INFLUENCE OF VEGETATION HEIGHT ON THE DISPERSAL OF HOUSE FLIES (*MUSCA DOMESTICA*) FROM LIVESTOCK FACILTIES AND FILTH FLY ACTIVITY ASSOCIATED WITH COMPOSTED AND NON-COMPOSTED BEEF CADAVERS

By

TRISHA ROSE DUBIE

Bachelor of Science in Forensic Chemistry
Bachelor of Science in Criminal Justice;
Criminalistics
Lake Superior State University
Sault Sainte Marie, Michigan
2006

Master of Science in Entomology and Plant
Pathology
Oklahoma State University
Stillwater, Oklahoma
2010

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Oklahoma State University
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Dissertation Approved:

Dr. Justin Talley

Dissertation Adviser

Dr. Astri Wayadande

Dr. Tom Royer

Dr. Joshua Payne

Dr. Chris Richards

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Abstract: House flies, *Musca domestica* L., are well known pests inexorably linked to livestock operations, and they also serve as mechanical vectors of various human pathogens including *E. coli* 0157:H7. Changes in the landscape from anthropogenic activity have resulted in increased human exposure to house fly pests, and monitoring their movements is paramount in assessing the risks associated with flies emigrating from livestock facilities. This study focused on edge following behavior and the use of habitat corridors by house flies. Artificial structures designed to represent tree and shrub lines were constructed in a field south of a beef feedlot facility. Marked house flies were released at individual sites containing 3m walls, 1.5m walls and a control site with no wall and a substantially higher number of flies were trapped at the 3m site than both the control and 1.5m sites. Immunomarking techniques were also evaluated for this study using chicken ovalbumin as an antigen. A false negative rate of 0% was determined using unmarked wild flies as negative controls. Livestock facilities are also faced with the challenge of disposing of a significant number of cadavers each year. One increasingly popular way of dealing with dead animals is composting. Animal mortality compost is designed to facilitate decomposition without the aid of carrion feeding insects and reduce the presence of common pathogens associated with animal waste and dead tissue. The goal of this study was to evaluate insect activity associated with composted and exposed beef cadavers, specifically filth flies that can serve as mechanical vectors of important human pathogens such as E. coli 0157:H7. The number of filth flies was significantly lower at the composted site than the exposed site. Volatile organic compounds were also sampled in this study, and known fly attractants such as dimethyl disulfide were inhibited by the composting process. Lastly, carrion feeding insects were collected from each of the exposed animals and documented. These species are also important in forensic entomology. Implementing composting programs and using walls of vegetation at livestock facilities could reduce the risk of flies spreading harmful pathogens to surrounding areas including farms that grow fresh produce.

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CHAPTER I

INTRODUCTION

Filth flies including house flies, *Musca domestica*, are well known pests inexorably linked to commercial livestock operations. They develop in various types of decaying organic matter and animal manure and facilities such as beef feedlots provide excellent resources for flies. These substrates also contain important microorganisms implicated in many human and other animal illnesses. A variety of these microorganisms can be transmitted by filth flies such as house flies and blow flies (Diptera:Calliphoridae). House flies are competent mechanical vectors of various pathogenic protozoa, bacteria and viruses including but not limited to *Escherichia coli 0157:H7, E. hystolytica, Samlonella spp., Camplyobacter jejuni, Giarda spp., Trichomonas spp., Sarcocystis spp., Toxoplasma gondii, Isospora spp., Endolimas nana, and Cryptosporidium parvum and Polio* (Graczyk 2001). Blow flies have been implicated in the spread of avian influenza (H5N1), *E. coli* 0157:H7 and other pathogens (Sawabe et al. 2006, Talley et al. 2009). Both types of flies have also been shown to carry a variety of antibiotic resistant bacteria including *E. coli*, *Staphylococcus* spp., *Enterococcus faecium*, and *Enterococcus faecalis*

(Graham et al. 2009, Blaak et al. 2014, Zurek and Ghosh 2014).

Changes in the landscape from anthropogenic activity have resulted in increased exposure to filth fly pests. Homes and business are being built in closer proximity to livestock operations that were once isolated which increases the likelihood of complaints from residents of nuisance flies and potentially the prevalence of pathogens in surrounding areas. Residents in areas where house flies are a nuisance pest have not only filed formal complaints but have also filed lawsuits against livestock producers in the area where they believe the flies originated. These lawsuits have resulted in costly settlements paid to the residents and have even caused some animal production facilities to close (Thomas 1993) or resulted in firmer "right to farm" legislation designed to protect such facilities (Reinert 1998). Research associated with house fly dispersal from livestock facilities and filth fly activity associated with livestock carcasses will aid in the development of pest management and waste management programs that may help decrease the nuisance created by large numbers of house flies invading residential areas. Pest management strategies applied in an integrated manner are necessary to keep fly populations below problematic levels. House flies have also become resistant to most chemical insecticides. A survey testing flies from dairies in nine states across the country found evidence of resistance to six different insecticides (Scott et al. 2013).

Livestock operations large and small must manage waste regularly including manure and dead animals. Manure in pens and barns are an ideal site for house fly larval development, and many of the traditional means of manure disposal allow

fly populations to proliferate throughout the landscape if not properly managed. This includes application to crop lands for fertilizer which has been a popular method among commercial animal facilities but will not eliminate fly activity and comes with additional public health risks (Graham and Nachman 2010). Animal mortalities can also be challenging to dispose of as the environmentally sound methods that have been available to producers, such as rendering, are becoming more costly and less readily available (Gwyther et al 2011). Composting is a method for manure and animal carcass disposal that has become popular in many countries. This simple, economical method facilitates the decomposition of waste and animal tissue with the aid of increased temperatures and thermophilic microorganisms (Berge et al. 2009). This study includes evaluations of this composting process focused primarily on filth fly activity. These experiments may also help to assess the ability of flies to transfer human pathogens to the surrounding area including facilities that grow fresh produce such as leafy greens and fruit. Filth flies have been captured in fields of leafy greens, some of which tested positive for the virulent, zoonotic pathogen *E. coli* 0157:H7 (Talley et al. 2009). These bacteria have been shown to be successfully deposited onto the surface of spinach leaves by house flies and even replicate in the drops of fly excreta (Wasala et al. 2013). Composting has been shown to efficiently degrade and eliminate a variety of pathogenic organisms (Wilkinson 2007). This research addresses several questions related to house fly dispersal behavior and filth fly activity associated with the disposal of animal mortalities by composting at beef feedlots.

OBJECTIVES

1. **Determine if house flies, *Musca domestica*, exhibit edge-following behavior and/or corridor use in a feedlot setting.**

   House flies may utilize habitat corridors when dispersing from environments such as commercial livestock facilities. They may also follow these corridors or edges while traveling toward a suitable oviposition source. Habitat corridors could potentially be used to guide house flies traveling toward and dispersing out of beef feedlots to specific areas for the purpose of pest control.

2. **Examine the efficacy of protein markers to determine dispersal behaviors of house flies, *Musca domestica*, out of beef feedlots**

   Mark, release and recapture studies in areas where house flies are problematic can provide valuable information regarding dispersal behaviors. Using data collected from field studies, densities of house flies within particular areas surrounding beef feedlots can be predicted. Any study evaluating fly dispersal requires a reliable and persistent marking technique. Protein markers detected using enzyme-linked immunosorbent assay may be a viable option for house fly-beef feedlot systems.

3. **Evaluate the fly activity and volatile organic compounds associated with exposed and composted livestock mortalities.**

   Commercial livestock facilities and even smaller animal farms must dispose of mortalities regularly. Composting animal cadavers is a new technique

developed to facilitate the disposal through high temperatures and beneficial microorganisms. Filth flies are always associated with decaying animal tissue. Composting carcasses may reduce the prevalence of pathogens and abundance of flies able to transport pathogens to other areas. The composting process may also inhibit the release of important decomposition odors that are known to attract filth flies.

4. **Identify the species of arthropods visiting carrion collected from exposed beef cadavers throughout the entire period of decomposition.**

   Hundreds of species of arthropods utilize decomposing animal tissue as a resource. Some consume the decaying tissue directly and others are predators and parasitoids taking advantage of the abundance of prey and hosts available at a carcass. Many of these species, especially flies, are used in forensic entomology which uses insect evidence to contribute valuable information to legal investigations. Cataloging the carrion feeding species will help forensic entomologists in future investigations.

CHAPTER II

REVIEW OF LITERATURE

THE HOUSE FLY, *Musca domestica*

The Dipteran family Muscidae contains several species of filth flies including the common house fly, *Musca domestica* L. These flies thrive in decomposing organic matter and substrates such as animal manure and household garbage (Moon 2009). House flies are considered to be synanthropic or having close association to human activity (Moon 2009). The life history of the house fly was not thoroughly studied and documented until 1896 when L.O. Howard shifted his attention to house flies, originally dubbed "typhoid flies" (Howard 1911). Subsequent research done by C.G. Hewitt (1914) emphasized the importance of house flies as nuisance pests and potential carriers of pathogenic organisms, then referred to as "disease carriers". While house flies are non-biting insects, they do have the ability to transmit a variety of pathogens from one place to another. Large numbers of house flies and their exposure to harmful pathogens associated with animal waste makes them more than simply a nuisance pest.

Biology and Ecology. House flies are ubiquitous in terrestrial environments with exception to the southernmost region of the globe that consistently reaches temperatures below freezing. Adult house flies are grey and black with four black vittae (longitudinal stripes) on the thorax ranging from 6mm to 9mm in length in the adult stage, and female adult flies have an ivory colored region on either side of the typically black and grey abdomen (Figure 1.1) (West 1951). House flies are non-biting muscoid flies that have haustellate mouthparts with modifications commonly referred to as sponging style mouthparts (Figure 1.2) (Moon 2009). The distended labellum of the house fly contains several sets of grooves on either side called pseudotracheae (Moon 2009). These structures include setae that aid in the manipulation and direction of food substrates, mechanoreception and chemoreception (Moon 2009).

Adult flies require sugar and protein in their diets in order to mature and reproduce (Adams and Nelson 1990). They are considered anautogenous, meaning they require protein for egg production (Moon 2009). Female flies also have specific dietary needs for the production of cuticular hydrocarbons that act as sex pheromones necessary for mating (Carlson et al. 1971, Adams and Nelson 1990). Female house flies synthesize a pheromone containing (Z)-9-tricosene present largely in the abdominal integument that attracts male flies and is highly correlated to oogenesis (Dillwith et al. 1983). Males sense the compounds using sensory structures located on the tibia (Schlein et al. 1981). Female house flies tend to be monogamous, and this is primarily because once

they have mated they become less receptive as a result of components found in the seminal fluid from the male house fly (Riemann et al. 1967). Receptivity can return once the female has laid her eggs and some will actually mate again (Riemann et al. 1967).

The fecundity of female flies is dependent on a variety of factors, and the amount of eggs that each fly will produce varies. These factors include ambient temperature, diet of the adult flies, quantity and quality of the diet as larvae, photoperiod, age and availability of oviposition substrates (Zvereva and Zhemchuzhina 1988, Pastor et al. 2011). In general, adequate amounts of both sugar and protein will allow for sufficient egg production (Pastor et al. 2011). Oviposition sites must be suitable for larval development, and the female house fly will lay very few or no eggs at all if appropriate factors are not present (Pastor et al. 2011). When eggs are laid, chemical cues are released involving symbiotic bacteria that illicit a response in conspecific females promoting further oviposition (Lam et al. 2007). These same bacteria proliferate and over time olfactory cues result in an inverse response from other female flies eventually inhibiting oviposition (Lam et al. 2007). House fly eggs usually hatch within 24 hours depending on the ambient temperature. The house fly larvae can feed on a wide range of organic substrates. This includes waste from various livestock animals such as poultry and cattle (Barnard et al. 1998, Calvert et al. 1970, Pastor et al. 2011). The diet of house fly larvae has a significant impact on the overall development of the flies through pupation and into adulthood including egg production (Hogsette 1992,

Krafsur et al. 1985, Pastor et al. 2011). Certain bacteria are also essential to larval nutrition (Watson et al., 1993). These bacteria can be associated with both the resource in which eggs were deposited by the female as well as the surface of the eggs themselves (Lam et al. 2009). Depending on the temperature, larvae will feed for three to four days before beginning to pupate. Pupal stage development is also dependent on temperature and can last for five to seven days under normal conditions. House flies commonly overwinter as adults in buildings, and they may do so in other life stages if they are located in a microhabitat that remains above -5°C (Rosales et al. 1994, Moon 2009). Eclosion from the pupae occurs after seven days on average depending on ambient conditions. The adult fly emerges from the puparium by pushing the anterior end where predetermined lines aid in the detachment of the cap (West 1951, Hall and Gerhardt 2009). They accomplish this by inflating an area of the head known as the ptilinum, which is subsequently deflated and retracted into a fissure near the antennae (Figure 1.3) (Hall and Gerhardt 2009). Adults can live for several weeks although the average for females is approximately 30 days and for males approximately 20 (Raglang and Sohal 1973).

Behavior and Sensory. House flies sense their environment in a variety of ways while foraging for food, searching for mates and locating adequate substrates for oviposition. They detect chemical compounds in the air, see certain colors and shapes, and even taste when they encounter a resource. These stimuli then

influence the behavior of house flies. Although control of house flies remains challenging, much is known about the physiology and biochemistry associated with these sensory mechanisms. All insects are equipped with different types of photoreceptors, mechanoreceptors and chemoreceptors. Photoreception and chemoreception are both involved in the location of a resource. Olfactory senses hone in on long range chemical cues emitted from a desired resource and prompt the flies to orient themselves in a specific direction to begin searching for that resource. Once the insect has oriented appropriately and begun following the chemical trail leading to the source from which it is emitting, visual cues help the insect pin point the location of the resource and where to land. Once they have landed and examined the substrate, further chemoreception aids in the flies' acceptance of the resource.

Olfaction in most insect groups is very similar with respect to the basic structural and functional components. Insects have various forms of olfactory sensilla including trichoid sensilla which are long, slender hair-like projections, basiconic sensilla that are thick, round projections commonly referred to as "pegs" and ceoliconic sensilla have structures located inside depressions in the cuticle and are given the "peg-in-a-pit" moniker (Chapman 1998, Nation 2008). The exact structure of these three types of sensilla can be quite diverse. They are classified using binomial nomenclature based on their specific structure and function, for example *Sensilla trichoidea* (Nation 2008). Each sensillum has a general structure regardless of its location or overall shape. They include one or more pores in the cuticle surrounding the main structure. Inside, there can

be one or more neurons surrounded by liquid media. The basic function of the sensillum can be explained as follows: A molecule of odorant, which can be as simple a carbon dioxide or as complex as a pheromone, enters through a pore on the sensillum. Pores vary in size and the compounds associated with the biochemical mechanisms that sense the odorant can be highly specific. The odorant molecule is met and bound to by an odorant binding protein (OBP). These proteins can have different forms such as, pheromone binding proteins or general odorant binding proteins (Nation 2008). The OBP then transports the odorant through the liquid media inside of the sensillum to a receptor located on the dendrite. The specific receptor is coupled with a G-protein that acts as a secondary messenger. These and many other sensory receptors in the insect system belong to a group referred to as GPCRs, g-protein coupled receptors (Nation 2008). Once the odorant is bound to the receptor site, the G-protein is activated and triggers the production of an enzyme, for example adenylate cyclase. The enzyme facilitates the production of small molecules that aid in the opening of ion channels, for example cyclic AMPs (Nation 2008). The change in electric charge from the movement of ions sends a signal down the axon, referred to as signal transduction. Another cascade that is activated by a G-protein on pheromone receptor sites is the phosphoinositide cascade in which inositol triphosphate, IP3, is the product that opens sodium and calcium ion channels (Nation 2008). The sensory reception is stopped by odorant degrading enzymes. These help to avoid overstimulation of the receptors in the specific area of the nervous system. These can be compared to enzymes such

as acetylcholinesterase which degrade the neurotransmitter acetylcholine in the insect nervous system to clear the pathways for another transmission and prevent the system from remaining in an excited state (Kent 1998).

Combinations of sensilla types can exist on the insect antennae, which are the main sites of long range olfactory reception. Filth flies typically have aristate antennae with a variety of sensilla covering the surface of the flagellum, the arista and the ventral and distal surfaces of the palps (Kelling et al. 2002, Kaupp 2010). This organization is similar to that of the Mediterranean fruit fly, *Drosophilia melanogaster,* and a substantial amount of information is available from this model insect (Figure 1.4) (Stocker 1994, Kelling et al. 2002). These sensory structures can detect a wide variety of volatile organic compounds emitted from decomposing organic matter including manure and decomposing animal tissue. Compounds that elicit a response in insects are collectively called semiochemicals, and they are further categorized by their specific function (Fig. 1.5). Some olfactory cues detectable by filth flies are emitted from non-living sources and are referred to as apneumones. These are different, for instance, from chemicals produced to communicate with another member of the same species referred to as pheromones. The physiological response of certain compounds emitted from swine manure has been documented and includes odorants like dimethyl trisulfide, indole, dimethyl disulfide, butanoic acid, 3-methylbutanoic acid, and phenol (Cosse and Baker 1996). There has also been extensive research on olfaction associated with mating pheromones as mentioned above. An interesting study by Boroczky et

al. (2012) revealed particular grooming behavior exhibited by house flies and other insects that actually increases the efficiency of the antennal olfactory sensilla. Semiochemicals emitted from substrates also play a key role in oviposition by female house flies (Lam et al. 2010). Additional research has also shown that larger adult house flies can have as much as double the number of sensilla on their antennae versus smaller adults house flies although no differences in behavior were observed (Smallegange et al. 2008).

Vision becomes important with locating resources once the olfactory cues have successfully drawn the insect to the vicinity. Flies can sense boundaries, contrast and outlines of certain shapes and are attracted to certain colors (Conlon and Bell 1991, Howard and Wall 1998, Geden 2006). Insects have three main types of photoreceptors: compound eyes and two types of simple eyes including ocelli and stemmata. Most adult insects have compound eyes and a varying number of ocelli located on the head. The ommatidia of the compound eyes are where most of the visual cues are interpreted (Nation 2008). Each facet is elongate with a corneal lens at the top followed by other dioptric structures including a cone, pigments cells, retinula cells and rhabdomeres where the active pigments are located (Fig. 1.6) (Nation 2008). Light enters each ommatidium axially through the corneal lens and passes down through the cone where it reaches the rhabdom. The rhabdom is the area that contains the retinula cells, rhabdomeres and light activated pigments. This area is surrounded by pigments cells, sometimes called shielding cells, which contain pigments that absorb excess light not coming down through the cone

allowing each individual facet to function independently (Nation 2008). The retinula cells are long and slender and on the inside is where the rhabdomeres are located. The rhabdomere consists of microvilli all along the length of the inside that contain rhodopsin, the pigments that interact with photons of light. Rhodopsin is a compound made of the protein opsin and a chromophore, molecules that react with light at specific frequencies and are responsible for color (Nation 2008). These can vary depending on which type of wavelengths and colors the insect can detect. The chemical process is similar to that of chemosensory of volatile organic compounds. Photons of light pass down through the ommatidia to the rhabdomere where they react with rhodopsin and activate the chromophores. This reaction triggers the activity of another G-protein which begins a cascade leading to depolarization and signal transduction down the axons that lead into the optic lobe (Nation 2008). Light energy is converted to chemical energy which is converted to electrical energy.

Experiments evaluating house fly vision have yielded somewhat inconsistent results with respect to color perception and/or attraction compared to those focused on contrast and particular patterns. House flies can learn to associate particular visual patterns with a food source (Conlon and Bell 1991). Fukushi (1976, 1989) also found that house flies and blows flies will learn to associate certain colors with a resource. The contrast between the surface of a substrate and background has been shown to be more attractive to house flies than the substrate alone with little to no contrast with the surrounding environment (Howard and Wall 1998). Numerous studies have

evaluated the usefulness of colored traps and the appeal of certain colors over others to house flies and other muscoid flies such as stable flies and tsetse flies. Few differences in fly preference were found among baited traps in one study that were painted red, yellow, blue, green and black and placed near poultry facilities to monitor house flies, although the yellow traps did have a higher number of flies that the white, unpainted traps (Burg and Axtell 1984). However, these results could not be attributed to the color alone due to the presence of additional lures within the jug traps. Another study compared the attractiveness to white vs. fluorescent yellow targets and found no significant differences although traps used in this study also contained (Z)-9-tricosense which likely influenced the ability to accurately evaluate the color alone (Chapman et al. 1999). Based on the substantial attraction of tsetse flies to blue colored substrates, Geden (2006) evaluated the effectiveness of Alsynite cylinders with white, black and blue colored fabrics on stable flies and house flies and concluded that the blue color does seem to attract both fly species. House flies have been shown to react to light ranging from 340 to 370nm (ultraviolet light) and 480 to 510nm (blue-green light) (Bellingham and Anderson 1993). Most recently, electroretinogram (ERG) has been used in addition to bioassays in a study designed to evaluate both the behavioral response and physiological response of house flies to different color targets. Results of these studies determined that attraction and physiological response are highly correlated with white and blue targets and although yellow targets elicited a strong physiological response they actually repelled the flies (Diclaro

et al. 2012). Contrast continues to be a major factor in house fly visual attraction and different colors can play a role.

Once the flies land they must confirm that the substrate is acceptable. They do this by "tasting" the source with gustatory receptors located on the surface of the labellum and on the foretarsi (Chapman 1998, Hansen et al. 1998, Nation 2008). These are usually trichoid type receptors with a single pore located at the end of the hair-like structure, and these sensilla can sense chemicals in solution such as water, sugars, and salts (Nation 2008). The chemical compounds entering the gustatory receptors are received similarly and involve similar biochemical pathways and activation of signal transduction as the olfactory mechanisms described above. When flies begin exploring substrates, they regurgitate fluid from the crop onto the surface facilitating the detection of ions from the food source (West 1951, Chapman 1998). Chemoreceptors on the tarsi allow for examination of the concentration of phagostimulants, and when high enough amounts are present the fly proboscis extends (Chapman 1998). Additional sensilla present on the labellum activate further response allowing the pseudotracheae on the surface of the mouthparts to contact the substrate and initiate feeding (Chapman 1998). These structures as well as the regurgitation behavior of flies play a role in the acquisition and retention of microorganisms present in the substrates on which the flies feed.

