Morgan S. Varn, Melissa Buchanan, Deb Midence, Aaryan Brooks, Alexis Barber
New College of Interdisciplinary Arts and Sciences
Arizona State University
4139 Delree Street
West Columbia, SC 29170

November 4, 2022

Dear Dr. Picard and Dr. Brundage,

We wish to submit an original research article entitled “A Comparison of Insect Succession on Burned and Unburned Porcine Cadavers” for consideration by the Journal of Forensic Entomology. We confirm that this work is original and has not been published elsewhere, nor is it currently under consideration for publication elsewhere.

In this paper, we show that arson damage to a cadaver would substantially impede oviposition by arthropods, thereby obstructing the determination of minimum post-mortem interval (PMI_{min}). This is significant because insect succession has been demonstrated to be a contributing factor in determining time of death (TOD), yet very few entomological studies exist on the subject of insect succession and arson. We believe that this manuscript is appropriate for publication by the Journal of Forensic Entomology because it formulates the intersection of forensic science and entomology by demonstrating the delay and reduced oviposition in arson cases, specifically in the cases of Crow-Glassman Scale burn levels 2 and 3. We are eager to share our findings with other forensic entomology researchers as well as practitioners and other members of the criminal justice system.

We believe that our research is a significant demonstration of the need for a standardized reduction scale of entomological successional variance utilizing the Crow-Glassman Scale of arson and similar studies to ours. This could potentially assist forensic entomologists to adjust for arson damage in cadavers when determining PMI_{min}, and by extension, TOD. We truly believe the readership of the journal would be interested in our research.

We have no conflicts of interest to disclose.

Please address all correspondence concerning this manuscript to me at msvarn@email.sc.edu.

Thank you for your consideration of this manuscript.

Sincerely,

Morgan S. Varn

A Comparison of Insect Succession on Burned and Unburned Porcine Cadavers

Morgan S. Varn[1]*, Melissa Buchanan[1], Deb Midence[1], Aaryan Brooks[1], Alexis Barber[1]

[1]School of Forensic Sciences, New College of Interdisciplinary Arts and Sciences, Arizona State University, 13591 N 47th Ave, Phoenix, AZ 85051
*Corresponding author: msvarn@email.sc.edu

Abstract: This study highlights the significance of entomological evidence in cases of arson by utilizing the Crow-Glassman Scale, a standardized measurement consisting of five levels (CGS-1 to CGS-5) used to depict different levels of destruction to the body relative to burn injury. CGS levels two (CGS-2) and three (CGS-3) were performed on two of the porcine carcasses, while the third remained as the control. The three carcasses were observed directly to measure variance in insect succession caused by intentional arson. As PMI_{min} can be determined by observation of insect succession, we hypothesized that arson damage to a cadaver would substantially impede oviposition by arthropods, thereby extending the PMI_{min} determination. Insect succession has been demonstrated to be a contributing factor in determining TOD, yet very few entomological studies exist on the subject of insect succession and arson. Our work demonstrates the need for further studies in this subject.

Keywords: Forensic Entomology, Arson, Time of Death (TOD), Time of Colonization (TOC), Insect Succession, Insect Oviposition, Post-Mortem Interval

Introduction

Insect and arthropod observations were first used as forensic indicators in the 13th century by Sung Tz'u, a Chinese physician and the first forensic scientist to record his entomological findings when he penned his medicolegal observations in *The Washing Away of Wrongs [Original Title: Hsi Yuan Chi Lu]*, a work some consider to be the world's first systematic treatise on forensic medicine.[1] Although forensic entomology found usage in medicolegal settings, the value of insect evidence was not largely recognized until the beginning of the twentieth century, when entomological studies proved to be one of the most important tools in determining time since death. This is especially true in the later stages of decomposition when other forensic medical approaches are no longer helpful in this determination.[6]

Entomological data has been shown to assist in determining time of death (TOD) by determining the minimum postmortem interval (PMI_{min}) via time of colonization (TOC).[5] Necrophagous arthropods have a life cycle that is split into predictable and distinct stages, each of which leave some physical evidence (adult flies, eggs, larvae, pupae, or a combination of all of these on or near the body or where a body has been).[4] The arthropod evidence is then collected and preserved so that identification of the species can be performed. This data is combined with environmental data, established information about arthropod eating preferences, and determination of larval stage. The environmental information aids in determining approximately how fast the arthropods were growing throughout the life stages, and the presence or absence of certain arthropods assists in determining the stage of decomposition. This data is then analyzed to determine the TOC, which provides information as to the shortest possible time of death, or the PMI_{min}. Unusual arthropod succession can also be used to determine perimortem or postmortem injuries that may no longer be visible due to latent stages of decomposition, as well as unusual methods of cadaver disposition.[4]

