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THE EFFECTS OF CLOTHING ON ARTHROPOD ACTIVITIES OF ADULT AND JUVENILE DECOMPOSING *SUS SCROFA DOMESTICUS* MODELS

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Abstract

Wide varieties of insects are attracted to decomposing remains and play an active role in the decay process. The present study aimed to compare arthropod succession patterns in decomposition in large and small clothed surface disposed *sus scrofa* models. A carrion succession study was conducted at DAFARF Okuku, Nigeria from June to August 2023. Two carcasses of domestic pigs, *Sus scrofa*, 55kg and 25 kg in weight were used to compare insect succession patterns in decomposition in large and small clothed surface disposed *sus scrofa* models. A total of 16 arthropod taxa were recovered during this study. In the beginning of the study, large numbers of female adults of C. albiceps began oviposition at day 3 after placement of the small cadaver. The second and third instars of C. albiceps larvae subsequently almost monopolized the cadaver. Larvae and adults of *Chrysomya marginalis* outnumbered all other blow fly species. No size-related differences were observed between carcasses with respect to composition of the arthropod fauna or patterns of succession. A greater number of arthropods were observed attracted to the 55kg carcass, and the rate of decomposition observed was more rapid for the 55kg carcass than for the 25kg carcass. The presence of clothes was not shown to influence the invasion of arthropods, and their presence did not affect the oviposition sites. Moreover, this study demonstrates, that arthropod mediated decomposition of a large exposed pig carcass will occur at a faster rate compared to a small carcass.

Keywords: Arthropod succession, Maggot migration, Decomposition, Clothing, Carcas size.

1.0 Introduction

Insects make up the biggest class of metazoans, with around one million species described (Price 2008). Nearly every type of ecosystem is home to them. A vertebrate corpse is one such habitat that serves as a great food supply for a community of insects that are more or less specialized (Anderson and Cervenka, 2012). An essential ecological function of insects is the breakdown of organic materials. They frequently arrive at the scene of a death first and follow a predictable pattern that is impacted by the biological, physical, and chemical changes that carrion undergoes during its breakdown (Smith, 2019).

Forensic inquiries can be addressed by analyzing the insect fauna once the order in which the insects colonize the carrion is established. A pig carcass in various states of decay has revealed almost 400 different insect species (Payne, 2017). Apart from their ecological significance in the process of decomposition, these insects could be valuable resources for criminal investigations (Erzinclioglu, 2016; Catts and Goff, 2015), as they can be used to determine the time of colonization of a deceased body (Greenberg, 2021). Specifically, blowflies (Calliphoridae), which were among the first to inhabit corpses, may act as a biological clock to determine the exact time of death over a period of two or more weeks. The medical examiner's estimate, which is only valid for a day or two after death, may be significantly less accurate than an entomologically based estimate (Greenberg and Kunich, 2022).

Soon after a death, usually within minutes, insects get drawn to the corpse (Haskell *et al*., 2019). However, oviposition may not occur. Many taxa which appear very early at a death scene are late colonizers or even non-necrophagous species (Anderson,2022). Recent research has expanded on the use of insect succession as a means of establishing time of death. Multiple stages of decomposition influence identifiable and predictable successional stages of insect diversity and activity (Goff 2007). As it is not possible to deduce directly the period of death, the estimation of the post-mortem interval was only possible by the determination of the chronological succession in the insect colonization process. It is called maximum PMI (Amendt *et al*., 2007). Once a cadaver has been found, temperature data from the dead body and also from the environment are collected (Amendt *et al.*, 2007).