House Flies and Pathogens. House flies have been implicated in the transmission of a variety of pathogens that can infect humans and other

animals. They are among several species known as filth flies that breed and develop in various types of animal waste including manure (Graczyk et al. 2001, Greenberg 1973). A diverse group of filth fly species have been implicated in the mechanical transmission of microorganisms, many of which are zoonotic pathogens important in food safety. Doctors and other scientists associated flies with disease centuries before the various modes of transmission were elucidated through that are examined by modern research conducted by medical and veterinary entomologists. Mechanical transmission of microorganisms by flies was described in the literature as early as 200 A.D. The Talmud, ancient Babylonian text, describes the spread of a skin disease by flies, perhaps caused by a pathogenic bacterium, in this statement from Kethuboth 77b: "Beware of the flies of the man afflicted with ra'athan..." (Greenberg 1973, Preuss 2004). Although the Babylonians were convinced that eating beets and drinking beer would prevent this particular illness, the implication of flies as mechanical vectors was perceptive. We now know that a variety of flies are capable of spreading microorganisms, and because of the discovery of germ theory we have a better knowledge of the transfer of these organisms and which of them causes disease in humans. House flies are likely one of the most important mechanical vectors of disease. Numerous pathogens have been linked to house flies and other non-biting flies including types of protozoa, bacteria, and viruses. Flies as nuisance pests and carriers of "disease" have been the subject of experiments and publications for more than two centuries. In 1776, Montfils speculated that flies could spread *Bacillus*

anthracis, the bacterium that causes anthrax (cited in West 1951). This hypothesis was tested nearly a century later when a scientist named Raimbert pulverized non-biting flies and caused the disease in guinea pigs using a suspension of the fly material (cited in West 1951). Since these early experiments, countless studies have implicated house flies in the mechanical transmission of harmful microorganisms. In Thailand a correlation was established between increases of enterohemorrhagic *Escherichia coli* 0157:H7 infections in villagers with the prevalence of the bacteria carried by synanthropic flies (Echeverria et al. 1983). The same pathogen, *E. coli* 0157:H7, was also recovered from house flies outside of cattle farms in Japan and the United States (Iwasa et al. 1999). Another study in Japan isolated verotoxin-producing *E.coli* O157:H7 from house flies collected near a facility where humans had been infected with the bacterium (Moriya et al. 2002). House flies from dairy and turkey facilities were shown to carry *E. coli* 0157:H7, *Campylobacter spp.*, and *Cochlosoma anatis* and successfully deposit the bacteria by defecation and regurgitation onto filter paper placed within each facility (Brazil et al. 2007). Another study examining house flies as mechanical vectors of *Campylobacter jejuni* concluded that these bacteria could potentially be transmitted to poultry facilitating subsequent infection in humans that consume the contaminated meat (Skövgard et al. 2011). After isolation of *E. coli* 0157:H7 from flies collected at a nursery school where an epidemic of enterohemorrhagic colitis had recently occurred, it was shown that the bacteria could possibly replicate on the fly and accumulate in the crop

resulting in a more complex interaction that the authors describe as "biomechanical transmission" (Figure 1.7) (Kobayashi et al. 1999). Wasala et al. (2013) showed that once *E. coli* 0157:H7 is acquired by flies from contaminated cow manure it can survive and proliferate on external fly surfaces, supporting Kobayashi's biomechanical transmission hypothesis. Additionally, Wasala et al. (2013) demonstrated bacterial replication in the regurgitation spots that the fly deposits after landing on substrates including fresh produce such as spinach leaves. Additional research has shown that *E. coli* from the house fly and stable fly larvae can persist through the pupal stage suggesting that larval exposure to pathogens could also be a factor in the transmission (Rochon et al. 2005). Retention of *E. coli* 0157:H7 by house flies from the larval stage to the adult stage was shown once again by Schuster et al. (2013) under laboratory condition substantiating the claim that the pathogen can survive fly metamorphosis. Other pathogens that have been isolated from and could potentially be spread by house flies and other synanthropic flies include *Salmonella* sp., *Shigella* sp., *Vibrio* sp., *Staphylococcus aureus*, *Yersinia enterocolitica*, *Pseudomonas* sp., *Acinetobacter* sp., *Chlamydia* sp., *Klebsiella* sp., *Enterobacter* sp., *Enterococcus* sp., *Proteus* sp., *Acinetobacter* sp., *Sarcocystis* sp., *Proteus* sp., *Toxoplasma gondii*, *Isospora* sp., *Giardia* sp., *Entamoeba coli*, *Entamoeba histolytica*, *Endolimax nana*, *Trichomonas* sp., *Hammondia* and *Cryptosporidium parvum Polio* (Greenburg, 1973, Graczyk et al., 2001, Nazni et al., 2005). House flies may also contribute to the spread of antibiotic resistant

bacteria. A review by Zurek and Ghosh (2014) asserts that the use of antibiotics in the livestock industry has likely driven the natural selection of antibiotic resistant bacteria, and filth flies can serve as carriers of these organisms from farms into urban areas. Multiple studies have found flies that tested positive for antibiotic resistant strains of bacteria (Table 1.1). One study tested house flies collected from two separate confined swine facilities and found that they carried a variety of antibiotic resistant *Enterococcus* spp. (Ahmad et al. 2011). Flies have also been collected form urban areas and tested for antibiotic resistant strains of bacteria. House flies collected from multiple fast food restaurants in Kansas tested positive for Enterococcus spp. resistant to tetracycline, erythromycin, streptomycin, and ciprofloxacin (Macovei and Zurek 2006).

INSECT DISPERSAL

A tremendous number of experiments have focused in the movement and dispersal patterns of different insect groups to examine their behaviors, predator-prey interactions, life history, etc. These experiments must utilize some form of mark recapture technique; marking on the insect at a known location and then trapping at additional locations in order to determine where that insect has been, how far it has traveled and perhaps how much time has gone by during those periods. The marking techniques used by scientists vary, and some methods may work very well with one system but not so well with

others. Novel and traditional methods of marking insects continue to benefit research examining insect movement.

Marking insects. Marking animals of any kind is commonly a vital component of experiments conducted to study the animals' biology, ecology and behavior (Hagler and Jackson 2001). Studies involving marked insects began in the early twentieth century and involved stains, paints and dyes (Dudley and Searles 1923, Geiger et al. 1919, Hagler and Jackson 2001). Studies in which insects are marked to determine movements fall into two categories: 1) mark-release-recapture and 2) mark and capture. The first technique involves marking insects in the laboratory either from colonies or live specimens collected in the field, and the second involves trapping insects that have been marked after contacting a marking material in the environment that has been provided for the study (Nazni et al. 2005). A variety of insect groups have been studied using techniques involving release and recapture of marked individuals. Insects can be successfully marked with many different materials; however, ensuring that these markers will persist in the field once the insects have been released can be considerably challenging. Fluorescent pigments are commonly used to mark insects for experiments associated with dispersal. Some studies in which fluorescent powders were used as markers include the dispersal of stable flies from larval development sites by Taylor et al. (2010) and estimating population densities of *Lucilia sericata* by Smith and Wall (1998). Mark-release-recapture studies do not always result in substantial catch rates. Smith and Wall (1998)

reported recapture of just 4% to 14% of their blow flies marked with fluorescent pigment. While fluorescent powders are presently used for studies evaluating insect movement, new marking methods continue to emerge that may be more accurate and cost efficient. Immunomarking techniques are the most recent method of marking insects to be used for dispersal and movement experiments. These techniques use proteins from soy, cow's milk, wheat gluten and chicken egg whites to mark the insects and then detect the marker using an enzyme-linked immunosorbent assay (ELISA) (Jones et al. 2006, Jones et al. 2011). These markers can be detected at very low levels, and persist in the environment even through light rainfall (Jones et al. 2006). The lower detection limits for these markers were tested and results showed that as little as 1 ppb can be detected using soy milk and soy flour, 1.9 ppb using powdered egg whites and 7.8 ppb with liquid egg whites, and bovine casein was detected at 7.8 ppb in powdered nonfat milk and 31.2 ppb from whole milk (Jones et al. 2006). Protein markers are commonly applied as a solution and sprayed onto the surface of vegetation to monitor insect movement. This technique was assessed using *Hippodamia convergens* and *Lygus hesperus* and result showed that both chicken ovalbumin and bovine casein can successfully mark the insects after exposure to residues on plants, and the marker can be retained by the insect for up to 2 days (Hagler and Jones 2010). Honeybees were effectively marked with powdered protein using an apparatus fixed to the hive entrance filled with the powder which marked the honeybees as they crawled through (Hagler et al. 2011). Protein markers have also been used successfully

with coddling moth, horn-faced bees, psyllids, and ants and their use continues to increase in popularity (Boina 2009, Buczkowski and Bennett 2009, Basoalto et al. 2010, Biddinger et al. 2013).

Dispersal of Synanthropic Flies. Tracking the movement of synathropic flies is of particular interest to scientists not only for purposes of more efficient pest control but also as a matter of public health. The distinction between the rural and urban landscape has become far less defined, and the amount of flies dispersing away from animal production facilities has become more problematic with these changes. Determining the distances and directions in which Dipteran pests will travel is vital to the success of pest management programs and assessments of the risks associated with contamination of areas by filth flies that can transmit important pathogens. One mark-release-recapture study evaluating the flight range of *Musca domestica* released marked flies and placed traps at various points within a 13 km radius of a poultry farm and stable farm. Recapture rates of the released house flies were approximately 0.05% for the poultry farm and 0.016% for the stable farm, and with these data the authors determined a flight range of 7 km and 5 km, respectively (Nazni et al. 2005). Winpisigner et al (2005) observed house flies originating from commercial poultry facilities and determined that significant proportions of house flies can invade areas and become a nuisance within 3.2 km of the operation. House fly populations that carry antibiotic resistant bacteria may also be a concern. Studies that evaluated the population

dynamics of house flies and their interactions between livestock facilities and urban areas demonstrated that antibiotic resistant gut fauna could be transferred by house flies to urban areas within 125 km of their origin (Chakrabarti et al. 2010). House flies are predictably attracted to and can flourish within the grounds of livestock production facilities. The distance in which house flies will disperse from these facilities to other areas such as suburban communities, nearby businesses and food production facilities as well as the ability of these flies to contaminate these areas by mechanically transmitting pathogenic bacteria, viruses and protozoa is a major concern.

Habitat Corridors. Different animals use different means to move within their particular ecosystems. Certain aspects of the landscape can encourage or inhibit movement. One type of landscape structure is a corridor. The "traditional corridor hypothesis" states that animals will utilize corridors to move between "patches" in the landscape (Fried et al. 2005). These corridors can exist in many forms including rows of vegetation such as trees and shrubs. Some insects have been shown to utilize corridors or hedge rows while moving throughout the landscape (Tewksbury et al. 2002, Haddad et al. 2003). Edge following has been observed in field studies of butterflies provided with an artificial hedge with specific visual cues (Dover and Fry 2001). The "drift fence hypothesis" proposes that animals, because they tend to follow corridors in the landscape when they are encountered, can be diverted into patches with corridors (Fried et al. 2005). Concurrent with this hypothesis is that corridors

could be used to direct filth flies away from areas where they are pestiferous and likely to introduce harmful pathogens. The use of habitat corridors or edge-following behavior by house flies and other filth flies has not been thoroughly studied. Fried et al. (2005) evaluated house fly dispersal behavior between landscape patches in forest settings and confirmed both edge-following and corridor use. This study focused on the impact of artificial walls of vegetation on the dispersal behavior of house flies near a beef feedlot facility.

LIVESTOCK PRODUCTION

Livestock facilities in the United States produced 72,371,000,000 pounds of livestock in 2010 according to the USDA National Agricultural Statistics Survey (www.nass.usda.gov). The majority of these facilities involve beef cattle, swine and poultry production. Beef feedlots alone in January of 2012 contained 11.9 million head in the United States, and this total includes only major facilities that house greater than 1000 individuals (USDA, National Agricultural Statistics Service 2012). The majority of cattle on feed in the United States reside in feedlot facilities located in Texas, Kansas, Nebraska and Iowa (Thomas 1993). Each animal can produce approximately 50 pounds of waste in just one day on average depending on the animal's specific diet (Runov 1977, Spiehs and Varel 2009). Thus, cattle on feed could potentially excrete 595 million pounds of waste per day in the United States. This not only provides a tremendous task for waste management but establishes a substantial amount of resource for insect pests such as filth flies. The flies are also

exposed to countless pathogens that are commonly associated with livestock and readily picked up from the animal feces. Changes in the urban and rural landscape have resulted in increased tension between livestock operations and the surrounding community (Tomberlin and Talley 2010). Regulations for livestock operations are implemented at the state level and many areas have adopted right-to-farm laws designed to avoid litigation against producers while following the regulations established by the U.S. Environmental Protection Agency (EPA) (Donham et al. 2007, Tomberlin and Talley 2010). The socioeconomic and health issues associated with livestock production are incredibly complex and legislation should include the interests of all parties (Donham et al. 2007). Integrated pest management programs designed to limit filth fly activity will help both producers and the surrounding community overall.

Filth Flies. Insects are ubiquitous and will take full advantage of all suitable resources that they encounter. Livestock concentrated on farms and feedlots provide increased amounts of manure and other substrates that are perfect for filth fly development. These fly groups can include house flies (*Musca domestica* L.), face flies (*Musca autumnalis* (DeGeer)), little house flies (*Fannia canicularis* Linnaeus), latrine flies (*Fannia scalaris* Fabricius), garbage flies (*Hydrotaea leucostoma* Weidemann), dump flies (*Hydrotaea aenescans* Weidemann) and a variety of blow flies including *Lucilia* sp., *Calliphora* sp., *Cochliomyia macillaria* (Fabricius), *Phormia regina* (Meigen), and *Chrysomyia*

rufifacies (Macquart). Outside of biting flies, the primary species of filth fly requiring the implementation of control measures in livestock operations is the house fly. The average cost to control flies reported in Texas facilities has been as much as $1.00 per head in past years accumulating to billions of dollars in total losses across the United States (Byford et al. 1992, Thomas 1993). Feedlot operations must also combat complaints of flies and odor coming from the facilities and reaching urban areas. Complaints often evolve into lawsuits in which the producers must make significant changes to their facility or ultimately close. One facility in Nebraska was ordered to pay $50,000 to homeowners who filed a lawsuit against them siting problems of dust, rodents, flies and odor (Thomas 1993).

Waste Management. Waste management protocols are an essential part of any livestock production facility. The traditional methods of disposal that formerly included utilization of waste in agriculture by applying it to the land are no longer sustainable, in some areas, given the changes in the rural and urban landscape as well as the dramatic increases in production (Figure 1.8) (Graham and Nachman 2010). If mismanaged, the benefits of applying animal waste for fertilization may not be great enough to justify the risks associated with soil and water contamination (Mallin and Cahoon, 2003, Kachnic et al. 2013). Additionally, filth flies will be attracted to any area where animal waste is stored or applied and as noted above can readily spread harmful microorganisms commonly found in manure and other waste to the surrounding

area. The primary factor in controlling fly populations in confined animal feeding operations is sanitation (Williams 2010). Common nuisance and ectoparasitic fly species can thrive in indoor environments that accumulate suitable habitat like animal waste and other decaying organic matter. Odors also attract other filth flies that do not develop within the facility but can also spread microorganisms such as blow flies. It is virtually impossible to completely eliminate the odor of animal excrement at a feedlot, poultry or swine facility. Adequate management of manure and other waste coupled with eliminating habitat and reducing odors can hold filth fly numbers to low, non-nuisance levels. Buildings and pens should be kept clean and free of built up manure, old feed and other wastes that can accumulate and filth fly propagation will be greatly reduced or eliminated. Feedlots have many different areas where these wastes can build-up so managers and employees should inspect the grounds regularly. Filth flies do not need more than a one or two liter sized area of material to develop so even that smallest areas need to be identified and cleaned regularly (Williams 2010). Deficiency of habitat and removal is much more economical than implementing chemical, cultural or biological pest control measures to suppress filth fly populations.

Land application of manure as fertilizer is still a common way to utilize the manure that has been removed from pens and other areas (Graham and Nachman 2010). This is commonly spread in liquid and solid form and should be carefully applied. Paying close attention to the wind speed and direction is important so that the manure is applied where it was intended. Moist manure

that is too thick will promote fly activity in the area, and these flies will likely acquire any pathogens that are present and potentially spread them to the surrounding area. Solid waste storage should be done in a manner that keeps the waste as dry as possible. Moist areas around or within the pile could allow for fly oviposition and maggot development. The odor will attract flies; however, they will not lay eggs and larvae cannot develop if there is not sufficient moisture. Liquid storage in lagoons will attract flies as well due to the pungent nature. Similar to the solid waste, if suitable oviposition and larval development substrates do not exist, they shouldn't become problematic. While lagoons don't typically promote larval development, instances could occur where construction is poor and muddy edges that contain other decaying organic matter could provide habitat (Williams 2012).

Disposal of Mortalities. Commercial livestock facilities are faced with the challenge of disposing of a significant number of cadavers at their facilities each year. In the state of Oklahoma, the average number of cattle and calf mortalities exceeds 5 million head annually (Table 1.2) (Payne and Pugh 2010). One increasingly popular way of dealing with dead animals is composting. Alternative methods of disposal may include burial, landfilling, rendering or incineration. Although leaving animal carcasses completely exposed to the environment is illegal, abandonment is also a common practice among producers. Burial will not contain the odors that attract flies, but the animal may be inaccessible when the fly arrives avoiding any acquisition of pathogens

directly from the body. Abandonment or above ground burial with a thin layer of soil (which is uncommon but has been used as a treatment in some experiments) is not an acceptable means of waste disposal in terms of limiting insect activity. Liquids from the carcass commonly soak through to the surface and outer edges of the pile creating areas that are very attractive to filth flies and could also be contaminated with pathogenic organisms (Eamens et al. 2011). The majority of harmful human pathogens are zoonotic, and exposed livestock mortalities do contain some of these pathogens (Lloyd-Smith et al. 2009). Moist areas such as the areas around above ground burial sites may also result in larval development. Coverage that is too thin may also allow for blowflies to lay eggs and larvae to develop that feed on the carcass. Other common methods of carcass disposal such as rendering and incineration do not promote fly activity in the general processing; however, transportation of the waste is a biosecurity risk. Incineration is an acceptable means of disposing tissue that leaves behind no pathogenic microorganisms (Gwyther et al. 2011). Rendering is also an approved method of waste disposal although emissions from this process are of some concern (Gwyther et al. 2011). Hauling the waste, by a variety of means still exposes the carcass or carcasses to filth flies that are easily drawn to the location. Sanitation once the dead animal waste arrives at the incineration and rendering facilities is a separate issue.

Composting cadavers may reduce the risk of flies spreading microorganisms such as *E. coli* 0157:H7 throughout the surrounding landscape. The cadavers are buried in a carbon source creating a barrier between the

dead tissue and the surrounding environment (Payne and Pugh 2010). Dead tissue can release compounds that not only contaminate the soil environment but also the groundwater and nearby surface water (Elwell et al. 2001, Glanville et al. 2009, Kalbasi et al. 2005). Arthropods and microbes aid significantly in the decomposition of exposed animal cadavers, and animal compost is designed to facilitate decomposition without the aid of carrion feeding insects and risk of common pathogens associated with animal waste and dead tissue (Payne 2009). An ideal composted cadaver will reach temperatures too high for arthropods and harmful pathogens to survive allowing other microbial activity to breakdown the tissues (Payne 2009).

Composting process. Unlike garden compost, animal compost does not begin as a homogenous mixture. The animal serves as a central, concentrated nitrogen source and the carbon source is the bulking agent surrounding the carcass (Figure 1.9). Appropriate bulking agent is one of the major components of an animal compost pile. Several variables should be considered with the selection of carbon sources including the contribution to the proper carbon to nitrogen ratio (C:N), size of particle, strength, ability to retain moisture and heat, and ability to absorb leachate and odors (Keener 1993, Ahn 2007, Morse et al. 2001). Of course, regular use of composting techniques should incorporate a bulking agent that is readily available and economical, and several options should be available to producers at low cost. The most important aspects of an efficient carcass compost pile are proper C:N, air movement and oxygen within

the pile, moisture levels and temperature (Haug 1993, Keener 1993, Ahn et al. 2007). Conditions inside of the pile and on the immediate surface of the animal are primarily anaerobic and the aerobic activity extends further out into the envelope of materials (Berge et al. 2009). As temperatures increase and the decomposition process begins, microbial activity increases and the anaerobic areas begin to decrease (Berge et al. 2009). Turning the pile once this initial cycle of activity begins to decrease is customary and incorporates more oxygen into the pile. Because of the concentrated nitrogen source, the carbon source should result in a ratio of at least 25:1 and optimal C:N recommended in the literature should be between 25:1 and 30:1 (Keener et al. 2006, Berge et al. 2009, Morse et al. 2001). The C:N is available for most common bulking agents including hay, wood chips, horse manure, and many more (Table 1.3) (Keener et al. 2006, Morse 2009, Payne and Pugh 2010).

A bulking agent that combines fine material and larger materials works best to promote optimal conditions. The area of bulking agent around the animal, referred to as the envelope, should allow some air flow but not too much. If the pore space within the pile is too great, the air flow will dry out the pile and moisture levels will be too low for efficient composting within the pile (Keener et al. 2006). The same deficiency in particle size and strength of the substrate may also allow for more compaction over time and inhibit oxygen levels (Kalbasi et al. 2005). Oxygen levels of roughly 10% are recommended as the important microorganisms that contribute to the composting process are aerobic, and if the oxygen levels in the pile become too low, they will not

survive (Keener et al. 2006, Berge et al. 2009). The material must also be able to absorb and retain moisture. Research has shown that moisture levels should be between 50% and 60%, and microbial activity will decrease under conditions that are too dry or too moist (Keener et al. 2006, Berge et al. 2009). Anaerobic bacteria are prevalent in the compost pile but only in the innermost areas around the animal and they do not contribute a great deal to the overall composting process (Berge et al. 2009). Adequate moisture is important in a properly constructed compost pile. Low moisture levels will inhibit beneficial bacterial growth and too much moisture will decrease oxygen levels required for the microorganisms that contribute to the composting process (Richard et al. 2002, Ahn et al. 2008). The foundation of bulking agent under the animal should be thick and absorptive enough to prevent liquid decomposed material from leaching in to the soil beneath the compost pile (Kalbasi et al. 2005). Most noticeable odors are also kept contained within the envelope. The bulking agent acts as a filter between the decomposition activity and the surrounding environment. These conditions are not conducive to filth fly development in general, although a poorly constructed pile may result in increased insect activity. The temperature inside the compost pile also has to be high enough to inhibit insect activity, and some flies can tolerate increased temperatures.

Research evaluating various carbon sources and combinations of substrates has been done using a variety of materials. Bulking agent materials and their respective water holding capacities have been evaluated as well as

microbial respiration rates within compost piles constructed from each type of material. Ahn et al. (2008) examined these properties associated with corn stalks, silage, oat straw, wood shavings, alfalfa hay, soybean straw, wheat straw, leaves, turkey litter, beef manure, sawdust and a soil compost blend. They established that no single material is ideal for mortality compost although alfalfa hay, silage, oat straw and turkey litter may improve temperatures inside of the compost pile which is an important factor in pathogen degradation. Old hay is popular because it is readily available once it is no longer suitable as feed. Manure and litter is a component that there is an obvious surplus of in livestock and poultry facilities. Mixtures using hay and manure have increased the efficiency of the compost likely due to the ability of the manure to fill some of the disproportionate amount of pore space and reduce the air flow through the pile (Ahn et al. 2007). Manure alone may not be an ideal carbon source perhaps because C:N is already lower than what is suggested with exception to horse manure (Morse 2009). Glanville et al. (2013) compared corn silage, straw/manure and ground corn stalks as envelope materials for bovine compost and determined that ground corn stalks alone did not preserve high enough internal temperatures. This is likely due to the large particle size that results in too much pore space. Those conditions could also potentially allow for flies to crawl through the envelope to the cadaver and proliferate. Wood shavings and wood chips from shredded trees and shrubs are suitable and are often available at no cost from the city, county or other facilities that shred fallen trees, brush trimmings, etc. These can be effective without adding

manure if there is a sufficient amount of small particles to fill the large pore space from the wood chips.

Overall, composting animal cadavers can be a simple and economical technique to include in a waste management program. Many of the components that fit the recommended procedures can be acquired at little to no cost. An assortment of material can be used as a bulking agent and can be selected based on availability and the guidelines available through local waste management sources. This technique could reduce filth fly activity by decreasing the odors that attract them as well as eliminating the ability to locate and gain access to the carcass.

Pathogen and Odor Degradation. Biosecurity is the primary concern of legislators and the general public surrounding the disposal of dead livestock on both small and large scales. Producers have few options for the disposal of carcasses in a safe and economical manner. Composting small and large amounts of animal cadavers can be both environmentally sound and cost effective. The temperatures inside of the compost pile can remain between 55-60°C for several days and this is considered sufficient to kill or "inactivate" the majority of harmful pathogens (Wilkinson 2007, Berge et al. 2009). This can occur even in static piles, compost piles that remain undisturbed throughout the period of decomposition, if properly constructed (Eamens et al. 2011).

