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The effects of insect on soft tissue decomposition

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THE EFFECTS OF INSECT REPELLANT ON SOFT TISSUE DECOMPOSITION

by

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Abstract

A primary goal of the forensic anthropologist is assisting in the estimation of a post-mortem interval. This assessment is largely based upon the degree and quality of soft tissue decomposition, influenced by factors including temperature, humidity, insect activity, carnivore and rodent activity, perimortem trauma and the depositional environment. While the effects of temperature on decomposition have been long appreciated and initially studied, little or no research has been conducted on the disruption of insect activity and how that disturbance may affect the decomposition process. This study was designed to determine if the exposure of skin surface of porcine remains to insect repellant (specifically, DEET) has an effect on the presence and overall activity of insects during decomposition. Two experiments were conducted in the spring and fall with results indicating that insect repellant slows the rate of decomposition. Such findings are important for criminal investigators requiring an accurate estimation of post-mortem time to appreciate those factors that may adversely affect the process and rate of soft tissue deterioration.

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1.1 *Introduction*

The medico-legal investigation of mutilated, fragmented, decomposed and skeletonized remains is often facilitated by a forensic anthropologist. While medical examiners are highly trained in the analysis of soft tissues (such as skin and organs) and using alterations in these tissues for determining the cause and manner of death, when remains are incomplete and soft tissues are absent or cannot be analyzed, they often request the assistance of a forensic anthropologist. Forensic anthropologists specialize in the analysis of skeletal remains, but also frequently examine those bodies that are somewhere between fresh and skeletonized.

The goals of these anthropological analyses are highly varied, and include the determination of whether the material in question is bone, determining whether the remains are human versus some other animal, estimating the number of individuals represented, estimating the post-mortem interval (PMI or time since death-TSD), estimating the individual's biological profile (such as their age, sex, ancestry and stature), analyzing the intensity and character of any trauma, to include antemortem trauma, perimortem trauma, and postmortem damage, and comparing antemortem and postmortem information to determine whether they could have originated from the same individual (Byers, 2008). This information assists the medical examiner, pathologist or coroner with determining the victim's identity, assessing the cause and manner of death (Megyesi et. al., 2005), and offers insights pertaining to the perimortem circumstances of the victim and any suspects (Geberth, 2007; Goff, 2000). Establishing the PMI in cases involving skeletal, decomposed, or unidentified human remains is difficult because decomposition rates can be significantly altered by such factors as the perimortem conditions of the remains (Marks and Love, 2003), temperature, depositional contexts (Mann et al., 1990), degree of insect infestation, and level of carnivore and rodent disturbance.

It has been demonstrated repeatedly that temperature and insect activity have the greatest impact on both the rate and process of decomposition. While the effects of temperature and insect activity have been heavily studied, little research has been aimed at determining how these factors affect decomposition when the normal processes are disrupted. For example, it is not known how decomposition rates are affected if the otherwise normal access to the remains by insects is disrupted by the addition of chemicals. This study was designed to determine whether the treatment of a skin surface with insect repellant has an effect on the presence and activity of insects during decomposition, which could alter the overall rate of decomposition. Artificially altered decomposition rates due to chemicals not found in the natural environment could potentially affect the ability to accurately determine the post mortem interval.

It is hypothesized that the insect repellant will decrease the amount of adult and larval insect activity on a carcass by inhibiting or deterring access to the carcass by the insects. This is an important variable to consider because insect infestation of a carcass serves as a prominent external factor involved in decomposition. A recent case report described the dousing of the victim in "bug spray' in an attempt to mask of the odor of decomposition (Vass, 2001). The application of insect repellant may, however, have also inadvertently altered the rate of decomposition (Vass, 2001). Although it was known in this case that insect repellant was applied, it is not currently known how this affects the rate of decomposition. Knowledge of the effect of insecticide in arresting or enhancing decomposition would have been vital to the investigation and could assist in determining whether such evidence should affect the postmortem interval.

1.2 *Decomposition*

Soft tissue decomposition begins at death as a continual microscopic and biochemical process occurring both internally and externally. Internal decomposition is initiated microbiologically at death continuing until the remains are skeletonized (Marks et al., 2003). Cell autolysis (or cell self-destruction, the destruction of a cell through the action of its own enzymes) represents the earliest biochemical process causing the initial microscopic tissue deterioration. The generally accepted order in which the main body tissues decompose is well-described by Gill-King (1997) progressing from intestines, stomach, accessory organs of digestion, heart, blood, circulation, heart muscle, air passages, lungs, kidneys, bladder, brain, nervous tissue, skeletal muscle, connective tissue and integument and eventually, bone.

When the body initiates decomposition, the processes of autolysis and cell death begins to degenerate the skin tissue causing the skin to exhibit a pale color. Breakdown of the cells between the epidermis and dermis causes skin slippage, or removal of the epidermal layer. Understanding the continuous process of decomposition and related factors that can alter the rates of decay is the concerted role of the pathologist, the anthropologist and entomologist. This collaboration is crucial for forensic evidence in death investigations (Mann et al., 1990).

Internally, algor, livor and rigor mortis are the initial pathophysiological processes that breakdown and alter the tissues of the body immediately upon death. Algor mortis is the cooling of the body after death, as it approached the ambient temperature. Livor mortis refers to the settling of the blood in gravity-dependent areas; when the heart is no longer forcing blood to circulate through the body, it succumbs to gravity and settles in the lowest parts. Rigor mortis is the stiffening of the muscles caused by chemical changes. While the role of each of these processes during decomposition is understood, many external factors that can alter the onset, rate, and process of decomposition, have not been as widely studied. Further, the presence of various chemicals on the remains and their impact on the rate and process of decomposition has not been studied.

It is known that chemicals such as the embalming fluid, formalin, flammable substances, lime and insecticides, among others, may affect decomposition. These chemicals have the effect of delaying the onset of insect activity by hours or days or may even permanently prevent insect colonization. It has been shown, for example, that certain insects will not land on a hand removed from the body for fingerprinting and placed in a jar of formalin. Additionally, when the hand was put back with the still decaying exposed body, the maggots would not feed on it (Mann et al., 1990). Of specific interest for this study is the potential effect of insecticides on decomposition. While few studies have investigated this question, Gunatilake and Goff (1989) and Goff (2009) demonstrated that the presence of insecticides will not permanently delay insect colonization on the body and that the immature insects can survive on a body with insecticide concentrations that to an adult of the same species would be fatal.

General stages of the decomposition process based on the physical appearance and internal temperatures of the remains and presence and nature of insect populations have been widely documented and generally accepted (Kreitlow, 2010; Payne, 1965; for review see Marks et al., 2009; Morris and Dadour, 2005). However, investigators need keep in mind that these stages are approximations since decomposition can be significantly affected by numerous factors.

During the fresh stage (approximately 1-2 days after death) there are no physical signs of decomposition even though internal chemical breakdown has begun. No odor is usually associated with the carcass at this time (Anderson et al., 1996, Grassberger 2004 and Megyesi et al., 2005). The fresh stage is further described as the beginning of decomposition following death with no visible maggot activity and will continue until visible signs of bloating begin (Galloway et al., 1989). This stage begins at the time of death and ends when bloating is recognizable. Insects discover remains and begin first ovipositing (laying eggs). Internal decomposition is occurring during this stage, with initial autolysis and putrefaction, but little notable external surface deterioration observed.

The first insects to arriving during the fresh stage are usually blowflies (Calliphoridae). These have been reported to arrive within minutes of exposure (Marks, 2012 personal communication), and begin egg deposition within hours. Eggs are typically laid in or near the natural orifices of the head, anus and ground body interface as well as well as at the site of perimortem wounds (Anderson, 1996). Depending on the rate of decomposition and the developmental chronology of particular blowfly species, eggs hatch and first stage/instar larvae begin to ingest liquids while the carcass is still classified in the fresh stage (Rodriguez and Bass 1983). Adult ants are not uncommon on the body surface during the fresh stage possibly feeding on eggs and young larvae (Payne, 1965).

The early decomposition stage (approximately 2-7 days after death) is characterized by bloating, skin slippage, hair loss, and discoloration (Galloway et al., 1989). There will be an increase in insect activity Calliphoridae (blow flies) and Sarcophagidae (flesh flies), the forming of maggot masses (Goff, 2009). Also, at this stage there will be the purging of gases and fluids leading to a strong increase in odor (Payne, 1965). Putrefaction is recognized first in this stage as gases are produced that cause noticeable inflation of the abdomen. Arthropod activities combined with the putrefaction processes create increased internal temperatures. Bloating is a byproduct of the putrefaction process initiated by decomposition of internal tissues by bacteria and other microorganisms with a marked green discoloration of the skin due to the accumulation of hydrogen sulfide in the blood (Goff, 2009, Vass et al., 2002). Vass et al., (2002) documented that the accumulation of gases such as methane, carbon dioxide, ammonia and hydrogen sulfide in decomposing remains may cause rotation.

The most significant quantity/activity of insects during this time is the adult Diptera. By the fourth day, first-instar and early second-instar stage Diptera are typical. Blowflies usually remain in great numbers during the bloat stage and blowflies, flesh flies and muscids continue egg laying. Also during this stage, ants feed on the eggs and young larvae of flies (Payne 1965; Grassberger 2004). The first species of Coleoptera arrive during the bloat stage of decomposition, including members of the families Staphylinidae (rove beetles), Silphidae (carrion beetles) and Cleridae. These beetles feed on fly eggs and larvae (Anderson, 1996; Gennard, 2007; Grassberger 2004).

During the advanced decomposition stage (approximately 5-23 days after death) there is “moist sagging” of the tissue and increased maggot activity (Galloway et al., 1989). Thoracic and abdominal body cavities are penetrated during this the decay stage resulting in the deflation of the thoraco-abdominal surfaces. Decaying odors are high with a significant weight decrease by 10th day. The larvae, reaching and surpassing their final instar stage begin to depart or migrate from the corpse seeking to pupate. The carcass still has a wet appearance due to the continued liquefaction of tissues. Skin covering in the head, perineal and abdominal region is mostly removed by larval feeding (Payne, 1965). Feeding larvae of the Calliphoridae are the dominant insects on remains during this stage (Anderson 1996). Adult calliphorids and muscids, decrease in numbers during this stage, and are not observed mating (Payne, 1965). Members of Sepsidae, black scavenger flies, and members of Coleoptera, beetles, become the dominant adult insects at the site of remains (Byrd and Castner, 2010). More fat on the body appears to accelerate decomposition (Gonzales et al., 1954) due to the composition of fat, which is high in water content.

By the end of this stage, Diptera larvae have removed most of the flesh from the carcass, showing skin and cartilage and dry post-bloat changes occur which may show signs of mummification (Galloway et al., 1989). The mummification process may be altered in “non-desert regions of the United States” (Galloway et al., 1989, Megyesi et al., 2005 and Goff 2009). The majority of Diptera larvae leave the carcass during this stage. The remains are typically skeletonized at this stage with some remaining joint or intervertebral disc cartilage, hair and small portions of soft tissue at this stage. This drying stage signals the first mass migration of third instar calliphorid larvae from the carcass. Few adult Calliphoridae are attracted to carcasses in the advanced, post-decay stage unless moisture initiates re-strikes. Adult Dermestidae (skin beetles) begin to colonize the remains (Grassberger, 2004) and while adult beetles may be common, the larval stages are not (Anderson, 1996).

During the early skeletonization phase (approximately 18-90+ days after death), the majority of soft tissue has decomposed to expose bone and insect activity is extensively reduced (Megyesi et al., 2005). As the stage progresses, the majority of the bones that are exposed become dry and any remaining odor is faint (Galloway et al., 1989). The remains become fully skeletonized at this stage with trace amounts of connective soft tissue and contain little or no insect activity. Very few adult calliphorids are attracted to the carcass at this stage (Grassberger, 2004) and adult piophilids, skipper flies, emerge (Anderson, 1996; Byrd and Castner, 2010). Non-carrion insects that commonly arrive at remains in this stage are centipedes, millipedes, isopods, snails and cockroaches (Payne, 1965). The Coleoptera (beetles) provide significant entomological evidence in forensic cases with reference to the dry stage of decomposition. These beetles are also used in the estimation of the minimum PMI and colonization can vary according to geographical regions (Kulshrestha et al., 2001).

1.3 *Forensic Entomology*

Forensic entomology is the application of the study of insect and other arthropods to criminal or missing person cases involving severely decomposed or skeletonized human remains (see Byrd and Castner, 2009). In this context the discipline is associated with death investigations providing the medico-legal community with relevant information about post-mortem time and the circumstances surrounding perimortem wounds based on insect involved. The forensic entomologist uses insect species identification, succession, larval morphology, size, and weights, in combination with calculated accumulated degree day (ADD) for estimations of time of colonization and period of insect activity. When estimating the PMI, forensic entomologists use maggot development and the identity of fly species collected from the remains. This entomologically-based estimation is commonly called the "Time Since Colonization." Entomologists agree that insects colonizing remains arrive in a predictable sequence at different times during the decomposition process and have well studied developmental stages necessary to estimate postmortem time (Anderson et. al., 1996; Catts, 1992, Goff, 1993, Anderson et al., 2002).