Improvements in our knowledge of grave soils have mostly come from empirical observations or research on the activities of insects and/or scavengers (DeVault *et al.*, 2019). These observations and research revealed that the entrance of cadaveric material into the soil is mostly determined by the activities of insects and scavengers, as well as the bulk of the cadaver. During the 'Bloated' stage, internal pressure from gas accumulation causes fluids to leak out of cadaveric orifices (mouth, nose, and anus) and into the earth. The impact of purge fluid on the belowground environment is unknown. This amendment is anticipated to cause a localized flush of microbial biomass, a shift in soil faunal communities, carbon mineralization (CO2-C evolution), and an increase in soil nutrient status (Swan et al., 2010). This flux of cadaveric material into the soil will connect any fertile islands created by purge fluid, resulting in the creation of a single cadaver decomposition island (CDI). This research documented the sequence of insect succession on clothed pig carcasses of varying sizes (big and small) in one habitat, during one season and on one geographic location.

2.0 Materials and methods

The study was carried out in the rainy season of 2023 at the Department of Anatomy and Forensic Anthropology Research Facility (DAFARF) University of Cross River State, Okuku Campus; located at 6°35′35″N 8°38′01″E. The ethical standards of the committee on animals and human subjects use in scientific research of Faculty of Basic Medical Sciences were followed.

Figure 1: Image showing (DAFARF) Research Facility

Two healthy pigs, (Sus scrofa) with adult(55kg) and juvenile(25kg) were purchased from a pig farm, were used for the study. Pig cadaver was chosen for the study as alternative for human model because of its analog to human cadaver and has been reported to attract similar arthropod fauna recorded for human cadavers in studies relating to forensic entomology (Tomberlin et al. 2012). The experimental study was carried out for thirty days, from June 1st to June 30th, 2023. Throughout the experiments, the decomposition patterns for the adult and juvenile carrions were examined for visible post-mortem changes, decomposition rates, the impact of temperature, insect succession pattern and its activities, PMI, and ADD.

The pigs were sacrificed immediately via blunt force trauma to the head. Death was confirmed using the principles of the triad of Bichat -when there was no heart beat recorded by the stethoscope and via the pupillary reflex. The two experimental animals (big and small) were clothed in cotton shirts and enclosed in scavenger-proof weld mesh carcass cages (25mm mesh) which prevented disturbances from large scavengers, about 20 centimeters away from each other. Data on the observable decomposition changes of the animals were collected by the researchers. However, the first procedure involved in

carrying out this experiment was to ascertain the body statistics of the animals in order to confirm if the animals are fully matured. This was immediately followed by recording the perimortem body temperatures of the animals.

2.1 Insect collection

Activities of insects on and around the carrion were monitored while samples of insects were collected using three methods: Aerial sweep net, handpicking, and pitfall traps. Flying insects were collected using sweep nets by making 12 sweeps using side to side sweeping motion for fifteen minutes per carrion. Some adult flies present on the carrion surface were gathered by performing the swatting technique twice. During this method, the researcher holds the tail of the net and swats the substrate with the net to collect specimens. Crawling insects were handpicked using long forceps from the carrion surface while others were collected into pitfall traps made of a 500-mL glass jar half filled with a soap solution. The pitfall traps were positioned 2 m away from the carrion at the four edges of the metal cages. Insects were sampled from the big and small pig every day for four weeks. Samples were taken less frequently after the period of peak maggot activity for each trial, which was denoted by the dispersal of the maggots away from the carcasses. All insects were collected into vials of 70% ethanol for preservation and returned to the lab for identification. Appendix 1 is a list of all the taxa collected from both pigs. However, due to the enormous numbers of insects collected, only species having recognized forensic relevance were used to assess insect succession on pigs. It should be noted that relative abundances of insects hand-picked from the pig carrion were based on personal observations taken at the time of sampling. Insects collected were identified using keys by Velasquez *et al*. 139(2010) (for diptera), Almeida and Mise (2009) (for Coleptera) and Flint (2012) (for hymenoptera). For confirmation, the collected insects were were compared and identified with reference to insects of Nigeria checklist and bibliography (Medler 1980). Since insect activity and carrion decomposition are influenced by temperature, HOBO temperature data loggers were used to record the ambient and carcass soil temperatures influenced by decomposition fluid.