Laboratory research evaluating the ability of composting to effectively degrade *Salmonella* Enteritidis and *E. coli* 057:H7 established a reduction

below detectable levels of these bacteria after 48 and 72 hours, respectively (Lung et al., 2001). The time required to inactivate green fluorescent protein-labeled E. coli 0157:H7 inoculated into commercial cow manure compost ranged from 8 hours to 1 minute in response to regulated temperatures between 50°C and 70°C (Jiang et al. 2003). Static compost piles constructed with suboptimal moisture and materials can be sufficient in inactivating pathogens under certain circumstances where biosecurity is less of a concern. Field trials examining the presence of pathogens throughout the period of decomposition in crude static compost piles containing whitetail deer road kill resulted in decreased prevalence within months and no detectable pathogens after 12 months (Schwarz et al. 2010). Additional research has shown that minimally managed pile could degrade *E. coli* 0157:H7 after extended periods but were not as effective as piles that had been turned consistently which effectively degraded *Camplyobacter* sp., *Salmonella* sp., and *Listeria monocytegenes* (Berry et al. 2013). Static pile composting was compared to above ground burial with a thin layer of soil, a treatment used for experimental purposes but not a common practice with producers. Results showed effective degradation of *E. coli* within 28 days from composting but no effect from the above ground burial (Eamens et al. 2011). The majority of events, especially catastrophic losses from disease epidemics, necessitate careful monitoring of temperatures and periodic turning of the compost piles to maintain high temperatures. Avian influenza (H7N2) was eliminated from poultry compost constructed inside of the poultry houses within 14 days after an epidemic in

Maryland (Malone et al. 2004). Research conducted on 16 cattle using manure and straw compost designed to assess conditions similar to a large scale disease outbreak successfully inactivated both *E. coli* and *C. jejuni* (Xu et al. 2009). Another study that simulated large scale loss of turkey broilers and in-house disposal methods showed that Newcastle disease virus could be eliminated after 2 days by composting (Benson et al., 2008). Pigs that were infected with pseudorabies virus, *Actinobacillus pleuropneumoniae,* and *Salmonella* serovar Choleraesius, euthanized and then composted were tested and showed no pathogen after 7 days (Garcia-Siera et al. 2001). Municipal solid waste and other biowastes have been effectively sanitized by composting as well. Meat was inoculated with *Salmonella enterica* ssp. *enterica* serotype Senftenburg and composted along with garden waste and was inactivated after just 10 hours above 60°C (Ceustermans et al. 2007). Municipal solid waste that had been stored and then composted was shown to be adequately disinfected after composting and free of *Salmonella, Shigella, Ascaris* eggs, *E. coli*, faecal Streptococci and faecal coliforms (Desportes et al. 1998). Some pathogenic microorganisms are able to withstand conditions in the compost pile environment. Spore-forming bacteria such as *Bacillus anthracis* and infective prions may be more difficult to inactivate through composting and additional research is needed (Kalbasi et al. 2005, Wilkinson 2007, Xu et al. 2014). A comprehensive study examining bacteria, yeasts and fungi in municipal solid wastes showed that spore forming bacteria such as micrococcus and bacilli could persist through the high temperatures generated by the composting

process, but less tolerant organisms such as *E. coli*, faecal Streptococci, *Shigella*, and *Salmonella* were all degraded or eliminated (Hassen et al. 2001). Very recent experiments examining prion activity in laboratory scale composters was unable to detect scrapie prions after 14 and 28 days, although the authors note that the prions could have changed beyond the detection limits of the western blot technique that was used (Xu et al. 2013). Optimal conditions within a well-constructed compost pile can be incredibly effective against pathogen growth and encourage heightened tissue decomposition.

Odors may be degraded or otherwise inhibited by the composting process as well through both degradation of tissues and microorganisms and by acting as a filter between the carcass and the environment. Some research has shown that volatile organic compounds may serve as indicators of the progression of decomposition within the compost piles, and suggest that these may be an alternative means to monitor activity without disturbing the pile (Akdeniz et al. 2009). More than 400 different compounds associated with animal decomposition have been isolated and compiled into the decompositional odor analysis database (Vass et al. 2004). This research was done primarily for the purpose of detection of human remains, although the majority of these compounds are consistent with other animals such as cattle, swine and other dead livestock (LeBlanc 2008). These odors often persist well into the post decay stages of decomposition and can be detected from buried cadavers (Vass et al. 2008). Although there is rarely an obvious smell associated with decomposition within a compost pile, the progression of certain indicator

compounds can be detected through different types of sampling methods specifically designed for volatile organic compounds such as solid-phase microextraction. According to Akdeniz et al. (2010, 2011) compounds than can be used as indicators include dimethyl disulfide, dimethyl trisulfide, and pyrimidine and the concentration of these gases over time can provide information on the progression of biosecure animal compost. The inhibition of these odors by composting is also beneficial to the area surrounding the facility where the mortalities are kept, even if it is off-site from the main production grounds as well as potentially decreasing olfactory cues that attract filth flies.

CARRION FEEDING ARTHROPODS

Select insect groups are directly involved in the decomposition of animal tissue. The most abundant insect groups that are found on the carcass and in the immediate vicinity of a decomposing cadaver include the flies and beetles (Krinsky 2009). Some of these fly groups (including house flies and blow flies) have also been implicated in the spread of pathogens associated with livestock. Animal decomposition can be divided into several specific intervals based on biological and chemical processes that take typically place as tissues are broken down, and five main stages are commonly used including fresh, bloated, active decay, post decay and skeletal (Goff 1993, Vass 2001, Gennard 2012). Arthropods are present and continue to feed on an animal throughout the entire period of decomposition. Adult flies are attracted to the body very soon after it has been exposed to the environment, in many instances within

minutes (Anderson 2010). Oviposition occurs after the fly has utilized a variety of olfactory and tactile cues including pheromones, moisture, and ammonia-rich compounds (Ashworth and Wall 1994, Byrd and Castner 2010). The larvae hatch and consume tissue throughout all larval instars until they move down into the area underneath the cadaver to pupate. Insect groups that feed on the freshly dead tissue move away from the area once they have completed their life cycle along with predaceous insects that feed on the eggs and larvae of flies. Different groups of insects are attracted to the body in later stages of decomposition. The interactions between these different groups of insects and the changes that take place throughout the entire period of decomposition are known as patterns of succession (Table 1.4) (Anderson 2010).

Early stages of animal decomposition attract large amounts of flies in the families Calliphoridae, Muscidae and Sarcophagidae. Beetles arriving at this stage include a variety of Staphylinidae and Silphidae. Other families of flies and beetles that can be found include Tephritidae, Ulidiidae, Psychodidae, Cleridae, and Histeridae. As the microbial and insect activity changes the environment in and around the carcass, the environment is no longer suitable for the first group of insects and new groups are attracted including Piophilidae, Phoridae, Trogidae, Dermestidae. Representatives of other taxa can of course be found as this type of ecosystem provides resources for predators, parasitoids, detritivores and many more.

Adult blow flies and house flies will lay eggs on and in the carcass and complete their entire life cycle in the area (Figure 1.10). Blow flies are diurnal

and historically thought to only oviposit during the day, although research has shown that alternative conditions for oviposition throughout the night and other instances of limited lighting can occasionally transpire (Greenburg 1990, Anderson 2010). In addition to time of day and lighting, some species of blow flies will inhabit a carcass that is indoors more commonly than other species (Frost et al. 2010, Pohjoismäki et al. 2010). The activity of select blow fly species can vary between seasons and geographical locations as well (Catts and Goff, 1992, Byrd and Castner, 2010). Sarcophagidae, flesh flies, will also complete their entire life cycle on the carcass, but they are viviparous and deposit first instar larvae rather than eggs (Byrd and Castner 2010) (Figure 1.11). The flies tend to lay eggs in open orifices such as the eyes, mouth, anus and any wounds (Byrd and Castner 2010). These areas are softer, usually contain more bacteria and fluids, and can more easily be consumed by early instar larvae (Kreitlow 2010). The blow fly and flesh fly larvae feed directly on the soft decaying animal tissue and also the bacteria that are associated with the carcass. Many flies require certain bacteria in their diet although it has been suggested that this is not true of blow flies (Ahmad et. al. 2006) House fly larvae mainly feed on the fluids, bacteria and other smaller substrates on the animal. The larvae feed continually in large groups usually referred to as a "maggot mass". Tomberlin et al. (2012) recently examined the relationships between bacterial chemical ecology and the attraction of blow fly maggots. The exact mechanisms are not well known; however, it is already very apparent that filth flies and bacteria are closely associated. Once the last

instar larvae are nearing time for pupation, they stop feeding, defecate to evacuate all of their gut contents and then crawl away from or underneath of the cadaver into the soil to pupate (Evans 1932, Denlinger and Zaark 1994). Once the adult flies emerge they typically disperse from the carcass as it is no longer in the same condition and is now more suitable for other groups of insects.

Many different types of beetles are attracted to decomposing animal tissue. Rove beetles (Family Staphylinidae), clown beetles (Family Histeridae), and checkered beetles (Family Cleridae) are all attracted to the veritable buffet of prey insects (Byrd and Castner 2010). Carrion beetles (Family Silphidae) can also be found at a decomposing carcass. These beetles can be observed as adults in the early stages and feed on fly eggs and larvae (Byrd and Castner 2010). The larvae feed on the decomposing tissue although very little is currently known about their ecology and behavior (Byrd and Castner 2010). Silphid beetles also include members called burying beetles. Burying beetles are similar in life cycle and behavior, but occur more commonly on smaller animals primarily because this group will bury and prepare small animal carcasses for their offspring (Byrd and Castner 2010). Larger mammals may not attract these types of species as readily, and this notion poses a variety of questions as to the olfactory and visual cues associated with decomposing large mammals vs. small rodents, birds or reptiles.

During the later stages of decomposition there are completely different groups of flies and beetles found on the cadaver. Some of the predaceous

beetles exist in fewer numbers; however, the majority of the insects found in the post decay and skeletal stages are skin or larder beetles (Family Dermestidae), hide beetles (Family Trogidae), cheese skippers or skipper flies (Family Piophilidae), and hump backed or coffin flies (Family Phoridae) (Byrd and Castner 2010). All of these groups feed on the dryer substrates that are left once the carcass begins to dry out and most of the soft tissue has been consumed by flies and bacteria such as dry skin, hair and other tissues.

The carrion feeding insect fauna present on an animal cadaver can vary across seasons and regions, although the patterns of succession in general remain consistent (Byrd and Castner 2010). Some research suggests that the species composition may vary between different animal taxa as well as the size of the carcass (Kneidel 1984). Studies comparing the insect fauna colonizing porcine vs. human carcasses could find no significant differences between samples collected throughout the period of decomposition (Schoenly et al. 2007). Overall, the ecosystem created by a decomposing animal provides resources for a tremendous variety of arthropod species.

Significance of Carrion Feeding Insects in Forensic Entomology. The term "forensic" is a broad designation assigned to a variety of sciences when applied to the legal system (Greenberg and Kunich 2002). When legal investigations involve insect or other arthropod-related evidence, they become part of the discipline forensic entomology. Forensic entomology is divided into three main areas: stored products, urban entomology, and medicolegal or medicocriminal

entomology. Medicolegal entomology involves criminal and other legal investigations where insect evidence collected from a human or other animal cadaver is used (Villet et al. 2010). The same species of insects that are found on cattle and other livestock cadavers are similar to those that would likely feed on a human cadaver in the same region. Insect evidence is used in many ways, primarily as a method of calculating post mortem intervals (PMI), period of insect activity (PIA) and evaluation of succession patterns among carrion feeding insects to propose a timeline (Byrd and Castner 2010, Villet et al. 2010). Weather data in addition to life cycle information for various carrion feeding insects, primarily flies, can be used to calculate the minimum amount of time the insects could have inhabited the decomposing body and this time frame can be very closely related to time of death in animals (Wells and LaMotte 2010).

Forensic entomologists have helped to solve a variety of cases through their knowledge of insect life histories and ecology. Investigations must often rely on entomological evidence taken from human remains to determine a post-mortem interval as decomposition can vary between different ecosystems and ambient conditions (Goff et al. 1988). Larvae of *C. rufifacies* were used to establish the age of a human corpse found in Thailand and how long the victim had been in the area (Sukontason et al. 2001). In another case involving a murdered woman that had been wrapped in a thick blanket, forensic entomological investigations helped determine PMI by determining the development time of the blow flies that were found consuming the body that

included additional experimentation that examined how the blanket affected the flies' access to the body (Goff 1992).

Cases of abuse and neglect often involve myiasis, infestation of living tissue by insect larvae, and can also involve filth fly species that feed primarily on waste. People that are cared for in their homes, by family, at hospital and assisted living facilities that have wounds from surgery or other medical conditions can be susceptible to larval infestation if not properly cared for. Flies that can be involved in myiasis include species of flies such as the primary screwworm, *Cochliomyia homnivorax* (Coquerel), and a variety of bot flies in the family Oestridae which are obligate parasites, and certain species of carrion feeding flies which can exhibit facultative or accidental myiasis (Greenburg 1984, Hall et al. 1986, Shcoll et al. 2009, Goff et al. 2010)). In Chicago, nosocomial infestations with *Lucilia* (*Phaenicia*) *sericata* (Meigen) larvae occurred in terminally ill patients in two different hospitals (Greenberg 1984). Since the infestations occurred in the hospitals, the facilities could be held responsible in a criminal or civil court. Flies causing myiasis can also be utilized to establish a timeline for neglect of a living person. A child found in the Hawaiian wilderness was infested with *Chrysomya megachephala* (Fabricius), and the development time was used to aid in the investigation of how long the child had been exposed (Goff et al., 2010). Some cases of myiasis in companion animals could also be a useful toll in forensic entomology for determining animal abuse and neglect (Anderson and Huitson 2004). Children, elderly, and persons with special needs that are abused and neglected can

often be left with soiled diapers and clothing, and filth fly species attracted to waste such as urine and feces. Benecke et al. (2004) conducted investigations for two cases where an elderly person was found deceased with infestations of *L. sericata* larvae that were present pre-mortem based on the development time and post-mortem interval calculations, and other was infested with *F. canicularis* and *Muscina stabulans* (Fallén) due to the accumulation of feces and urine. A child was found in their home with *M. stabulans* and *F. cannicularis* in the genital region as well as *Calliphora vomitoria* (Linnaeus) on the face, and after completing the analysis for the development time of each species it as determined that the child had not been cleaned in approximately 14 days but had likely died only 6 to 7 days prior to discovery (Benecke and Lessig 2001).

Other areas of forensic entomology can involve homes or businesses infested with arthropods, nuisance pest lawsuits and issues of food safety. Civil action is commonly brought against business for damages caused by arthropods such as bed bugs, *Cimex lectularius* (Linnaeus), that result in financial settlements awarded to patrons that were harmed (Murphy 2003, Cassidy et al. 2011). Entomologists can even isolate human DNA from the blood meal of a bed bug to identify hosts and contribute to forensic investigations (Szalanski et al. 2006). Stored product pests can become the subject of legal investigations if they have been found in food at levels beyond the allowable limits determined by the U.S. Food and Drug Administration. Microanalytical entomology is a sub-area that focuses on the detection and identification of insect material in

commodities and aid in legal investigation associated with insects in food (Olsen 1995). Pathogenic bacteria including *E. coli* 0157:H7 have been shown to contaminate fresh produce, and the implication of livestock production facilities as the origin of filth flies that can mechanically transmit these microorganisms to fresh foods is currently under investigation (Talley et al. 2009, Wasala et al. 2013). The potential for civil or criminal action against livestock producers is amplified when issues of food safety are added to the existing problems of nuisance flies and odor.

Research associated with regional species of forensic importance, their ecology, behavior and significance to public health is rapidly accumulating although still lacking in many areas of the United States. Studies documenting the carrion feeding species of arthropods and succession patterns on carrion have not yet been explored in Oklahoma.

FIGURES

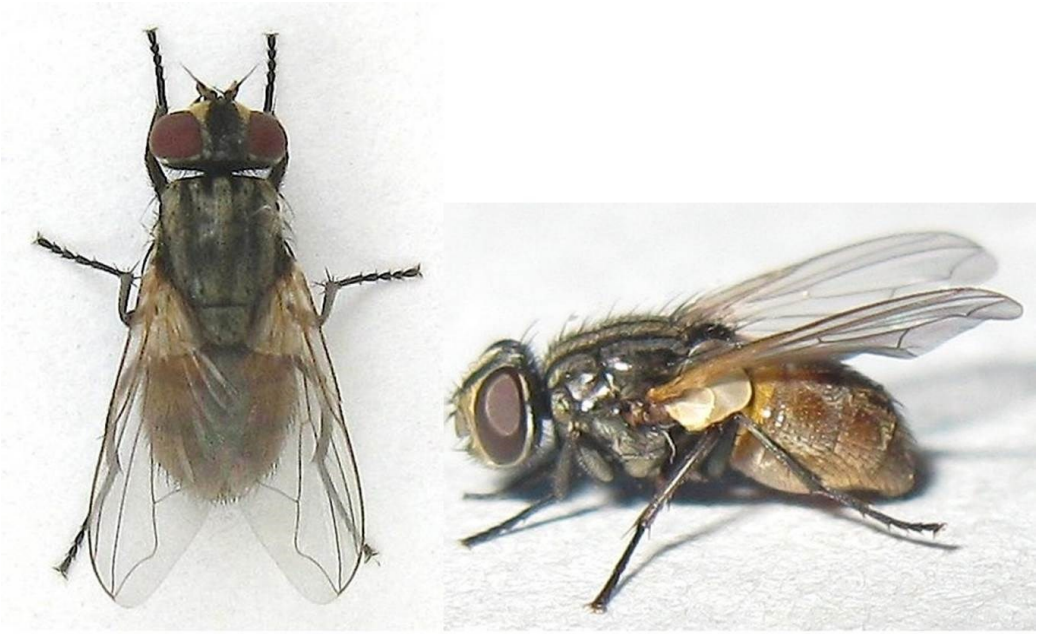


Figure 2.1 – Dorsal and lateral view of *Musca domestica* L. ©Jim Moore

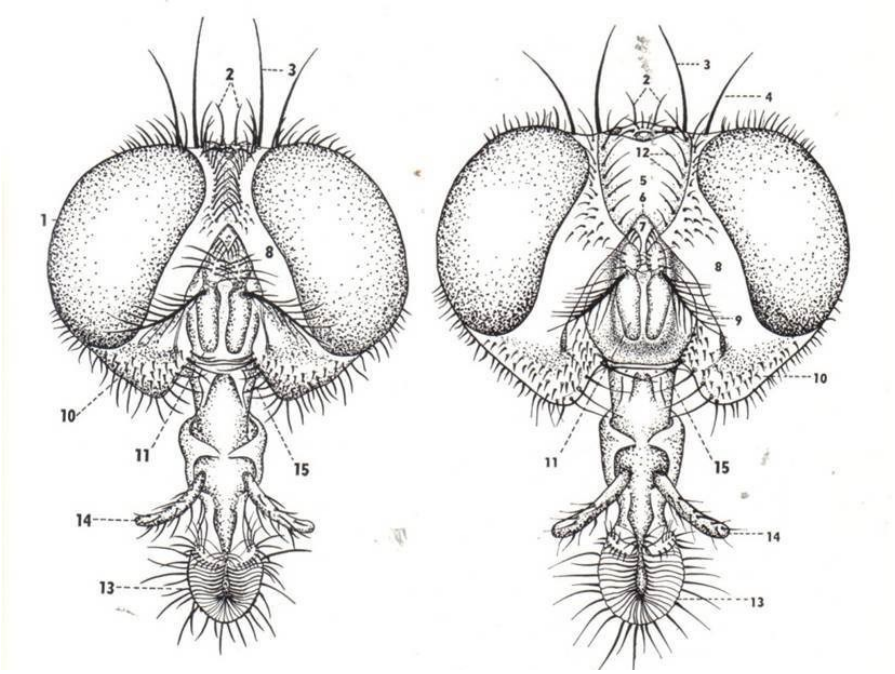


Figure 2.2 - Musca domestica.Cephalic views of head. Left: Male. Right: Female.1) compound eye,2) postvertical bristles,3) inner vertical bristle,4) outer vertical bristle,5) front,6) frontal suture,7) frontal lunule,8) sides of face,9) facial ridge,10)cheek(gena),11) antennae,12) inner vertical row of frontal bristles,13) labella,14) palpus,15) oral vibrissae. (from West 1951)

Figure 2.3 – House fly with inflated ptilinum emerging from the puparium, Pictures taken by Alex Wild.

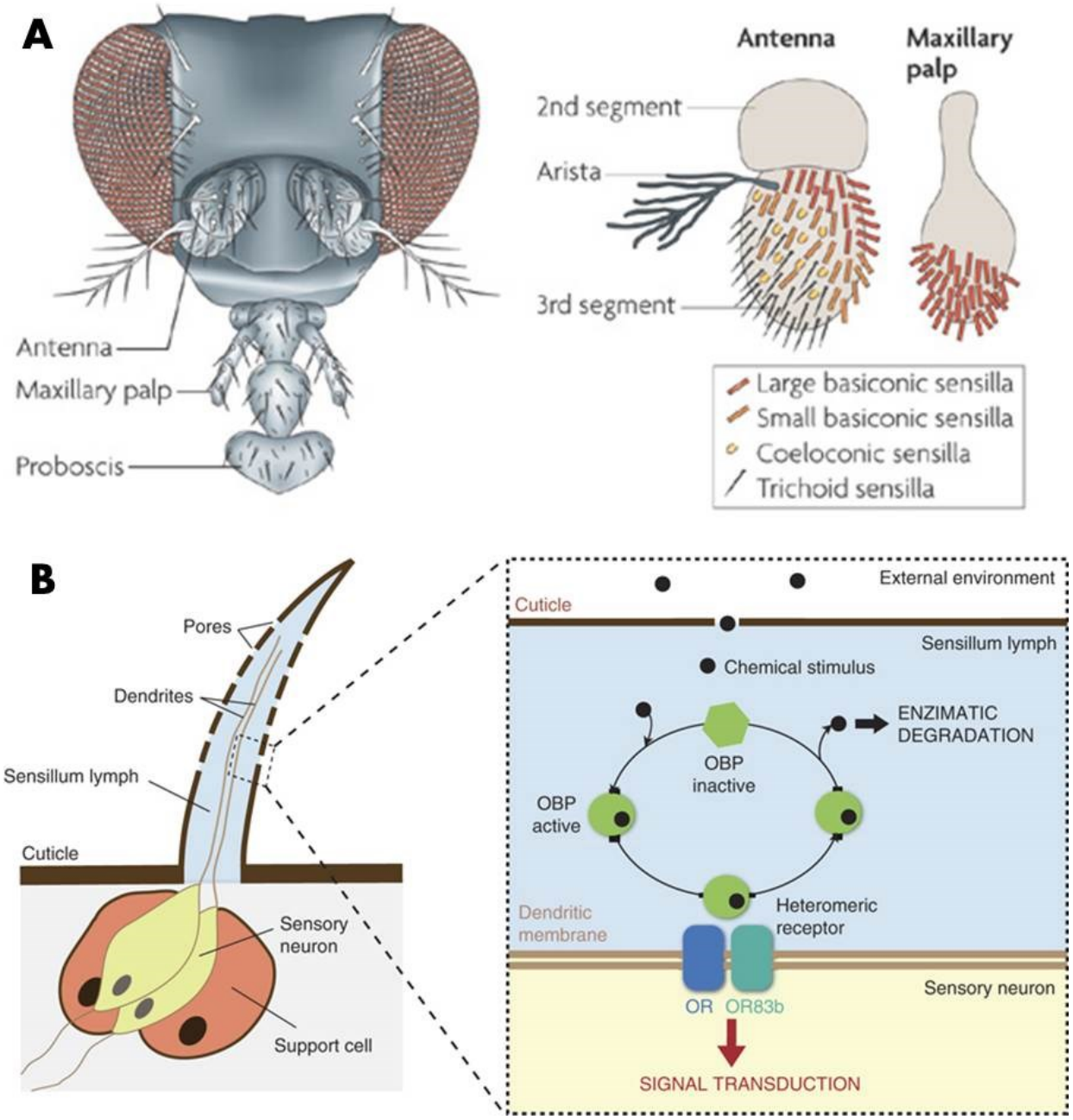


Figure 2.4 - The arrangement of olfactory sensilla on aristate antennae and palps of *Drosophila melanogaster* (Taken from Kaupp 2010) B) Schematic of an olfactory sensillum and the subsequent biochemical reaction following the reception of volatile organic compounds (Taken from Sanchez-Gracia et al. 2009).

