented-chamber for translocation.

Pigs were spaced 25 m apart in the transfer field and removed from the chambers for a 15-min rest period. Two additional 15-min sampling periods (vented-chamber and hand-collections) separated by a 15-min rest period on the ground were then performed.

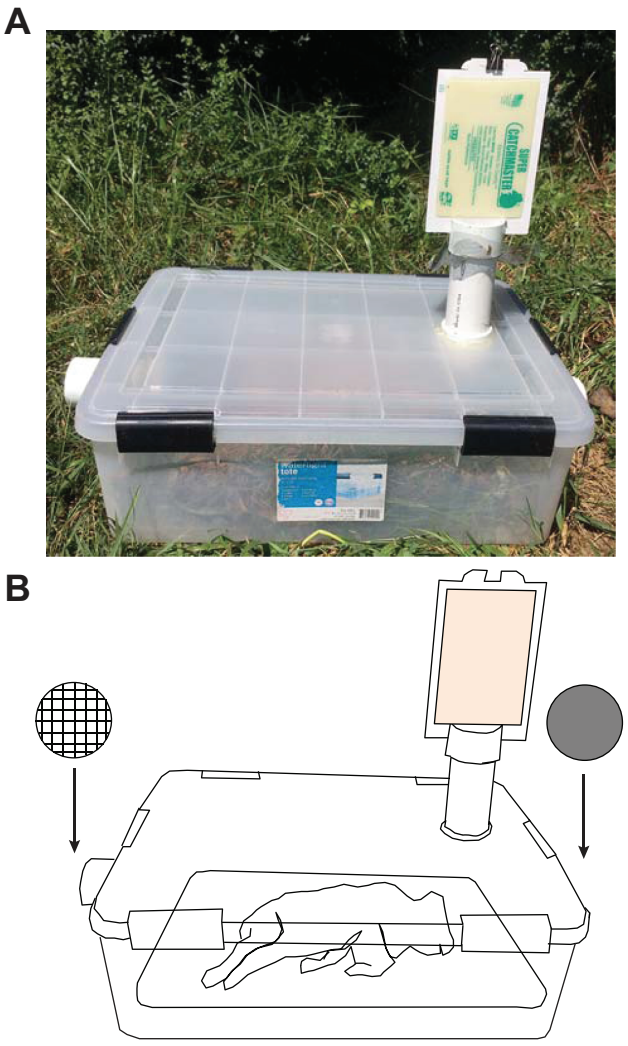

Fig. 2. The vented-chamber method, as described in Cruise et al. (2018b). The collection unit consists of a 39-liter airtight chamber with PVC ports. Ports on the left and top of the box, with the orientation as above, were kept open with mesh window screening. Thus, air could flow freely through the chamber, but insects could not enter it. The right port was capped. The top port, or chimney, opened to back-to-back unscented glue traps. Pig orientation in reference to the ports was always as shown above.

Insect Identification

Collected insects were categorized based on their forensic significance. Orders Diptera and Coleoptera were the main sampling targets, with emphasis on those necrophilous families commonly used in forensic entomology for postmortem interval determinations (Table 2). The local fauna of interest was also reported in Cammack et al. (2016) and in Cruise et al. (2018b). Because of their significant role as primary colonizers of decomposing bodies, calliphorids (blow flies) were further identified to species level using Whitworth's taxonomic key (Whitworth 2006). Beetles were identified using Almeida and Mise's forensic Coleoptera key (Almeida and Mise 2009). Insects on sticky traps were identified in situ.

Insect Community

Linear discriminant analysis (LDA) was conducted in JMP (SAS Institute 2016) on absolute number of each taxon sampled 1) in the decomposition field across day 2–5 of the experiment (decomposition ages 1–4 d), 2) in the decomposition field on the day of transfer, and 3) in the transfer field after transfer. If no insects were sampled from a pig on a particular day, that data point was excluded from the analysis. Pig decomposition ages were denoted as day 1 to day 4, corresponding to the pig's time spent in the field (Table 1). For example, pigs placed in the field 24 h before the transfer day were considered day 1 pigs. Beetles were excluded from the linear discriminant analysis because low numbers of beetles were trapped both before and after transfer.

Differences in insect numbers by pig decomposition age both within and between fields were analyzed with one-way ANOVA followed by Tukey's HSD test (SAS Institute 2012). For these analyses, all beetle species were grouped together as 'Coleoptera'.

Table 2. Forensically significant insects sampled from all pigs and both experimental fields

Order	Family	Genus and species
Diptera	Calliphoridae	*Lucilia illustris* Meigen[a,b]
		Lucilia coeruleiviridis Macquart[a,b]
		Lucilia sericata Meigen[a,b]
		Lucilia cuprina Wiedemann[a,b]
		Phormia regina Meigen[a,b]
		Cochliomyia macellaria Fabricius[a,b]
		Calliphora vicina Robineau-Desvoidy[a]
		Chrysomya rufifacies Macquart[a]
	Sarcophagidae	Sarcophagidae[a,b]
	Muscidae	*Musca domestica* Linnaeus[a,b]
Coleoptera	Histeridae	Histeridae[a]
	Staphylinidae	Staphylinidae[a,b]
	Dermestidae	*Dermestes* spp.[a]

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

[a]Taxa sampled in the decomposition field.

[b]Taxa sampled in the transfer field.