2.2 Entomological assessment and analysis

Individual specimens from various sampling methods were used to establish the total number of beetle and fly taxa that visited hanging and ground pig carcasses. We solely used individual trap-collected specimens to assess insect abundance. Species numbers were analyzed for all beetle families as well as flies from the families Calliphoridae, Sarcophagidae and Muscidae. Only taxa with a minimum abundance of 10 individuals on at least one carcass per treatment were evaluated. In most taxa, the minimal abundance was determined using those captured using traps. In certain species, however, fewer individuals were detected in traps than were observed on carcasses. In these species, data on the number of individuals were compiled via written reports and traps. Separate analyses were performed on adults and larvae of the same taxon. The study reported the relative abundances of adult species collected from the individuals. We used nonparametric tests in our analyses due to sample size limitations. We utilized the Kruskal-Wallis test to investigate how size affected the number of minimally abundant insects. To determine the influence of the carcass treatment on the number of minimally abundant taxa and

species abundances. The level of significance in all analyses was set at 5%.

3.0 Results

A total of 16 taxa of arthropods, representing four 4 families were collected during both experiments. Only two species were not insects: *Diplopoda juliforma* and *Arachnida Araneae*. We collected 350 individual specimens from adult carcasses (54% flies, 46% beetles) and 709 from juvenile carcasses (69% flies, 31% beetles). During the trial, the total quantity of adult flies on juvenile carcasses was more than double that on adult carcasses. During the trial, adult Diptera abundances were similar across both treatments.

3.1 Qualitative observations

3.1.1 Fresh stage

The fresh stage commenced directly after the animal was sacrificed, and it was associated with soft torso and flexible limbs and no foul odor. The arrival of adult calliphorids and oviposition occurred within 30 ad 35 minutes of placements of adult and juvenile pigs. Adult calliphorids had colonized and oviposited the carrion.

Fig 2 A&B: Images showing big and small carrion respectively in the fresh stage of decay

3.1.2 Bloated Stage (2-4 days)

The first signs of the bloat stage appeared on day two. At this point, the carcass's body color changed from white to darker, and abdominal distension was accompanied by gas escape, resulting in a typical bloated appearance and a foul odor. First instar calliphorid larvae were established on the orifices. Calliphorid eggs, early and late first instar larvae extensively covered the head areas. In the adult carcass, Silphids and Staphylinids were observed crawling on top of and underneath the carcass. Dipteran species observed and recorded during the fresh stage persisted on the carcasses to the bloated stage.

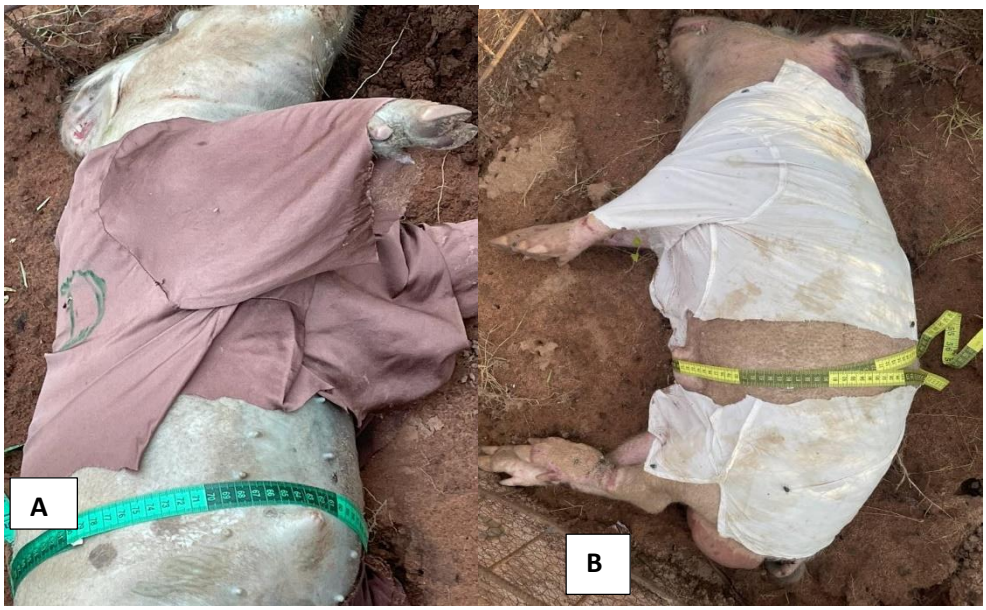


Fig 3 A&B: Images showing big and small carrion in the bloat stage of decay

3.1.3 Active Decay Stage (5-10Days)

This stage was characterized by intense foul odour, exudates were discharged by the body,