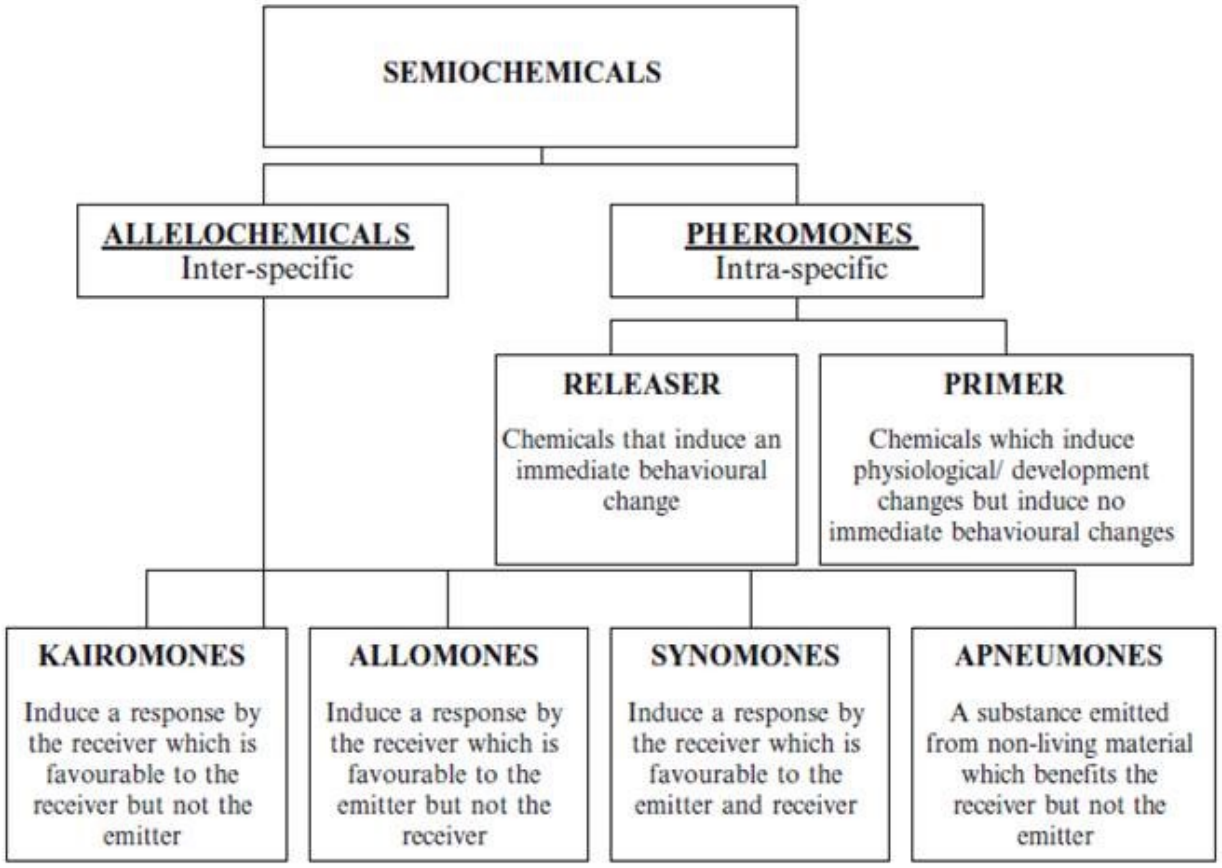


Figure 2.5 – Semiochemical classification (Norlund and Lewis 1976, Howse et al. 1998)

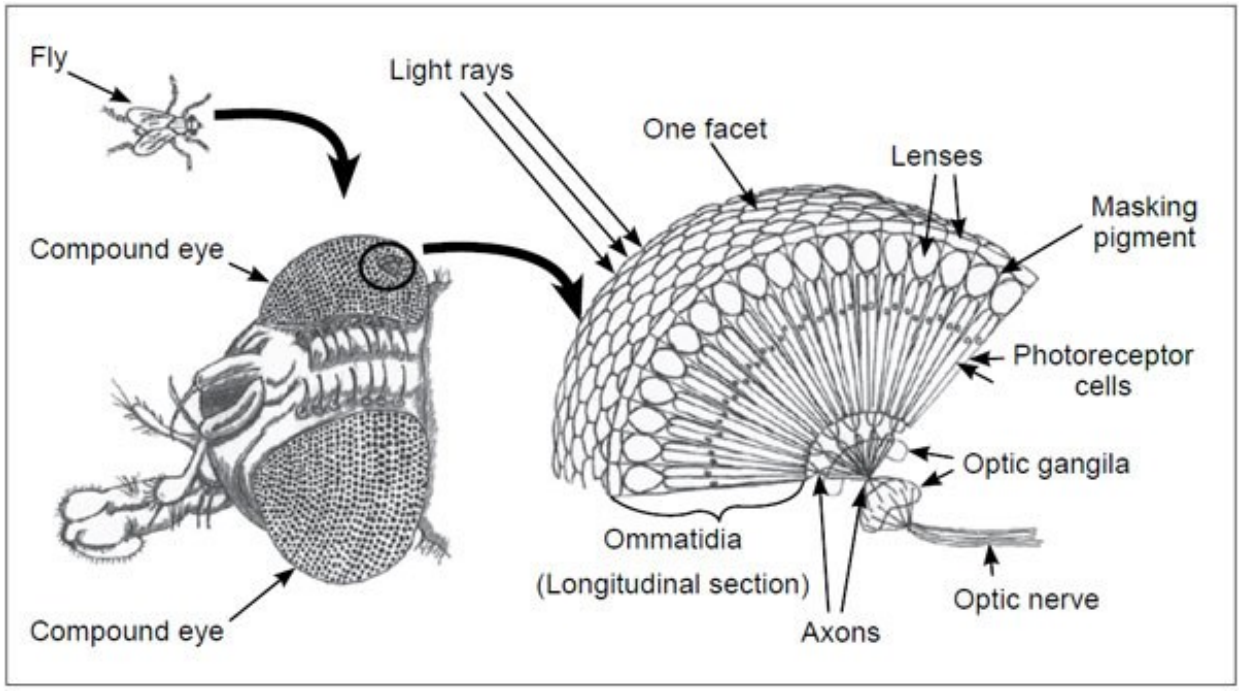


Figure 2.6 – A close-up of the fly compound eye and individual ommatidia (Taken from Mitchell et al. 1988)

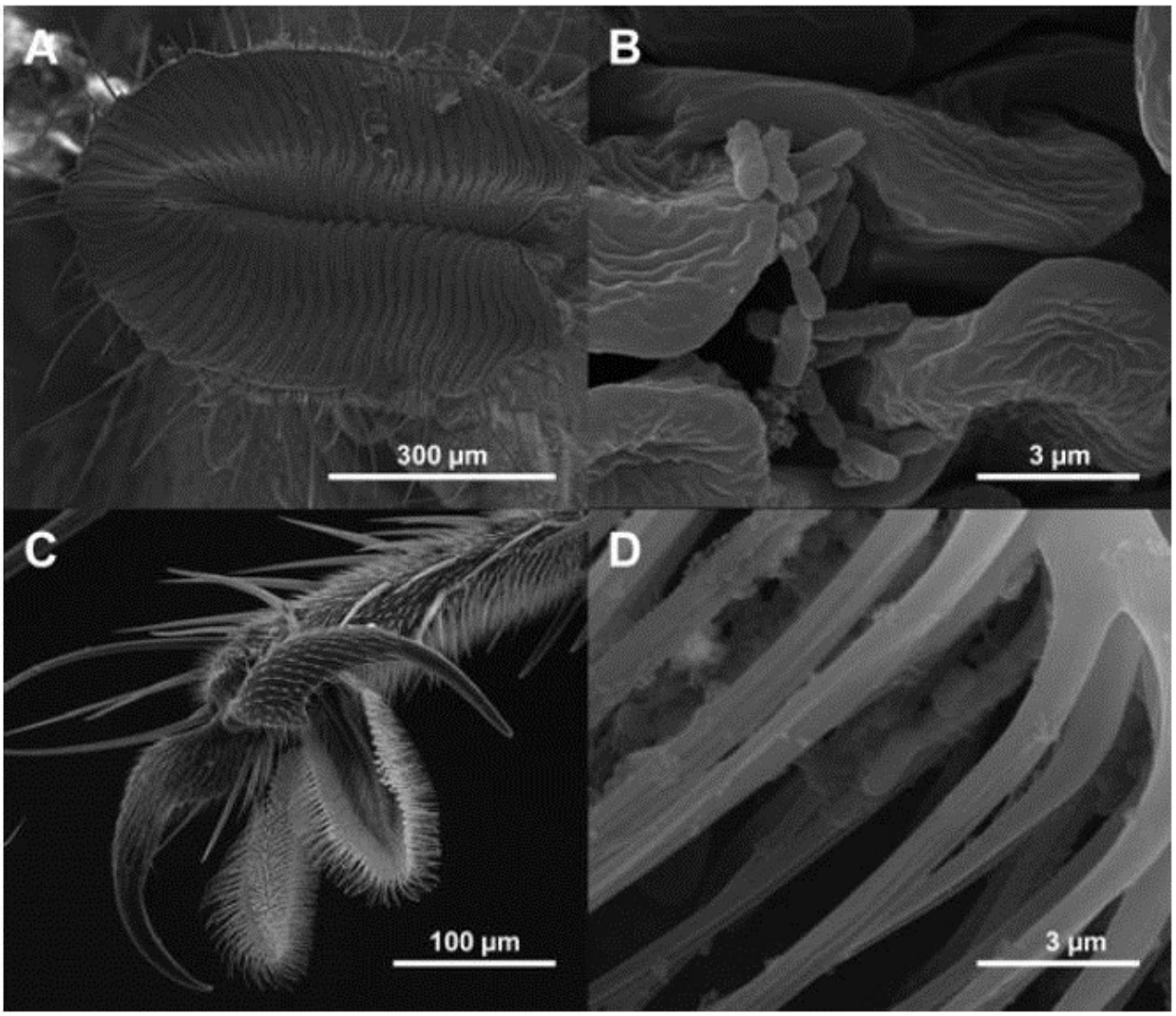


Figure 2.7 – Scanning electron micrographs of fly body parts. A) House fly labellum B)Higher magnification showing bacteria adhering to and dividing on pseudotracheae C) House fly tarsus D) Higher magnification of glandular hairs on the pulvillus of the tarsus showing bacteria adhering to individual hairs. (SEM by Lakmini Wasala, from Wasala et al. 2013)

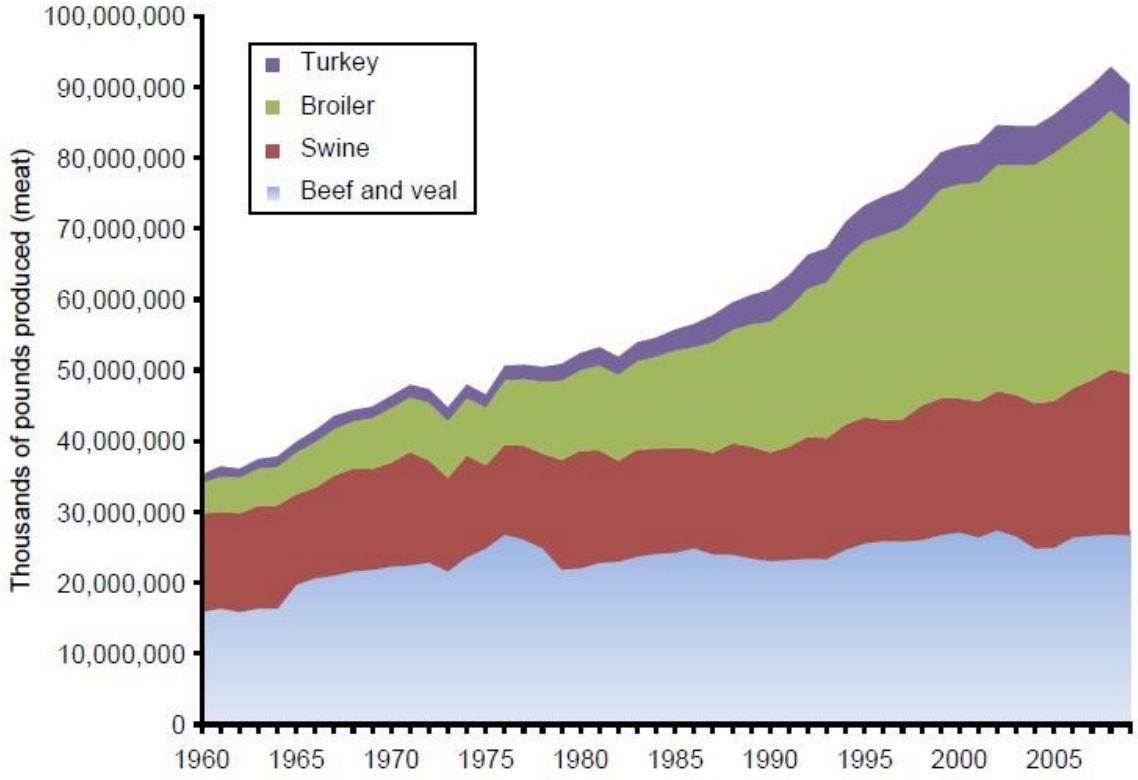


Figure 2.8 – Increase in livestock production from 1960 through 2009, given in pounds of meat (Taken from Graham 2010).

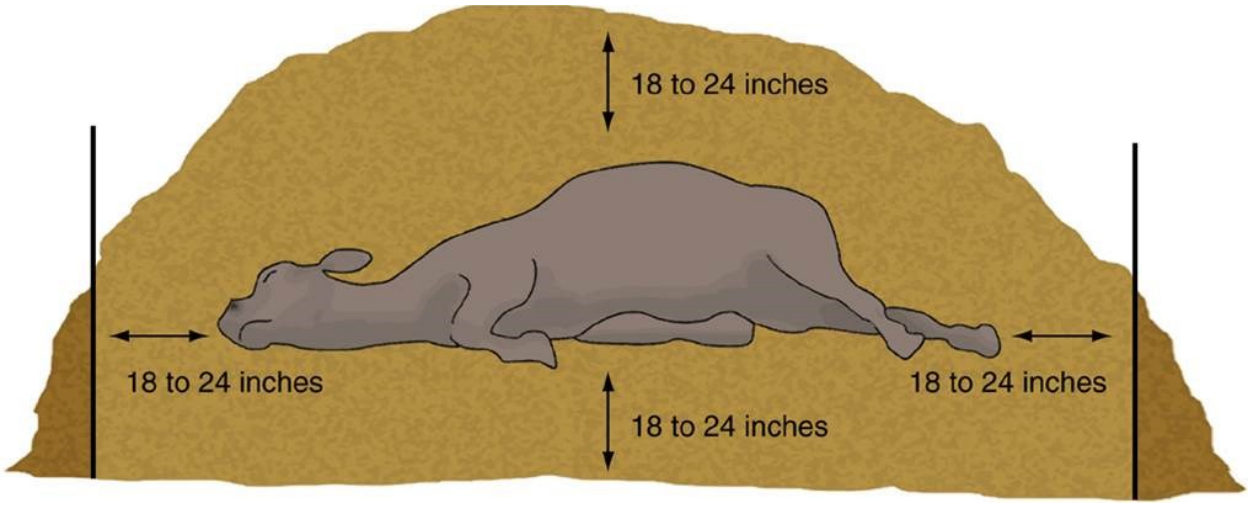


Figure 2.9 – Proper construction of a large animal carcass compost pile (Taken from Payne and Pugh 2010).

Figure 2.10 – Adult green bottle flies, *Lucilia sericata* laying eggs on decomposing animal tissue ©Susan Ellis

Figure 2.11 – Flesh fly, *Sarcophaga haemorrhoidalis* (Fallén), depositing first instar larvae onto decomposing animal tissue ©Jerry Butler, University of Florida

TABLES

Table 2.1 – Studies isolating antibiotic resistant bacteria from animal farms and urban environments (Recreated from Zurek and Ghosh 2014).

Insects	Bacteria	Antibiotic resistance**	Environments	References
Cockroaches				
German cockroach (Blattella germanica)	*Enterococcus faecalis* *Enterococcus faecium* *Enterococcus hirae* *Enterococcus casseliflavus*	AMP, CHL, CIP, ERY, KAN, STR, TET	Swine farms	Ahmad et al. 2011
Flies				
House fly (*Musca domestica*)	*Enterococcus faecalis* *Enterococcus faecium* *Enterococcus casseliflavus*	CIP, ERY, KAN, STR, TET	Fast food restaurants	Macovei and Zurek 2006
House fly (*Musca domestica*) Blow fly (*Lucilia* spp.) Bottle fly (*Phaenicia* spp.)	*Enterococcus faecalis* *Enterococcus faecium* *Staphylococcus spp.*	CLN, ERY, PEN, SYN, TET	Poultry farms	Graham et al. 2009
House fly (*Musca domestica*)	*Enterococcus faecalis* *Enterococcus faecium* *Enterococcus hirae* *Enterococcus casseliflavus*	AMP, CHL, CIP, ERY, KAN, STR, TET	Swine farms	Ahmad et al. 2011
House fly (*Musca domestica*)	*Enterococcus faecalis* *Enterococcus faecium*	DOX, ERY, GEN, STR, TET	Wastewater treatment facilities	Doud et al. 2014
House fly (*Musca domestica*)	*Escherichia coli 0157:H7*	AMP, CER, CTE, GEN, NEO, OXY, SPC, SXT	Cattle farm	Alam and Zurek 2004
House fly (*Musca domestica*)	*Escherichia coli*	AMP, STR, SUL, TET	Swine farms	Literak et al. 2009
House fly (*Musca domestica*) Stable fly (*Stomoxys calcitrans*)	*Escherichia coli*	AMP, AMX, CHL, CEP, CIP, GEN, NAL, SUL, STR, SXT, TET	Dairy cattle farm	Rybarikova et al. 2010
House fly (*Musca domestica*) False Stable fly (*Muscina stabulans*)	*Escherichia coli*	AMP, CED, CEZ, STR, TET, TRM	Cattle farm	Usui et al. 2013
House fly (*Musca domestica*) Blow fly (*Lucilia* spp.)	*Escherichia coli*	CAZ, CEF	Poultry farms	Blaak et al. 2014
Australian bush fly (*Musca vetustissima*)	*Escherichia coli* *Salmonella* sp. *Shigella* sp.	AMX, CLR, ROX	Cattle farm Urban area Outdoor eateries	Vriesekoop and Shaw 2010

**AMP, ampicillan; AMX, amoxicillan; CAZ, ceftazidime; CED, cefpodoxime; CEF, cefotaxime; CEP, cephalotin; CER, ceftiofur; CEZ, cefazolin; CHL, chloramphenicol; CIP, ciprofloxacin, CLN, clindamycin; CLR, cefaclor; CTE, chlortetracycline; DOX, doxycycline; ERY, erythromycin; GEN, gentamicin; KAN, kanamycin; NAL, nalidixic acid; NEO, neomycin; OXY, oxytetracycline; PEN, penicillin; ROX, roxythromycin; SPC, spectinomycin; STR, streptomycin; SUL, sulfamethoxazole/trimethoprim; SYN, quinupristin-dalfopristin; TET, tetracycline; TRM, trimethoprim.

Table 2.2 – Annual Oklahoma cattle and calf death loss and carcass nutrient data (Taken from Payne and Pugh 2010).

	Cattle	*Calves*	*Total*
OK Inv. (# head)	2.1 million	3.3 million	5.4 million
Death loss (%)	2.1§	6.4§	4.8
Mortalities (#head)	44,100	212,850	256,950
Average Wt. (lbs)	1246‡	460Ω	–
Avg. Mortality (lbs)	54.9 million	97.9 million	152.8 million
Projected carcass C (lbs/head)	180	66.5	–
Projected carcass N (lbs/head)	36	13.3	–
Total Projected C (lbs)	7.9 million	14.1 million	22.1 million
Total Projected N (lbs)	1.5 million	2.8 million	4.4 million
Projected value of N†	$556,500	$990,817	$1.5 million

† Based on a conservative value of $0.35 per pound of N as Urea

* This does not include the added value of increased organic matter, Ca,P,K or other nutrients.

§ National Death Loss Survey, USDA: 1996-2005

‡ Livestock Marketing Information Center, LMIC. 1999-2008

Ω National Stocker Survey, BEEF. 2008

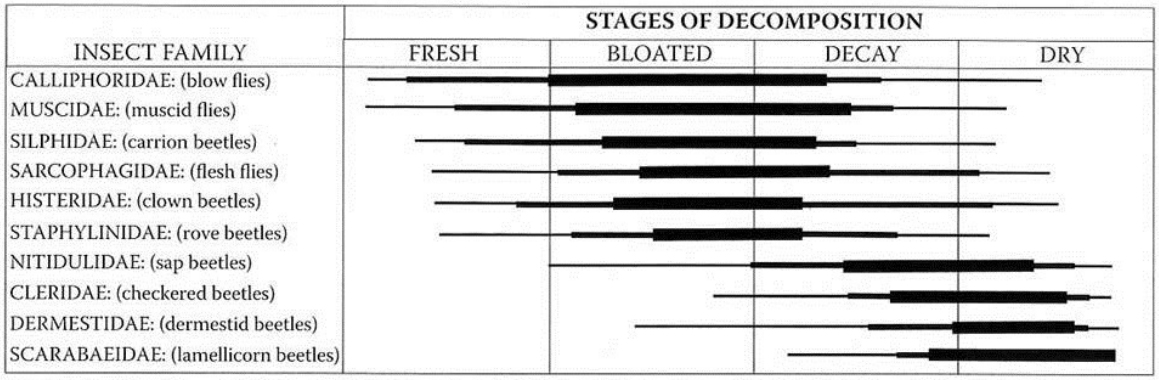
Table 2.3 – The carbon to nitrogen ration of common composting materials (Taken from Payne and Pugh 2010).

Compost material	*C:N*
Sawdust[1]	442:1
Straw-wheat[1]	127:1
Rice hulls[1]	121:1
Straw-general[1]	80:1
Corn stalks[1]	60-73:1
Finished compost[1]	30-50:1
Hay-general[1]	15-32:1
Horse manure-general[1]	30:1
Cattle manure[1]	19:1
Grass clippings[1]	17:1
Sheep manure[1]	16:1
Turkey litter[1]	16:1
Broiler litter[1]	14:1
Swine manure[2]	14:1
Cottonseed meal[1]	7:1
Soybean meal[1]	4-6:1
Animal carcass[2]	5:1

[1]On-Farm composting Handbook. Agriculture and Engineering Service, NRAES-54, Natural Resource, Ithaca, New York

[2]Compost Materials, 1996 EBAE172-93, North Carolina Cooperative Extension Service, Raleigh, North Carolina.

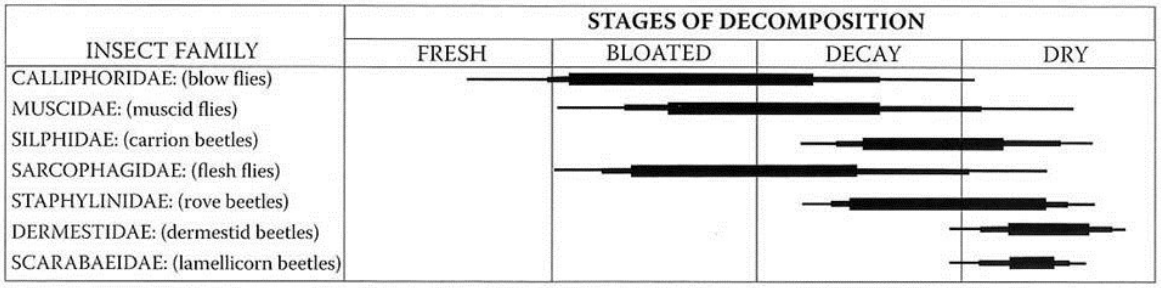
Table 2.4 – Succession of insect families on human cadavers in Tennessee throughout the spring and summer a) adults b) larvae (Taken from Byrd and Castner 2010).



* Each stage of decomposition is given the same amount of space in this table.

——— Indicates a small number of individuals present.
Indicates a moderate number of individuals present.
Indicates a large number of individuals present.

(a)



* Each stage of decomposition is given the same amount of space in this table.

——— Indicates a small number of individuals present.
Indicates a moderate number of individuals present.
Indicates a large number of individuals present.