Results

Decomposition

Pig decomposition progressed at different rates in the September and October replicates (i.e., seasonal differences), with slower decomposition during October than in September. All but day 1 pigs in the September study had progressed to the stage of abdominal opening, whereas no pigs in the October study reached this stage. Throughout this study, we use 'decomposition age' to refer to a pig's age in the field and its daily progression through the decomposition process, rather than to denote discrete physical and insect community markers. Pigs spent several days in each of the discrete decomposition stages, as defined by Kreitlow (2010), so defining decomposition ages as daily changes accounted for differences among pigs without necessarily synchronizing them to discrete decomposition stages (Cruise et al. 2018b).

Succession of the Insect Community in the Decomposition Field

The overall undisturbed ecological succession in the decomposition field is shown in Fig. 3. Linear discriminant analysis of the insect community structure across all four sampling days showed significant overlap between day 1 and day 2 pigs, but day 3 and day 4 pigs had significantly different community structures, indicated by the lack of overlap of their 95% confidence ellipses with each other and with any other groupings (Fig. 3A). Day 1 and day 2 pigs were

relatively tightly grouped, indicating minimal variation in their insect community structure. The groupings became weaker, however, with increasing pig decomposition age (Fig. 3A).

The number of insects collected on the first 2 d of sampling pigs (24–48 h after placement, decomposition ages 1 and 2 d) was lower than on the subsequent two decomposition ages ($F_{3, 38}$ = 7.27, P = 0.0006) (Fig. 3B). Insect diversity was also low with five taxa trapped on these pigs, but only three representing the vast majority of newly arriving insects (*Lucilia coeruleiviridis* Macquart [Diptera: Calliphoridae], *Lucilia illustris* Meigen [Diptera: Calliphoridae], and *Phormia regina* Meigen [Diptera: Calliphoridae]). Samples from decomposition ages 3 and 4 d were much more diverse, but were again dominated by the same three species, with the addition of a late dominant species, *Cochliomyia macellaria* Fabricius (Diptera: Calliphoridae), on day 3 pigs. Beetles, while low in abundance, were collected only on day 2–4 pigs.

The relative abundance of taxa changed over the 4-d experiment. The relative abundances of *L. coeruleiviridis* and *L. illustris* were high on day 1 and declined over the next 2 d, whereas the relative abundances of *P. regina* and *C. macellaria* increased concomitantly (Fig. 3C). Surprisingly, this pattern reversed on the fourth day of decomposition, on which the relative representation of these three taxa was similar to day 1.

Because the pigs were sampled daily for 4 d in the decomposition field before they were relocated to the transfer field, we also conducted LDA of insect community structure on these pigs only on the day of transfer to minimize the effects of climate variation and to enable a direct comparison to the transfer field on the same day. Results showed significantly different community structures, indicated by the lack of overlap of their 95% confidence ellipses, for the four decomposition ages sampled simultaneously on the same day (Fig. 4A). Although differences between the two trials (September and October) contributed to variation, this pattern held for both trials, as pigs in the two trials grouped with the respective pigs of the same decomposition age.

Like the patterns observed across all four sampling days of the experiment, those from the transfer day alone showed dominance of *L. coeruleiviridis*, *L. illustris*, and *P. regina* on all pig decomposition ages, as well as dominance of *C. macellaria* on day 3 pigs. Again, day 1 pigs had lower insect diversity and significantly lower abundance than any other decomposition age, and Coleoptera was sampled from day 2–4 pigs. Mean abundance of insects did not differ significantly

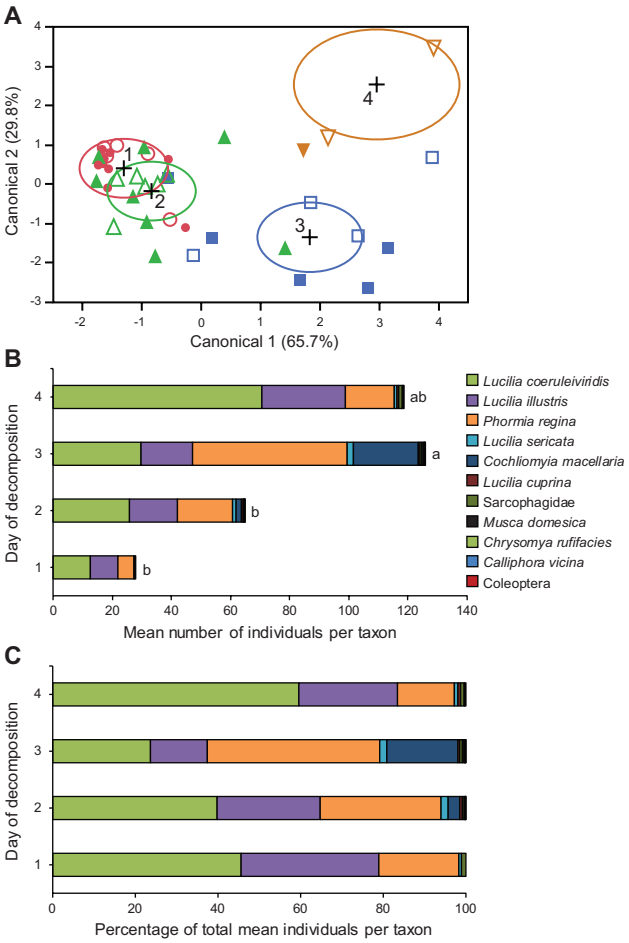

Fig. 3. Insect activity in the decomposition field (field 1) across all days of the experiment. (A) Linear discriminant analysis of community structure by day of decomposition (i.e., pig age) and season (September = solid symbols, October = open symbols). (B) Mean number and (C) relative abundance of taxa trapped or hand-collected daily from pigs during the 4-d decomposition process. N = 20 pigs. In (A), the percentage adjacent to each canonical variable represents the proportion of the sum of the eigenvalues. In (B), unique letters indicate significantly different total mean insects by day using one-way ANOVA ($F_{3, 38}$ = 7.143, P = 0.0006) and Tukey's HSD.