Insect succession on carrion is highly variable in that geographical location, local ambient temperature, stage of decomposition, endemic species, access to the carrion matter, and excess perimortem or postmortem damage to the carcass can affect the initial time of colonization. As arthropods are ectothermic, the rate of development from egg to adult is temperature-dependent; therefore, tracking the local temperature by using the nearest weather station information is critical to the determination of time of colonization.[4] Arthropods are attracted to carrion and other decaying matter via detection of volatile organic compounds (VOCs). Arthropod species have differentiated preferences for VOCs released during each stage of decomposition, which ensures resource partitioning to limit arthropod competition for resources.[9] Upon discovery, adults will oviposit in any available orifice or wound, sometimes laying hundreds of eggs at a time. As the eggs hatch, the larval stage will feed continuously as it matures. During the last stage of larval maturation, the larvae will migrate (in most cases), to pupate safely away from other species, leaving the carrion for the next arthropod group in the succession. Although the pupal stage is largely stationary, pupal evidence is frequently missed during crime scene investigation.[8] The adult emerges from the pupa and may oviposit on the existing cadaver, or may find new, fresher carrion for oviposition.

As fire is an effective method for concealing evidence, burning is one of the most prevalent methods for cadaver disposal. This method can obscure the identity of the decedent, the manner and cause of death, and the location and time of death.[5] Despite this, few entomological studies on the subject are available.[5] In forensic studies, the Crow-Glassman Scale

(CGS) has been used to standardize descriptions of levels of burn damage to human bodies.[3] The extent of burn injury is delineated into five levels as described by Glassman and Crow: [5]

a. CGS-1: Death by fumes. Visual identification possible. First and second degree burns on the body.

b. CGS-2: Dental or DNA used for identification. Significant carbonization of the body and possible mutilation of tarsals and carpals.

c. CGS-3: Possible identification with dental records or DNA. Significant carbonization of body, skull intact, but mutilation of limbs possible.

d. CGS-4: Possible identification with dental records or DNA. Total fragmentation of skull and limbs.

e. CGS-5: Highly complex/problematic identification of remains. Purely skeletal with no soft tissue or remaining identifiable skeletal component.

The purpose of this study is to analyze the growth and development of blowflies on burned and unburned pig cadavers to determine if burning a cadaver affects insect succession and oviposition.

Materials and Methods

Three porcine feet were obtained from a local butcher shop to serve as cadavers. The fresh porcine remained covered while the other two were burned to simulate arson victims using CGS-2 and CGS-3 of the Crow-Glassman Scale (CGS). The cadavers were placed in a tinfoil “boat” inside of a 12ft. x 16ft. covered outdoor enclosure simultaneously. This served as the time of death (TOD). The enclosure was lined with chicken wire to allow access for the insects, but also served as protection from local wildlife and other scavengers (**Figure 1**). The accelerant for the arson simulation was approximately 237 mL of Publix QuickLight Lighter fluid, which does not serve as a deterrent to the necrophagous species.

(Figure 1). Outdoor enclosure the cadavers were placed in.

All cadavers were observed via a Youtube livestream for insect activity. Direct observations were also performed daily. The local weather was recorded daily from the Weather Underground (WU)- Columbia Metropolitan Airport Station, located approximately 5 km from the experimental site.

Adult insect and larval samples were collected for preservation and rearing throughout the observation period (13 days). The larvae selected for rearing observations were placed in an indoor, temperature-controlled enclosure with raw beef liver until pupation and hatching occurred. Other larval specimens were killed in hot water and preserved in 70% reagent-grade alcohol. Larvae and caught adult specimens were examined microscopically for identification to assist in the completion of three comparative succession charts (**Tables 1, 2, 3**).

After identifying the necrophagous species and comparing their growth to existing data which utilized the temperature and stage of larvae, a numeric range demonstrated the difference in succession between a fresh, CGS-2, and CGS-3 cadaver.