Insects begin arriving on deceased remains usually within minutes of death (Goff 2009) with flies (order Diptera) being the first to colonize the body with egg laying behavior. Four categories of insects can be found on decomposing remains: 1) necrophagous species that feed on the remains; 2) predator insects and parasites that feed on the necrophagous species; 3) omnivorous species feeding on the remains and other arthropods such as ants, wasps and some beetles; and 4) other species such as springtails and spiders that use the corpse as part of their environment (Smith, 1986).

As stated, the flies or Diptera are the first insects to arrive at the body and the regular areas of egg laying (ovipositing) are natural body openings and wounds. Once developed, eggs hatch into larva (maggot) that grow rapidly passing through three instars or stages prior to reaching full size. Once full size is reached, feeding stops and the maggots migrate away from the body to begin pupa formation. The outer layer of the maggot's exoskeleton hardens to form a protective encasement. The insect eventually emerges from this encasement as a fly (Joseph et al., 2011; Goff and Lord, 1994).

Low(er) temperatures decrease the presence and activity of blow-flies (Lopes de Carvalho et al., 2001). Higher summer temperatures favor larger maggot masses whereas there is very little insect activity in cold and frozen environments. Payne (1965) demonstrated that frozen fetal porcine carcasses (*Sus scrofa*) placed outside and allowed to thaw were initially free from insects suggesting that insects are more attracted to fresh remains than frozen ones. As thawing commenced, calliphorids arrived and oviposited while the carcasses were partially frozen. Once fully thawed several ant species began feeding and transporting eggs away from the carcasses during thawing.

The ability and interest of insects to lay eggs and feed is affected by body access and other circumstances of body deposition. For example, remains in brightly lit areas are generally inhabited by green blowfly (*Lucilia illustris*). This contrasts to a black bottle fly (*Phormia regina*), which prefers more shaded areas. An aquatic submerged corpse varies in core and ambient temperature being colonized by few terrestrial insects. Flies do frequent exposed body surfaces in drowned bodies after bloating provides floating. Buried bodies have limited colonization by most surface insects. The coffin fly (*Megaselia scalaris*) is one fly species observed on buried bodies given the ability to dig up to six feet underground to reach a body and oviposit. The damage created by scavengers such as coyotes, dogs, rodents and cats, beetles, and other insects feeding on remains can make estimating the timing of insect colonization much more difficult because their feeding and dismemberment patterns may affect the ability of the flies to access the body.

Drugs in the body at death can also affect how quickly the insects can break down the corpse. For example, insect development has been shown to be increased in corpses that contain cocaine, and slowed down by bodies containing chemicals such as arsenic. Studies relating to poison detection have demonstrated that maggots feeding on tissue containing cocaine displayed a shorter total development time compared with cocaine-free maggots (Beyer et al., 1980 and Goff et al., 1991).

Wraps, garments, and clothing also affect the rate of decomposition. Tight fitting tarps can advance decay during warm weather by increasing the temperature around and within the body cavities, yet may inhibit insect access. Loose(r) fitted coverings with open ends may aid colonization of by providing protection from the outside environment, sunlight and predators and serving to "incubate" the remains. Clothing also provides a protective barrier between the body and insects that alter the rate of decomposition. If a corpse is contained within a heavy jacket, this can slow down decomposition in that particular area since insects will usually elect to colonize elsewhere (Voss et al., 2011). During the extreme bloated stages, insect access between the skin and garments is compromised (Marks, personal communication, 2012).

Finally, it has been observed that repellants such as gas, other petroleum-based spirits, perfume, mosquito citronella repellant and insecticides result in a delay of as many as several days in the appearance of the first Diptera species (Charabidze et al., 2009). In particular, the presence of insect repellent and insecticide is the most probable means of a significant reduction or complete cessation of insect activity during the initial stages of decomposition. Understanding a disruption of the normal insect succession and fauna would be critical for forensic entomologists in determining the PMI. In fact, a recent criminal case (Vass, 2001) involved a fully clothed victim in summer with no indication of decomposition or insect activity. Investigators obviously believed the condition of the body signaled a recent homicide. It was eventually determined that the victim was deceased for almost four months. In an attempt to mask the odors of decomposition, the perpetrator had sprayed the body with insect repellant. The "bug spray" altered the rate of decomposition by delaying and/or severely limiting attraction and colonization of the body by insects. The autopsy revealed some internal decomposition, and the chemicals from the repellant were found in the lungs and also to have spread to the rest of the body (Vass, 2001). This case raises very interesting questions regarding the impact of chemical alteration of insect activity slowed down the decomposition rate.

1.4 *Repellents and Pesticides*

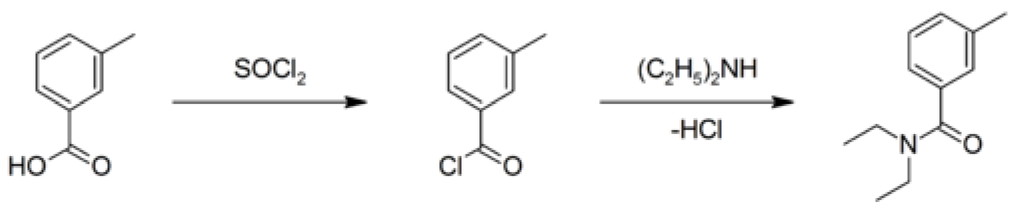
From the time of the first oviposition to the time of discovery of remains represents a minimum PMI estimation. The absence of commonly recognized early colonizers may be due to body placement, or an attempt to conceal the remains during the initial stages of deposition (Archer, 2003) with substances such as insect repellent or insecticide. The presence of repellant substances, if undetected, could lead to an under-estimation of the PMI resulting from an inaccurate age estimation of insect species found on the remains (Campobasso et al., 2001). As described in the earlier case study, certain chemical compounds may have been used in an attempt to conceal the odor of the remains, and these chemicals may have prevented or limited insect colonization.

Pesticides are substances or combinations of substances intended for repelling, halting or killing certain pests (National Resource Council, 2003). A pesticide may be a chemical, a biological agent (such as a viral or bacterial agent), an antimicrobial, a disinfectant, or a device used against any pest. Insecticides are a certain type of pesticide used in agriculture and farming, industry, medicine and residential applications. Pesticides include ovicides (which are used against insect eggs) and larvicides (which are used against insect larvae). Farming insecticide use is one of the biggest factors increasing 20^{th} century agricultural productivity in this country (National Resource Council, 2003). Nearly all insecticides have the potential to alter ecosystems and many are poisonous to humans.

Classification of insecticides is either systemic or contact. Systemic insecticides are incorporated through the interior of treated plants with pest ingestion during feeding. Contact insecticides are toxic to insects via direct contact with the poison. The effectiveness of the pesticide is related to the application quality. Small aerosol droplets are considered the most effective (Pesticide Fact Sheet, 2008). Naturally produced plant insecticides such as nicotine, pyrethrum and neem extracts are secreted by some plants as natural defenses against insects.

N,N-Diethyl-meta-toluamide, better recognized by the abbreviation, DEET, is the most commonly used active ingredient in insect repellents for personal use. It is a topical substance for skin or clothing contact, and provides protection against tick, mosquito and chigger bites and other insects capable of transmitting blood specific diseases. Usually a yellow liquid at room temperature, DEET can be prepared by chemically converting m-toluic acid (3-methylbenzoic acid) to the corresponding acyl chloride and allowing it to react with diethylamine (Wang, 1974; Pavia, 2004) (Figure 1).

Figure 1: Preparation of DEET



As initially developed, DEET was thought to block olfactory receptors for 1-octen-3-ol, a volatile substance that is often contained in human sweat and breath. The prevailing knowledge was that DEET effectively blocks an insect's senses so that the biting/feeding instinct is not triggered by humans or other animals that are associated with these chemicals. DEET does not appear to hamper the insect's ability to smell carbon dioxide as had been earlier suspected (Petherick, 2008; Ditzen et al., 2008). Current evidence shows that DEET serves as a true insect repellent whereby mosquitoes are intensely repelled by the odor of the repellent (Syed and Leal, 2008; Center for Disease Control and Prevention, 2009). A type of olfactory receptor is triggered by DEET along with other well-known repellents such as eucalyptol, linalool, and thujone. In behavioral tests, DEET had a strong repellent activity in the absence of body odor attractants such as 1-octen-3-ol, lactic acid, or carbon dioxide and both female and male mosquitoes show the identical response (Syed and Leal, 2008; Ditzen et al., 2008).

DEET is commercially sold in spray or lotion concentrations up to 100%. Concentrations of 100% DEET provide up to 12 hours of protection while lower concentrations (20%-40%) offer 3–8 hours of protection (National Pesticide Information Center, 2008). The Center for Disease Control recommends a 30-50% DEET concentration to block the spread of pathogens carried by insects (Pavia, 2004). Other research has corroborated the effectiveness of DEET (Wang, 1974). While these chemicals reduce or eliminate insect activity on any surface to which they are applied, there have been no published results at this time from studies investigating the effect of insect repellents or insecticides on the rate and process of soft tissue decomposition. Accordingly, this study of porcine carcasses was performed to qualitatively and quantitatively assess the impact of DEET application on insect activity during decomposition. These findings can provide medicolegal death investigators valuable information to assist in determining the PMI in human remains that have been exposed to these types of chemicals.

Chapter 2 Materials and Methods

This study consisted of two experiments. Each of the two experiments involved the observation of decomposing domestic porcine carcasses, some of which were treated with DEET, and some of which were untreated controls. The two experiments were conducted at two different locations in Virginia during the spring of 2009 (Experiment 1: May 23-June 13) and fall of 2011 (Experiment 2: September 27-October 23). The porcine carcasses were placed in observation housings at each site for protection from carnivore activity.

2.1 *Test specimens*

Domestic porcine carcasses (*Sus scrofa*) were utilized because they are known to be somewhat anatomically and physiologically similar to humans and are used extensively in biomedical and forensic research as proxies for human remains in studies of skeletal trauma and decomposition. For mammals they are relatively hairless and being omnivorous, have a similar constitution of intestinal micro fauna. Moreover, domestic porcine carcasses are easily obtainable, affordable and do not stimulate the same ethical considerations as using human subjects (Swindle and Smith, 1998; Michaud and Moreau, 2011; Matuszewski et al., 2008; Anderson and VanLaerhoven, 1996; Shean et al., 1993; England, 2006; Payne, 1965). When studying decomposition from an entomological perspective for application data to human death investigations, the domestic is considered the preferred human analog (Catts and Goff, 1992) which is why it was selected for this study.

Eight domestic bred porcine carcasses (*Sus scrofa linneaus*) were obtained for this study. Specimens for experiment 1 were obtained from

Nebraska Scientific in Omaha, Nebraska. Specimens for experiment 2 were obtained from a local livestock farmer from Summerduck, Virginia. The specimens used in experiment 1 were euthanized by the captive bolt process with the time of death was recorded. The captive bolt euthanasia process is a humane process in which the domestic porcine carcass is rendered insensible with minimal pain and distress until death. The captive bolt works on the principle of a gun and fires a blank cartridge, which propels a short bolt from the barrel into the midline of the forehead (American Association of Swine Euthanasia, 2008). Captive bolt swine euthanasia is an accepted method by the American Veterinary Medical Association. The specimens used for experiment 2 were euthanized by cranial gunshot, as is typical of farm-raised livestock for food.

2.2 *Observation housing*

Observation housing was used in both experiments. These observation houses were intended to allow insect access and permit easy observation of the specimens by the researcher, while at the same time restricting carnivore access. Carnivore access was restricted in order to prevent disturbance (including consumption, dismemberment and displacement) of the test specimens during the study. Observation houses for the specimens used in experiment 1 were hand constructed by the researcher. The side walls were made from natural stock, non-pressure treated lumber in order to prevent any ancillary chemical interference with insect activity. The tops and bottoms of the housings were constructed of untreated bare metal ½ inch latte. The tops were secured with bare metal wire to allow opening for photography. Due to the fetal sized remains for experiment 1, the housings were compact and portable measuring approximately 18 inches x 18 inches x 10 inches. The structures were secured to the ground using bare metal wire and rebar to prevent any carnivore disturbance.

The observation housings for containing the larger porcine carcasses in experiment 2 were dog kennel cages purchased from the commercial vendor BestPet in Excelsior, Minnesota. These containers were selected for allowing the remains to be easily accessed by insects while restricting carnivore disturbance. The sides and top were black epoxy coated metal wire and the tops were secured with a sliding latch for photography measuring approximately 30 x 24 inches. These containers came shipped with a removable bottom pan that was removed to facilitate exposure of the skin to the ground surface.