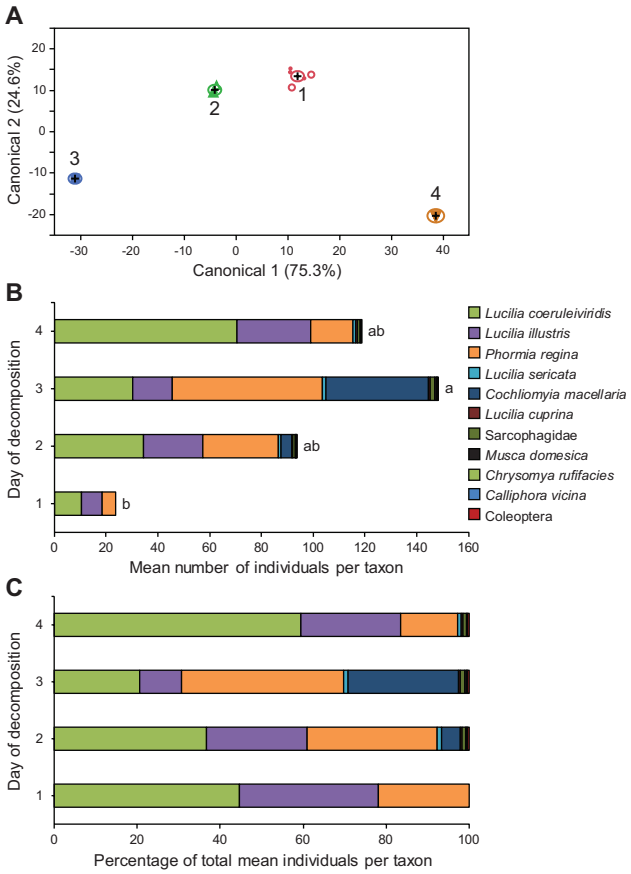

Fig. 4. Insect activity in the decomposition field (field 1) on the day of transfer (day 4), just prior to pig relocation. (A) Linear discriminant analysis of community structure by day of decomposition (i.e., pig age) and season (September = solid symbols, October = open symbols). (B) Mean number and (C) relative abundance of taxa trapped or hand-collected from pigs in field 1 on the day of transfer. N = 19 pigs. In (A), the percentage adjacent to each canonical variable represents the proportion of the sum of the eigenvalues. In (B), unique letters indicate significantly different total mean insects by day using one-way ANOVA ($F_{3,14}$ = 3.8042, P = 0.0348) and Tukey's HSD.

in a comparison of all four sampling days and the transfer day only in the decomposition field (t_{42} = 1.2328, P = 0.2245). The relative abundances of the four major taxa (*L. coeruleiviridis*, *L. illustris*, *P. regina*, and *C. macellaria*) were also similar on the day of transfer and the preceding 3 d. Therefore, the community composition on a single day (transfer day) in the decomposition field satisfactorily represented the asynchronous succession in the same field during the previous 3 d.

Succession of the Insect Community in the Transfer Field

After relocation of the decomposing pigs of different ages to a nearby field, the clear separation in LDA that we observed in the decomposition field became much weaker with broad overlap among different decomposition ages (Fig. 5A). The greatest overlap was between day 1 and day 2 pigs, whereas day 3 pigs separated significantly from day 1 and day 2 pigs. There was no significant difference in the number of insects sampled by pig decomposition age in field 2 ($F_{3, 14}$ = 1.176, P = 0.3540) (Fig. 5B).

Insect Arrival in the Decomposition Field Versus Transfer Field

More insects were trapped and hand-collected in the decomposition field (93.7 ± 17.3 insects) than in the transfer field (51.3 ± 11.7 insects) (t_{30} = 2.0289, P = 0.0514) across pigs of all decomposition ages on the transfer days. A higher diversity of insects was also sampled in the decomposition field, with several rare (with respect to Raleigh, NC) fly species (*C. vicina*, *Chrysomya rufifacies* Macquart [Diptera: Calliphoridae]) only sampled on pigs prior to relocation. The numbers of coleopterans sampled were low (<10 total) in both fields across pigs of all decomposition ages on the transfer days, with only one taxon, Staphylinidae, sampled in both fields. The relative abundance of taxa over decomposition was different in the transfer field. The pattern of change of the three major taxa was less consistent than in the decomposition field, but the increase in *C. macellaria* mirrored the increase in decomposition field.

Insect diversity sampled from day 1 pigs after relocation was higher than earlier in the decomposition field. *Lucilia sericata*, *C. macellaria*, and Sarcophagidae were sampled on pigs after relocation in the transfer field, although overall numbers and relative percentages of these taxa on day 1 pigs were low. Greater insect diversity after transfer occurred only with day 1 pigs. The relative abundance of the three major taxa also varied between the decomposition and transfer fields. *Lucilia illustris* was equally represented in the decomposition field throughout the experiment, including on the transfer day, representing ~22% of the three major taxa (Figs. 3C and 4C). After translocation, however, *L. illustris* represented slightly more than 10% of the insect diversity, as the relative abundance of *P. regina* was higher in the transfer field.