(b)

CHAPTER III

INFLUENCE OF VEGETATION HEIGHT ON THE DISPERSAL OF HOUSE FLIES, MUSCA DOMESTICA, FROM BEEF FEEDLOTS

ABSTRACT

House flies, *Musca domestica* L., play an integral role in food safety. Contamination of fresh produce by flies carrying virulent strains of bacteria, such as Shiga toxin producing *E. coli*, is a major concern in the United States. Changes in the landscape from anthropogenic activity have resulted in increased exposure of urban areas to house fly pests, and monitoring their movements is paramount in assessing the risks associated with flies emigrating from livestock facilities. Artificial structures designed to represent tree and shrub lines were constructed in a field south of a beef feedlot facility. Marked house flies were released at individual sites containing 3 m walls, 1.5 m walls and a control site with no wall. Movement of the house flies was monitored using sticky traps placed within each corridor and at the top of the camouflage walls. Throughout 2012, the number of flies trapped at the control site was higher overall although not significantly different from the 3 m and 1.5 m sites.

The wild flies trapped showed a contrasting trend with the mean numbers of flies trapped at the 3m site were significantly higher than both the control and 1.5m sites. Evaluating whether or not minor manipulations to the landscape of livestock facilities will help to discourage fly movement to urban areas and food production facilities nearby could have tremendous influence on house fly management programs. Studying the movements of house flies that disperse from feedlot facilities is also important in assessing the risk of transmission of harmful human pathogens to the surrounding landscape.

INTRODUCTION

House flies (*Musca domestica* L.) are well known pests inexorably linked to commercial livestock operations. Although house flies have long been considered a nuisance pest, they impact public health and food safety as well. The waste generated from commercial livestock facilities such as beef feedlots is an excellent resource for house flies, and these substrates contain important microorganisms implicated in many human illnesses. House flies are known mechanical vectors of pathogens including *Escherichia coli*, *E. hystolytica, Giarda* spp., *Trichomonas* spp., *Sarcocystis* spp., *Toxoplasma gondii*, *Isospora* spp., *Endolimas nana, Hammondia*, *Cryptosporidium parvum* and others (Greenburg 1973, Graczyk 2001). One study in Thailand established a correlation between increases of enterohemorrhagic *E. coli* 0157:H7 infections in villagers with the prevalence of the bacteria carried by synanthropic flies (Echeverria et al., 1983). The same pathogen, *E. coli* 0157:H7, was also

recovered from house flies captured in the immediate vicinity of cattle farms in Japan (Iwasa et al. 1999). A study examining house flies as mechanical vectors of *Campylobacter jejuni* concluded that these bacteria could potentially be transmitted to poultry facilitating infection in humans that consume the contaminated meat (Skövgard et al. 2011). House fly populations that carry antibiotic resistant bacteria may also be a concern. Animal production facilities contain antibiotic resistant bacteria, and filth flies dispersing from the facility can carry these dangerous organisms into surrounding urban areas (Zurek and Ghosh 2014). House flies collected form a cattle farm in Kansas tested positive for *E. coli* 0157:H7 with resistance to ampicillin, ceftiofur, chlortetracycline, gentamicin, neomycin, oxytetracycline, spectinomycin, and trimethoprim-sulfamethoxazole (Alam and Zurek 2004). Other pathogens resistant to multiple antibiotics have also been isolated from house flies in both rural and urban settings including *Enterococcus faecalis*, *E. faecium* and *E. casseliflavus* (Macovei and Zurek 2006, Graham et al. 2009, Doud et al. 2014).

Changes in the landscape from anthropogenic activity have resulted in increased exposure to house flies emigrating from beef feedlot facilities. Homes and businesses are being built in closer proximity to livestock operations that were once isolated. This increases the likelihood of complaints of nuisance flies and could potentially increase the prevalence of pathogens in surrounding areas. Residents in areas where house flies are problematic have filed formal complaints and even filed lawsuits against livestock producers in the area

where they believe the flies originated. These lawsuits have resulted in costly settlements paid to the residents and have caused some animal production facilities to close (Thomas 1993) or resulted in firmer "right to farm" legislation designed to protect these types of facilities (Reinert 1998). Limited research has focused on the dispersal of house flies from livestock facilities and has yielded varying results on the distance they are likely to travel. Even fewer studies have examined how house flies interact with the surrounding landscape. Marked house flies originating within the grounds of various livestock facilities have been captured up to 32 km away although the majority of flies were recovered within 2 – 4 km of livestock facilities (Nazni et al. 2005). Winpisigner et al. (2005) observed marked house flies originating from commercial poultry facilities and determined that significant numbers of house flies can invade areas and become a nuisance within 3.2 km of the operation. Additional research evaluating the population dynamics of house flies in relation to livestock facilities and urban areas asserts that antibiotic resistant gut fauna could be transferred to areas within 125 km of their origin (Chakrabarti et al. 2010).

Collectively, research on house fly dispersal will contribute to risk assessments and protocols outlining safe proximity to livestock production facilities. Manipulation of the vegetation around the perimeter of livestock facilities may help to influence the dispersal of house flies and ultimately reduce the risk of invasion into the urban landscape. Some animals, including insects, have been shown to utilize corridors, hedge rows or exhibit edge-

following behavior while moving throughout the landscape (Tewksbury et al. 2002, Haddad et al. 2003). The use of hedgerows by carabid beetles has also been observed (Tischendorf et al. 1998). These types of arrangements in the surrounding landscape that impact insect dispersal can exist in a variety of forms including lines of trees and shrubs. The "drift fence" hypothesis proposes that animals can be diverted into patches with corridors because they tend to follow corridors in the landscape when they are encountered (Haddad and Baum 1999). Concurrent with this hypothesis is the possibility of affecting the movement of filth flies in order to inhibit their dispersal to areas where they are pestiferous and likely to introduce harmful pathogens. Edge-following behavior and corridor use by flies has not been widely studied. This type of behavior has been observed in field studies of butterflies provided with an artificial hedge equipped with specific visual cues (Dover and Fry 2001). Fried et al. (2005) evaluated house fly dispersal behavior between landscape patches in forest settings and confirmed both edge-following and corridor use. Continued research associated with house fly dispersal from livestock facilities will aid in the development of pest management programs that may reduce the nuisance created by large numbers of house flies invading residential areas. Studying the movement of house flies from livestock facilities may also help to assess the ability of house flies to transfer zoonotic pathogens to residential areas and facilities that grow fresh produce such as leafy greens and fruit. The objective of this study was to determine the impact of artificial walls of vegetation on the dispersal behavior of house flies near a beef feedlot facility.

METHODOLOY

This research was conducted at Willard Sparks Beef Research Center near Oklahoma State University's main campus in Stillwater, OK. Three individual experimental sites were assembled in a field on the south side of the feedlot grounds. Each site consisted of one artificial corridor structure designed to simulate a corridor of vegetation such as trees or shrubs. The structures were comprised of four walls assembled in the formation of a corridor and were made from camouflage netting attached to metal t-posts. Each wall was 4m long and the height of the camouflage netting varied by treatment. The three treatments consisted of a control site with no walls, a site with 1.5 m tall walls, and a site with 3 m tall walls. This experiment was organized into a randomized complete block design, and each treatment was randomly assigned to one experimental site at each sampling date. Blocks were represented by successive dates where marked flies were released serving as replicates of each treatment throughout the duration of the field season. During season one, the walls were set up parallel to one another with two on each side with approximately 2 m of space in between and a 0.5 m break in the center of each side (Figure 3.1). Traps with adhesive were placed in specific areas of the artificial corridors in order to monitor the movement of marked house flies released at the end of each artificial corridor. Sticky traps were made from sheets of 0.003 mm clear acetate sheets coated with Tanglefoot®Tangle-trap™. The sheets of adhesive were attached to 30 X 30 cm pieces of particle board

mounted on posts approximately 1.5 m high. The traps were painted in shades of green and brown in order to blend in with both the artificial vegetation and surrounding area. These trap structures were placed in the center of each break in the walls and at the end of each wall. The traps were placed inside between and at the end of each artificial corridor wall in order to determine any attraction the house flies may have to the artificial vegetation as well as any edge-following type of behavior. Additional traps were placed at the top of each camouflage wall to monitor marked flies moving over the top of the walls.

House flies were used from colonies maintained at the Medical Veterinary Entomology building at Oklahoma State University. Larvae were reared on a diet consisting of a moistened mixture of 80% wheat bran and 20% calf-manna® (MannaPro® Products, LLC., Chesterfield, MO) and allowed to pupate in the material. Plastic tubs containing approximately 5000 house fly pupae in rearing media were placed at a release point just inside the corridor between the two walls at the end opposite the traps. Marking powder was applied to the entire surface of the media at a rate of approximately 50mg/cm^2. The marking substrate contained a 1:1 ratio of fluorescent powder (DayGlo® eco pigment, DayGlo® Color Corp., Cleveland, OH) and powdered egg white (Honeyville Food Products Inc., Rancho Cucamonga, CA). As the adult flies emerged and crawled up through the media and marking powders, they became thoroughly coated with both fluorescent and protein markers (Fig. 3.2). Traps were collected seven days after each release and examined using ultraviolet light.

The construction of each corridor site was modified after the first field season. At the beginning of season two, the walls at each site were moved to eliminate the 0.5m gap that previously existed in the middle. This resulted in two continuous walls approximately 8 m in length set 2 m apart. Trap structures were also moved and placed approximately 3 m of the way down each of the two walls on each side of the corridor. Flies were once again released just inside the corridor at the opposite end of the traps. Marking powder was applied in the same manner as season one. Additional traps were added to each artificial corridor site and placed approximately 4m away from the release point at the end of the camouflage walls (Fig. 3.3). The base of the traps placed at the top of each wall was changed from camouflage colored sheet of particle board to clear acrylic squares. Traps were once again collected seven days after each release and examined using ultraviolet light.

STATISTICAL ANALYSIS

Mean total house fly abundance for both marked and wild flies was compared among the different treatments (no wall, 1.5m, & 3m artificial walls) by using the PROC MIXED procedure with an LSMEANS test (SAS. 9.3; SAS Institute, 2013). The mean total abundance of marked flies on outside traps at each site (no wall, 1.5m & 3m artificial walls) and the mean total abundance of flies trapped at the top of the 3m and 1.5m walls were also compared using the same procedure. All p-values of 0.05 or lower were considered significant.

RESULTS

Based upon counts of marked house flies trapped throughout the 2012 field season (season one) there was no significant difference among house fly abundance in the control, 1.5 m and 3 m corridor sites. The mean total abundance of house flies trapped at the control site was however numerically higher than both the 1.5 m and 3 m sites ($p=0.0620$) (Fig. 3.4). The number of wild flies trapped at the 3m site was significantly higher than the control site ($p=0.0023$) and the 1.5m site ($p=0.0549$). Results for the 2013 season (season two) following changes to the corridor design and trap placement were dramatically different. The mean total abundance of flies trapped at the 3 m site was significantly higher than the 1.5 m and control sites ($p=0.0003$, $p=0.0014$ respectively) (Fig. 3.5). Wild house flies showed a similar trend to the season one results. A significantly greater number of wild house flies were trapped at the 3m site than the 1.5m and control sites ($p=0.0032$, $p=0.0140$ respectively). Traps placed 4 m outside of the artificial corridor area revealed differences between the control site and the sites with walls. Significantly more house flies were caught on the traps placed outside of the corridor area at the control site than the 3 m and 1.5 m sites ($p=0.0002$ for both treatments) (Fig. 3.6). The traps placed outside of the artificial corridors at the 3 m and 1.5 m sites also caught considerably fewer flies than traps placed within the artificial corridor. The flies captured on outside vs. inside traps at the 1.5 m site represented 27.66% and 72.34% of the total flies captured, respectively. At the 3 m site, 13.79% of lies were captured on outside traps compared to

86.21% captured on traps inside of the corridor. The proportion of flies captured on outside traps at the control site was 52.94% compared to 47.06% captured on traps within the corridor area. Marked flies also appeared to fly over the 1.5 m walls much more readily than the 3 m walls throughout season one and season two ($p=0.0107$ and $p=0.0013$ respectively) (Fig. 3.7).

DISCUSSION

Mark-release-recapture studies have been used for nearly a century to monitor insect movement and continue to be a popular means of studying flight patterns and behavior (Dudley and Searles 1923, Geiger et al. 1919, Hagler and Jackson 2001). Studying the movement of mechanical insect vectors of pathogenic organisms is incredibly important to understanding the epidemiology of foodborne illness as well as the control of house flies as nuisance pests that invade the landscape surrounding livestock facilities. This study on house fly movement revealed a strong interaction with walls of artificial vegetation and perhaps landscape corridors. The 3 m high synthetic walls appear to influence the direction in which house flies move immediately following eclosion as they begin to forage. After the initial field trials, the design of each field site was modified. Since the control sites contained no artificial vegetation and only traps, the flies may have been attracted to the traps resulting in a disproportionate representation of the possible interaction with the artificial walls. Once the gap in each wall was eliminated and traps were placed inside and outside of the simulated corridors, the attraction of the

artificial vegetation became more apparent. Traps placed outside the artificial corridor areas resulted in greater numbers of house flies at the control site than the 3m or 1.5m sites suggesting that the house flies utilize the artificial wall structures in their movement. In contrast, similar numbers of marked flies were recovered at the control site on traps placed outside of the corridor areas as well as traps placed within the corridor area (Fig. 3.8). In contrast, recovery of marked house flies on traps placed outside of the 3m artificial corridor was considerably lower than traps placed within the walls (Fig. 3.9). These results imply that the marked house flies were more likely to fly in the direction of the camouflage walls rather than the direction away from the area containing walls. The interactions of house flies and artificial vegetation established by this study suggest that altering the landscape within feedlot grounds may help to decrease fly invasion to neighboring areas. These data will be useful in continued research evaluating the dispersal behavior of house flies originating from livestock production facilities. Additional field studies investigating these behaviors could provide insight on the influence of vegetation on native house flies developing within the feedlot.

Fly management protocols at confined animal feeding operations continue to evolve in response to challenges of insecticide resistance and increased urban development. The most effective method for suppressing house fly populations is by reducing habitat for house fly development through adequate sanitation, but eradication of house flies is unrealistic. An integrated approach to house fly management is essential and increased knowledge of

their behavior and ecology will facilitate the development of novel tactics that can benefit control overall. Edge-following behavior and landscape corridor use by house flies has been established in forest settings (Fried et al. 2005). House flies have also demonstrated behaviors in response to the visual stimuli of high contrast resource boundaries in laboratory assays, and were more likely to stay within a patch that contained a desired resource such as sucrose (Conlon and Bell 1991). The high contrast that exists between taller vegetation and the surrounding sky is consistent with the light and dark colors eliciting a response from house flies. Contrast between light and dark areas has been proven to be the primary visual attractant for house flies in both laboratory and field experiments (Conlon and Bell 1991, Collins and Bell 1996, Howard and Wall 1998, Diclaro et al., 2012). These behaviors have not yet been explored together in association with confined animal feeding operations. Additional experiments at the field scale looking at push-pull strategy (Cook et al. 2007) by combining a resource such as sucrose as well as lines of vegetation or corridors is also lacking. Foraging house flies dispersing from animal farms in search of sugar containing substrates may be influenced by the presentation of these substrates within boundaries created with the intention of impacting fly movement. Experiments examining these phenomena within the grounds of a livestock facility could further enhance knowledge of house fly behavior and the implications for novel pest management strategies.

CONCLUSIONS

While feedlots provide valuable resources for house flies, large numbers emigrate from the grounds and become a nuisance pest to homeowners and business owners nearby. House flies were shown to interact with corridors constructed of 3 meter camouflage walls significantly more readily than 1.5m meter walls and were captured consistently in greater numbers within the corridor than at the control sites with no artificial corridor. This research is consistent with findings previously published on house flies and corridor use (Freid et al. 2005). Smaller scale laboratory studies demonstrated that house flies are alerted to boundaries with high contrast such as light and dark, and they are more likely to remain within the boundary if the area contains a resource such as sucrose (Conlon and Bell 1991). The issue with urban encroachment with livestock facilities is increasing the likelihood that house flies will become problematic, even at levels previously considered manageable. House flies interact with the landscape while foraging for food, mates and oviposition sites suitable for larval development. Manipulating the landscape surrounding livestock operations could result in more favorable pest management programs that incorporate house fly behavior and ecology. Currently, the relationship between filth flies and insects producing sugary excreta known as honeydew is being explored after preliminary field examination of filth flies in leafy greens fields resulted in the recovery of several filth fly species, some of which tested positive for *E. coli* 0157:H7 (Talley et al 2009). This may present additional opportunities for field

experiments utilizing phytophagous insects that produce honeydew and potentially attract house flies. It may be possible to inhibit house fly dispersal from livestock facilities by providing sugar containing resources to adult flies within boundaries created by tree and/or shrub lines. Future research should utilize the data obtained from these projects as well as those previously mentioned to further address the use of vegetation in feedlot pest management. Influencing the movement of house flies away from neighboring homes and business could decrease the occurrence of nuisance flies and subsequent complaints leading to legal action against producers. Determining what proportion of mechanical vectors such as house flies under particular conditions will pose a risk to fresh produce production facilities and other areas surrounding feedlot facilities is vital to increasing food safety overall.

FIGURES

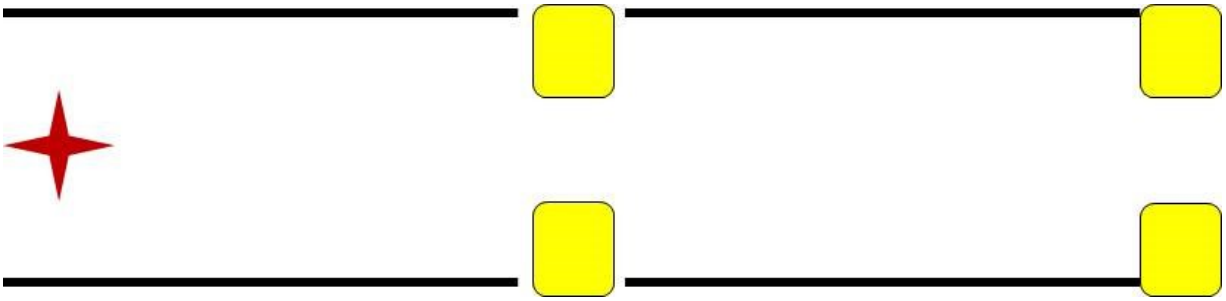


Figure 3.1 - Diagram of the artificial corridor structures with camouflage netting walls (black lines), sticky trap placement (yellow boxes) and release point of marked flies (red star) for season one (April through October 2012).

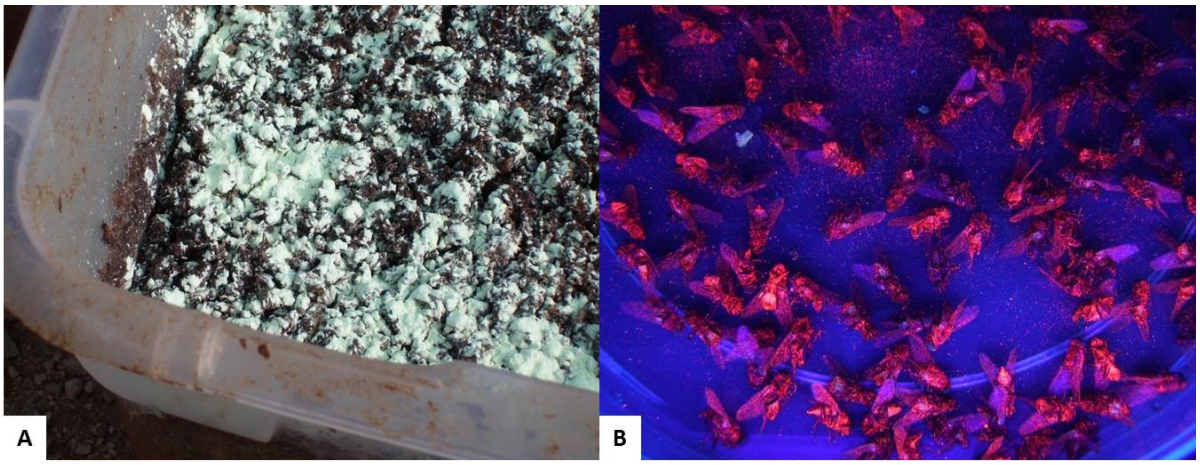


Figure 3.2 – House flies marked with 1:1 mixture of fluorescent powder and powdered egg whites A) Powder applied to the surface of bin filled with house fly pupae and rearing media B) Marked house flies illuminated with UV light

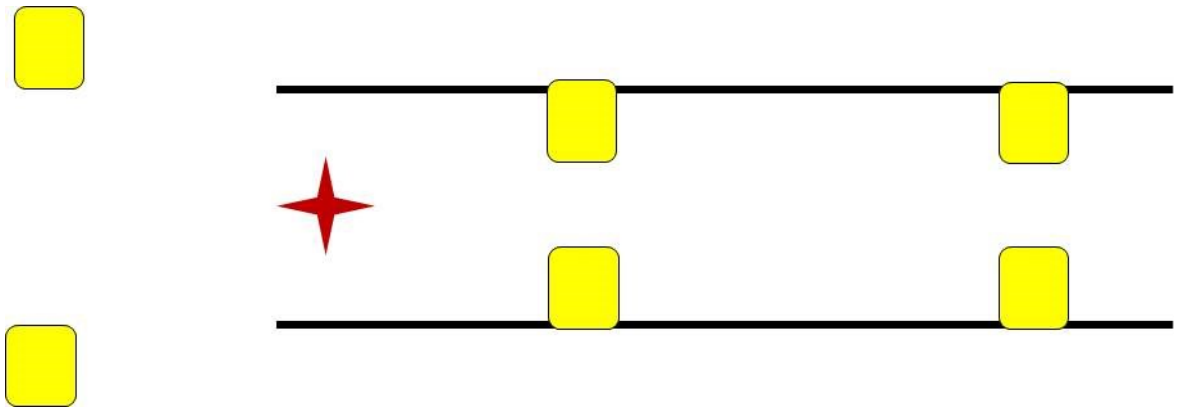


Figure 3.3 – Diagram of artificial corridor structures with camouflage netting walls (black lines), sticky trap placement (yellow boxes) and release point of marked flies (red star) for season two (April through October 2013).

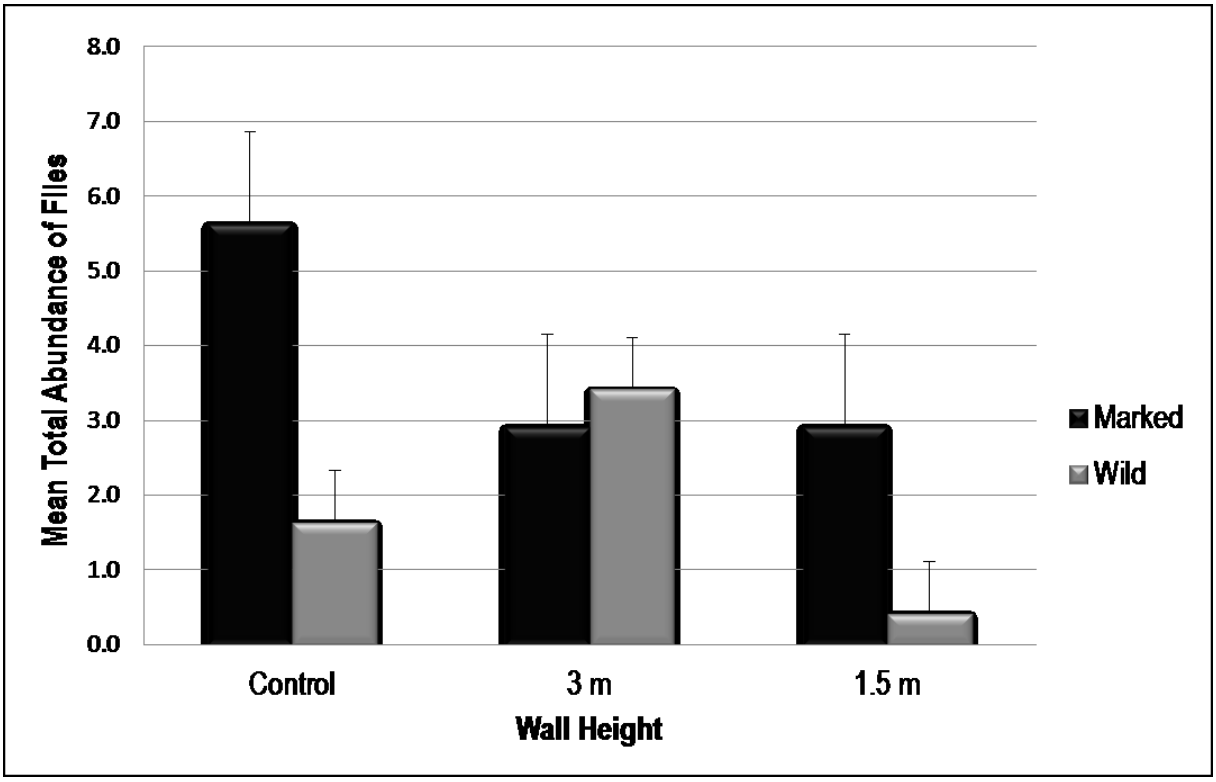


Figure 3.4 – Mean total abundance of marked house flies trapped at each artificial corridor site throughout the period of April through October 2012. The number of flies trapped at the control site was higher, although not statistically significant from the 3 m or 1.5 m sites (p=0.0620). Wild flies showed a trend opposite the marked flies and were significantly higher at the 3 m site than the 1.5 m site (p=0.0023).