Chapter 3 DEET Treated Test Specimens

3.1 *Experiment Number 1*

Experiment 1 utilized four fetal porcine carcasses (*Sus scrofa domesticus*) (Figure 2). The specimens were sealed in plastic and shipped frozen directly to the research site from Nebraska Scientific in Omaha, Nebraska. Each fetal porcine carcass was approximately nine (9) inches long and exempt of internal or external preservatives and unremarkable in appearance, exhibiting a fresh pink color. Two "control" carcasses were placed in the hand constructed observation housings with no DEET insect repellent. One control was placed in the shade and one control was placed in full sun.

Two fetal porcine carcasses were sprayed with Colemans® 40% DEET Insect Repellent Aerosol, prior to placement in the observation housing. The insect repellant was sprayed directly onto the surface of the two porcine carcasses' skin until the skin surface was covered completely. The 40% DEET insect repellent was applied twice to ensure a complete coating. Approximately 2.5 ounces of the 40% DEET was used, per specimen. One DEET treated specimen was placed in the shade and one was placed in full sun.

Figure 2: Specimen from Experiment 1

Research site 1 was located at GPS coordinates 38.426803 N, 77.584596 W, had an approximate elevation of 75 feet, and was located 11.9 miles (19.1 kilometers) northwest of downtown Fredericksburg, Virginia, on privately owned residential land (see aerial image in Figure 3). The property is 10.62 forested acres with a residence. The land consists of mixed timber surrounding the residence. The timber includes northern red oak (*Quercus rubra L.*), sugar maple (*Acer saccharum Marsh*), black walnut (*Juglan nigra L.*), Eastern white pine (*Pinus Strobus L.*) and Virginia pine (*Pinus virginiana*). Various local mammalian species are known to inhabit the land in the vicinity of the research site including red fox (*Vulpes vulpes*), raccoon (*Procyon lotor*), white-tailed deer (*Odocoileus virginianus*), coyote (*Canis latrans*), Virginia opossum (*Didelphis* *virginiana*), black bear (*Ursus americanus*), and skunk (*Mephitidae spp*.). There are a significant number of rodents including field mice (*Apodemus spp*.), old world rats (*Rattus spp.*), ground squirrels (*Spermophilus spp*.) and a variety of moles (*Scapanus spp*.) near the residential property. The area is also home to several species of birds including the American robin (*Turdus migratorius*), American crow (*Corvus brachyrhynchos*), and bluebird (*Sialia sialis*). There is a large variety of insects at the residential property including various flies and beetles, e.g., American Carrion Beetle (*Necrophila americana*).

Figure 3: Aerial View of Site 1

The research site for experiment 1 covers an area approximately 40 feet in its north-to-south dimension and 20 feet in its east-to-west dimension. The site is located 40 yards northwest of Rainwater Lane on the west side of the property. The research site was selected because it provides a mixture of vegetation and access to a large area with both full shade and full sun. The porcine carcasses were placed in their observation housings and positioned approximately three feet apart within the research site (see Figure 4 and Figure 5).

Figure 4: Specimens in Observation Housing at Site 1

Figure 5: DEET Sun Specimen at Site 1

3.2 *Experiment Number 2*

Experiment 2 utilized four porcine carcasses (*Sus scrofa domesticus*) obtained fresh from a local livestock farmer (Figure 6). All experiment 2 specimens were euthanized with a single gunshot to the head. The specimens were retrieved from the farmer’s food processing facility on September 27, 2012 at approximately 12:00 pm EST. Each specimen was wrapped in a clean plastic tarp to transport them to the rear of a large pickup truck. The specimens were then immediately driven to the research site and placed in their observation housings by approximately 2:00 pm EST (see Figures 7 and 8). Each specimen was approximately 32-40 inches in length weighing approximately 40-50 pounds and was three to four months old. At this site, as in experiment 1, two “control” carcasses were placed in their observation housing with no DEET applied. One control specimen was placed in shade (see Figure 9) one was placed in full sun. Two carcasses were treated with Colemans® 40% DEET Insect Repellent Aerosol. Because these pigs were significantly larger than the fetal porcine carcasses at site 1, they required larger quantities of repellent to cover the larger skin surface. Approximately 30 ounces were used to completely cover the surface area of the two specimens, or approximately 15 per specimen.

Figure 6: Specimens from Experiment 2

Figure 7: Aerial View of Site 2

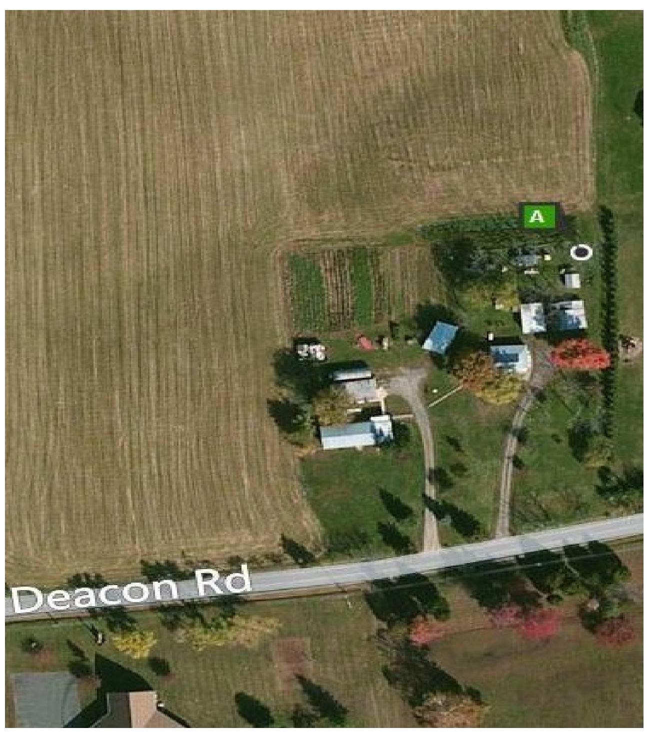


Figure 8: Specimens in Observation Housing at Site 2

Figure 9: DEET Shade Specimen at Site 2

Research site 2 was located at GPS coordinates 38.330306 N, 77.426939 W, had an approximate elevation of 80 feet, and was located approximately 4.3 miles (7.0 kilometers) northeast of Fredericksburg, Virginia on privately owned 20 acreage farm and forestland, The farmland is utilized to grow assorted vegetables, as well as hay. The farmland contains multiple storage structures and two residential houses. The timber at this location includes northern red oak (*Quercus rubra L*.), black walnut (*Juglan nigra L*.) and sugar maple (*Acer saccharum marsh)* reported from the Virginia Department of Forestry.

The many varieties of mammalian species at the farmland location included red fox (*Vulpes vulpes*), raccoon (*Procyon lotor*), coyote (*Canis latrans*), white-tailed deer (*Odocoileus virginianus*), Virginia opossum (*Didelphis virginiana*), black bear (*Ursus americanus*), and skunks (*Mephitidae spp.*). The farmland has a large number of rodents including field mice (*Apodemus spp.*) old world rats (*Rattus spp.*), ground squirrels (*Spermophilus spp.*), and a variety of moles (*Scapanus spp.*). The research area is home to species of birds to include the American robin (*Turdus migratorius*), bald eagle (*Haliaeetus leucocephalus*), American crow (*Corvus brachyrhynchos*), and bluebird (*Sialia sialis*). There is a large variety of insects including flies and beetles.

The second research site covered approximately 40 feet in its north-to-south dimension and 20 feet in its east-to-west dimension. The site was located 60 yards north of Deacon Road to the east side of the property. The site was selected because it offered an ideal mixture of vegetation and access to a large area with areas of full shade and full sun. The area also offered ease of observation and monitoring with last hay harvesting in July, 2011.

3.3 *Temperature Monitoring*

Temperature monitoring for this study included both ambient temperature and individual specimen temperatures, and both were collected daily throughout the study. The specimen temperatures were monitored using a Micro Temp MT-Pro pistol grip infrared thermometer with a range of -76 degrees F to 932 degrees F. This thermometer allowed skin temperature recordings without physical contact. Temperatures were taken twice daily during each experiment at approximately 6:30 a.m. and 6:00 p.m. All temperature data was hand recorded.

Ambient temperature was monitored using a National Oceanic and Atmospheric Administration (NOAA) climate-radar station located at the Fredericksburg, Virginia sewage plant, GPS coordinates 38.2875 N, 77.4507 W. This climate-radar site, the National Climatic Data Center (NCDC), can be monitored via their website (NOAA Satellite and Information Service). The station is located approximately 15.7 miles (25.2 kilometers) from the site of experiment 1 and approximately 5.4 miles (8.6 kilometers) from the site of experiment 2.

Ambient temperature data was collected daily. This temperature data was used to calculate the accumulated degree days (ADD). This unit of measure is an important value reflecting the external thermal energy which is a major factor influencing the rate of decomposition. As calculated, the ADD is a uniform unit that takes into account the sun's total accumulated thermal energy despite the time of day and the seasonal and regional ambient changes. The ADD standardizes time and temperature as a unit of thermal energy allowing data to be collected in a specific context of environmental conditions for comparison with similarly collected data from other specific environments. The recorded temperature data were used to calculate the ADD following Megyesi et al. (2005). Utilizing the NOAA weather data from the Fredericksburg, Virginia sewage climate-radar station, maximum and minimum temperatures were averaged to arrive at a daily average temperature.

3.4 *Photographic Documentation*

All carcasses were photographed twice daily at 6:30 a.m. and 6:00 p.m. Photographs were taken using a Nikon Coolpix P80 digital camera. This camera was selected based on the simple functionality of the camera. A total of 580 digital images were collected over the course of the two experiments. Photographs were sorted according to whether they depicted a control (untreated) specimen or a chemically treated (DEET-treated) carcass. The images were used not only to document the progress of the study, but were also directly utilized to qualitatively and quantitatively assess decomposition.

3.5 Decomposition Assessment

The carcasses were qualitatively and quantitatively assessed using the total body score (TBS) method (after Megyesi et al, 2005). This method allows a standardized and systematic evaluation of the decomposing remains that can be quantified and used in metric analyses if desired (Tables 1-3). Using this method, the TBS is determined by individually assessing three anatomical regions and summing the scores to derive a cumulative number. The three assessment regions are (1) the head/neck, (2) the abdomen, and (3) the limbs. They are scored separately because various body regions decompose at different rates and produce different amounts of decomposition. The method proceeds by determining which description best fits with the level of decomposition observed, noting features and observations such as discoloration, skin slippage, mummification, and exposure of the skeleton.

Table 1: Scoring of Decomposition of the Head and Neck
(adapted from Megyesi, 2001)

Feature(s) Observed	**Score**
No discoloration	1
Pink to white appearance with skin slippage and some hair loss	2
Gray to green discoloration, some flesh still relatively fresh	3
Discoloration or brown shades at edges, drying of nose, ears, lips	4
Face may be present	5
Brown to black discoloration	6
"Sinking in" of eye and throat tissues	7
Moist decomposition with bone exposure less than half of the area	8
Mummification with bone exposure less than half that of the area	9
Bone exposure more than half of the area with greasy decomposition tissue	10
Bone exposure of more than half the area with mummified tissue	11
Bone largely dry but retaining some grease	12
Dry bone	13

Table 2: Scoring of Decomposition of the Trunk
(adapted from Megyesi, 2001)

Feature(s) Observed	**Score**
No discoloration	1
Pink to white appearance with skin slippage and marbling	2
Gray to green discoloration, some flesh still relatively fresh	3
Bloating with green discoloration and purging	4
Post bloating following release of abdominal gas; green to black discoloration	5
"Sinking in" of the abdominal cavity	6
Moist decomposition with bone exposure less than half of the area	7
Mummification with bone exposure less than half that of the area	8
Bones with decomposed tissue and possibly body fluids and grease	9
Bones with mummified tissue covering less than half of the area	10
Bone largely dry but retaining some grease	11
Dry bone	12

Table 3: Scoring of Decomposition of the Limbs
(adapted from Megyesi, 2001)

Feature(s) Observed	Score
No discoloration	1
Pink to white appearance with skin slippage of hands and/or feet	2
Gray to green discoloration, marbling, some flesh still relatively fresh	3
Discoloration or brown shades at edges, drying of fingers, toes and other projecting extremities	4
Brown to black discoloration skin having a leathery appearance	5
Moist decomposition with bone exposure less than one half that of the area scored	6
Mummification with bone exposure of less than one half of the area being scored.	7
Bone exposure over one half the area being scored, some decomposed tissue and body fluids remaining	8
Bones largely dry, but retaining some grease	9
Dry bone	10

As can be seen from Tables 1-3, a score of “1” denotes the lowest score for any region, while the lowest possible TBS is 3 (if, for example, there is no discoloration present in any of the three regions assessed). The highest score possible in any individual region is “10” for the limbs, “12” for the trunk and “13” for the head. The greatest TBS possible is equal to “35”, which would indicate that the remains are completely dry bone across all three areas of assessment. Utilizing these tables and following this method, individual scores were recorded based on the photographs, and the TBS was then calculated by summing the individual recorded daily observations.