Unlike with the day 1 pigs, a lower diversity of insects was sampled from day 2 pigs after relocation. Dermestid beetles were sampled on day 2 pigs before transfer only. Staphylinids were sampled from day 2 and day 3 pigs in the decomposition field but after transfer they were sampled on day 3 and day 4 pigs. These staphylinids represented the only coleopterans sampled in the transfer field (Fig. 5B; Table 2). Two fly species, *Lucilia cuprina* Wiedemann (Diptera: Calliphoridae) and *C. rufifacies*, were sampled in the decomposition field but were not sampled on the same pigs after they were transferred to another field. The relative percentage of *L. illustris* sampled from pigs after relocation was also lower than in the decomposition field (Fig. 5C).

Day 3 pigs had very similar community structures in the two fields. Only one taxon, *C. rufifacies*, was sampled in the decomposition field but not in the transfer field. Relative percentages of taxa on pigs were also very similar across fields for day 3 pigs, with *P. regina* having the highest representation, and other taxa, like *L. sericata* and *L. cuprina*, in low relative abundance.

Day 4 pigs attracted similar taxa in both fields in terms of the relative representation of the most abundant taxa, but they differed slightly in terms of diversity of rare taxa. One fly species, *C. vicina*, was sampled on day 4 pigs in field 1 but was not sampled after their relocation to the transfer field. This was also the case for histerid beetles. Another fly species, *Musca domestica* Linnaeus (Diptera: Muscidae), was sampled on day 2–4 pigs in field 1 but was only found on day 2 and day 3 pigs after relocation. As in the decomposition field, the pattern of insects on day 4 pigs in the transfer field was inconsistent with the pattern of changes in relative abundance seen on day 1–3.

Discussion

Complex biological processes that are climate-dependent and site-specific, like decomposition and ecological succession, are subject to inherent variability (Tomberlin et al. 2011b). To minimize variation

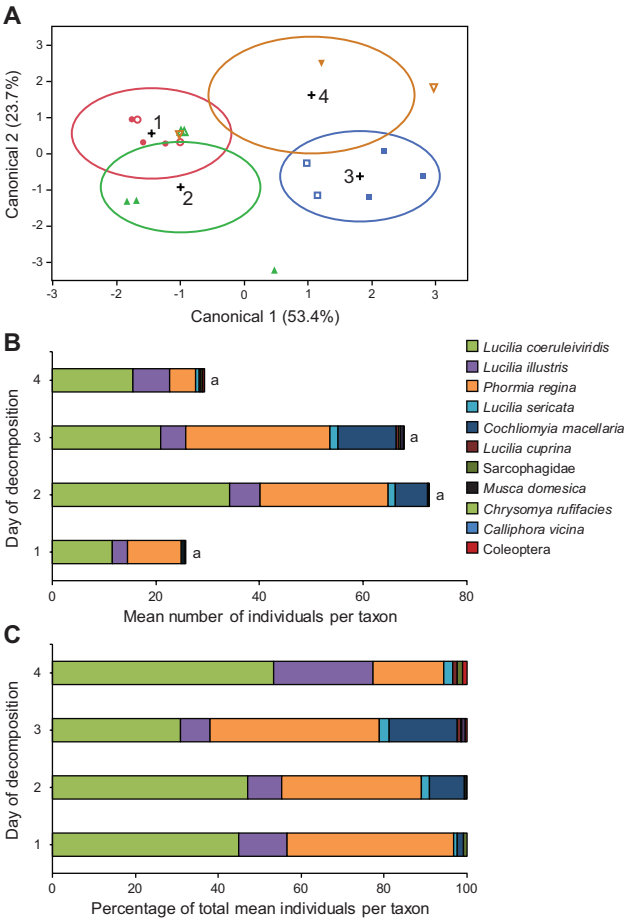

Fig. 5. Insect activity in the transfer field (field 2) on the day of transfer (day 4), just after pig relocation. (A) Linear discriminant analysis of community structure by day of decomposition (i.e., pig age) and season (September = solid symbols, October = open symbols). (B) Mean number and (C) relative abundance of taxa trapped or hand-collected from pigs in field 2 on the day of transfer. N = 19 pigs. In (A), the percentage adjacent to each canonical variable represents the proportion of the sum of the eigenvalues. In (B), the same letter indicates no significant differences in total mean insects by day using one-way ANOVA ($F_{3, 14}$ = 1.176, P = 0.3540).

in our study, we ensured that pigs were of the same size (~1.5 kg) and temperature (≤0°C) at their time of placement in the field, as the rate of decomposition is highly influenced by mass and temperature (Komar and Beattie 1998). We also minimized potential sampler bias by using the vented-chamber, a passive sampling method that operates independently of the investigator, unlike the sweep net (Cruise et al. 2018c).

While we sought to minimize environmental influences, several factors contributed substantially to variation in our study, including 1) differences between the two fields, 2) daily variation related to the number of days that each pig spent in the decomposition field, and 3) seasonal variation. In selecting two similar fields for this study, we considered proximity, vegetative characteristics, and level of sun exposure to be the most important factors to keep consistent across fields. Insect succession differs between shaded and full sun exposure, as some necrophilous insects like *L. illustris* are more commonly sampled from sun-exposed carrion (Byrd and Castner 2000, Cammack et al. 2016), and silphid and staphylinid beetles are more likely to be sampled from shaded carcasses (Hobischak et al. 2006). We ensured that sun exposure was consistent across fields in the locations where pigs were placed. We also aimed to keep vegetation in the two fields similar, as insect populations vary based on habitat type, perhaps even on a microgeographic scale (Hwang and Turner 2005, Pechal and Benbow 2015). Proximity of the two fields ensured minimal differences in their microgeographic characteristics.