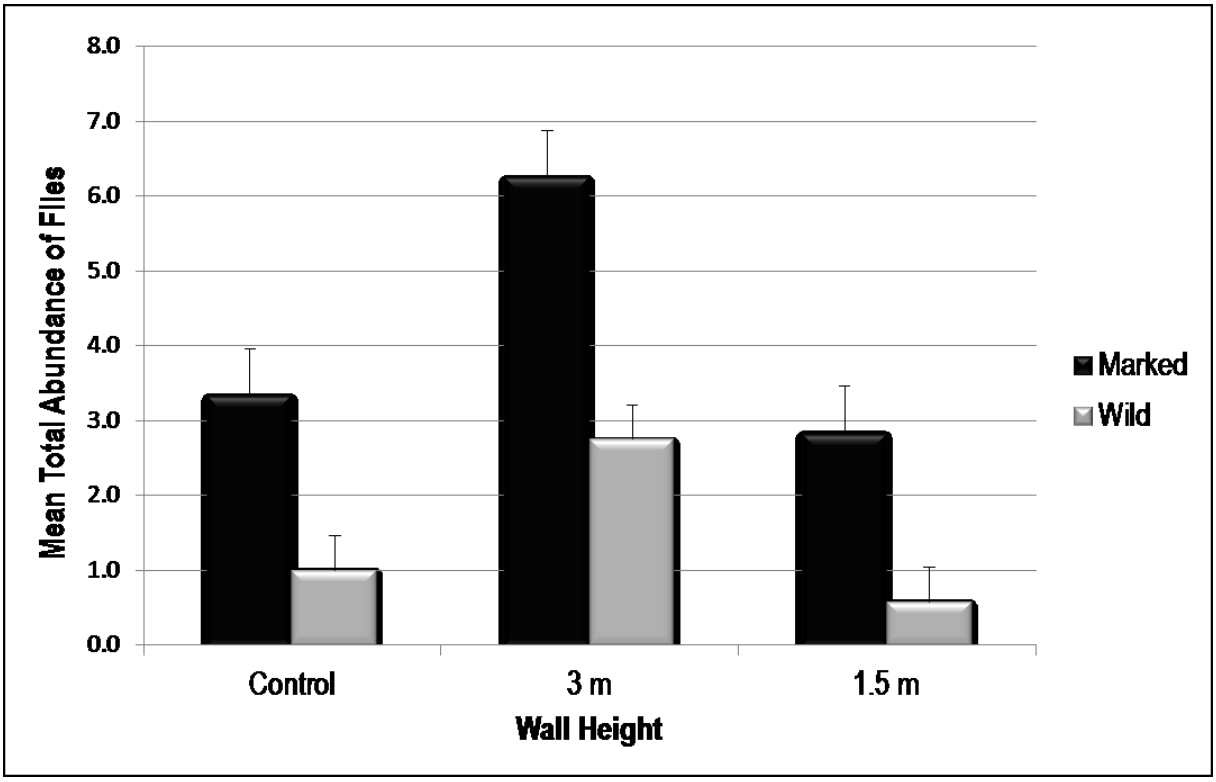


Figure 3.5 – Mean number of marked house flies trapped at each artificial corridor site throughout the period of April through October 2013. The mean number of flies trapped at the 3 m site was significantly higher than the control and 1.5 m sites ($p=0.0003$, $p=0.0014$ respectively). The mean number of wild flies at each corridor site showed a similar trend throughout the same time period in 2013 with a higher number at the 3 m site than both the control and 1.5 m sites ($p=0.0140$, $p=0.0032$ respectively).

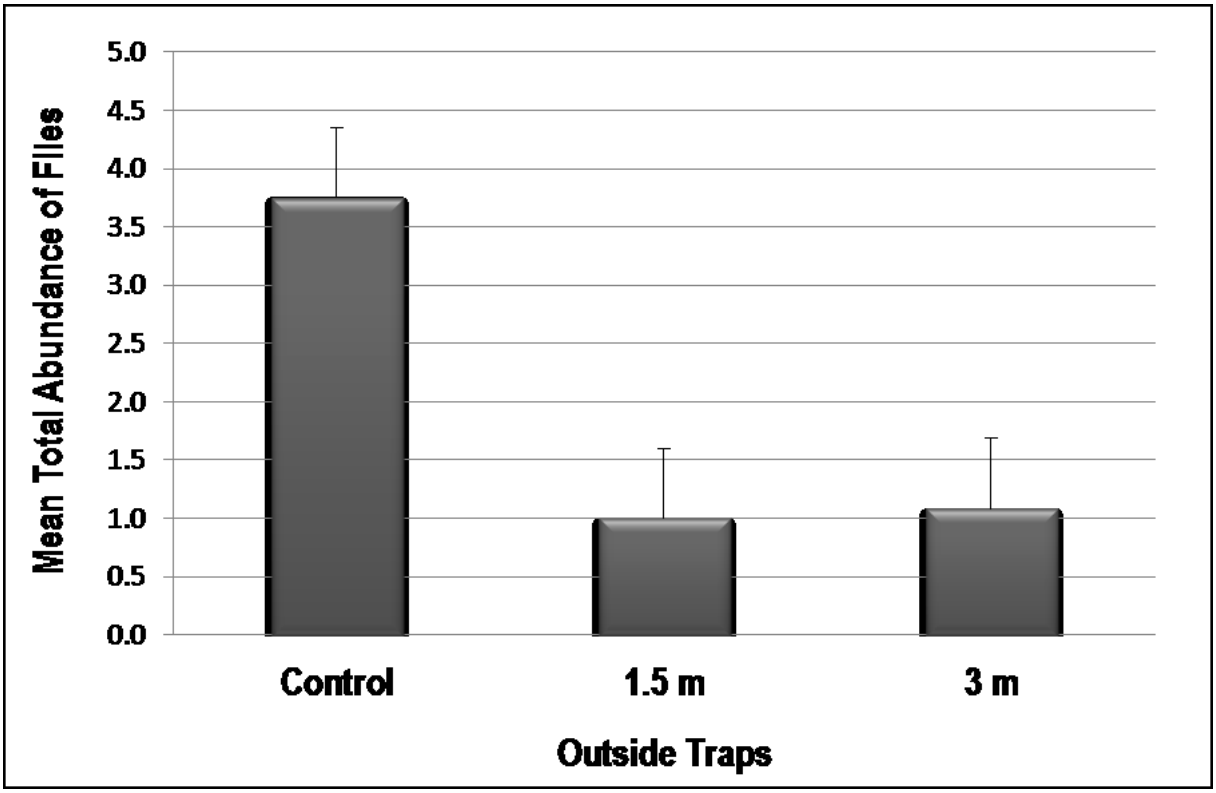


Figure 3.6 – Total number of marked house flies trapped outside of each artificial corridor throughout the period of April through October 2013. The overall number of marked flies trapped outside of the artificial corridors was significantly higher at the control site than the 3 m and 1.5 m sites (p=0.0002 for both treatments).

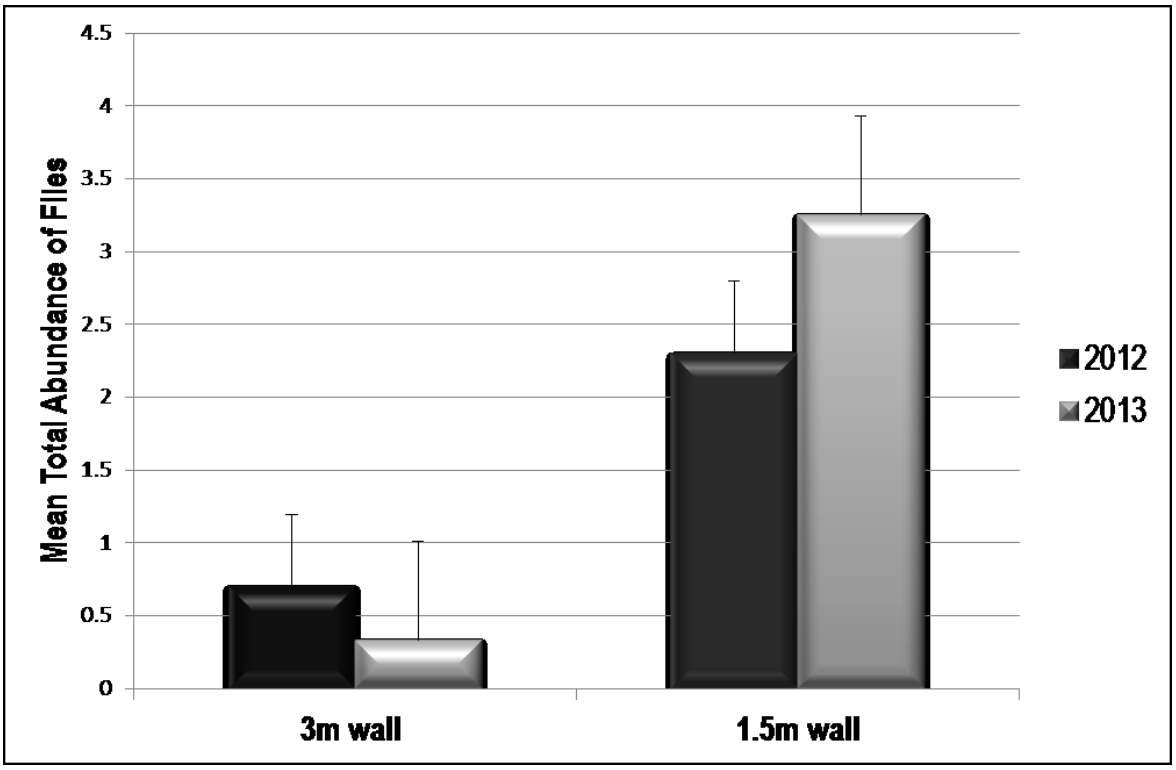


Figure 3.7 – Mean total abundance of house flies trapped at the top of each camouflage wall. Flies were significantly more likely to fly over the 1.5 m walls than the 3 m walls throughout both the 2012 and 2013 field seasons ($p=0.0107$ and $p=0.0013$ respectively).

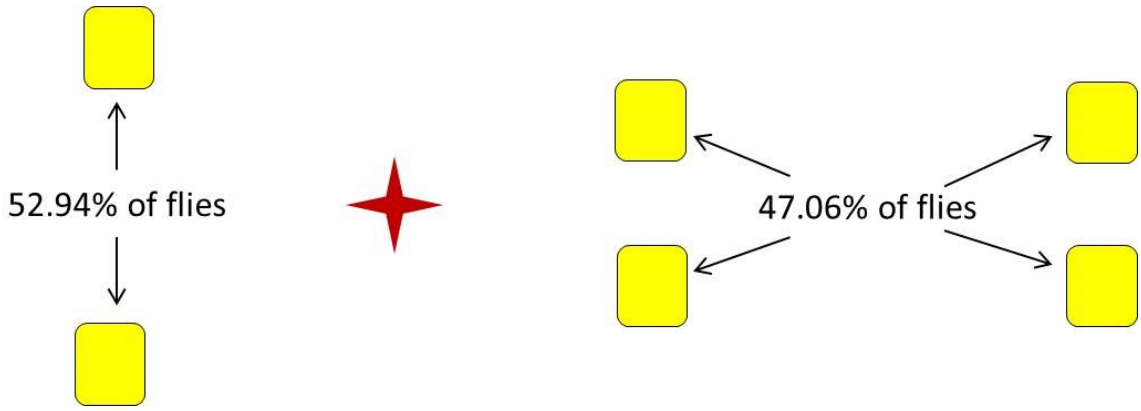


Figure 3.8 – Diagram of the control site and the resulting proportions of marked house flies (red star) recovered from traps outside and trapped within the corridor area (yellow boxes).

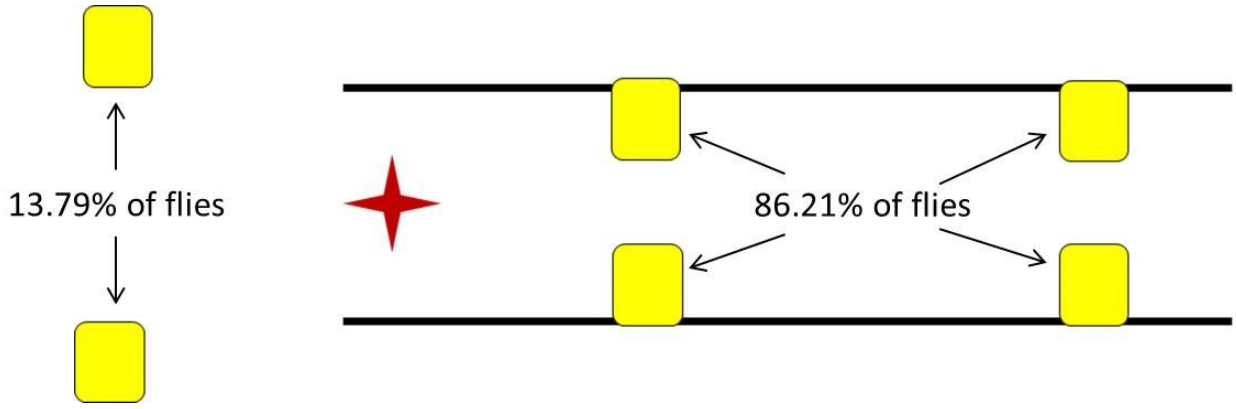


Figure 3.9 - Diagram of the 3 m site and the resulting proportions of marked house flies (red star) recovered from traps outside and trapped within the corridor area (yellow boxes).

CHAPTER IV

EVALUATING AN IMMUNOMARKING TECHNIQUE TO STUDY HOUSE FLY (DIPTERA:MUSCIDAE) DISPERSAL BEHAVIOR

ABSTRACT

House flies (*Musca domestica* L.) are ubiquitous nuisance pests and mechanical vectors of countless pathogens. Examining the dispersal and foraging behavior of house flies in relation to livestock production facilities is an integral component of developing sustainable pest management programs. In order to examine the interaction of house flies with the surrounding landscape, a reliable and economical marking technique must be implemented. This study was designed to examine the usefulness of immunomarking techniques in house fly-feedlot systems. A combination of fluorescent pigments and dried chicken egg whites was used to determine if house flies could be adequately marked with the chicken ovalbumin protein and detected by an enzyme-linked immunosorbent assay (ELISA). All flies that were known to contain both markers based on the presence of fluorescent powder yielded positive optical density values substantially higher than the negative control house flies that did not contain marker. House flies consistently retained enough protein

to yield optical density values higher than the optical density values obtained from the 10 ppm control solutions after they were marked, released and captured on sticky traps at a local beef feedlot facility. The optical density values of all marked house flies were substantially higher than the wild, unmarked flies. Based on the results of this study, immunomarking techniques may serve as a reliable and cost efficient marking technique in certain studies focused on house fly utilization of the landscape and their dispersal from animal farms.

INTRODUCTION

Marking animals is a common method used to study the animals' biology and ecology, especially for population size estimates (Hagler and Jackson 2001). Utilization of novel marking techniques has been integral for monitoring insect movement for centuries. Studies involving marked insects began in the early twentieth century and involved stains, paints and dyes (Dudley and Searles 1923, Geiger et al. 1919, Hagler and Jackson 2001). Studies in which insects are marked to determine movements fall into two categories: 1) mark-release and recapture and 2) mark and capture. The first technique involves marking insects in the laboratory either from colonies or live specimens collected in the field, and the second involves trapping insects that have been marked after contacting a marking material in the environment that has been provided for the study (Nazni et al. 2005). A variety of insect groups have been studied using techniques involving release and recapture of marked individuals. These

include *Hippodamia convergens*, *Lygus hesperus*, *Cacopsylla pericola*, and *Cydia pomonella* (Hagler and Jones 2010, Jones et al. 2006). Insects can be successfully marked with many different materials, however, ensuring that these markers will persist in the field once the insects have been released can be challenging. The use of fluorescent pigments is a common method of marking insects for experiments associated with dispersal. Studies that used fluorescent powders as markers include the dispersal of stable flies from larval development sites by Taylor et.al, (2010) and estimating population densities of *Lucilia sericata* by Smith and Wall (1998). Mark-release-recapture studies do not always result in substantial catch rates. Smith and Wall (1998) reported recapture of just 4% to 14% of their blow flies marked with fluorescent pigment. While fluorescent powders are still used for studies evaluating insect movement, new marking methods are being developed that may be more accurate and cost efficient. Immunomarking techniques are the most recent method of marking insects to be used for dispersal and movement experiments. Insects are marked with proteins derived from soy, cow's milk, wheat and egg whites which are then detected an enzyme-linked immunosorbent assay (ELISA) (Jones et al. 2006). These proteins can be detected at levels as low as 1.9 ppb, and persist in the environment even through light rainfall (Jones et al. 2006).

House flies are well-known nuisance pests and can be problematic when invading homes and business in the area surrounding livestock facilities. As the interface between the urban and rural landscape becomes less defined,

filth flies originating from livestock production facilities become more challenging to control. Along with the obvious nuisance caused by house flies, they have been implicated as mechanical vectors of various harmful foodborne pathogens. Pathogenic bacteria such *as E. coli 0157:H7*, *Salmonella* sp. and *Camplyobacter jejuni* that are commonly associated with livestock can be readily acquired and spread by house flies (Graczyk et al. 2001). A study in Japan isolated *E. coli* 0157:H7 from house flies collected from four different cattle farms (Iwasa et al. 1999). Skövgard et al. (2011) found that house flies associated with poultry production were capable of transmitting *Camplyobacter jejuni* over short distances. Limited research has focused on the distances that house flies will travel once they leave a livestock facility. A study on house flies originating from poultry and horse operations determined that their flight range was 7km and 5km respectively (Nazni et al 2005).

Because house flies are capable of transmitting human pathogens, reliable marking techniques are needed to accurately monitor their movement and ultimately determine the distances needed to maintain safety for homes, business and other facilities such as those growing fresh produce. Immunomarking has been a successful option in several insect systems, and could also be useful in house fly systems. This study was designed to evaluate the usefulness of protein markers to monitor house fly movement in association with beef feedlots.

METHODOLOGY

Mark-release-recapture Study. House flies were obtained from colonies maintained at the Medical Veterinary Entomology building at Oklahoma State University. House flies in the pupal stage were allowed to emerge at field sites and caught using sticky traps coated with Taglefoot®Tangle-trap™ adhesive for a period of seven days. Several replicates of the seven day trapping period occurred throughout the spring, summer and fall of 2012 (n=14) and 2013 (n=12). Flies were removed from each sticky trap and placed in 1.5 ml microcentrifuge tubes. All samples were kept frozen until the end of each field season when the assays were performed.

The house flies were marked using a combination of fluorescent powder and powdered egg white. This mixture included a 1:1 of DayGlo® eco pigment (DayGlo® Color Corp., Cleveland, OH) and powdered egg white (Honeyville Food Products Inc., Rancho Cucamonga, CA). House flies were allowed to pupate within the media in which the larvae were reared. The mixture of marking powders was applied liberally (approximately 40mg/cm^2) to the surface to the media, and when the adult flies emerged from their puparia they were coated with the markers as they crawled up through the media and out of the container.

Anti-egg Albumin Enzyme-linked Immunosorbent Assay. Enzyme linked immunosorbent assays were used to detect chicken egg albumin on the surface of the house flies. Protocols for indirect ELISA were adapted for use with

house flies from Hagler et al. (2011) and Jones et al. (2011) (Fig. 4.1). Microcentrifuge tubes were removed from the freezer and placed in the refrigerator to thaw overnight. In order to remove the protein from the surface of each house fly, 1.0 ml of Tris buffered saline (pH 7.4) extraction buffer was added to each tube. The tubes were each vortexed for five seconds to submerge the flies and then allowed to soak at 37ºC for one hour. The buffer was then siphoned out of each tube using a pipette and transferred to a new 1.5 ml microcentrifuge tube. Additional samples were also diluted by 10% and 6.25% as suggested by Jones et al. (2011) to examine the possibility of stearic inhibition caused by high concentrations of protein marker extracted from the house flies. Individual wells of an untreated 96-well assay plate (Falcon® Pro-bind, BD Biosciences, San Jose CA) received 100µl aliquot of each sample. The plates were then allowed to incubate for 1 hour at 37ºC. Contents of each well was discarded and washed five times with phosphate buffered saline tween-20 (0.5% tween, pH 7.4) (PBST) (No. P3563, Sigma Aldrich Co. LLC, St. Louis MO). A 360 µl aliquot of Tris-buffered saline with bovine serum albumin (BSA 1%, pH 7.4) (TBS-BSA) blocking solution was added to each well and allowed to incubate for 2 hours at 37ºC or overnight at 4ºC. The contents of each well was discarded and then washed twice with PBST. A 50 µl aliquot of primary antibody, rabbit anti-chicken ovalbumin (No. C6534 Sigma Aldrich Co.) diluted in a buffer solution of TBS-BSA (1%) (1:8000) was added to each well and incubated for 1 hour at 37ºC. The contents of each well was discarded and washed five times with PBST. A 50 µl aliquot of secondary antibody, goat anti-

rabbit IgG (No. A6154, Sigma-Aldrich Co.) conjugated to horseradish peroxidase diluted in a buffer solution of TBS-BSA (1%) (1:2000) was added to each well and incubated for 2 hours at 37ºC. The contents of each well was discarded and washed five times with PBST. The 3,3',5,5' – Tetramethylbenzidine substrate (TMB) enzyme substrate for horseradish peroxidase (No. T0440, Sigma-Aldrich Co.) was then added to each well in 50 µl aliquots and allowed to develop on the bench at room temperature for 3 minutes. A solution of 2N H_2SO_4 was added to stop the reaction, and the plates were read using a dual-wavelength microplate reader (ELx800 BioTek Instruments Inc., Winooski, VT) at 450 nm using 490 nm as the reference standard. All optical density readings were corrected using the mean value of the (blank) wells containing only TBS buffer with no antigen.

House Fly Positive Controls. House flies were marked in the laboratory in mesh cages for use as positive controls. The flies were reared in the same manner as previously described and the same 1:1 mixture of marking powder was applied to the surface. Flies were allowed to desiccate in the mesh cages and then transferred to individual microcentrifuge tubes. The tubes were then frozen until the flies were assayed. Additional flies marked in the same manner were allowed to emerge in a laboratory observation box. The flies were provided with sucrose, water and an egg laying substrate and were allowed to feed and mate for approximately 3 weeks until they expired naturally. The flies were marked "extended flight", transferred to individual

microcentrifuge tubes, and frozen. The “extended flight” flies served as a control to represent flies traveling greater distances.

House Fly Negative Controls. House flies used for negative controls on each microplate (n=8) were captured from Willard Sparks Beef Research Center, Stillwater, OK prior to the beginning to the field study before any marked flies were released. The house flies were examined for the presence of fluorescent powder and assayed following the protocols described above. The mean optical density values were calculated, and a positive threshold determined. Positive thresholds were calculated using the mean of the negative control flies plus 6 standard deviations, the mean plus 4 standard deviations and the standard normal variate transformation outlined by Sivakoff et al. (2011).