Hazards and Safety Precautions

Appropriate fire safety measures were taken when the pig cadavers were burned, including placing the pig cadavers into a burn pit for the simulated arson. Masks were worn to avoid inhalation of any hazardous fumes while burning. Gloves were worn when handling the decomposing flesh and live insect specimens.

Results

FIGURE 2. *Images of insect succession on burned and unburned porcine cadavers.* Photographs were taken between 09/05/22 and 09/18/22 to demonstrate the insect succession between fresh, CGS-2, and CGS-3 porcine cadavers.

FIGURE 3 - *Days postmortem vs. number of flies observed*

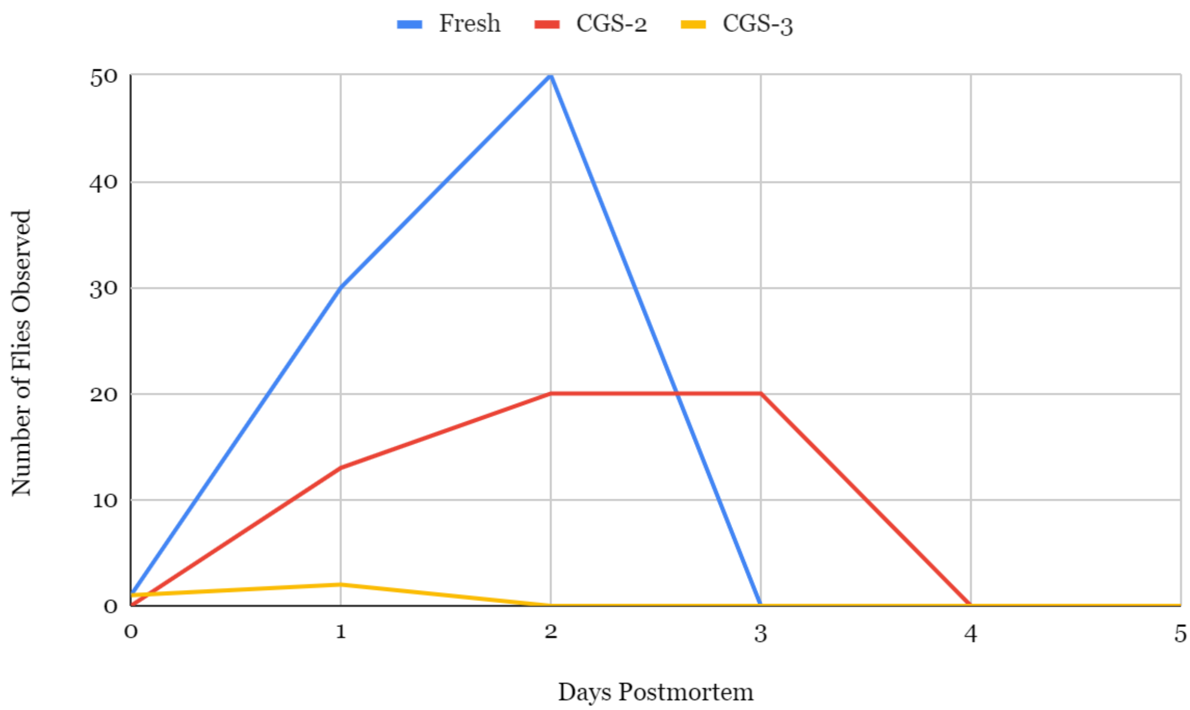

FIGURE 4 - *Days postmortem vs. number of beetles observed*

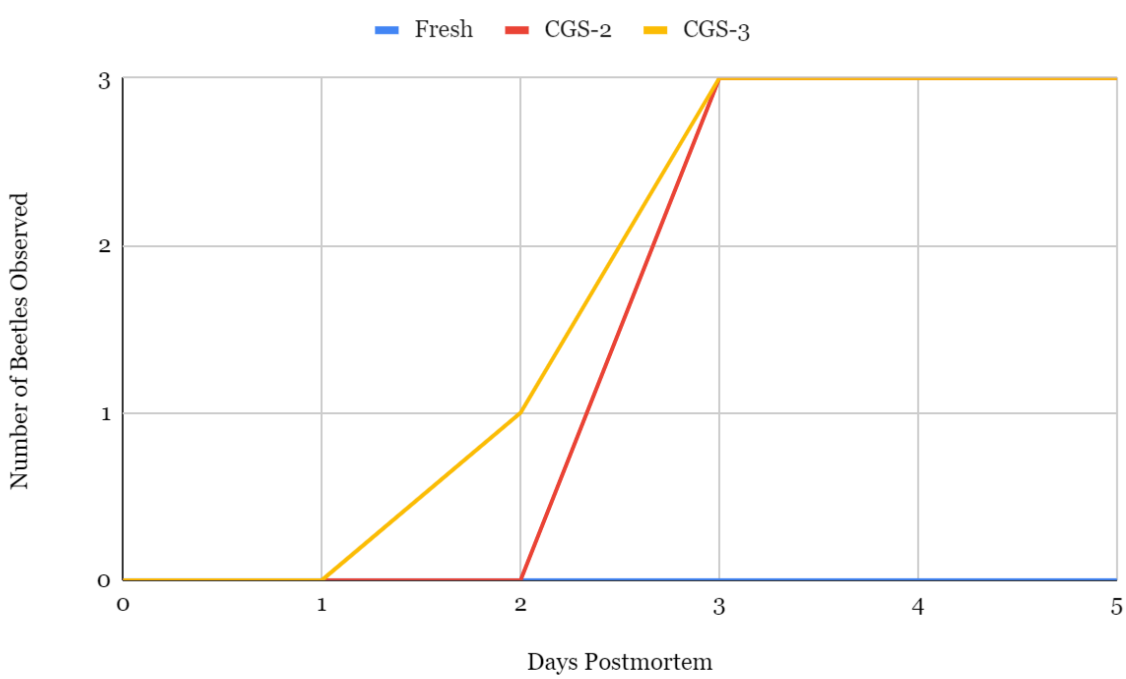

FIGURE 5 - *Days postmortem vs. number of earwigs observed*

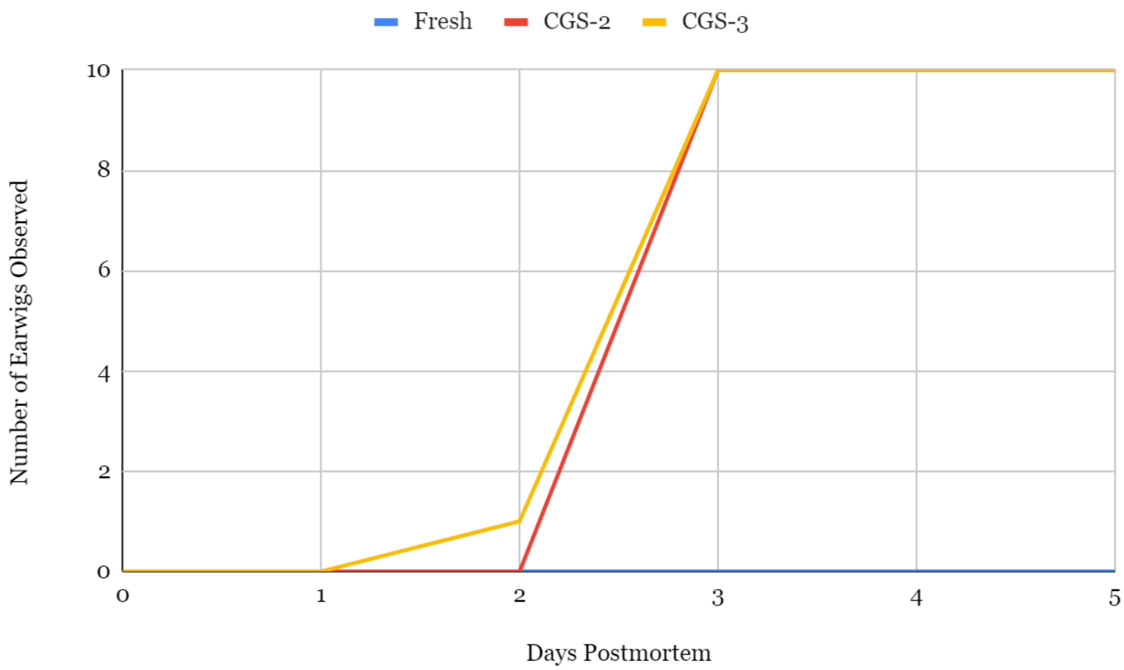

TABLE 1 - *Insect succession on fresh porcine cadaver*

		Days Postmortem					
Insect Family	**Common Name**	0	1	2	3	4	5
Calliphoridae	blowflies	■	■	■			
Muscidae	muscid flies		■	■			
Sarcophagidae	flesh flies		■	■			
Histeridae	clown beetles						
Forficulidae	earwig						