Chapter 4 Data Analysis and Results

The two experiments in this study lasted a total of 49 days. As previously indicated, experiment 1 was carried out over 22 days from May 23, 2009 through June 13, 2009 using 4 fetal porcine carcasses as subjects. Experiment 2 was carried out over 27 days from September 27, 2011 through October 23, 2011 using 4, 3-4 month old porcine carcasses as subjects. The results of each of these two experiments are detailed below, including observations of the DEET-treated specimens and control specimens, as well as specimens positioned in the sun and shade for each of the two experiments.

4.1 *Experiment 1*

Maximum and minimum ambient temperature data was collected for each day of the experiment and is shown in the leftmost columns of Table 4. Ambient temperatures ranged from lows in the low 50s to highs in the low 90s. This experiment was carried out in the late spring in northern Virginia, and these temperatures reflect typical temperatures for that area and season. The temperatures of the porcine carcasses were collected twice daily (AM and PM) and are also shown in Table 4. The average temperature of control specimens in the AM sun was 61 degrees; the average temperature of control specimens in the PM sun was 68.5. The average temperature of control specimens in the AM shade was 61.2 degrees; the average temperature of control specimens in the PM shade was 68.4. The average temperature of DEET specimens in the AM sun was 61 degrees; the average temperature of DEET specimens in the PM sun was 68 degrees. The average temperature of DEET specimens in the AM shade was 61.5 degrees; the average temperature of DEET specimens in the PM shade was 67 degrees. During the duration of this experiment rainfall was observed on several days, though observations of the specimens continued. The heaviest periods of rainfall with moderate winds were 5/27/09, 6/11/09 and 6/12/09 respectively (NOAA).

Table 4: Recorded Temperatures for Experiment 1

Day	Max Temp ˚F	Min Temp ˚F	Control Sun AM	Control Sun PM	Control Shade AM	Control Shade PM	DEET Sun AM	DEET Sun PM	DEET Shade AM	DEET Shade PM
23-May	83	57	36	71.5	38	70	35	70	46	69
24-May	85	64	63	71	62	71	62	71	62	70
25-May	80	65	67	70	66	70	66.5	70	66	69
26-May	83	65	67	57	70	60	67	58	68	58
27-May	77	59	59	66	59	67	59	65	58	65
28-May	73	59	64	70	63	69	63	68	62	69
29-May	83	59	68.5	62	70	66	68	62	68.5	60
30-May	87	55	59	71	56	70	56	72	56	69
31-May	82	58	61	73	58	66	58	65	58	65
1-Jun	84	51	53	70	51	68	54	70	52	68
2-Jun	80	52	60	66	59	69	60	66	61	68
3-Jun	91	62	64	71	64	71	63	72	63	71
4-Jun	85	60	59	60	60	60	61	60	59	59
5-Jun	64	60	60	57	60	57	61	57	61	56
6-Jun	67	58	57	63	57	64	58	64	56	54
7-Jun	76	58	58	69	59	68	59	66	59	68
8-Jun	83	60	62	77	62	77	63	78	63	77
9-Jun	89	64	70	75	69	75	71	75	69	75
10-Jun	88	62	65	75	63	75	65	75	65	75
11-Jun	82	62	67	71	67	71	67	72	67	71
12-Jun	84	62	66	70	66	70	66	70	66	70
13-Jun	85	68	64	71	67	71	67	72	67	71

There was no significant difference between the average temperatures of the controls and the DEET treated specimens over the course of the experiment. The greatest difference in average temperature was 1.4 degrees. These results are not surprising since there is no obvious reason to think that treatment with DEET would directly impact the temperature of the specimen. Also, because of the seasonality and location of the study (spring time in Northern Virginia), it was not unexpected that temperatures of specimens in the sun were not significantly different from those that were located in the shade.

Decomposition assessments were collected each day for each of the three regions. Decomposition scores including individual scores and summed total body scores (TBS) are shown in Table 5. Average daily temperatures were calculated by taking the mean of daily maximum and minimum temperatures over a 24 hour period from the NOAA climate radar station located in Fredericksburg VA. The average ambient daily temperature during the duration of experiment 1 varied from a high of 78 degrees to a low of 60 degrees. Temperature data was used to calculate accumulated degree-days (ADD), which is shown along with TBS in Table 6, and graphs of TBS by date and ADD (for shade and sun separately) are shown in Figures 10-11. Photographs were taken daily during the entire experiment. Selected photographs taken on the same dates for the control and DEET specimens in the shade and in the sun are shown in Figures 12 and 13, respectively.

Table 5: Individual and Total Body Scores from Experiment 1

	Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Control Sun																							
	Head/Neck	1	1	1	2	7	7	7	7	7	10	10	10	12	12	12	12	12	12	12	12	12	12
	Trunk	1	3	3	4	4	5	5	6	7	9	9	9	11	11	11	11	11	11	11	11	11	11
	Limbs	1	1	1	1	2	3	4	4	4	5	8	8	9	9	9	9	9	9	9	9	9	9
	Total	**3**	**5**	**5**	**7**	**13**	**15**	**16**	**17**	**18**	**24**	**27**	**27**	**32**	**32**	**32**	**32**	**32**	**32**	**32**	**32**	**32**	**32**
DEET Sun																							
	Head/Neck	1	1	1	2	2	2	2	7	7	7	7	7	7	7	7	7	7	7	10	10	10	10
	Trunk	1	2	2	4	4	4	4	4	4	4	6	6	7	7	7	10	10	10	10	10	10	10
	Limbs	1	1	1	1	2	2	3	3	3	4	4	4	4	4	4	4	4	4	5	8	8	8
	Total	**3**	**4**	**4**	**7**	**8**	**8**	**9**	**14**	**14**	**15**	**17**	**17**	**18**	**18**	**18**	**21**	**21**	**21**	**25**	**28**	**28**	**28**
Control Shade																							
	Head/Neck	1	1	2	2	2	7	7	7	7	8	8	8	8	10	10	11	12	12	12	12	12	12
	Trunk	1	2	3	4	4	5	5	6	6	6	6	6	7	9	9	9	11	11	11	11	11	11
	Limbs	1	1	1	1	2	2	2	4	4	4	5	5	6	6	6	8	9	9	9	9	9	9
	Total	**3**	**4**	**6**	**7**	**8**	**14**	**14**	**17**	**17**	**18**	**19**	**19**	**21**	**25**	**25**	**28**	**32**	**32**	**32**	**32**	**32**	**32**
DEET Shade																							
	Head/Neck	1	1	2	3	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
	Trunk	1	2	3	3	3	4	5	5	5	5	5	6	6	6	6	6	6	6	9	9	9	9
	Limbs	1	1	1	1	1	2	3	3	3	5	5	5	6	6	6	6	6	6	6	8	8	8
	Total	**3**	**4**	**6**	**7**	**11**	**13**	**15**	**15**	**15**	**17**	**17**	**18**	**19**	**19**	**19**	**19**	**19**	**19**	**22**	**24**	**24**	**24**

Table 6: Accumulated Degree Days (ADD) and Total Body Scores (TBS) for Experiment 1

Date	Max Temp ˚F	Min Temp ˚F	Avg Temp ˚F	ADD	Control Sun TBS	Control Shade TBS	DEET Sun TBS	DEET Shade TBS
23-May-09	83	57	70	70	3	3	3	3
24-May-09	85	64	74.5	144.5	5	4	4	4
25-May-09	80	65	72.5	217	5	6	4	6
26-May-09	83	65	74	291	7	7	7	7
27-May-09	77	59	68	359	13	8	8	11
28-May-09	73	59	66	425	15	14	8	13
29-May-09	83	59	71	496	16	14	9	15
30-May-09	87	55	71	567	17	17	1	15
31-May-09	82	58	70	637	18	17	14	15
1-Jun-09	84	51	67.5	704.5	24	18	15	17
2-Jun-09	80	52	66	770.5	27	19	17	17
3-Jun-09	91	62	76.5	847	27	19	17	18
4-Jun-09	85	60	72.5	919.5	32	21	18	19
5-Jun-09	64	60	62	981.5	32	25	18	19
6-Jun-09	67	58	62.5	1044	32	25	18	19
7-Jun-09	76	58	67	1111	32	28	21	19
8-Jun-09	83	60	71.5	1182	32	32	21	19
9-Jun-09	89	64	76.5	1259	32	32	21	19
10-Jun-09	88	62	75	1334	32	32	25	22
11-Jun-09	82	62	72	1406	32	32	28	24
12-Jun-09	84	62	73	1479	32	32	28	24
13-Jun-09	85	68	76.5	1555.5	32	32	28	24