By Day 5, the carcass had deflated and the active decay stage began. Adult calliphorids were still abundant and the carcass was literally bubbling with maggot activity. Thousands of first and second instar maggots shrouded the pig carcass. A few adult Muscids were also observed and collected. Presence of black ants and houseflies were noticed. Some maggots had developed into 2nd instar larvae. Cadaver decomposition Island began to form due to purging of decomposition fluid. Red ants predated on some of the maggots thereby impeding the development of CDI at the back region. The clothed areas provided a conducive environment for maggots to thrive. By the end of the 2nd week in the adult pig, maggot activity had greatly reduced but was not restricted to beneath the cloth. Some of the maggots migrated outside the cage. Activity of blowflies had reduced as well. There was also complete exposure and detachment of the tail bone. A huge number of maggots had begun to wander away from the carcass. This indicated the beginning of the advanced decay stage.

Fig 4 A&B: Images showing big and small carrion in the active decay stage of decomposition

3.1.4 Advanced Decay stage (11-21 Days)
On Day 14, there was still maggot activity on the juvenile carcass; however, the largest portion of the maggots had moved away from the carrion. Pre-pupae and pupae were observed in the layers of clothing and were found in the soil near the cages. Many adult sarcophagids, muscids, Cleridae and Meloidae were observed on the carrion and vespids continued to feed on adult flies. For the majority of the days during the advanced stage, the total number of necrophagous flies collected from both carcasses declined at this stage in comparison to the previous stages. *M. domestica* was the only Dipteran species which persisted on pig carcass to this stage. In the adult, there were still small masses of third instar maggots associated with pig carcasses on Day 17. The second generation of S. calcifera was denoted by the presence of teneral adults. Newly emerged S. calcifera were collected from the adult carrion until the last sample day before the end of the study.

Fig 5 A&B: Images showing big and small carrion in the advanced decay stage

3.1.5 Dry Stage (21-60days)

No significant changes occurred in relation to the rate of carrion decay and insect diversity, abundance and/or activity during this period of time. Observations and samples were, therefore, taken on a less frequent basis. The carcass was still moldy, wet and pasty in places. These areas and the drier regions hosted species of insects that were present in the advanced stage. Most insects had retreated within the clothes, underneath the carcass and within skeletonized portions of the head.

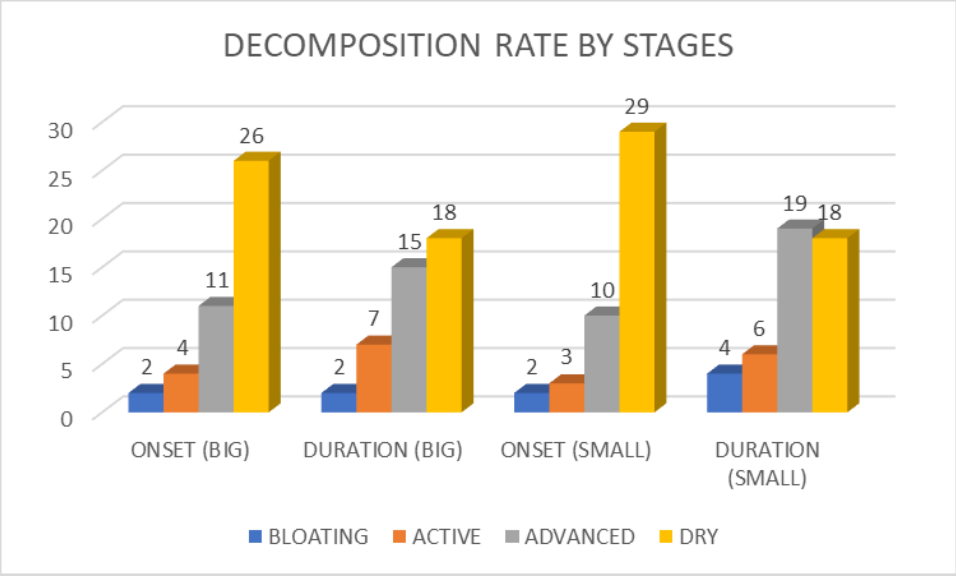
Fig 6 A&B: Images showing big and small carrion in the dry decay stage of decomposition