The length of time each pig spent within the decomposition field was another source of variation. Because pig placement was staggered over 4 d, more successive observations were made on day 1 pigs than on day 4 pigs (Fig. 3A). When data points from all 5 d of the experiment were used in LDA, there was not a clear separation among pig decomposition ages. Samples from day 1 pigs were represented over 4 d of the experiment, contributing to substantial variation in the trapping results. This variation was largely eliminated when succession was assessed only on the last day (transfer day) across pigs of different decomposition ages (Fig. 4). Average ambient temperature increased >7°C during the 4 d in the decomposition field in September and varied between nearly 0 and 25°C in October, further contributing to variation in trapped insects (Fig. 1).

Seasonal differences (September vs October) probably contributed most to variation. The rate of decomposition is greatly influenced by ambient and soil temperature (Gill-King 1996). Three replicates were conducted in late September, when mean ambient temperature was ~21°C, and two replicates were in mid-October under unusually low ambient temperatures of ~12°C. Thus, decomposition and succession proceeded much faster in September. Combining these replicates resulted in greater variance, particularly in late stages of decomposition—day 4 in September represented a substantially more advanced stage of decomposition than day 4 in October. This was evident in the apparent 'reversal' of the successional pattern on day 4 (Figs. 3C and 4C) where declining representations of *L. coeruleiviridis* and *L. illustris*, and increasing representation of *P. regina* and *C. macellaria* were reversed on day 4. Closer examination of the data revealed that while the September replicates trapped more insects than the October replicates (September: 1,695 insects on three pigs, 565/pig; October: 921 on two pigs, 460/pig), the October replicates caught substantially more insects on day 4 than the September replicates in both fields. In both fields ~88-fold more insects were trapped in October than in September (decomposition field: 353 vs 3, transfer field: 87 vs 1). Since succession in October was substantially slower, day 4 in October likely corresponded to an earlier decomposition day in September as evidenced by trap catches and community structure. In addition, some cool-weather species like *C. vicina* were found only in the October replicates.

To minimize these sources of variation, we focused our analyses on the day of transfer only. It is important to note, however, that while daily temperature variation was minimized and all pig decomposition ages received the same number of observations, the seasonal effect remained a major source of variation in this analysis as well. When the transfer day alone was analyzed in LDA, there was clear separation among pig decomposition ages in the decomposition field (Fig. 5A). Representations of taxa were strikingly similar between the 4 d of trapping in the decomposition field (Fig. 3B and C) and the transfer day only (Fig. 4B and C), justifying a comparison of the decomposition and transfer fields on a single day.

While the community structure differed by pig decomposition age on the day of transfer in the decomposition field, the distinct separation among pig decomposition ages was not as well defined after the pigs were moved to the transfer field (Fig. 5A). The community structures on day 1 and day 2 pigs were not significantly different. Surprisingly, neither was the difference in structure of day 1 and day 4 pigs, despite their decomposition age differences and significant separation in the decomposition field. This 'reversal' on day 4 to a pattern reminiscent of earlier decomposition stages again highlighted the dominance of the October replicates, which represented a much slower decomposition rate. LDA became less effective for assessing community structure because our sample size declined while seasonal variation increased.

Considering only the transfer day, generally more insects were trapped per pig in the decomposition field than in the transfer field. However, the relative abundance of taxa varied more substantially between the two fields. In the decomposition field, we observed the expected pattern of declining relative abundances of *L. coeruleiviridis* and *L. illustris*, and increasing representation of *P. regina* and *C. macellaria* between day 1 and 3 (Fig. 4C). In the transfer field, on the other hand, the only clear pattern was an increase in *C. macellaria* (Fig. 5C).

As indicated earlier, decomposition progressed more rapidly in September. Thus, total trap catch per pig in the decomposition field varied from 21.3 on decomposition day 1 pigs to 131.0, 172.3 and 3.0 on day 2, 3, and 4, respectively. In October, the corresponding numbers were 27.5, 38.0, 112.5, and 176.5. Clearly, pigs in their fourth day of decomposition in October were still in a rather early decomposition state as indicated by the large numbers of insects trapped and the early successional community structure. Because LDA was conducted on the absolute trap catch, the day 4 October catches dominated the data set.

Calliphora vicina and *C. rufifacies* were trapped only in the decomposition field, and in low numbers (<10) (Fig. 4B). This is the first record of *C. rufifacies*, the hairy maggot blow fly, in central North Carolina. This invasive fly species poses significant challenges to forensic entomologists because its predatory larvae can consume other first responders and thus create a false picture of succession. This can lead to potentially incorrect postmortem interval estimations. The unique feeding behavior of *C. rufifacies* may also affect the arrival pattern of native insects, further complicating postmortem interval estimations (Brundage et al. 2014).