Figure 10: Total Body Score (TBS) for Experiment 1 Shade Specimens

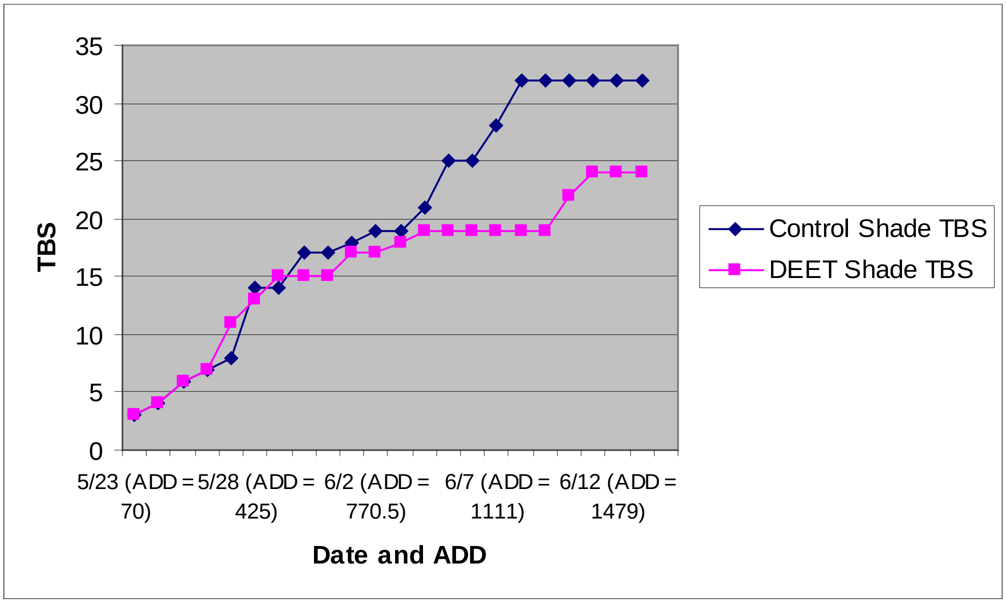


Figure 11: Total Body Score (TBS) for Experiment 1 Sun Specimens

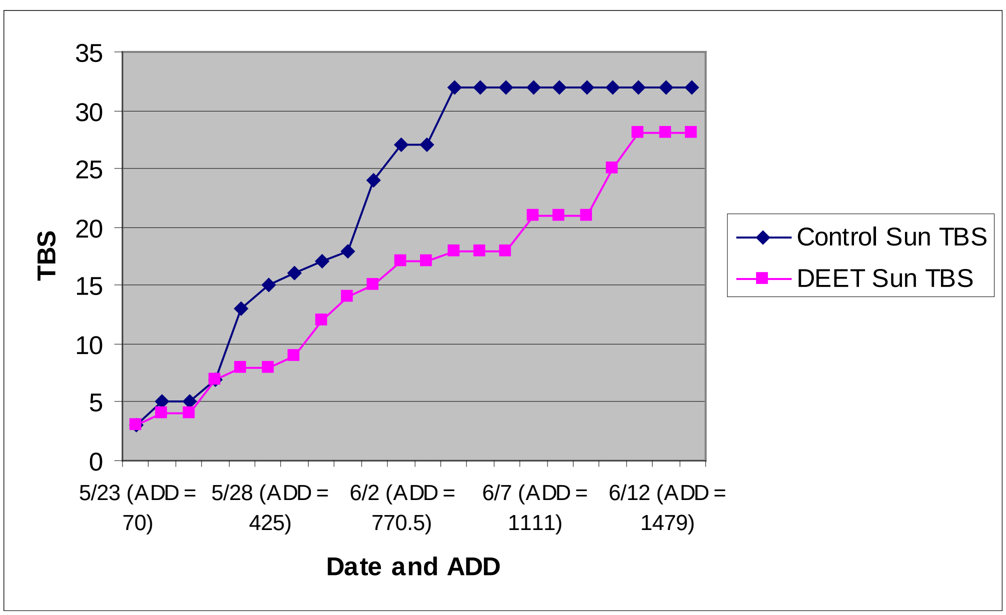


Figure 12: DEET and Control Shade Specimens from Experiment 1 on Selected Dates

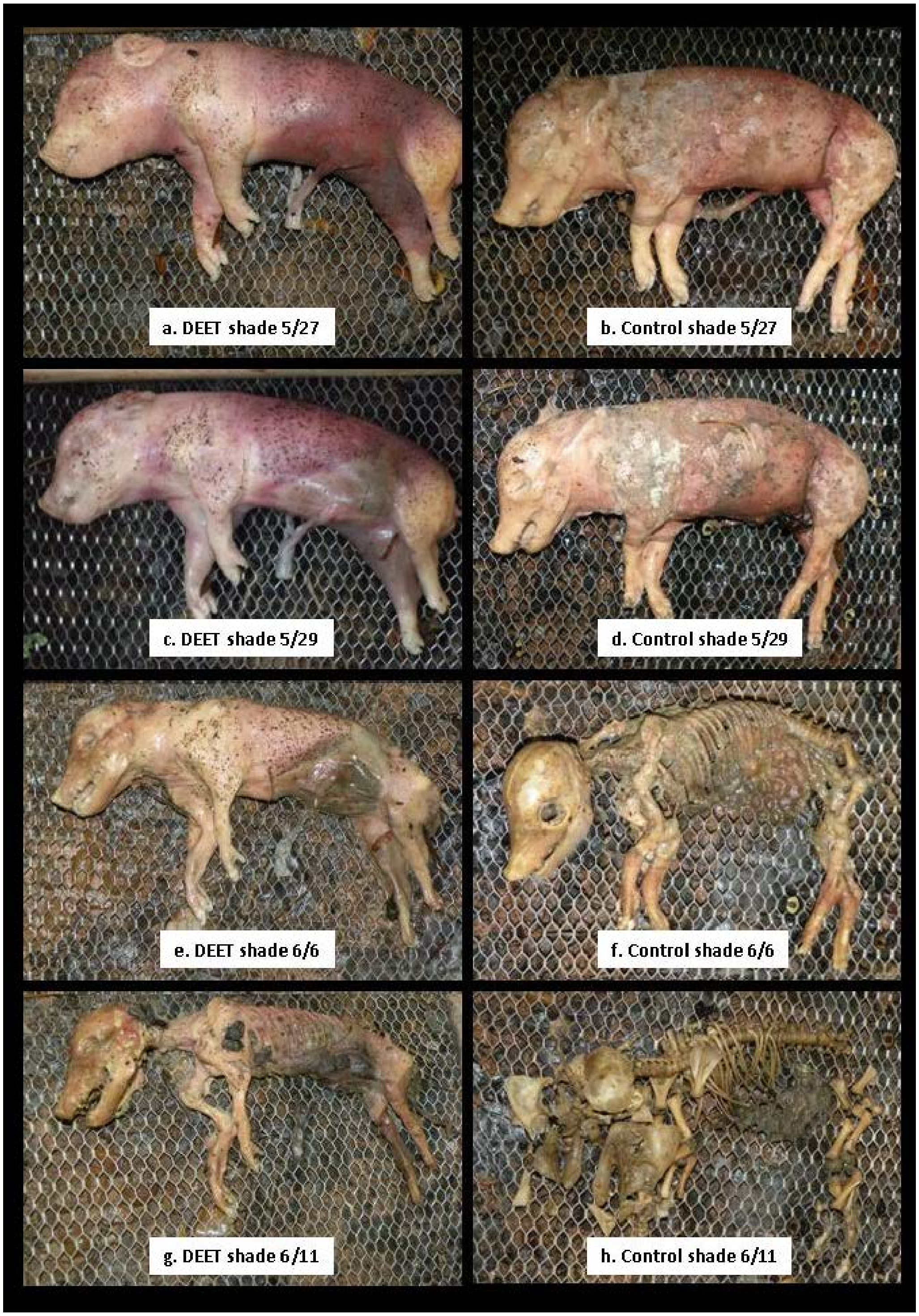


Figure 13: DEET and Control Sun Specimens from Experiment 1 on Selected Dates

It can be seen both quantitatively and qualitatively in the preceding tables and photographs that the DEET specimens showed reduced decomposition as compared to the control specimens. The data in Table 6 and graphic representation of TBS in Figure 10 and 11 clearly show that the TBS for the DEET specimens was lower for almost every day in the study. Qualitatively, these differences can be seen in the photographs shown in Figure 12 and 13. These results suggest that DEET was indeed inhibiting or slowing insect activity resulting in decreased decomposition rate. Some general observations made during the course of the study described below.

The specimens remained fresh for the first couple of days after which bloating was noted. The small size of these fetal specimens made bloating somewhat difficult to initially detect, but bloating was eventually apparent, as can be seen, for example in Figure 12b. Again probably due to their small size, there was very little obvious decomposition odor. Autolysis was already occurring when the carcasses were placed in their housing containers, observed as minor external discoloration that could be seen due to their thin skin. The control and DEET porcine carcasses exhibited an equal amount of change early on.

Approximately one week into the experiment, control and DEET specimens exhibited decay with blowflies noted around the carcasses and numerous egg masses in the eyes, nose, mouth and face, which was more pronounced in the control specimens. There were differences in the insect colonization of the DEET and control specimens, which is especially apparent in

Figure 13 c, d, e and f, where there are clearly more insects present on the control specimens. The abdomens of both controls and DEET specimens became very bloated, lasting approximately 5 days. The carcasses displayed dark purple discoloration of abdominal skin that spread to the groin and back (Figure12c and d) and some rolled on their backs due to bloating pressures. This was accompanied by odor from purging of gas and very little maggot activity was noted prior to gas purging. Blowfly ovipositing activity increased on warm days. Elevated temperatures were noted to characterize the maggot masses that eventually enveloped the decomposing carcasses. During the terminal bloat stage, large amounts of skin slippage were noted on all specimens.

Approximately 1.5-2 weeks into experiment 1, the gases were completely purged from the specimen's abdomens resulting in a distinct flattening. Around this time, some of the carcasses showed defined ribs through the skin (Figure 12e). Blowfly larvae were still active on the remains during this time, and each carcass possessed a very strong odor. Moist soft tissue decomposition was ongoing and the maggots began to migrate away from the specimens, leaving their characteristic trail in the nearby soil. As the tissues were shrinking/receding, there was a marked color change to gray (Figure 12e). The soft tissues, however, were still moist and a majority of the bones became exposed (Figure 12g and Figure 13g). Some carcasses showed internal organs breaching the abdominal wall from maggot activity. Portions of the skeleton disarticulated due to loss of the bodies' connective muscles and ligamentous support, especially in the control specimens (Figures 12h and 13h).

Two to three weeks into the experiment, there was a major departure of the maggot mass with just a few scattered maggots remaining. The majority of soft tissue had been removed from the specimens. There was a decrease odor level, though odor was still noticeable on warmer days. Some of the carcasses became complete skeletons by this time (Figures 12h, 13g and h). The control specimen placed in shade mummified rather than decomposed. This carcass became covered with some type of fungus. The reason for the development of this fungus is unknown, but may possibly be related to the external DEET application.

4.2 *Experiment 2*

Maximum and minimum ambient temperature data was collected for each day of experiment 2 and is shown in the leftmost columns of Table 7. Ambient temperatures ranged from lows in the mid 40s to highs in the upper 70s, reflecting typical temperatures for that area and season. The temperatures of the porcine carcasses were collected twice daily (AM and PM) and are also shown in Table 7. The average temperature of control specimens in the AM sun was 58 degrees; the average temperature of control specimens in the PM sun was 65. The average temperature of control specimens in the AM shade was 62 degrees; the average temperature of control specimens in the PM shade was 67. The average temperature of DEET specimens in the AM sun was 64 degrees; the average temperature of DEET specimens in the PM sun was 68 degrees. The average temperature of DEET specimens in the AM shade was 66 degrees; the average temperature of DEET specimens in the PM shade was 68 degrees.

Heavy rainfall was observed during the duration of experiment 2. On 9/27/11, the first day that the porcine carcasses were sprayed with the 40% DEET and placed in the observation housing, rainfall began within 30 minutes and continued for 48 hours. This likely caused the DEET application to be diluted or washed away. During the duration of experiment 2, the following days produced periods of heavy rain: 10/2/11, 10/12/11, 10/13/12 and 10/19/11 (NOAA), but observations of the specimens continued on these dates. On 10/20/11 (day 23), heavy rainfall continued with high winds for 24 hours. No recordings or observations could be made regarding the specimens on that date due to the rain.

Table 7: Recorded Temperatures for Experiment 2

	Max T ˚F	Min T ˚F	Cont. Sun AM	Cont. Sun PM	Cont. Shade AM	Cont. Shade PM	DEET Sun AM	DEET Sun PM	DEET Shade AM	DEET Shade PM
Day										
27-Sep	77	60	83	80	81	80	82	78	70	76
28-Sep	78	58	63	80	61	80	61	81	59	81
29-Sep	75	55	65	73	67	71	67	73	67	72
30-Sep	78	57	55	63	55	61	54	64	55	63
1-Oct	78	52	43	38	42	38	44	38	42	39
2-Oct	60	45	60	60	61	61	60	61	60	60
3-Oct	53	46	82	80	81	82	82	80	80	80
4-Oct	54	46	80	81	81	82	82	80	80	80
5-Oct	69	46	78	80	81	81	81	80	80	80
6-Oct	75	46	78	79	81	80	80	80	80	80
7-Oct	71	45	70	68	80	80	82	81	81	81
8-Oct	73	45	58	61	78	80	80	82	81	80
9-Oct	73	46	44	60	77	77	81	81	81	80
10-Oct	78	46	48	64	77	76	80	82	80	80
11-Oct	79	46	48	65	50	65	81	65	80	75
12-Oct	70	59	59	60	59	61	80	61	81	77
13-Oct	66	64	63	60	63	61	63	61	81	60
14-Oct	78	63	58	64	58	64	58	64	82	63
15-Oct	78	46	48	64	48	64	48	63	80	64
16-Oct	70	46	48	60	48	61	48	60	48	60
17-Oct	72	54	57	61	58	61	58	60	58	60
18-Oct	73	53	49	62	49	61	49	61	49	62
19-Oct	75	53	48	62	48	62	48	62	48	61
20-Oct	66	51	47	59	48	60	49	60	47	59
21-Oct	59	43	42	55	42	55	42	55	42	55
22-Oct	63	42	44	57	44	57	44	57	44	57
23-Oct	63	39	42	55	42	55	42	56	43	56

There was no significant difference between the average temperatures of the controls and the DEET treated specimens over the course of the experiment. The greatest difference in average temperature was 3 degrees.

Decomposition assessments were collected each day for each of the three regions. Decomposition scores including individual scores and summed total body scores (TBS) are shown in Table 8 (with the exception of day 23 when no recordings could be made due to rain). Average daily temperatures were calculated by taking the mean of daily maximum and minimum temperatures over a 24 hour period from the NOAA climate radar station located in Fredericksburg VA. The average ambient daily temperature during the duration of experiment 2 varied from a high of 70 degrees to a low of 50 degrees. Temperature data was used to calculate accumulated degree-days (ADD), which is shown along with TBS in Table 9, and graphs of TBS by date and ADD (for shade and sun separately) are shown in Figures 14-15. Photographs were taken daily during the entire experiment. Selected photographs taken on the same dates for the control and DEET specimens in the shade and in the sun are shown in Figures 16 and 17, respectively.