TABLE 2 - *Insect succession on CGS-2 porcine cadaver*

		Days Postmortem					
Insect Family	**Common Name**	0	1	2	3	4	5
Calliphoridae	blowflies		■				
Muscidae	muscid flies		■	■			
Sarcophagidae	flesh flies		■	■	■		
Histeridae	clown beetles				■	■	■
Forficulidae	earwig				■	■	■

TABLE 3 - *Insect succession on CGS-3 porcine cadaver*

		Days Postmortem					
Insect Family	**Common Name**	0	1	2	3	4	5
Calliphoridae	blowflies	■	■				
Muscidae	muscid flies		■				
Sarcophagidae	flesh flies		■				
Histeridae	clown beetles			■	■	■	■
Forficulidae	earwig			■	■	■	■

Table 4 - *Daily and average yearly temperatures*

Days Post Mortem	0	1	2	3	4	5	6	7	8	9	10	11	12	13
Daily Temperature (°C)	33.1	32.8	31.7	27.8	27.8	29.4	30.5	31.7	29.4	29	28.3	29.4	28.9	30
Average Yearly Temperature (°C)	33.2	33.1	31	30.9	36.7	31.7	30.5	30.3	30.3	30.2	30	30	29.7	29.6

Discussion and Conclusion

The unburned cadaver (control) had visible egg masses by day one and developed large maggot masses within two days post mortem. Blowflies were attracted to the cadaver within minutes of being placed in the outdoor enclosure. Flies overlooked CSG-2 and CSG-3 in favor of the fresh control. Within three days, one small maggot mass was present on CGS-2, and no maggot activity was observed on CGS-3 (**Table 2, Table 3**). By day five, there was a decrease in maggot activity on the dorsal surface of the fresh porcine, however, a few Histeridae (Gyllenhal, 1808) beetles were observed on CGS-2 and CGS-3 (**Figure 4**). It was also observed that the flies favored the fresh cadaver over the burned cadavers CGS-2 and CGS-3 (**Figure 3**); this was likely due to the moisture and softs fats that remained on the unburned cadaver and the difference in VOCs released by the burned cadavers. CGS-3 was the most severely burned and therefore did not attract many flies or develop maggot masses. The flies most likely avoided cadaver CGS-3 because it lacked the moisture and soft fats that are preferable for the larvae.

The temperature, although fairly consistent, could have affected the study. Since flies are ectothermic, the rate of development from egg to adult is temperature-dependent. Data collection began with a few days that were warmer than the yearly average. It is unlikely that this greatly impacted the study. When the preserved maggots were studied microscopically from the masses on the control and CGS-2 cadaver, the maggots were aged at third instar and second instar stages, respectively, indicating a clear deviance in development despite identical placement and environmental conditions (**Figure 2**). It is extremely probable that the CGS-3 level burn is severe enough to prevent maggot masses entirely, in which case, increased study of other necrophagous insects must be conducted in order to determine a method for estimating the TOD scale for arson victims with CGS-3 level burns and below.

This study found that insect succession and oviposition are highly affected by arson. The act of burning a corpse severely inhibits the development of larvae on the corpse and the severity of the burns delays the development of the larvae. Furthermore, Diptera (Linnaeus, 1758) did not oviposit on CGS-3, causing insect succession to be non-conforming with the predictable succession on the fresh carcass. This would disrupt the TOC calculation, as general oviposition was inhibited or negated all together and succession produced arthropods typically attracted to later stage decomposition, possibly giving the impression that the carcass had been exposed for a much longer period of time. If TOC is used to determine TOD, entomological analysis would provide a TOC that was much later. This would impede forensic investigations due to the remaining question of TOD.

The found delay in insect succession and oviposition and colonization by late-stage decomposition feeders, requires further study to determine the PMI_{min} based on entomological evidence in found arson cases, possibly utilizing the Crow-Glassman scale on succession studies to standardize the difference in PMI_{min} between burned and unburned cadavers in the Southeastern United States.

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