Coleoptera numbers were low both before and after transfer for all pigs (Figs. 4B and 5B). This observation is not uncommon for decomposition of fetal pigs in our area (Cruise et al. 2018b). While fetal pigs attract the same successional pattern of taxa, the mean number of each taxon is typically much lower than present on larger pigs (Kuusela and Hanski 1982, Hewadikaram and Goff 1991). Of the beetle taxa sampled, only staphylinids were sampled in both the decomposition

and transfer fields (Fig. 5B; Table 2). All other beetle taxa were sampled only in the decomposition field. We suspect this is due to the flight behavior of these necrophilous beetles, which are not as agile as necrophilous flies (Charabidze et al. 2017). *Necrophila americana* Linnaeus (Coleoptera: Silphidae), a necrophilous silphid beetle sampled in previous studies, searches for carrion from low heights and is not generally trapped above 2 m of the ground (LeGros and Beresford 2010; Cruise et al. 2018b,c). While similar studies, to our knowledge, have not been conducted for those beetle taxa sampled in the decomposition and transfer fields, we observed these beetles flying low to the ground and often walking to the resource after dropping from flight in nearby vegetation (Cruise et al. 2018a, A.M.C., personal observations). Perhaps these strategies slow resource detection and/or pursuit, as vegetation and other physical obstacles may impede odor dispersal near the ground or slow flight and subsequent walking to the resource. Further studies should extend the observation and sampling period for beetles after carrion relocation to determine whether the observed differences in Coleoptera taxa between the decomposition and transfer fields were due to olfactory cues affecting assemblage patterns or simply because a longer time was needed for these insects to locate and/or travel to the resource. It is also important to note that despite the measures taken to ensure that all beetles were hand-collected prior to pig transfer, it is possible that one or both of the staphylinids sampled after transfer were transported along with the pig due to the cryptic nature of these beetles.

From our analyses, we are able to draw several conclusions. First, the inherent variability in decomposition and ecological succession created a challenge as climate changed over the course of the experiment. Although we attempted to minimize several sources of variation in our experimental design, seasonal differences dominated the variation. This effect can be mitigated with larger sample sizes in a single replicated experiment conducted during a minimum number of days. Second, it was clear that different insects were differentially attracted to pigs of different ages of decomposition. Differential attraction was also observed in the transfer field after relocation but was less pronounced. Third, the general community structures of trapped insects on decomposing pigs were similar before and after relocation, with the exception of several rare taxa, indicating that the predicted insect succession continues in locations that are geographically and ecologically similar. All major and some minor (i.e., rare) taxa were sampled from pigs in both fields, although a few rare taxa were only found prior to relocation. Fourth, necrophilous insects locate a newly relocated host within minutes, guided by olfactory cues. Insects were sampled from pigs of all four decomposition ages in field 2 within 15 min after placement. Although other studies have documented necrophilous insect host location within minutes (Payne 1965, Grassberger and Frank 2004), our vented-chamber trap excluded all sensory modalities but olfaction. While other cues, such as on-carcass interactions or even visual cues may be important for insect oviposition, we focused solely on olfactory attraction with the use of the vented-chamber, as it sampled insects before they were able to interact with the carcass. Finally, olfactory cues alone can shape the community structure of necrophilous insects. These differences may be related to differing VOC profiles from the carrion itself or from other insects on the decomposing body (Spivak et al. 1991, Frederickx et al. 2012b).

Literature surveys indicate that research using necrophilous insects is usually categorized as medical- (including medico-legal) or veterinary-focused. Learning more about the basic biology of these insects, especially regarding host-finding, provides a framework for future research involving trapping/monitoring, control, or the utility of these insects in criminal investigations. Our findings provide insight into the significance of olfaction for these transient-resource specialists and should motivate researchers in typically necrophilous insect-focused fields, like forensic entomology, as well as chemical ecologists and neuroethologists to use these insects as models for olfaction studies.

Acknowledgments

We would like to thank the North Carolina State University Lake Wheeler Field Lab Swine Unit for providing the neonate pigs, Rick Santangelo (NC State) for his help with field sampling and pig relocation, and Ann Ross for critical comments on an earlier draft. Partial funding for this work was received from a Graduate Research Fellowship from the National Science Foundation (GRFP Award DGE-1252376) and the Blanton J. Whitmire Endowment at NC State.

References Cited

Almeida, L. M., and K. M. Mise. 2009. Diagnosis and key of the main families and species of South American Coleoptera of forensic importance. Rev. Bras. Entomol. 53: 227–244.

Anderson, G. S., and S. L. VanLaerhoven. 1996. Initial studies on insect succession on carrion in southwestern British Columbia. J. Forensic Sci. 41: 617–625.

Archer, M. 2004. Annual variation in arrival and departure times of carrion insects at carcasses: implications for succession studies in forensic entomology. Aust. J. Zool. 51: 569–576.

Battán Horenstein, M., A. Xavier Linhares, B. Rosso de Ferradas, and D. García. 2010. Decomposition and dipteran succession in pig carrion in central Argentina: ecological aspects and their importance in forensic science. Med. Vet. Entomol. 24: 16–25.

Brodie, B., R. Gries, A. Martins, S. VanLaerhoven, and G. Gries. 2014. Bimodal cue complex signifies suitable oviposition sites to gravid females of the common green bottle fly. Entomol. Exp. Appl. 153: 114–127.

Brundage, A., M. E. Benbow, and J. K. Tomberlin. 2014. Priority effects on the life-history traits of two carrion blow fly (Diptera, Calliphoridae) species. Ecol. Entomol. 39: 539–547.

Byrd, J. H., and J. L. Castner. 2000. Insects of forensic importance, pp. 43–79. *In* J. H. Byrd and J. L. Castner (eds.), Forensic entomology: the utility of arthropods in legal investigations. CRC Press, Boca Raton, FL.

Cammack, J. A., A. C. Cohen, K. L. Kreitlow, R. M. Roe, and D. W. Watson. 2016. Decomposition of concealed and exposed porcine remains in the North Carolina Piedmont. J. Med. Entomol. 53: 67–75.

Campobasso, C. P., G. Di Vella, and F. Introna. 2001. Factors affecting decomposition and Diptera colonization. Forensic Sci. Int. 120: 18–27.