3.2 Insect Succession

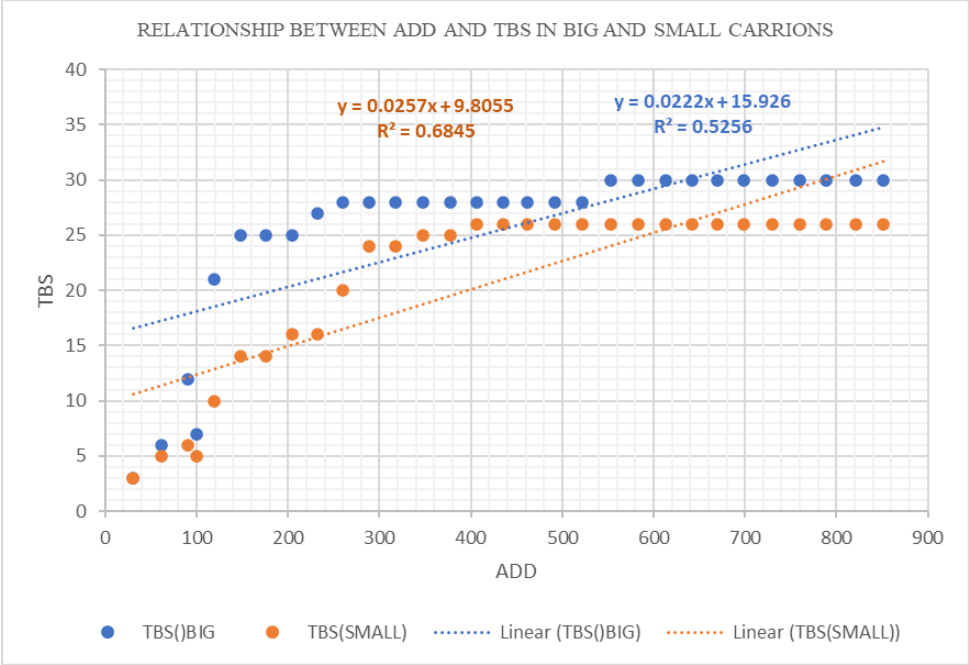
A total of 16 taxa of arthropods, representing four 4 families were collected during both experiments. Only two species were not insects: *Diplopoda juliforma* and *Arachnida Araneae.*

Table 1: Summary of the physical characteristics of decomposition of pig (Sus scrofa domesticus) carcass, sampling times, and the presence of Dipteran and Coleopteran species in big and small pigs

Decomposition Stages	Period (days)	Sampling time	Physical Changes	Foul Odour	Presence of Dipteran Species	Presence of Coleopteran Species
Fresh (0–1 d)	2	9:00 – 11:00	Soft torso Flexible limbs	None	Yes	No
Bloated (2–4 d)	3	9:00 – 11:00	Body color darkened	Present	Yes	Yes
Active (5-10d)	6	9:00 – 11:00	Skin peeling commenced	Present and intense	Yes	Yes
Advanced (11-21d)	11	9:00 – 11:00	Extensive peeling of skin Dry skin Loss of soft tissue	Present	Yes	Yes
Dry (21-60)		9:00 – 11:00	No moisture on carcass Dry skin	None	Yes	Yes