Analysis of Trapped House Flies. House flies trapped on sticky traps were examined for fluorescent powder and assayed for the presence of chicken egg protein following the ELISA protocols outlined previously. The efficacy of the immunomarking technique was determined by the percentage of flies marked with fluorescent powder that yield a positive ELISA optical density. Flies marked with fluorescent powder that did not result in a positive optical density value were considered false negatives. The rate of false positive house flies was determined by unmarked house flies that yielded a positive ELISA optical density value. Flies from the study site were assayed and the data was subjected to three separate positive threshold values based on common

methods used in immunomarking studies (Jones et al. 2006, Hagler et al. 2011, Sivakoff et al. 2011).

STATISTICAL ANALYSIS

Samples and positive control flies were diluted to examine any differences in the resulting optical density values. Controls and field samples were diluted by 10% and 6.25% and compared to the 100% buffer solutions by using the PROC GLM procedure with an LSMEANS test (SAS. 9.3; SAS Institute, 2013).

RESULTS

All house flies assayed from the field study and from laboratory marked positive controls yielded positive optical density values after background correction. All flies were positive for fluorescent powder indicating that they should be positive for egg protein as well. Field samples and positive controls that were diluted to 10% and 6.25% showed no significant difference in optical density values from the 100% solutions ($F=2.63$, $df=21$, $p=0.0800$ and $F=1.48$, $df=21$, $p=0.2358$ respectively) (Fig. 4.2). The methods of calculating positive thresholds based on the negative control flies yielded varying results. Based on a positive threshold value calculated using the mean optical density of negative control flies (n=8) on each plate plus 6x the standard deviation, 23 of 262 flies were assigned a negative score. This resulted in an 8.78% false negative rate as all flies assayed were known to contain ovalbumin protein indicated by the

presence of fluorescent powder. The second and third methods of calculating the positive threshold value both lowered the false negative rate to 0%. Using the mean optical density of negative control flies (n=8) on each plate plus 4x the standard deviation, 0 of 262 house flies scored negative for protein marker. These results were consistent with the false positive rates based on a positive threshold calculated using the standard normal variate method (0/262) (Sivakoff et al. 2011). The chicken ovalbumin protein was detectable in solutions at levels as low as 5 ppb. The mean optical density value resulting from positive flies after background correction was substantially higher than the mean optical density values of negative control flies obtained from the feedlot which yielded a mean optical density value of 0.299. Based on serial dilutions of powdered egg white and TBS buffer, the mean level of protein retained by the marked house flies exceeded 10 ppm (Figure 4.3). These ELISA results indicate that house flies marked with chicken egg protein can be distinguished from native house flies trapped in immediate vicinity of beef feedlots.

DISCUSSION

The impact of monitoring house fly dispersal and their interaction with the landscape surrounding livestock operations has increased tremendously with the spread of urban environments and the implication of filth flies as mechanical vectors of virulent, zoonotic pathogens such as *E. coli* 0157:H7. In order to produce reliable data from monitoring house flies, an adequate

marking technique must be employed. Fluorescent powders, also referred to as dusts, have been widely used for marking a variety of insects and remain a useful tool to monitor insect movement (Hager and Jackson 2001, Hagler et al. 2011). In studies focused on the long distance dispersal patterns of flies, these pigments could become too sparse to detect even under a microscope. Other methods such as radioactive isotopes marking are becoming much less favorable due to the increased expense and decreased efficiency (Hagler and Jackson 2001).

Immunomarking is a novel method that utilizes different types of proteins as markers on the insect or on the vegetation in a given area and detects them through ELISA. First generation and second generation protein markers have been effectively used to mark dozens of different arthropods. Marking insects with IgG proteins is considered first generation, and while it is a reliable marker it is also substantially more expensive than the second generation proteins such as chicken egg ovalbumin, bovine casein, soy and wheat gluten (Slosky et al. 2012). First generation markers used by Hagler (1997) were approximately $500/liter. The price of second generation markers is between $0.12 and $0.26/liter depending on the concentration and specific protein used (Jones et al. 2006). The low cost of the second generation markers allows researchers to conduct field studies on a much larger scale, and these have included primarily methods where the insects acquire the protein markers when contacting vegetation in a given areas that was previously sprayed with a solution of the protein (Jones et al. 2006, Hagler and Jones

2010). One particular study was successful in marking bees with powdered protein as they exited the nest and the protein marker was sensitive enough to be detected directly from the petals of flowers that the bees had visited (Biddinger et al. 2013). This study used a marking method similar to Halger et al. (2011) where honeybees were marked by crawling through powdered protein, captured and then assayed. Another study marked spined soldier bug, *Podisus maculiventris* by incorporating the protein marker into the artificial diet of their prey, *Manduca sexta* (Kelley et al. 2012). The potential for use of these novel methods for marking and detection of second generation proteins in insect systems is enormous.

Protein markers have not been evaluated for use with filth flies until now. House flies were marked with a mixture of powdered fluorescent pigment and chicken egg white in order to monitor the retention and recovery of the ovalbumin protein from house flies trapped in a feedlot setting. One of the most important factors in the success of an immunomarking technique where the protein is applied directly to the insect is the ability to distinguish the marked insects from the native population. These proteins were detected at levels as low as 5 ppb consistently by the assay utilized in this study and they are able to persist for extended periods of time and even through light rainfall (Jones et al. 2006). These factors become less accommodating if the native population of insects comes in contact with the protein in their environment or otherwise yield high optical density values. The efficacy of protein markers must be evaluated for the insect system being examined before using them

with any field study (Slosky et al. 2012). The selection of a positive threshold value can also impact the data set. In this study, the data obtained from marked house flies was subjected to three methods. The methods using 6x the standard deviation above the mean optical density of the negative control flies has been used in other experiments, but perhaps not appropriate for this study (Hagler et al. 2011). The mean value of house flies that were marked but considered negative based on the calculated threshold were all substantially higher than the mean optical density of the negative control flies and much closer to the mean value of the flies considered positive (Fig. 4.4). This method of calculating the positive threshold with this particular set of data would have resulted in an unnecessarily high false negative value. Two other methods utilized (mean plus 4 standard deviation values and the standard normal variate methods (Sivakoff et al. 2011)) eliminated the false negative issue that occurred with the previous method (6x standard deviation above the mean optical density of the negative control) at the same rate of 0/262 false negative flies. Utilizing different methods to account for differences in optical density values of the wild, unmarked population and the positive control flies was necessary to determine which method may be best for analysis of field data.

The goal of exploring marking options for house flies originating from livestock production systems is to study long distance dispersal behaviors and use the data to contribute to risk assessments of house flies invading the surrounding landscapes. Having reliable insect markers that are economical

will also aid in determining the risk of urban or food production areas becoming contaminated with zoonotic pathogens acquired from animal waste by filth flies. The house flies trapped in this study contained an ample amount of the marking powder mixture seven days after they were released (Fig. 4.5). The persistence of fluorescent marking powders can decrease dramatically when flies are released and trapped at greater distances, and the detection of trace amounts even with the aid of a microscope and UV light may still result in high numbers of false negatives. The efficacy and sensitivity of protein markers indicate that they may be a viable option for long distance house fly dispersal studies and eliminate some of the issues faced with other marking techniques. The sensitivity of the assay, retention and persistence of the protein powder on the house flies and the optical density values that result from wild caught, unmarked house flies are the major factors influencing the success of immunomarking in house flies collected near beef feedlot systems. The anti-chicken ovalbumin assay is detectable at levels as low as 1.9 ppb (Jones et al. 2006). Samples assayed in this study consistently detected egg white powder solutions as low as 5 ppb. Based on the optical density values that resulted from each known concentration of powered egg whites and buffer, the house flies retained enough marking powder to extract over 10 ppm. Additional flies that were marked in the laboratory and allowed to feed on sucrose and water, mate, lay eggs and desiccate in observation boxes (approximately 2-3 weeks) were assayed and yielded a mean optical density value of 0.952. This value indicates protein concentrations just below 100 ppb (Fig. 4.6). Compared to

the values consistently obtained from wild, unmarked flies, the mean optical density is still substantially higher for the extended flight positive controls. This may imply that over extended periods of flight and through various behaviors, an adequate amount of marking powder can still be extracted from the flies. Although conditions in the field may further influence marker retention, these values initially indicate that chicken egg ovalbumin could be an efficient marker for house flies in long distance dispersal studies.

CONCLUSIONS

This research has shown that immunomarking techniques using chicken egg albumin could be used for monitoring house fly movement in feedlot systems. House flies retained large amounts of the 1:1 fluorescent with egg white marking powder after seven days in the field, and 100% of flies positive for fluorescent marker yielded positive optical density values with ELISA. Any study that has not previously evaluated this technique should thoroughly examine all aspects of the marking method, type of protein used and optical density values of the wild populations before employment at the field scale. In house flies collected from beef feedlot systems, the anti-chicken ovalbumin assay could be a valuable marking method in situations where large amounts of flies are trapped and assayed. Immunomarking could be useful for moderate size field sites, although the cost of materials and labor may not be justified with small samples sizes such as those obtained from this study. Despite typically having a smaller sample size, long distance field studies may rationalize the expense of

protein markers due to their persistence and sensitivity. An evaluation similar to this study should be implemented before collecting data for a long distance study using exclusively protein marker.

FIGURES

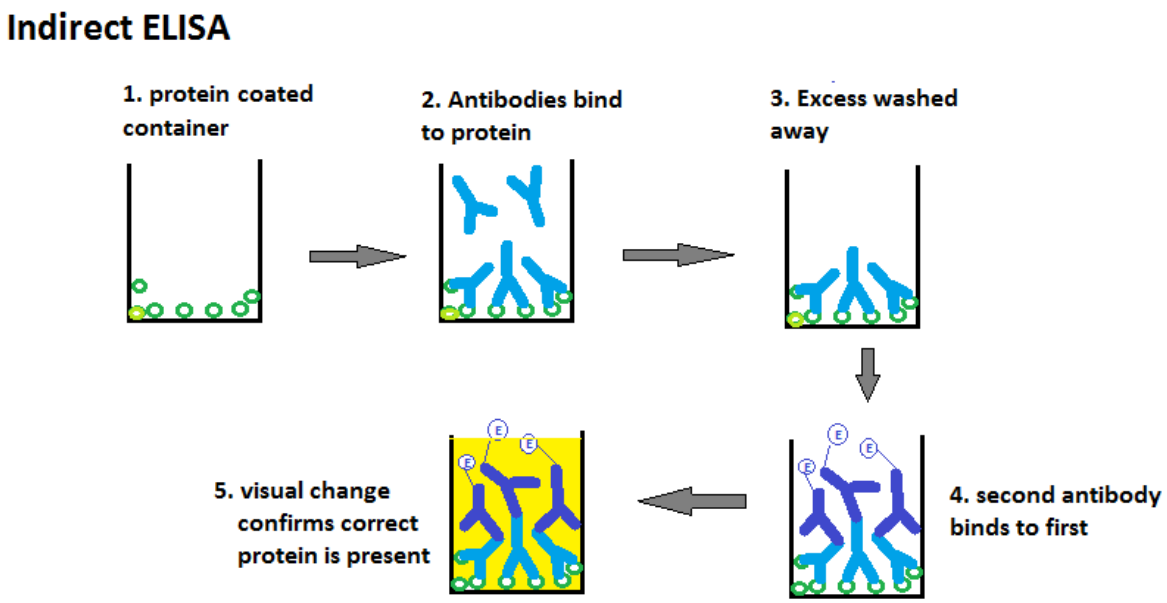


Figure 4.1 – Diagram of the steps associated with Indirect ELISA

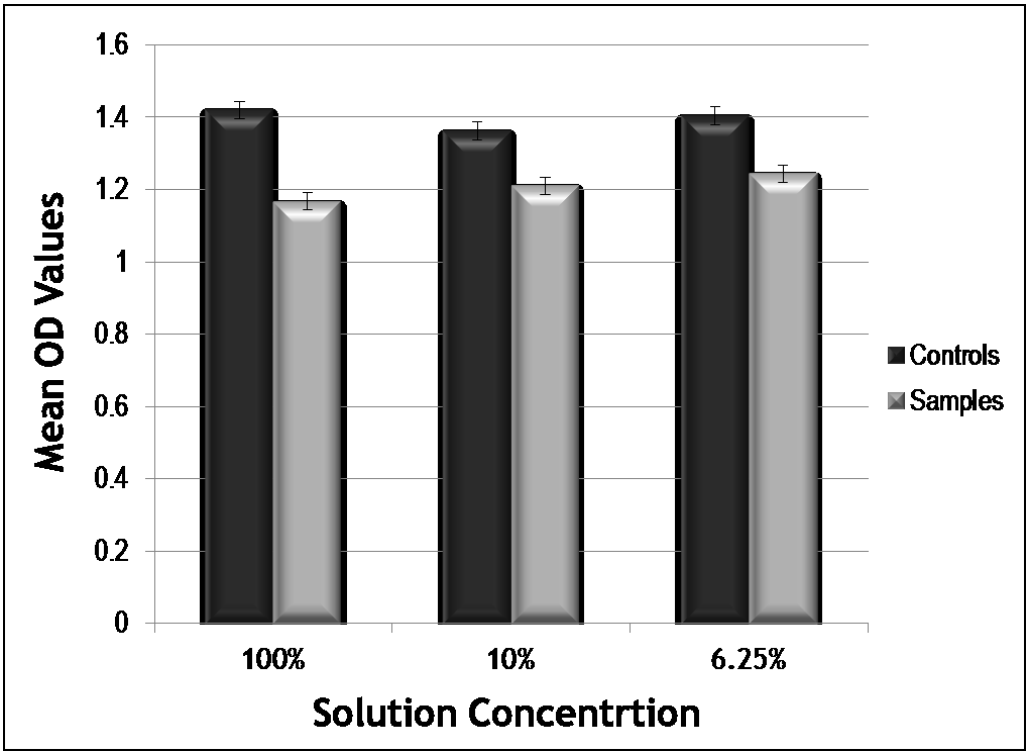


Figure 4.2 – Mean optical density values for positive flies marked in the laboratory as well as trapped at field sites, diluted by 10% and 6.25%.

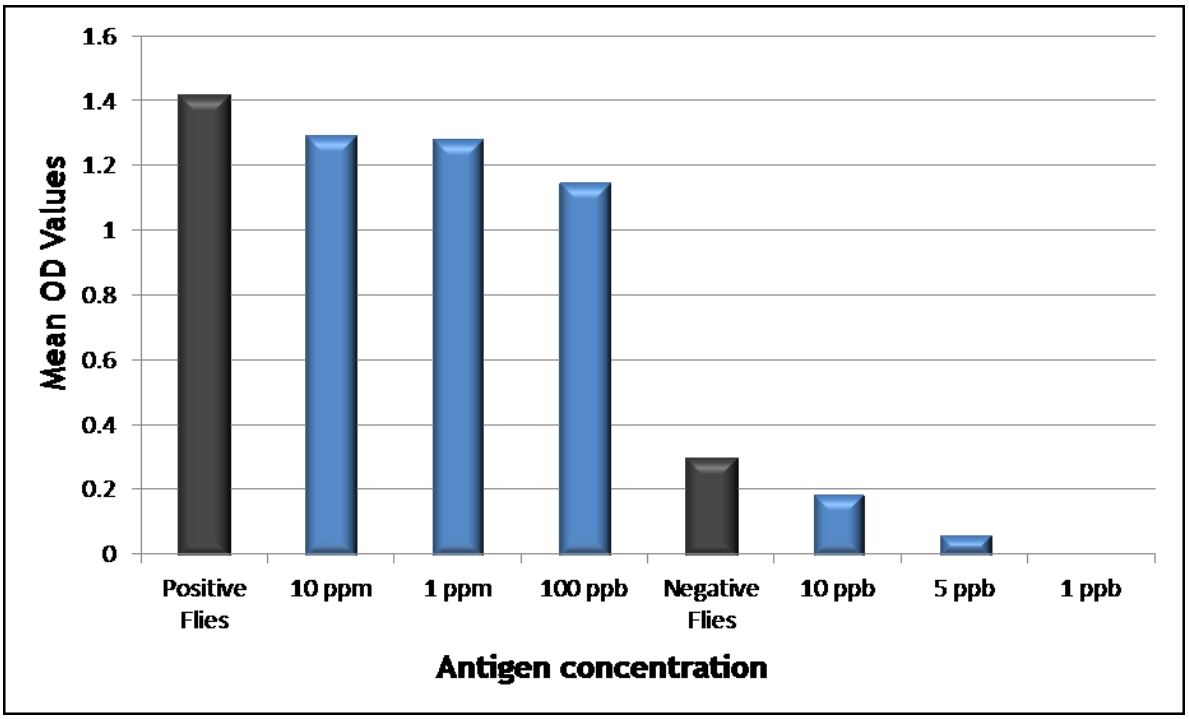


Figure 4.3 – Mean optical density value of positive house flies assayed after emerging from a bin with marking powder and allowed to remain in a mesh cage after one week in addition to flies collected from sticky traps and mean optical density value for negative control flies compared to serial dilutions of chicken egg white in TBS buffer.

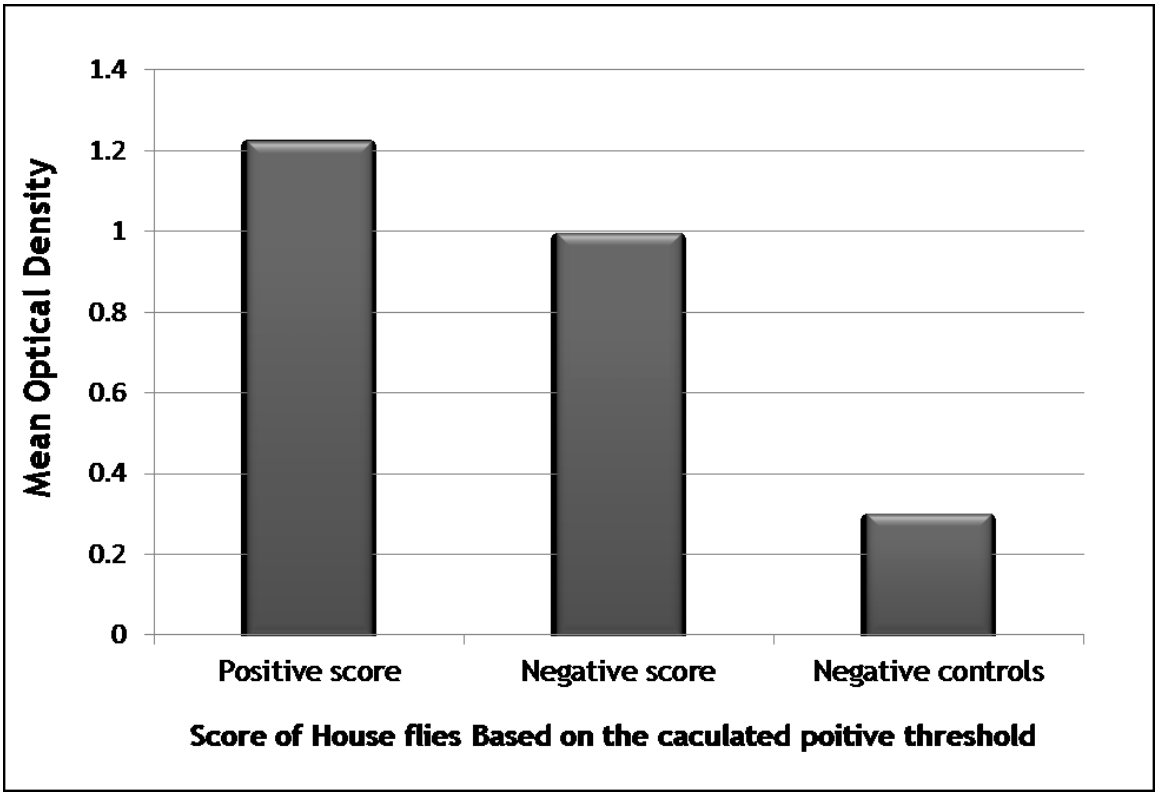


Figure 4.4 - The mean optical density values for flies that scored positive and negative based on the positive threshold calculated using the mean plus 6 standard deviations of the negative control flies.

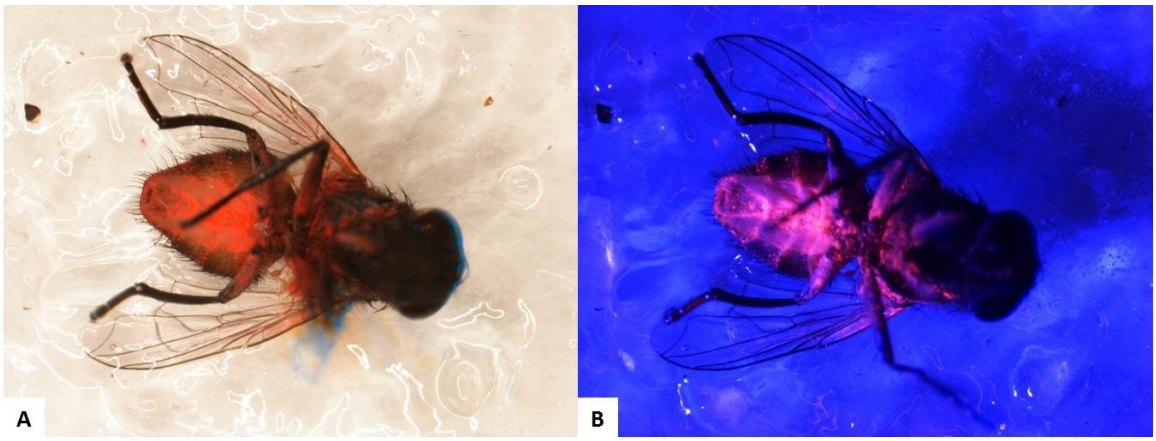


Figure 4.5 - House fly, *Musca domestica*, marked with fluorescent powder and powdered egg white (1:1 mixture) A) magnified 10x under normal light B) magnified 10x under ultraviolet light.

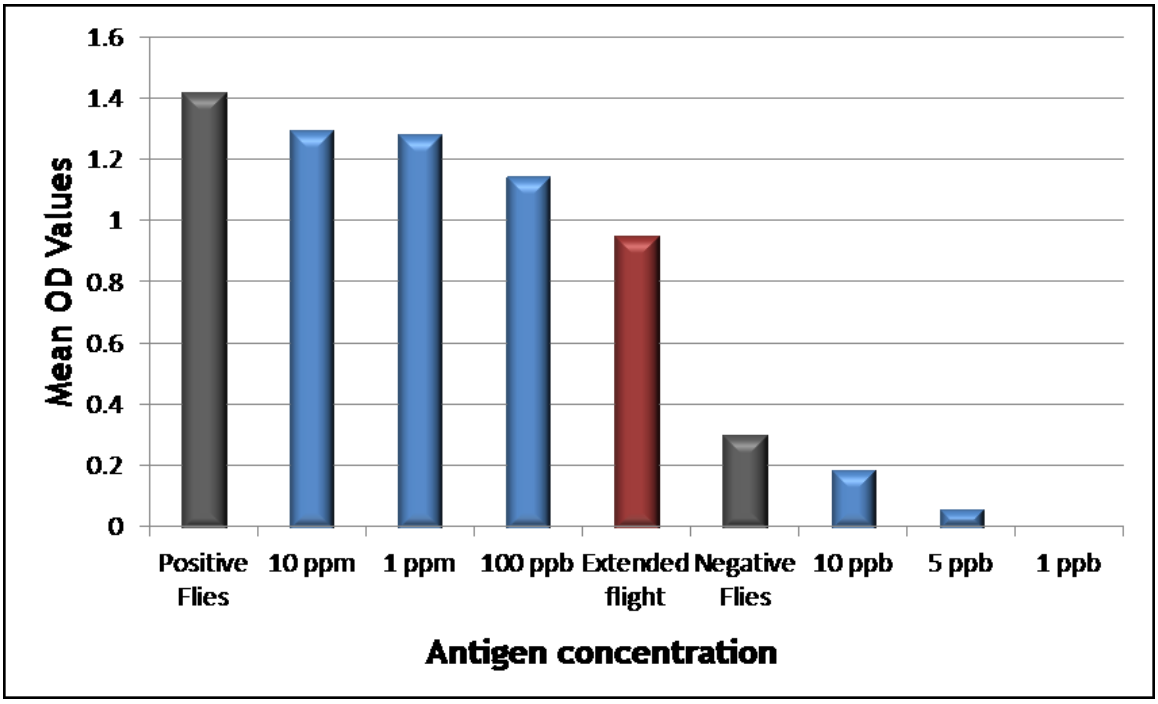


Figure 4.6 – The mean optical density values for house flies allowed to feed, mate and lay eggs in laboratory observation boxes (red bar) and mean optical density values of all positive flies and negative control flies (grey bars) compared to serial dilutions of chicken egg white in TBS buffer.