Carter, D. O., D. Yellowlees, and M. Tibbett. 2007. Cadaver decomposition in terrestrial ecosystems. Naturwissenschaften. 94: 12–24.

de Carvalho, L. M. L., and A. X. Linhares. 2001. Seasonality of insect succession and pig carcass decomposition in a natural forest area in southeastern Brazil. J. Forensic Sci. 46: 604–608.

e Castro, C. P., J. P. Sousa, M. I. Arnaldos, J. Gaspar, and M. D. García. 2011. Blowflies (Diptera: Calliphoridae) activity in sun exposed and shaded carrion in Portugal. Ann. Soc. Entomol. Fr. 47: 128–139.

Charabidze, D., V. Hedouin, and D. Gosset. 2015. An experimental investigation into the colonization of concealed cadavers by necrophagous blowflies. J. Insect Sci. 15: 149.

Charabidze, D., M. Gosselin, and V. Hedouin. 2017. Use of necrophagous insects as evidence of cadaver relocation: myth or reality? Peerj 5: e3506.

Clark, M. A., M. B. Worrell, and J. E. Pless. 1996. Postmortem changes in soft tissues, pp. 151–164. *In* M. H. Sorg and W. D. Haglund (eds.), Forensic taphonomy: the postmortem fate of human remains. CRC Press, Boca Raton, FL.

Cruise, A., D. W. Watson, and C. Schal. 2018a. A novel passive sampling technique for collecting adult necrophilous insects arriving at neonate pig carcasses. Environ. Entomol. 47: 1573–1581.

Cruise, A., D. W. Watson, and C. Schal. 2018b. Ecological succession of adult necrophilous insects on neonate *Sus scrofa domesticus* in central North Carolina. PLoS One. 13: e0195785.

Cruise, A., E. Hatano, D. W. Watson, and C. Schal. 2018c. Comparison of techniques for sampling adult necrophilous insects from pig carcasses. J. Med. Entomol. 55: 947–954.

Dekeirsschieter, J., F. J. Verheggen, M. Gohy, F. Hubrecht, L. Bourguignon, G. Lognay, and E. Haubruge. 2009. Cadaveric volatile organic compounds released by decaying pig carcasses (*Sus domesticus* L.) in different biotopes. Forensic Sci. Int. 189: 46–53.

Frederickx, C., J. Dekeirsschieter, F. J. Verheggen, and E. Haubruge. 2012a. Responses of *Lucilia sericata* Meigen (Diptera: Calliphoridae) to cadaveric volatile organic compounds. J. Forensic Sci. 57: 386–390.

Frederickx, C., J. Dekeirsschieter, Y. Brostaux, J. P. Wathelet, F. J. Verheggen, and E. Haubruge. 2012b. Volatile organic compounds released by blowfly larvae and pupae: new perspectives in forensic entomology. Forensic Sci. Int. 219: 215–220.

Gill-King, H. 1996. Chemical and ultrastructural aspects of decomposition, pp. 93–108. *In* M. H. Sorg and W. D. Haglund (eds.), Forensic taphonomy: the postmortem fate of human remains. CRC Press, Boca Raton, FL.

Goff, M. L. 1993. Estimation of postmortem interval using arthropod development and successional patterns. Forensic Sci. Rev. 5: 81–94.

Grassberger, M., and C. Frank. 2004. Initial study of arthropod succession on pig carrion in a central European urban habitat. J. Med. Entomol. 41: 511–523.

Haskell, N. H., R. D. Hall, V. J. Cervenka, and M. A. Clark. 1996. On the body: insects' life stage presence and their postmortem artifacts, pp. 415–448. *In* M. H. Sorg and W. D. Haglund (eds.), Forensic taphonomy: the postmortem fate of human remains. CRC Press LLC, Boca Raton, FL.

Hewadikaram, K. A., and M. L. Goff. 1991. Effect of carcass size on rate of decomposition and arthropod succession patterns. Am. J. Forensic Med. Pathol. 12: 235–240.

Hobischak, N. R., S. L. VanLaerhoven, and G. S. Anderson. 2006. Successional patterns of diversity in insect fauna on carrion in sun and shade in the Boreal Forest Region of Canada, near Edmonton, Alberta. Can. Entomol. 138: 376–383.

von Hoermann, C., J. Ruther, S. Reibe, B. Madea, and M. Ayasse. 2011. The importance of carcass volatiles as attractants for the hide beetle *Dermestes maculatus* (De Geer). Forensic Sci. Int. 212: 173–179.

Hoffman, E. M., A. M. Curran, N. Dulgerian, R. A. Stockham, and B. A. Eckenrode. 2009. Characterization of the volatile organic compounds present in the headspace of decomposing human remains. Forensic Sci. Int. 186: 6–13.

Hwang, C., and B. D. Turner. 2005. Spatial and temporal variability of necrophagous Diptera from urban to rural areas. Med. Vet. Entomol. 19: 379–391.

Hyde, E. R., D. P. Haarmann, J. F. Petrosino, A. M. Lynne, and S. R. Bucheli. 2015. Initial insights into bacterial succession during human decomposition. Int. J. Legal Med. 129: 661–671.

Kalinová, B., H. Podskalská, J. Růzicka, and M. Hoskovec. 2009. Irresistible bouquet of death–how are burying beetles (Coleoptera: Silphidae: *Nicrophorus*) attracted by carcasses. Naturwissenschaften. 96: 889–899.

Komar, D., and O. Beattie. 1998. Effects of carcass size on decay rates of shade and sun exposed carrion. Can. Soc. Forensic Sci. J. 31: 35–43.