Table 8: Individual and Total Body Scores from Experiment 2

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Control Sun														
Head/Neck	1	1	3	3	3	3	5	5	6	6	6	6	7	8
Trunk	1	2	4	4	5	5	5	5	5	6	6	6	6	7
Limbs	1	1	1	1	1	1	1	1	4	5	5	5	5	6
Total	**3**	**4**	**8**	**8**	**9**	**9**	**11**	**11**	**15**	**17**	**17**	**17**	**18**	**21**
DEET Sun														
Head/Neck	1	1	1	1	3	3	3	5	5	5	6	6	6	7
Trunk	1	2	4	4	4	5	5	5	5	5	6	6	6	7
Limbs	1	1	1	1	2	2	2	2	2	4	5	5	5	6
Total	**3**	**4**	**6**	**6**	**9**	**10**	**10**	**12**	**12**	**14**	**17**	**17**	**17**	**20**
Control Shade														
Head/Neck	1	1	3	3	3	3	5	5	6	6	6	6	7	8
Trunk	1	1	1	4	4	4	5	5	5	6	6	6	6	7
Limbs	1	1	1	1	2	2	2	2	4	5	5	5	5	6
Total	**3**	**3**	**5**	**8**	**9**	**9**	**12**	**12**	**15**	**17**	**17**	**17**	**18**	**21**
DEET Shade														
Head/Neck	1	1	3	3	3	3	5	5	6	6	6	6	7	8
Trunk	1	4	4	4	4	5	5	5	5	6	6	6	6	7
Limbs	1	1	1	1	1	1	1	1	1	1	1	5	5	6
Total	**3**	**6**	**8**	**8**	**8**	**9**	**11**	**11**	**12**	**13**	**13**	**17**	**18**	**21**

Table 8 (cont'd)

Day	15	16	17	18	19	20	21	22	23	24	25	26	27
Control Sun													
Head/Neck	8	8	8	8	9	9	9	9		9	9	9	9
Trunk	7	7	7	7	8	8	8	8	RAIN	8	8	8	8
Limbs	6	6	6	6	7	7	7	7		7	7	7	7
Total	**21**	**21**	**21**	**21**	**24**	**24**	**24**	**24**		**24**	**24**	**24**	**24**
DEET Sun													
Head/Neck	8	8	8	8	9	9	9	9		9	9	9	9
Trunk	7	7	7	7	8	8	8	8	RAIN	8	8	8	8
Limbs	6	6	6	6	6	6	7	7		7	7	7	7
Total	**21**	**21**	**21**	**21**	**23**	**23**	**24**	**24**		**24**	**24**	**24**	**24**
Control Shade													
Head/Neck	8	8	8	8	9	9	9	9		9	9	9	9
Trunk	7	7	7	7	8	8	8	8	RAIN	8	8	8	8
Limbs	6	6	6	6	6	7	7	7		7	7	7	7
Total	**21**	**21**	**21**	**21**	**23**	**24**	**24**	**24**		**24**	**24**	**24**	**24**
DEET Shade													
Head/Neck	8	8	8	8	9	9	9	9		9	9	9	9
Trunk	7	7	7	7	7	8	8	8	RAIN	8	8	8	8
Limbs	6	6	6	6	6	6	7	7		7	7	7	7
Total	**21**	**21**	**21**	**21**	**22**	**23**	**24**	**24**		**24**	**24**	**24**	**24**

Table 9: Accumulated Degree Days (ADD) and Total Body Scores for Experiment 2

Date	Max Temp ˚F	Min Temp ˚F	Avg Temp ˚F	ADD	Control Sun TBS	Control Shade TBS	DEET Sun TBS	DEET Shade TBS
27-Sep-11	77	60	68.5	68.5	3	3	3	3
28-Sep-11	78	58	68	136.5	4	3	4	6
29-Sep-11	75	55	65	201.5	8	5	6	8
30-Sep-11	78	57	67.5	269	8	8	6	8
1-Oct-11	77	52	64.5	333.5	9	9	9	8
2-Oct-11	60	45	52.5	3786	9	9	10	9
3-Oct-11	53	46	49.5	435.5	11	12	10	11
4-Oct-11	54	46	50	485.5	11	12	12	11
5-Oct-11	69	46	57.5	543	15	15	12	12
6-Oct-11	75	46	60.5	603.5	17	17	14	13
7-Oct-11	71	45	58	661.5	17	17	17	13
8-Oct-11	73	45	59	720.5	17	17	17	17
9-Oct-11	73	46	59.5	780	18	18	17	18
10-Oct-11	78	46	62	842	21	21	20	21
11-Oct-11	79	46	62.5	904.5	21	21	21	21
12-Oct-11	70	59	64.5	969	21	21	21	21
13-Oct-11	66	64	65	1034	21	21	21	21
14-Oct-11	78	63	70.5	1104.5	21	21	21	21
15-Oct-11	78	46	62	1116.5	24	23	23	22
16-Oct-11	70	46	58	1224.5	24	24	23	23
17-Oct-11	72	54	63	1287.5	24	24	24	24
18-Oct-11	73	53	63	1350.5	24	24	24	24
19-Oct-11	75	53	64	1414.5	RAIN	RAIN	RAIN	RAIN
20-Oct-11	66	51	58.5	1473.5	24	24	24	24
21-Oct-11	59	43	51	1524	24	24	24	24
22-Oct-11	63	42	52.5	1576.5	24	24	24	24
23-Oct-11	63	39	51	1627.5	24	24	24	24

Figure 14: Total Body Scores from Experiment 2 Shade

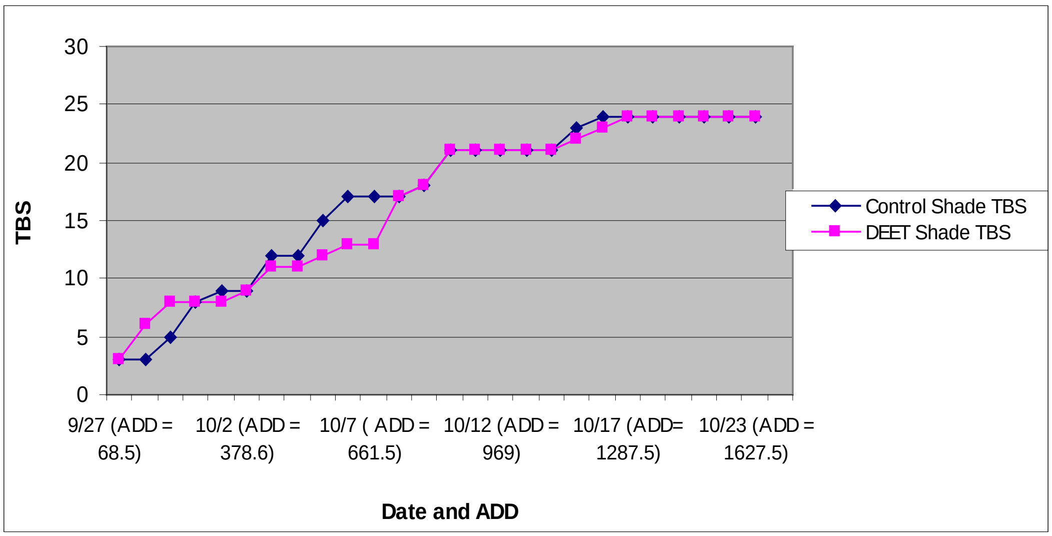


Figure 15: Total Body Scores from Experiment 2 Sun

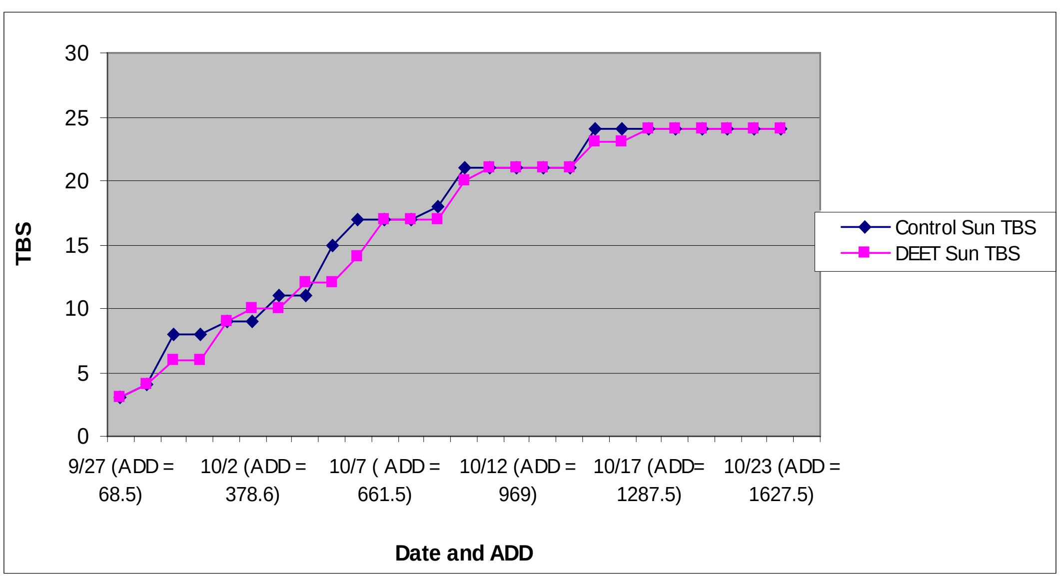


Figure 16: DEET and Control Sun Specimens from Experiment 2
on Selected Dates

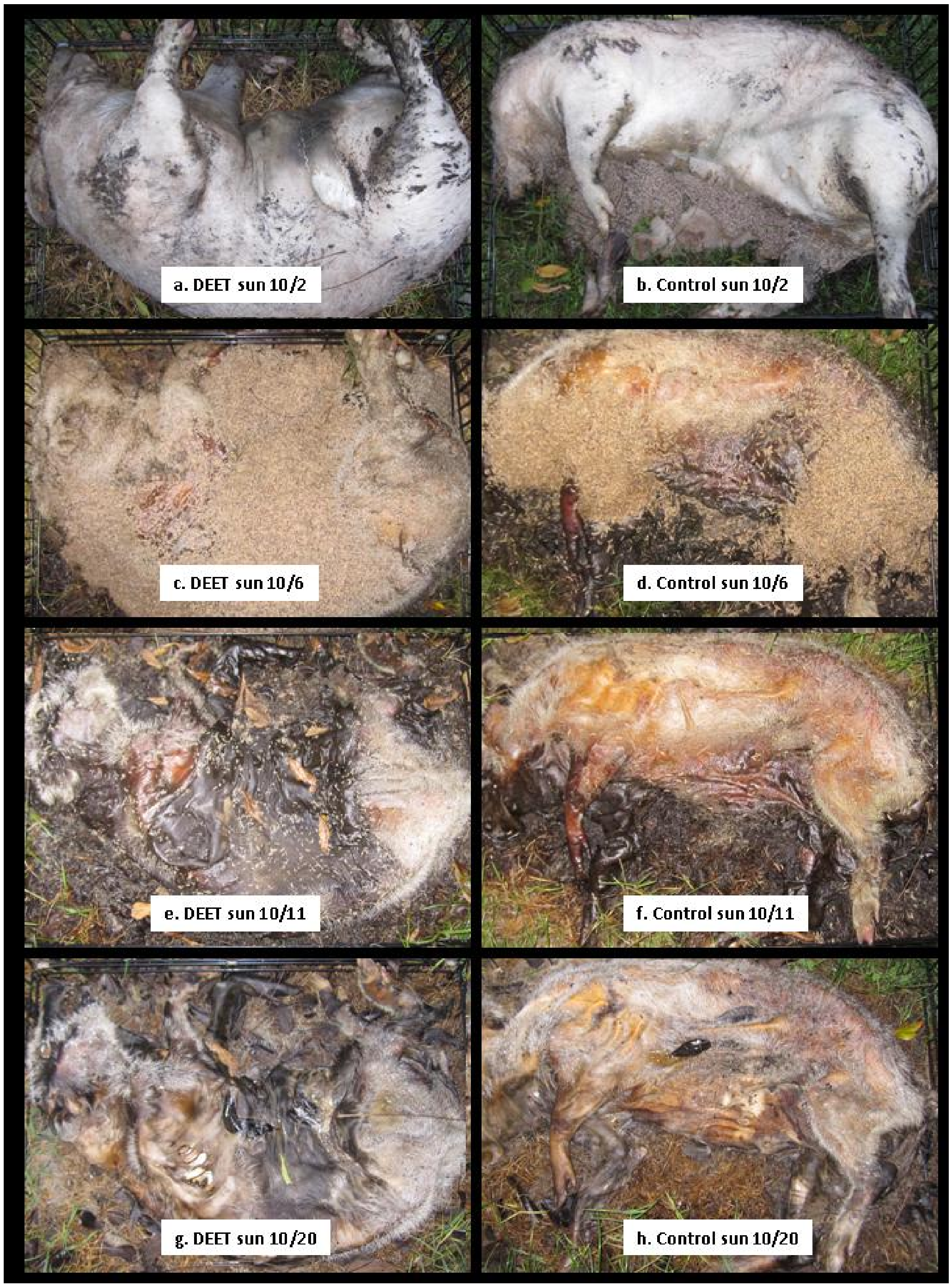


Figure 17: DEET and Control Shade Specimens from Experiment 2
on Selected Dates

The data in Table 9 and graphic representation of TBS in Figure 14 and 15 indicate that the TBS for the DEET specimens were approximately the same for almost every day in the study. Qualitatively, these similarities can be seen in the photographs shown in Figure 16 and 17. These results suggest that DEET was not inhibiting or slowing insect activity resulting in decreased decomposition rate (or that there was no DEET remaining due to the rain, which is addressed further below). Some general observations made during the course of the study are described below.

The specimens in experiment 2 remained fresh for approximately the first day only, with bloating apparent the next day. Decomposition odors were not obvious though skin slippage was occurring when the carcasses were placed in their observation housings. No external discolorations were noted though blowflies were ovipositing almost immediately after the carcasses were unloaded from the truck. Fresh blood around the head wound attracted the flies giving immediate access for egg laying.