Graph 1: Showing the rates of decomposition compared in onsets and durations of decomposition stages in big and small Sus Scrofa Domesticus models



Graph 2: Showing the relationship between Accumulated Degree Days (ADD) and Total Body Score (TBS)

Table 2: **Abundance of adult Dipteran and Coleopteran species associated with different stages of pig carcasses during different stages of decomposition in big and small carrion models**

Ecological category	Order	Family	Genus/Species	Fresh (0-1d)		Bloat (2-4d)		Active (5-10d)		Advanced (13-21d)		Dry (22-60d)		Total	
				BC	SC	BC	SC	BC	SC	BC	SC	BC	SC	BC	SC
Necrophagous	Diptera	Calliphoridae	Chrysomya marginalis	6	10	22	35	18	40	9	26	0	0	55	111
Necrophagous	Diptera	Calliphoridae	Chrysomya putoria	2	5	4	7	0	0	0	0	0	0	6	12
Necrophagous	Diptera	Calliphoridae	Chrysomya albiceps	4	16	14	38	9	30	7	15	0	0	32	99
Necrophagous	Dipterab	Calliphoridae	Chrysomya chloropyga	5	8	11	13	3	9	0	5	0	0	19	35
Necrophagous	Diptera	Calliphoridae	Lucilia cuprina	5	11	9	15	5	7	0	0	0	0	19	33
Necrophagous	Diptera	Muscidae	Musca domestica	11	22	26	38	26	42	13	19	5	10	81	131
Necrophagous	Diptera	Muscidae	Atherigona soccata	0	0	2	0	0	0	0	0	0	0	2	0
Necrophagous	Diptera	Sarcophagidae	Sarcophaga calcifera	1	4	4	6	1	3	2	7	1	4	9	24
Total Diptera				34	76	92	152	62	131	31	72	6	14	225	445
X2/Fisher exact P-value				P = 0.082a		P=0.150a		P=0.116a		P=0.10a		P=0.00*		P = 0.134a	
Necrophagous	Coleoptera	Dermestidae	Dermestes maculatus	0	0	0	11	18	28	22	34	26	39	66	112
Predators	Coleoptera	Cleridae		0	0	0	0	10	13	18	22	20	27	48	62

Predators	Coleoptera	Silphidae	Thanatophilus micans	0	0	0	4	0	9	2	13	0	18	2	44
Coprophagous	Coleoptera	Scarabaeidae	Onthophagus crassicollis	0	0	0	5	0	12	4	15	5	10	9	42
Incidental	Coleoptera	Meloidae	Hycleus lunatus	0	0	0	4	0	0	0	0	0	0	0	4
Total Coleoptera				0	0	0	24	28	62	46	84	51	94	125	264
X2/Fisher exact P-value				N/A		N/A		P=0.002*		P = 0.08a		P=0.002*		P < 0.001a*	

4.0 Discussion and conclusion

During the interval of June 1 and August 1, 2023, observations of the two pig cadavers yielded significant information concerning the sequence and composition of the local carrion visiting fauna as well as the duration of decay rates in Okuku Nigeria. The predominant orders of insects collected during this study were Diptera (8 species) and Coleoptera (5 species). All Diptera were necrophilic taxa with the predominant family of the Calliphoridae (seven species).

Large numbers of female Calliphoridae were attracted to the remains within minutes after cadaver placement, with oviposition beginning shortly thereafter. This observation is in line with other carrion studies (Payne 2017, Anderson and VanLaerhoven, 2022).

The described colonization of both carcasses by Calliphoridae species highlights the importance of collecting the oldest individuals when estimating the postmortem interval based on insect development. Ants were collected from both carcasses throughout the decomposition period, feeding on both carrion and insects. In some areas, the removal of eggs by ants, particularly fire ants (*Solenopsis sp*.), can have a major effect on decomposition rates (Greenberg, 2021, Payne, 2017). The ant species found in this study, although present throughout decomposition, did not appear to have a major impact on decomposition rates.