Kreitlow, K. 2010. Insect succession in a natural environment, pp. 251–270. *In* J. H. Byrd and J. L. Castner (eds.), Forensic entomology: the utility of arthropods in legal investigations. CRC Press, Boca Raton, FL.

Kuusela, S., and I. Hanski. 1982. The structure of carrion fly communities: the size and the type of carrion. Ecography. 5: 337–348.

LeGros, D. L., and D. V. Beresford. 2010. Aerial foraging and sexual dimorphism in burying beetles (Silphidae: Coleoptera) in a central Ontario forest. J. Entomol. Soc. Ont. 141: 3–10.

Merritt, R. W., and G. D. De Jong. 2015. Arthropod communities in terrestrial environments, pp. 65–92. *In* M. E. Benbow, J. K. Tomberlin, and A. M. Tarone (eds.), Carrion ecology, evolution, and their applications. CRC Press, Boca Raton, FL.

Paczkowski, S., F. Maibaum, M. Paczkowska, and S. Schütz. 2012. Decaying mouse volatiles perceived by *Calliphora vicina* Rob.-Desv. J. Forensic Sci. 57: 1497–1506.

Payne, J. A. 1965. A summer carrion study of the baby pig *Sus scrofa* Linnaeus. Ecology. 46: 592–602.

Pechal, J. L., and M. E. Benbow. 2015. Community ecology, pp. 347–360. *In* M. E. Benbow, J. K. Tomberlin, and A. M. Tarone (eds.), Forensic entomology: international dimensions and frontiers. CRC Press, Boca Raton, FL.

Pechal, J. L., M. E. Benbow, T. L. Crippen, A. M. Tarone, and J. K. Tomberlin. 2014. Delayed insect access alters carrion decomposition and necrophagous insect community assembly. Ecosphere. 5: 1–21.

SAS Institute. 2012. Statistical Analysis System computer program, version 9.4. SAS Institute, Cary, NC.

SAS Institute. 2016. JMP Pro computer program, version 13.1.0. SAS Institute, Cary, NC.

Sharanowski, B. J., E. G. Walker, and G. S. Anderson. 2008. Insect succession and decomposition patterns on shaded and sunlit carrion in Saskatchewan in three different seasons. Forensic Sci. Int. 179: 219–240.

Simmons, T., P. A. Cross, R. E. Adlam, and C. Moffatt. 2010. The influence of insects on decomposition rate in buried and surface remains. J. Forensic Sci. 55: 889–892.

Spivak, M., D. Conlon, and W. J. Bell. 1991. Wind-guided landing and search behavior in fleshflies and blowflies exploiting a resource patch (Diptera: Sarcophagidae, Calliphoridae). Ann. Entomol. Soc. Am. 84: 447–452.

Statheropoulos, M., C. Spiliopoulou, and A. Agapiou. 2005. A study of volatile organic compounds evolved from the decaying human body. Forensic Sci. Int. 153: 147–155.

Statheropoulos, M., A. Agapiou, C. Spiliopoulou, G. C. Pallis, and E. Sianos. 2007. Environmental aspects of VOCs evolved in the early stages of human decomposition. Sci. Total Environ. 385: 221–227.

Stensmyr, M. C., I. Urru, I. Collu, M. Celander, B. S. Hansson, and A. M. Angioy. 2002. Pollination: rotting smell of dead-horse arum florets. Nature. 420: 625–626.

Tomberlin, J. K., R. Mohr, M. E. Benbow, A. M. Tarone, and S. VanLaerhoven. 2011a. A roadmap for bridging basic and applied research in forensic entomology. Annu. Rev. Entomol. 56: 401–421.

Tomberlin, J. K., M. E. Benbow, A. M. Tarone, and R. M. Mohr. 2011b. Basic research in evolution and ecology enhances forensics. Trends Ecol. Evol. 26: 53–55.

Urech, R., P. E. Green, M. J. Rice, G. W. Brown, F. Duncalfe, and P. Webb. 2004. Composition of chemical attractants affects trap catches of the Australian sheep blowfly, *Lucilia cuprina*, and other blowflies. J. Chem. Ecol. 30: 851–866.

Vass, A. A., S. A. Barshick, G. Sega, J. Caton, J. T. Skeen, J. C. Love, and J. A. Synstelien. 2002. Decomposition chemistry of human remains: a new methodology for determining the postmortem interval. J. Forensic Sci. 47: 542–553.

Vass, A. A., R. R. Smith, C. V. Thompson, M. N. Burnett, D. A. Wolf, J. A. Synstelien, N. Dulgerian, and B. A. Eckenrode. 2004. Decompositional odor analysis database. J. Forensic Sci. 49: 760–769.

Watson, E. J., and C. E. Carlton. 2003. Spring succession of necrophilous insects on wildlife carcasses in Louisiana. J. Med. Entomol. 40: 338–347.

Watson, E. J., and C. E. Carlton. 2005. Insect succession and decomposition of wildlife carcasses during fall and winter in Louisiana. J. Med. Entomol. 42: 193–203.

Whitworth, T. 2006. Keys to the genera and species of blow flies (Diptera: Calliphoridae) of America North of Mexico. Proc. Entomol. Soc. Wash. 108: 689–725.

Zeariya, M. G., K. M. Hammad, M. A. Fouda, A. G. Al-Dali, and M. M. Kabadaia. 2015. Forensic-insect succession and decomposition patterns of dog and rabbit carcasses in different habitats. J. Entomol. Zool. Stud. 3: 473–482.