Blowflies were active during the first week, and numerous egg masses were noted within the gunshot shot wounds (GSW), eyes, noses, mouths and rectums and the carcasses took on a very bloated appearance. As can be seen in the photographs of the sun specimens on October 1, ovipositing did occur somewhat earlier on the control specimen than the DEET specimen (Figure 16a and b), but then appeared more similar just a few days later. The discoloration, first noted in the abdomen of the carcasses, spread to the legs, groin, neck and head. An obvious odor was noted due to the purging of the gases, and lividity was marked in the carcasses as purple discoloration. Maggot activity increased dramatically on warmer days and elevated temperatures were prevalent in the maggot mass concentration enveloping the decomposing carcasses. The maggot mass eventually almost completely engulfed the carcasses (Figure 16 c and d) and large amounts of skin slippage were noted on all carcasses. Body fluid runoff began to kill the grass (Figure17c and d).

After the first week, dramatic changes in the carcasses appeared when the gases were fully purged from the abdomens resulting in a flattening of the specimens' bodies. Some blowfly larvae were still active with very strong odor as moist soft tissues were still decomposing. The maggots began migrating during a majority of this process leaving a trail in the grass. The tissues were beginning to recede and marked color changes became black and leathery (Figure 16e). The tissues were still moist with some exposed bone (Figure 16f) and blackened grass. Some carcasses revealed internal organs from breaching of the abdominal wall by maggots. By the end of the first week or so, most of the maggots had migrated away in multiple directions. The skeleton began to disarticulate due to the muscular and ligamentous loss (Figure 16g).

After 2 weeks, there was near complete departure of the maggot masses from the remains with significant soft tissue loss. Decomposing odors were also less but still noticeable on the warmer days. No carcass in this experiment became completely skeletonized and there was desiccation of some of the outer skin surface. Longer exposed bone was both drier and lighter. Both control and DEET remains exhibited the same relative amount of decay throughout the experiment.

Chapter 5 Discussion

Interestingly, the results of the two experiments conducted in this study appear rather different, with experiment 1 suggesting that DEET inhibited decomposition, while the results of experiment 2 suggest that the DEET had little or no effect. This is most likely due to the fact that fetal remains were used in one experiment and juvenile remains were used in the other experiment. Despite this critical difference between the two experiments, several factors that affect decomposition will be discussed in relation to the present study.

Temperature: The temperatures for each of the two experiments were not drastically different. This was the case for both ambient temperature and also specimen temperature as recorded and documented in Table 4 and Table 7. This is likely due to the use of similar locales (both in northern Virginia) and similar seasons (spring and fall). The close dispersal of the subject carcasses was also likely responsible for the fairly consistent temperature reading for the carcasses. However, in experiment 2, it was observed that the porcine carcasses had a higher body temperature during the periods of increased maggot masses than that of the fetal porcine carcasses in experiment 1. This could be due to the fact that the 3-4 month old porcine carcasses had more body fat than that of the fetal carcasses (Mant, 1987), or may be related to greater insect activity inhibition in experiment 1.

Length of Experiment: Despite the fact that the two studies differed in the total number of days over which they were carried out (22 days for experiment 1 and 27 days for experiment 2), they had almost the same ADD by the end of each study. This is due to the somewhat cooler overall temperatures in the fall in northern Virginia versus the spring. Experiment 2 actually had a slightly higher ADD by the end of the study (1627.5 versus 1555.5 for experiment 1), though the pigs in experiment 2 showed less decomposition overall. This is likely related to the size of the specimens used. The smaller fetal carcasses would be expected to completely decompose more rapidly than the larger carcasses. Given somewhat more time and greater ADD, it is expected that the soft tissues in experiment 2 would have eventually decomposed.

Insect Activity: In experiment 1 it was observed that the blowflies appeared shortly after the control fetal carcasses were placed in the housing, but there was no interest around the 40% DEET-treated fetal carcasses. Even over the next few days as the blowflies found the control specimens a site for egg deposition, there was still no interest in the fetal carcasses sprayed with the 40% DEET.

The maggots became visible and progressed into larger masses on the porcine carcasses at approximately 10-12 days in experiment 1 and 7 days in experiment 2. The entire porcine carcasses became covered with maggot masses during certain peaks, especially in experiment 2. The majority of the insect infestation on both experiments occurred in the first 14-15 days, though it can be seen in the photographs in Figures 12-13 and Figures 16-17 that insects were far less prevalent on the fetal specimens in experiment 1 versus the larger specimens in experiment 2. In both studies, the large maggot masses left the carcasses after about 15 days. This observation was the same for the control carcasses as well as the porcine carcasses that were treated with DEET.

As the blow flies (Greenbottle, *Lucilia illustris*) and ants began to appear on the porcine carcasses it was noted in experiment 1 that the ants appeared on the fetal porcine carcasses treated with DEET before blowflies were present. This may be due to the DEET being a detractor for the blowflies, it was noted that the most of the concentrations of the insects were attracted to the carcasses' nose, mouth, eyes and other natural orifices. Since the 3-4 month old porcine carcasses were euthanized by a gunshot to the head, the wound site bleed and blood was also present on the facial region and the nostrils. This presence of blood attracted a faster infestation of insects than that of the fetal porcine carcasses. The presence of the insects appeared almost simultaneously as the 3-4 month old carcasses were being placed in the observation housing before the 40% DEET was applied. As the days progressed in both experiments the maggot masses began to cover more skin surface and in the later stages completely engulfed the abdomen region.

The greatest insect concentrations were observed by day 5 in experiment 1 for both controls and by days 10-12 for the DEET treated specimens. In experiment 2 for both the controls and the DEET specimens the greatest concentration was by day 4. It was also observed that even though the specimens for experiment 1 and 2 were placed in different locations and had different housing structures that this did not affect how long the insects were present on the porcine carcasses.

A rating of three (the lowest possible total body score) was recorded for all animals when first placed at the research site according to the Megyesi et al. (2001) scale. The DEET treated fetal carcasses remained unremarkable/fresh for longer than the DEET treated 3-4 month old carcasses. This could be attributed to the size of the porcine carcasses and the internal bacteria compared to the 3-4 month old carcasses. It was observed that the initial discolorations began in the abdominal regions for both experiment 1 and 2, showing the pinkish hue of the skin turning to a brownish color. Skin slippage was also noted for all of the porcine specimens, beginning in the areas around the eyes and lips. As can be seen in the charts in Figures 10-11 and 14-15, after about 20 days, there was little change in the total decomposition score, with the score appearing to plateau and no longer increase with increasing ADD.

In experiment 1 it was difficult at times to see significant signs of bloating of the fetal carcasses due to their smaller structure than compared to the 3-4 month old porcine carcasses in experiment 2. The 3-4 month old carcasses presented the greatest amount of bloating due to their greater mass and possibly having more naturally occurring bacteria which produces the gases that are part of process during the bloating phase (Gill-King 1997).

In both experiments 1 and 2 the migration of the maggot masses began departing off the porcine carcasses at approximately day 16. This was observed as a large mass migrating off the remains and trailing across the forestland grounds adjacent to the carcasses. The large maggot trails of maggots left a residual fluid trail in the grass, which later caused the grass to die. Not all of the maggots migrated away from the carcasses, but some remained still feeding off of the remaining tissue. Other insects observed during the decomposition phases were the American Carrion Beetle (*Necrophila Americana*) and the Carolina Carrion Beetle (*Nicrophorous carolinus*). In both experiments, before and after the disappearance of the maggots, the decomposing remains continued to stay moist. During both experiments 1 and 2 there were several days of heavy rain. In experiment 1 there were three days of heavy downpours the first being 4 days after the fetal porcine carcasses were placed in their housing.

In experiment 2, there were 6 rain days, with heavy downpours. The heaviest rain day was within 30 minutes of placing the 3-4 month old DEET treated porcine carcasses in their observation housing. It rained for approximately 48 hours. It seemed that the rain often caused the tissue in the remains to appear moist and full again making it difficult to gauge moisture content in the remains.

As the bones in each set of remains began to become exposed and disarticulated due to maggot activity, there was a noted color change from a darker brown to a medium lighter brown in the bones as they began to lose moisture and dry. Only in experiment 1 did the fetal control carcasses become completely skeletonized. The fetal control shade carcass became mummified as it decomposed. The control sun and shade for experiment 1 both developed an unknown type of fungus. No testing was done on the fungus growth. This fungus was not observed on any of the remaining specimens for both experiments. In experiment 2 the porcine carcasses exhibited a large area of fat with the outer skin still together. One possibility for this is that the DEET kept the outer surface of the skin from being degraded, causing the maggots and beetles to feed off of the internal sections of the porcine carcasses.

In both experiments the areas affected the most by insect activity were the face, neck and abdominal regions. The TBS of both experiments never reached the maximum possible score as referenced in Tables 1, 2 and 3 adapted from Megysi's, (2001) stages of decomposition. Since the fetal carcasses in experiment 1 were the only specimens to become skeletonized the TBS could only be recorded at a maximum of 32, due to the fact that the skeletal bones never completely dried. Experiment 2 had a recorded TBS of 24 and the experiment was discontinued after 27 days therefore the porcine carcasses did not reach the skeletal stage.

There are some factors that should be discussed regarding the differences in the results of the two experiments and the effects of decomposition rates overall. In comparing the scoring methods of the two experiments, and the subjective categorization for the scoring stages, some variations could factor in regarding the results, due to the observations of the researcher. These observational variations are present but remained constant throughout the experiments and were documented as such.

Another factor could be contributed to the different times of the year that the experiments were carried out, which may have an effect on the rate of decomposition. Even though the temperatures did not vary that significantly, there were more rain days and colder temperatures noted during the duration of experiment 2. In observing the rates of decomposition for both experiments, it was noted that the rates of decomposition did vary in the onset with the DEET-treated fetal specimens for experiment 1. This variation being solely attributed to the DEET-treated porcine carcasses and not a combination of temperature and seasonal variation has not been clearly established.

In this study, the specimens were contained in observation housing. The observation housing could be considered as a variation that had an effect on the rate of decomposition, since it kept the decomposing remains sheltered from carnivore activity. It is not known if the DEET would have been a deterrent to the carnivores since this study contained the porcine carcasses from this natural element.

Another factor that may have affected the rate of decomposition was the use of previously frozen thawed animals in this study in experiment 1. The fetal porcine carcasses were previously frozen and shipped to the location site. However, in experiment 2 the porcine carcasses were obtained immediately after being euthanized. In studies conducted by Micozzi (1986) and Archer (2004) it was determined that previously frozen thawed animals showed mostly decay (aerobic decomposition) and freshly killed animals showed mostly putrefaction (anaerobic decomposition). These studies also demonstrated that there were faster rates of disarticulation with the neonatal remains. Archer, (2004) furthered determined that neonatal remains already contained reduced gastrointestinal bacteria and this would ultimately affect the decomposition rates described in Micozzi's experiment. This could be an additional variation in the difference of the specimen's rates of decomposition, knowing that the previously frozen fetal carcasses in experiment 1 already possessed reduced gastrointestinal bacteria compared to the fresh carcasses used in experiment 2.

In regards to the DEET concentrations and its by-products, it has not been determined that the DEET itself or the by-products from the manufacturing of the DEET aerosol has a direct affect on the decomposing porcine carcasses. It is possible that a larger amount of DEET or a higher concentration would be required than is normally produced by a small can of the insect repellent. According to the manufacturer of Colemans® DEET Insect Repellent Aerosol products, 100% DEET only offers 12 hours of protection from mosquitoes. It is conceivable that a large enough exposure could deter insects, but it is unclear if it would still conceal the body from insect activity or at least delay the onset. In retrospect the DEET should have been spayed in the orifices and wounds of the porcine carcasses to possibly prevent insects from nesting inside the carcass.

As discussed earlier, environmental factors may also affect the post-treatment staying power of the DEET on the remains. In both experiments (and especially in experiment 2), the heavy rains in the early stages of the study may have removed or diluted some of the external DEET that was applied, washing away the most active ingredients of the DEET spray that was applied to the porcine carcasses skin. In experiment 2 after the porcine carcasses were placed at the site and the DEET was applied, and within 30 minutes of the application of the DEET to the specimens, it began to rain and continued through that day and night. This may have effectively removed all of the DEET from the experiment 2 specimens, effectively making the results less useful. This may also explain why decomposition was not slowed in experiment 2 – it rained much more frequently during experiment 2 than during experiment 1.

This experiment only touched on the idea of a certain chemical (DEET) may inhibit the process of decomposition. More studies regarding the effects utilizing different concentrations of DEET, different seasonal times, and different size porcine carcasses would collect essential data not found in this study. More research would be helpful to demonstrate why or how DEET can or cannot affect the decomposition rates and whether it is the DEET or a by-product of DEET that repels or delays insect activity.