Among the Hymenoptera, two parasitic species were observed during the study. A small number of the parasitic wasps were found at the cadaver during the second week Pteromalid wasps, ectoparasitic on-fly pupae of various species and worldwide in distribution (Reed, 2021), are commonly used and sold for control of pest flies on feedlots and dairies. Calliphoridae larvae and pupae was observed attacking second and third-instar larvae in the big pig because pupal parasitoids of blow flies could play a major role in the estimation of the postmortem interval (Forbes & Dadour, 2019; Anderson and Cervenka, 2012) future studies are desirable to investigate this matter. Maggot mass temperature peaked earlier in the big carrion because the temperature probe had to be inserted manually, whereas, in the small pig, the internal carcass temperature peaked when the maggot mass passed the probe.

Maggot migration has been observed in numerous studies on decomposition (Voss *et al*., 2011; Haskell *et al.,* 2022; Carter *et al*., 2017). Post-feeding larvae often migrate away from the body to pupate. After pupation, the next generation of Diptera (flies) become sexually mature and start to look for a carcass on which to deposit their eggs (Haskell *et al*., 2022). However, due to the small size of the facility, the rate of decomposition may have been influenced by the above-mentioned phenomenon. Even though the pigs had been placed a minimum of 10 m from each other and fresh pigs were never placed near pigs in either the fresh or early stages of decomposition, insect migration could have taken place. The overall insect succession, with Diptera and Coleoptera species bdominating the fauna, was consistent with other studies from around the world (Campobasso *et al*., 2016). It is accepted that Diptera numbers increase to a peak during the initial stages of decomposition and Coleoptera numbers only increase significantly during the advanced stages of decomposition (Greenberg, 2021). However, in this trial, the adult Diptera appeared to be consistently present with Calliphoridae and Sarcophagidae observed throughout the trial. Cleridae and Dermestidae represent the major Coleoptera species in most insect successional studies (8, 38, and 40). The

Dermestidae, D. maculatus, and Cleridae, N. rufipes, numbers were relatively high in the early stages of active decay and steadily increased to reach their peak during the later stages of active decay and in the advanced stages of decomposition. The D. maculatus larvae were observed in the mid to later stage of active decay. These larvae were recorded on the clothed control and all the wrapped carcasses before the control carcass. This suggests that the presence of the clothing and wrapping may have allowed the D. maculatus adults to breed sooner, as the larvae were observed feeding on the fibers of the clothing. They were, therefore, present when the maggot numbers were at their largest

Slight variation in the decay rates of the two studied cadavers was observed similar to that of other studies (Anderson and VanLearhoven, 2022, Rodriguez, 2020, Payne, 2017).

In addition, as the current study shows, egg patches or first instar larvae are not easily washed away by rain, as might be the case with unclothed cadavers (Anderson and VanLaerhoven, 2022). Clothing may also slow down postmortem body cooling and favor the onset of the putrefaction process one important observation was the ability of the maggot mass to alter the clothing pattern within a very short period of time. These findings are in line with the results of Komar and Beattie (2019), who found that postmortem insect activity frequently reproduces the sexual disarray in clothing patterns seen in cases of sexual assault and sexual homicide.

The presence of clothes was not shown to influence the invasion of arthropods, and their presence did not affect the oviposition sites. The use of large and small freshly killed and clothed pig carcasses as surrogate human models in this study resulted in a decomposition pattern similar to those observed in actual forensic cases. Clothes on a cadaver do not delay oviposition, but clothes permeated with lubricants, paint, or combustibles may double the time of initial colonization and retard decomposition. Conversely, as the clothing gets soiled with blood, urine or fluids leaking in the course of decomposition, it provides more sites for oviposition than a naked corpse, resulting in larger larval masses and, hence, faster decomposition.

Conflict of interest

The authors whose names are listed in this manuscript declare no conflict of interest. All Co-Authors have seen and agreed with the contents of this manuscript.

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