This experiment used both fetal and older porcine carcasses. It may have been more beneficial to have only used the older porcine carcasses and limit the placement in either of the carcasses in either the sun or shade. By doing so this would have limited some of these variables (size, sun and shade) observed in this experiment and focused on the effect of DEET in a more controlled study. Since only surface unclothed remains were studied in this experiment, it is unknown whether that DEET would have more or less of an effect on remains that have been buried or clothed. If the remains were contained this may allow the DEET to stay on the remains longer, or possibly permeate into the tissues of the clothed or buried remains. Also, remnants of the DEET may leave a residue on the clothing that could be tested. It is essential that future experiments should continue in this research area to understand the affects of DEET to help determine if the application of DEET after an extended exposure will cause additional changes to the decomposing remains.

Finally, in order for the potential effect of DEET to be useful in a forensic context, it would need to be confirmed whether the presence of DEET could be detected on decomposing remains. Studies would need to be conducted to see if, for example, body tissues or pupa cases could be tested for the presence of DEET. If it were known to be present, then it would be possible to adjust the time since death estimate to take the potential slowing of decomposition into account.

Conclusions

The results above, which in one experiment showed a qualitative and quantitative difference in the decomposition rates of control and DEET-treated porcine carcasses, suggest that insect repellants may have the effect of inhibiting insect infestation thereby slowing the rate of decomposition. While little difference was observed between the control and DEET-treated carcasses in the second experiment, this is almost certainly a function of the large amount of rain that occurred early in the study, which effectively removed the DEET from those specimens. This study was somewhat limited in scope, and additional research would improve our understanding of how the application of DEET affects the decomposition process and the estimation of PMI. Nonetheless, this study has the potential to benefit the medico-legal community by providing additional information on one factor that may affect the rate of decomposition. Such information is useful in determining the postmortem intervals and postmortem processes which are often critical to case resolution.

REFERENCES

American Association Swine Euthanasia (2008). Retrieved from http//www.aasv.org/aasv/documents/SwineEuthanasia.pdf

Anderson, G.S., Cervenka V.J. (2002).Insects associated with the body: their use and analyses. In: Haglund W.D., & Sorg M.H., editors. *Advances in Forensic Taphonomy: Method, Theory and Archaeological Perspectives* (173-200). Boca Raton: CRC Press.

Anderson, G.S., VanLaerhoven S.L. (1996). Initial studies on insect succession on carrion in southwestern British Columbia. *Journal of Forensic Sciences 41(4)*, 617-625.

Archer, M.S. (2004). Rainfall and temperature effects on the decomposition rate of exposed neonatal remains. *Journal of the Forensic Science Society 44(1)*, 35-41.

Archer, M.S. (2003). Annual variation in arrival and departure times of carrion insects at carcasses: implication for succession studies in forensic entomology. *Australian Journal of Zoology 51(6)*, 569-576.

Beyer, J.C., Enos, W.F., & Stajie, M. (1980). Drug identification through analyses of maggots. *Journal of Forensic Sciences 25(2)*, 411-412.

Byers, S.N. (2008). *Introduction to Forensic Anthropology*, third edition (1-27). Boston: Pearson Education, Inc.

Byrd, J.H., Castner, J.L. (2010). Insects of forensic importance in forensic entomology. In: Byrd, J.H., Castner, J.L., editors. *Forensic Entomology: The Utility of Arthropods in Legal Investigations* second edition (39-126). Boca Raton: CRC Press.

Campobasso, C.P., Vella, G.D., & Introna, F. (2001). Factors affecting decomposition and Diptera colonization. *Forensic Science International 120(1-2)*, 18-27.

Catts, E.P., Goff, M.L. (1992). Forensic entomology in criminal investigations. *Annual Review of Entomology 37,* 253-272*.*

Catts, E.P. (1992). Problems with estimating the PMI in death investigations. *Journal of Agricultural and Urban Entomology 9(4)*, 245-255.

Center for Disease Control and Prevention (2009). Insect repellant use and safety. www.cdc.gov/ncidod/dvbid/westnile/qa/insect_repellent.htm

Charabidze, D., Bourel, B., Hedouin, V. & Gosset, D. (2009). Repellent effect of some household products on fly attraction to cadavers. *Forensic Science International 189(1-3)*, 28-33.

Ditzen, M., Pellegrino, M. & Vosshall, L.B. (2008). Insect Odorant Receptors Are Molecular Targets of the Insect Repellent DEET. *Science Express 319 (5871)*, 1838–42. doi:10.1126/science.1153121.

England, D.B. (2006). Post mortem interval and decomposition rates: biological observations and mathematical analysis (master of science thesis). Corvallis (OR): Oregon State University.

Galloway, A., Birkby, W.H., Jones, A.M., Henry, T.E., & Parks, B.O. (1989). Decay rates of human remains in an arid environment. *Journal of Forensic Sciences 34(3)*, 607-616.

Geberth, V. (2007). Estimating the time of death. *Law and Order 55(3)*, 58.

Gennard, D.E. 2007). *Forensic Entomology: An Introduction* (1-70). England: Wiley & Sons Ltd.

Gill-King, H. (1997). Chemical and ultrastructural aspects of decomposition. In: Haglund, W.D., Sorg, M.H., editors, *Forensic Taphonomy: The Postmortem Fate of Human Remains* (93-108). Boca Raton: CRC Press.

Goff, M.L. (2009). Early post-mortem changes and stages of decomposition in exposed cadavers. *Experimental & Applied Acarology 49*, 21-36.

Goff, M.L. (2000). *A Fly For the Prosecution: How Insect Evidence Helps Solve Crimes* (61-80). Cambridge, Mass: Harvard University Press.

Goff, M.L., Flynn, M.M. (1991). Determination of postmortem interval by arthropod succession: a case study from the Hawaiian Islands. *Journal of Forensic Sciences 36(2)*, 607-614.

Goff, M.L. (1992). Problems in estimation of postmortem interval resulting from wrapping of the corpse: a case study from Hawaii. *Journal of Agricultural Entomology 9*, 237-243.

Goff, M.L. (1993). Estimation of the postmortem interval using arthropod development and successional patterns. *Forensic Science Review 81*, 81-94.

Goff , M.L., Brown, W.A., Hewadikaram, K.A. & Omori, A.I. (1991). Effect of heroin in decomposing tissues on the development rate of *Boettcherisca peregrina* (Diptera, Sarcophagidae) and implications of this effect on estimation of postmortem intervals using arthropod development patterns. *Journal of Forensic Sciences 36(2)*, 537-542.

Gonzales T.A., Vance M, Helpern M, Umberger C.J. (1954). Legal Medicine, Pathology and Toxicology. New York: Appleton-Century Crofts Inc.

Grassberger M., Frank, C. (2004). Initial study of arthropod succession on pig carrion in a central Europe urban habitat. *Journal of Medical Entomology 41(3)*, 511-523.

Gunatilake, K., M. L. Goff. (1989.) Detection of organophosphate poisoning in a putrefying body by analyzing arthropod larvae. *Journal of Forensic Sciences 34(3)*, 714-716.

Kulshrestha, P., Satpathy, D. K. (2001). Use of beetles in forensic entomology. *Forensic Science International 120(1-2)*, 15-17.

Kreitlow, K.L.T. (2010). Insect succession in a natural environment. In: Byrd, J.H., Castner, J.L., editors. *Forensic Entomology: The Utility of Arthropods in Legal Investigations* second edition. Boca Raton: CRC Press, 251-266.

Lopes de Carvalho, L.M., Linhares, A.X. (2001). Seasonality of insect succession and pig carcass decomposition in a natural forest area in southeastern Brazil. *Journal of Forensic Sciences 46(3)*, 604-608.

Lord, W.D., M.L. Goff, T.R., Adkins, & N.H. Haskell, (1994). The black soldier fly Hermetia illucens (Diptera: Stratiomyidae) as a potential measure of human postmortem interval: observations and case histories. *Journal of Forensic Sciences*, *39*, 215-215.

Mann, R.W., Bass, W.M. & Meadows, L. (1990). Time since death and decomposition of the human body: variables and observations in case and experimental field studies. *Journal of Forensic Sciences 35(1)*, 103-111.

Mant, A.K. (1987). Knowledge acquired from post-war exhumations. In Boddington A, Garland A.N., Janaway R.C., (eds.): Death, Decay and Reconstruction: Approaches to Archaeology and Forensic Sciences. Manchester, England: Manchester University Press, 65-80.

Marks M., and Love J., (2003). Taphonomy and Time: Estimating the Postmortem Interval. In: Steadman DW, editor. Hard Evidence: Case Studies in Forensic Anthropology. Upper Saddle River, NJ: Person Education, Inc. and Prentice Hall, 160-175.

Marks, M.K., Love J.C., & Dadour, I.R. (2003). Taphonomy and time: estimating the postmortem interval. In: Steadman, D.W., editor. *Hard Evidence: Case Studies in Forensic Anthropology*, second edition (160-175). NJ: Prentice Hall.

Matuszewski, S., Bajerlein, D., Konwerski, S. & Krzysztof, S. (2008). An initial study of insect succession and carrion decomposition in various forest habitats of Central Europe. *Forensic Science International 180*, 61-69.

Megyesi M (2001). The Effects of Temperature on the Decomposition Rate of Human Remains (Master Thesis/abstract). University of Indianapolis Archeology & Forensics Laboratory. (http://archlab.uindy.edu).

Megyesi, M.S., Nawrocki, S.P. & Haskell, N.H. (2005). Using accumulated degree-days to estimate the postmortem interval from decomposed human remains. *Journal of Forensic Sciences 50(3)*, 618-626.

Michaud, J.P., Moreau, G. (2011). A statistical approach based on accumulated degree-days to predict decomposition-related processes in forensic studies. *Journal of Forensic Sciences 55(2)*, 295-301.

Micozzi, M.S. (1986). Experimental study of postmortem change under field conditions: effects of freezing, thawing, and mechanical injury. *Journal of Forensic Sciences 32*, 953-961.

Morris, B., & Dadour, I. (2005). Forensic Entomology; The use of insects in legal cases. *Expert evidence. Australia: Lawbook Co*, 91A.

National Pesticide Information Center. (2003). DEET Technical Fact Sheet. Retrieved from http://npic.orst.edu.

National Research Council. (2003). Executive Summary. *Gulf War and Health: Volume 2. Insecticides and Solvents*. Washington, DC: The National Academies Press.

NOAA Satellite and Information Service. National Oceanic Atmospheric Administration/National Climate Data Center. Retrieved from http://www.ncdc.noaa.gov.

Pavia, D.L. (2004). *Introduction to Organic Laboratory Techniques* (370-376). Belmont: Thomas Learning. David Harris Publishers Brooks/Cole

Payne, J.A. (1965). A summer carrion study of the baby pig *Sus scrosfa* Linnaeus. *Ecology 46(5)*, 592-602.

Petherick, A. (2008). How DEET jams insects' smell sensors. *Nature News*. doi:10.1038/news.2008.672

Pesticide Fact Sheet (2008). United States Environmental Protection Agency Office of Prevention, Pesticides and Toxic Substances. (7505P). Retrieved from http://www.epa.gov/opprd001/factsheets/chloran.pdf.

Rodriguez, W.C., Bass, W.M. (1983). Insect activity and its relationship to decay-rates of human cadavers in east Tennessee. *Journal of Forensic Sciences 28(2)*, 423- 432.

Shean, B., Messinger, L., & Papworth, M. (1993). Observations of differential decomposition on sun exposed v. shaded pig carrion in coastal Washington State. *Journal of Forensic Sciences 38(4)*, 938-949.

Smith, K.G.V., (1986). A manual of forensic entomology. British Museum, London

Syed, Z., Leal, W.S. (2008). Mosquitoes smell and avoid the insect repellent DEET. *Proceedings of the National Academy of Sciences of the United States of America 105 (36)*, 13598–13603. doi: 10.1073/pnas.0805312105. http://www.pubmedcentral.nih.gov

Swindle, M.M., & Smith, A.C. (1998). Comparative anatomy and physiology of the pig. *Scand J Lab Animal Science*, *25* (Suppl 1), 1-10.

Vass, A.A. (2001). Beyond the Grave - understanding human decomposition. *Microbiology Today 28*, 1-3.

Vass, A.A, Barshick, S.A, Sega, G., Caton, J., Skeen, J.T., Love, J.C. & Synstelian, J.A. (2002). Decomposition chemistry of human remains: a new methodology for determining the postmortem interval. *Journal of Forensic Sciences 47(3)*, 14.

Voss, S.C., Cook, D.F. & Dadour, I.R. (2011). Decomposition and insect succession of clothed and unclothed carcasses in Western Australia. *Forensic Science International 211*, 67-75.

Wang, Benjamin J.S. (1974). An interesting and successful organic experiment (CEC). *Journal of Chemical Education 51 (10)*, 631. doi: 10.1021/ed051p631.2

Vita

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