CHAPTER V

FILTH FLY ACTIVITY ASSOCIATED WITH COMPOSTED AND NON-COMPOSTED BEEF CADAVERS AND THE EFFECT OF COMPOSTING ON VOLATILE ORGANIC COMPOUNDS

ABSTRACT

Commercial livestock facilities are faced with the challenge of managing large amounts of waste including manure and animal mortalities. One increasingly popular way of dealing with dead animals is composting. The cadavers are buried in carbon material that creates a barrier between the dead tissue and the surrounding environment. Dead tissue can release compounds that not only contaminate the soil but also the groundwater and nearby surface water. Animal mortality composting is designed to facilitate decomposition without the aid of carrion feeding insects and reduce the presence of common pathogens associated with animal waste and dead tissue. The goal of this study was to evaluate insect activity associated with composted and exposed beef cadavers, specifically filth flies that can serve as mechanical vectors of important human pathogens such as *E. coli* 0157:H7. Greater numbers of all types of arthropods were trapped overall at the exposed animal site than the composted animal site. Most importantly, the number of filth flies was

significantly lower at the composted site. Volatile organic compounds were also sampled in this study, and known fly attractants such as dimethyl disulfide were inhibited by the composting process. Implementing composting programs at livestock facilities could reduce the risk of flies spreading harmful pathogens to surrounding areas including farms that grow fresh produce.

INTRODUCTION

Filth flies are well known pests that are directly associated with livestock operations. They develop in various types of decaying organic matter including animal manure and decomposing animal tissue, and animal production facilities create ideal conditions for filth fly development. Many filth flies are competent mechanical vectors of pathogenic microorganisms that can be acquired from animal manure and animal carcasses (Greenberg 1973, Graczyk 2001). These include *Escherichia coli* 0157:H7, *Salmonella*, *Camplyobacter jejuni*, *Listeria monocytogenes* and other zoonotic pathogens that can cause serious illness and even death in humans (Alam and Zurek 2004, Szalanski et al. 2004, Pava-Ripoll et al. 2012). Flies have also been implicated in the transmission of antibiotic resistant bacteria. Livestock operations have been suspected of creating selection pressure for antibiotic resistance due to their widespread use of antibiotics, and filth flies can easily spread these organisms from animal facilities directly to urban environments (Zurek and Ghosh 2014). Filth flies are potential vectors for contaminating produce on-farm and therefore essential component of food safety.

Commercial livestock facilities are challenged to dispose of substantial amounts of manure as well as animal carcasses. In Oklahoma, the mean cattle and calf mortalities exceeds 5 million head annually (Table 5.1) (Payne and Pugh 2010). Catastrophic events such as disease outbreaks and natural disasters can dramatically increase the number of mortalities and requires a safe and effective means of disposal. Exposed livestock mortalities can contain harmful zoonotic pathogens as well as those than can be transmitted to other livestock animals (Lloyd-Smith et al. 2009). Some alternative methods of disposal may leave the area more accessible to flies that can pick up and carry off harmful microorganisms. Abandonment is illegal although many producers still leave exposed carcasses to decompose on their property. Above ground burial with a thin layer of soil was used in experiments examining fly activity and decomposition and proved to be an ineffective means of cadaver disposal and does not reduce insect activity or pathogen survival (Eamens et al., 2011). Incineration and rendering are additional options for carcass disposal. Both methods are acceptable although the biosecurity of transporting and preparing the dead animals for these methods can be problematic as well as expensive for producers (Bonhotal et al. 2002, Gwyther et al. 2011). One increasingly popular way of dealing with excessive amounts of dead animals is composting. Arthropods and microbes aid significantly in the decomposition of exposed animal cadavers. Animal compost is designed to facilitate decomposition without the aid of carrion feeding insects and common microorganisms associated with animal waste. An ideal composted cadaver will reach

temperatures too high for arthropods and harmful pathogens to survive allowing heat and the activity of thermophilic microorganisms to breakdown the dead animal tissues (Kalbasi et al. 2005, Berge et al. 2009). The composting process breaks down dead animal tissue in an efficient and biosecure manner (Wilkinson 2007).

Olfactory cues generally referred to as semiochemicals allow insects to locate resources, including flies that develop on carrion (Nation 2008, LeBlanc and Logan 2010). Chemical compounds attractive to filth flies emitted from non-living sources such as dead animals are more specifically referred to as apneumones (Norlund and Lewis 1976, LeBlanc and Logan 2010). These decomposition odors are not sufficiently degraded or suppressed when animal carcasses are buried (Vass et al. 2004). Burial up to 1 meter deep will allow odors from the carcasses to escape through the soil, and this attracts filth flies to the area (Vass et al. 2008). It is suspected that the composting process can reduce the emission of volatile organic compounds released from the cadaver interfering with the olfactory cues that attract flies but up to this point has never been proven. Proper conditions within a compost pile reach temperatures that theoretically should be too high for filth fly development, and the envelope of composting materials should prevent flies from accessing the carcass. This study is the first to our knowledge to evaluate filth fly activity and composted livestock mortalities. Decomposing livestock carcasses can pose a major health risk if they are left exposed and available to filth flies that can mechanically transmit pathogenic microorganisms such as *E. coli* 0157:H7 from

the dead animal to the surrounding area. Composting cadavers could reduce filth fly activity overall and lessen the public health risks associated with the disposal of livestock mortalities. This study was designed to evaluate the activity of filth flies associated with livestock mortalities and any effects that can be attributed to composting the carcasses.

METHODOLOGY

Insect Activity. Fly activity was evaluated by comparing composted beef cadavers to those left completely exposed. An individual experimental unit included one carcass acquired from the Oklahoma State University's Willard Sparks Beef Research Center and North Lake Carl Blackwell Beef Research Range in Stillwater, OK. The site containing the composted cadaver was constructed on the feedlot grounds at the Willard Sparks Beef Research Center, and the exposed cadaver site was located on the grounds at the Medical Veterinary Entomology building one mile southeast of the feedlot. Both field sites were equipped with a 3m x 3m fence surrounding the carcasses approximately 1.5m high. Malaise traps were suspended over the top of each animal and secured to the fence on one side in order to trap flying insects visiting the carcass (Figure 5.1). Each trap was equipped with an inverted 1 quart plastic jar fitted with a funnel made of clear acetate at the mouth of the jar. Insects were able to fly or crawl up through the funnel opening into the jar and remain trapped inside. Samples were collected every 2 to 4 days and

stored in the freezer for subsequent identification. A set of animals, *Bos taurus*, was observed and sampled throughout the fall/winter season (from October through December 2012) and the spring/summer season (from April through June 2013). The animals used in for the fall/winter period were approximately 181 – 226 kg (400-500 lbs.) and the animals used for the spring summer period were approximately 544 – 635 (1200-1400 lbs.).

Temperatures inside of the compost piles and under the exposed cadavers were monitored throughout the period of decomposition. A HOBO U23 Pro v2 External temperature/relative humidity data logger with a sensor on the end of a cable approximately 2m long was used for each animal and placed directly under the posterior end of each carcass (U23-002, Onset Computer Corp. Bourne, MA). A mixture of different types of wood chips, leaf litter and sawdust obtained from the OSU Botanical Garden was used for the composting media, and the material was moistened with water per the standard recommendations for mortality compost which is approximately 50% moisture (Payne and Pugh 2010). The composted animal sites were constructed by establishing a pad of composting media that was approximately 0.5 m thick. Then, the carcasses were placed in the center of the pad and the rumen was cut in order to keep the carcass from bloating excessively. The composting material was moistened and an envelope of approximately 0.5 m was built around the carcass to ensure adequate insulation of the compost pile (Fig. 5.2). In the interest of keeping the variables at both field sites consistent with

exception to the treatment being examined, the compost piles were not turned or otherwise disturbed throughout the period of decomposition.

Analysis of Volatile Organic Compounds. Laboratory studies using large feeder rats were designed to evaluate odors emitted from both exposed and composted animals. The rats were obtained frozen from a local pet supply company and allowed to thaw overnight in the refrigerator. Each animal was placed in an individual plastic container with a lid. Each lid contained a small area of nylon mesh to grant access to the sampling equipment, and parafilm was used to completely seal in all odors. Replicates of three animals were used for each of the two treatments, composted and exposed. All containers were kept in an insect colony room that was kept at 20°C to 24°C and 60% to 65% relative humidity.

Volatile organic compounds were sampled using 75 µm carboxen®/polydimethylsiloxane solid-phase microextraction (SPME) fibers (Supelco #57344-U, Sigma-Aldrich Co. LLC, Bellfonte, PA). Individual fibers were exposed in the headspace above each animal for a period of 20 minutes. Between each sampling period, odors were allowed to accumulate in the container for approximately 48 hours. The parafilm and lids were removed after sampling in order to release the compounds and purify the headspace between the stages of decomposition and sampling episodes. The samples were immediately analyzed using gas chromatography/mass spectrometry (GC/MS).

The instrument used was an HP6890 gas chromatograph (GC) with a 5973 mass selective detector (MSD) (Agilent Technologies, Palo Alto, CA). The instrument was equipped with a DB5-MS capillary column 30 meters long with an internal diameter of 0.25 mm and a film thickness of 0.25 µm and an SPME injection port liner operated at 250°C. The carrier gas used was Helium and the flow rate was set at 1.5mL/minute. The oven temperature was set at 40°C to begin, held for 1 minute, ramped to 80°C at 3°C/minute, then up to 120°C at 10°C/minute, and lastly raised to 260°C at 40°C/minute. The total run time for the program was 21.83 minutes. The MSD was scanned 10 to 700 amu at a rate of 2.94 scans per seconds. Protocols for this study were adapted and modified from Hoffman et al. 2009. Data was collected using ChemStation and the spectra deconvoluted using AMDIS32 software. Compounds were identified using the NIST library.

RESULTS

The mean total abundance of insects collected with the malaise traps throughout the fall/winter season and the spring/summer season was numerically higher at the exposed carcass site although not statistically significant from the composted site (df=1, F=1.05, p=0.2730) (Fig. 5.3). Overall abundance included many insects not commonly associated with carrion. Filth flies and families commonly associated with carrion were examined separately after non-relevant samples, those not commonly associated with carrion, were excluded. The mean number of filth flies trapped at the exposed cadaver site

was significantly higher than those trapped at the composted site (df=1, F=196, p= 0.0009) (Fig. 5.4). The major insect groups trapped at both sites belonged to the families Calliphoridae, Muscidae, Fanniidae and Ulidiidae (Fig. 5.5). Temperatures within the compost pile were consistently much higher than both the ambient temperature and temperature under the exposed carcass for both the fall/winter and spring/summer periods (Fig. 5.6 and 5.7). Temperatures reached only 48°C for one day during the fall/winter period within the compost pile however these temperatures were consistently higher than the exposed carcass which showed a brief spike up to 33°C but remained below 22°C for the majority of the decomposition period. During the spring/summer period the compost pile remained between 55°C and 59°C for over 6 days while the exposed carcass reach temperatures of only 38°C for a period of less than 12 hours.

The difference in decomposition and odor between the exposed cadavers and the composed piles were very apparent once the compost pile had completed the initial temperature cycle and was opened for examination (Fig. 5.8). During the initial cycle, and what would be referred to as the active decay stage in an exposed animal, odor was detectable only in close proximity to the compost pile. Although no odor could be detected from the compost pile over, flies were still attracted on some dates. The volatile organic compounds (VOCs) emitted from large rats that were either composted or left entirely exposed showed a qualitative difference in the compounds isolated from rats with each treatment (Fig. 5.9 and 5.10). Few compounds directly related to

odors of decomposition have been tested specifically with blow flies. The only compound that was isolated from the composted rats was dimethyl disulfide, and it was not present after the active decay stage of decomposition (Fig. 5.10, Table 5.2). The two compounds that were isolated from the exposed rats were dimethyl disulfide and dimethyl trisulfide (Fig. 5.9), and these were present throughout the active and post decay stages of decomposition (Table 5.2).

DISCUSSION

The relationship between livestock production and filth flies is inevitable, and the considerable amount of waste including animal mortalities generated annually by these facilities can have a profound effect on filth fly populations. Poorly managed animal waste and carcasses will result in increased filth fly proliferation and as a consequence intensifies the risk to public health. Decomposing animals are very easily located and colonized by insects when left uncovered and exposed to the environment. The detection of long range olfactory cues that attract insects to a resource or a mate varies with the concentration of material released, wind speed and direction, as well as vegetation or other surrounding structures (Nation 2008). Despite interference from the surrounding environment, insects are able to detect semiochemicals in minute amounts and locate the source from impressive distances (LeBlanc and Logan 2010). The composting process significantly decreases emission of odors from decomposition. Early in the active decay stages, unpleasant odor is mild and detectable only by persons in close proximity and quickly diminishes.

Nearly 500 volatile organic compounds have been isolated from decomposing human and other animal remains; however, studies evaluating odors from decomposing animal tissue and specific fly olfactory cues are limited (Vass et al. 2008). Specific volatile organic compounds emitted from animal cadaver compost have been studied for use as indicators of composting efficiency. Akdeniz et al. (2010) isolated 55 different compounds from swine carcass compost, and concluded that dimethyl disulfide, dimethyl trisulfide and pyrimidine could be measured throughout the composting process for use as indicators of carcass degradation. Two of the three compounds listed as indictors of carcass degradation are also known to be olfactory cues for blows flies. Odors of decomposition that have been specifically tested using electroantennogram (EAG) techniques and elicited a strong response in blow flies include dimethyl disulfide, dimethyl trisulfide, and dimethyl tetrasulfide (LeBlanc 2008). The highest concentrations of these semiochemicals are emitted throughout the active decay stages of decomposition (LeBlanc and Logan 2010). The active decay stage of animal decomposition is altered significantly by the composting process and likely results in decreased emission of fly olfactory cues. Results of experiments identifying compounds that indicate successful carcass degradation substantiate results of this study that showed a decrease in prevalence of two compounds that are also apneumones known to attract blow flies to carrion. Burial is still a popular method of livestock carcass disposal. This technique however does not inhibit insect activity as efficiently as properly constructed compost. The odors of

decomposition that attract flies are still emitted through the soil even after 230+ days at approximate depths of .5 to 1 m (Vass et al. 2004, 2008). This is likely related to the prolonged period of decomposition associated with buried remains. Research has shown that buried animal remains decompose at decreased rates compared to remains exposed to open air (Rodriguez and Bass 1985, Rodriguez 1997, Gaudry 2010). As well as extending the period of decomposition, burial of carcasses may not prevent fly colonization. As much as 0.3 meters of soil will not prevent blow flies from colonizing a carcass (Rodriguez and Bass 1985). The ability of some flies to develop on buried remains suggests that they may have the ability to navigate through the envelope of composting material to reach the cadaver inside. A properly constructed compost pile should have an envelope thick enough to prohibit fly activity. This factor as well as the dramatic increase in temperatures and suppression of odor will likely deter rather than promote filth fly activity. Further research examining various conditions of beef mortality compost and the volatile organic compounds emitted throughout the process coupled with the succession of insects attracted to the compost pile would be worthwhile. This research offers insight into the benefits of composting livestock mortalities by demonstrating the reduction in filth fly activity and the overall impact to food safety and public health.

CONCLUSIONS

Regular and catastrophic animal losses create additional challenges for animal waste management. The methods of disposal that have historically been popular and environmentally sound, such as rendering and incineration, are no longer cost efficient and alternative means such as composting are being explored. Composting is an economical, environmentally sustainable means of disposing of dead animal carcasses, and this study examined the associated insect activity as well as volatile organic compounds emitted from the compost pile. Composting livestock mortalities greatly reduces filth fly activity and inhibits propagation. Preliminary results from laboratory studies with volatile organic compounds released from exposed and composted animals indicate that the composting process may degrade or otherwise inhibit the release of important olfactory cues that typically attract flies to carrion. Compounds including dimethyl disulfide were found in samples taken at the beginning of the active decay period and not in other samples. This was significantly different from the large amounts of dimethyl disulfide and dimethyl trisulfide detected in the headspace of completely exposed animals throughout the majority of the decomposition period. The breakdown or suppression of chemical cues may contribute to the overall decrease in filth fly activity associated with composted beef carcasses. Reduction in filth fly activity within the grounds of livestock facilities by composting mortality waste could also reduce the risk of flies contaminating the surrounding area by spreading pathogenic microorganisms acquired from the dead animals.

TABLES

Table 5.1 – Annual Oklahoma cattle and calf death loss and carcass nutrient data (Taken from Payne and Pugh 2010).

	Cattle	*Calves*	*Total*
OK Inv. (# head)	2.1 million	3.3 million	5.4 million
Death loss (%)	2.1§	6.4§	4.8
Mortalities (#head)	44,100	212,850	256,950
Average Wt. (lbs)	1246‡	460Ω	–
Avg. Mortality (lbs)	54.9 million	97.9 million	152.8 million
Projected carcass C (lbs/head)	180	66.5	–
Projected carcass N (lbs/head)	36	13.3	–
Total Projected C (lbs)	7.9 million	14.1 million	22.1 million
Total Projected N (lbs)	1.5 million	2.8 million	4.4 million
Projected value of N†	$556,500	$990,817	$1.5 million

† Based on a conservative value of $0.35 per pound of N as Urea

* This does not include the added value of increased organic matter, Ca,P,K or other nutrients.

§ National Death Loss Survey, USDA: 1996-2005

‡ Livestock Marketing Information Center, LMIC. 1999-2008

Ω National Stocker Survey, BEEF. 2008

Table 5.2 – Volatile organic compounds present in samples taken from exposed and composted rat cadavers.

	Exposed Animals						Composted Animals					
	Fresh		*Active decay*		*Post decay*		*Fresh*		*Active decay*		*Post decay*	
Dimethyl sulfide	-	-	-	-	-	-	-	-	-	-	-	-
Dimethyl disulfide	-	✸	✸	✸	✸	-	-	✸	✸	-	-	-
Dimethyl trisulfide	-	-	✸	✸	✸	-	-	-	-	-	-	-

FIGURES

Figure 5.1 – Compost pile inside of 3 x 3 m fence equipped with malaise trap.

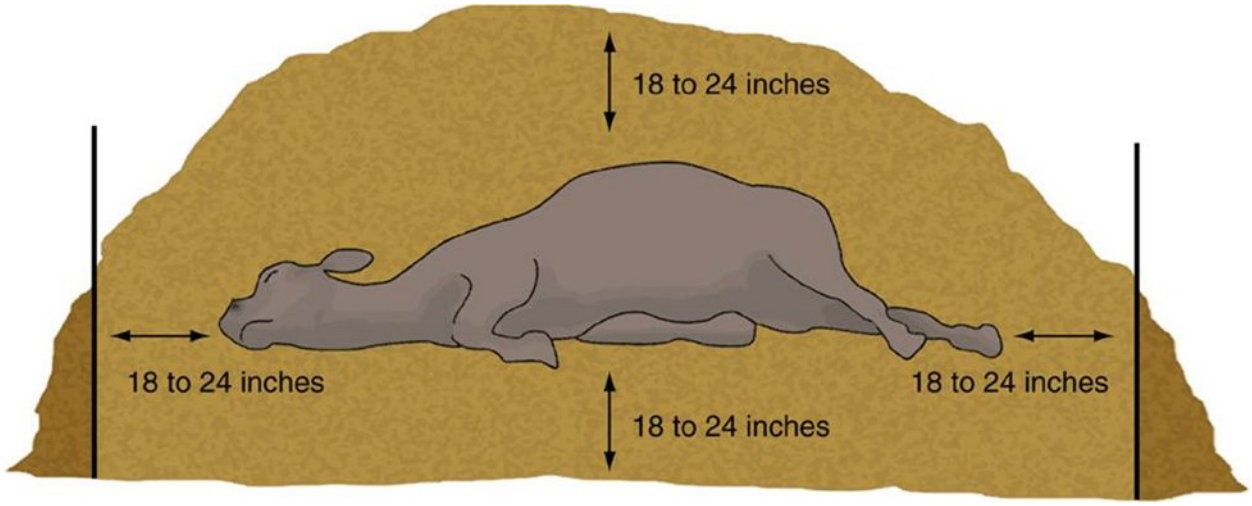


Figure 5.2 - Proper construction of a large animal carcass compost pile (Taken from Payne and Pugh 2010)

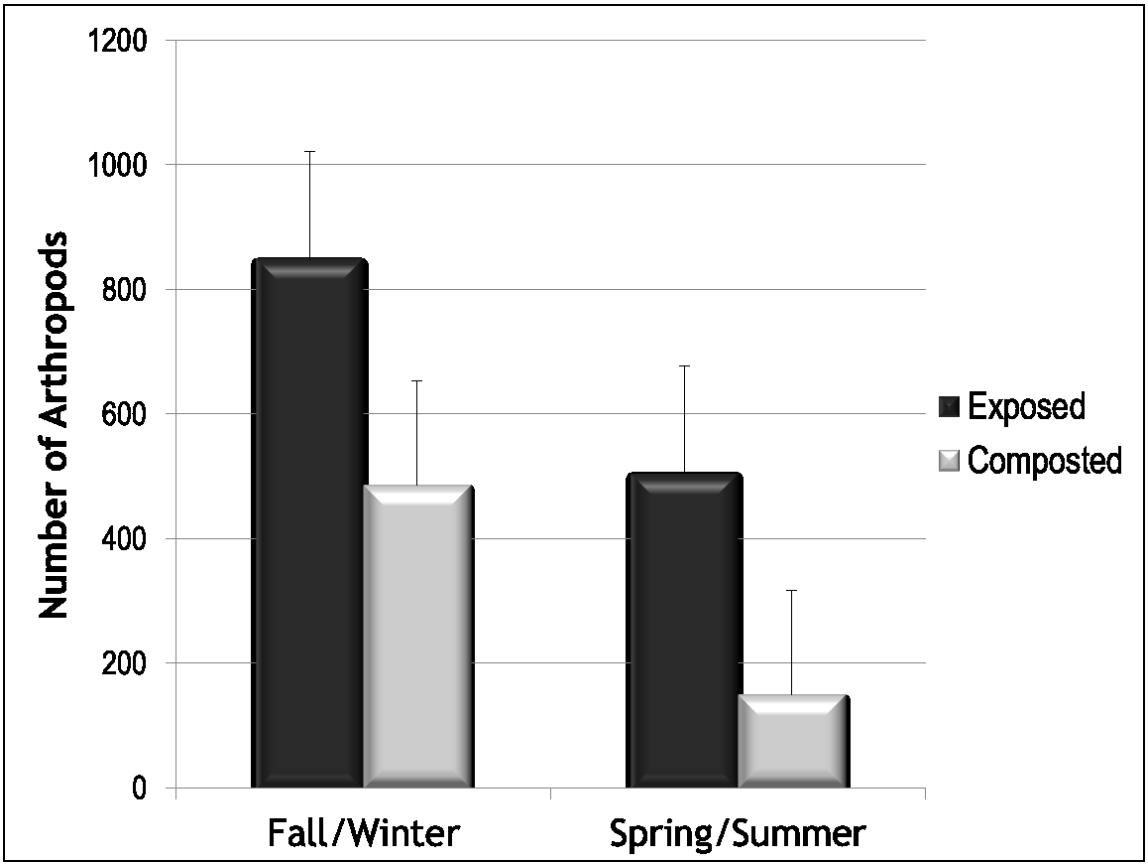


Figure 5.3 – Total abundance of arthropods trapped near composted and non-composted beef cadavers throughout the period of decompositions (n=4). The number of arthropods trapped at the exposed animal site was higher after both seasons than the composted site but not statistically different (p=0.02730)

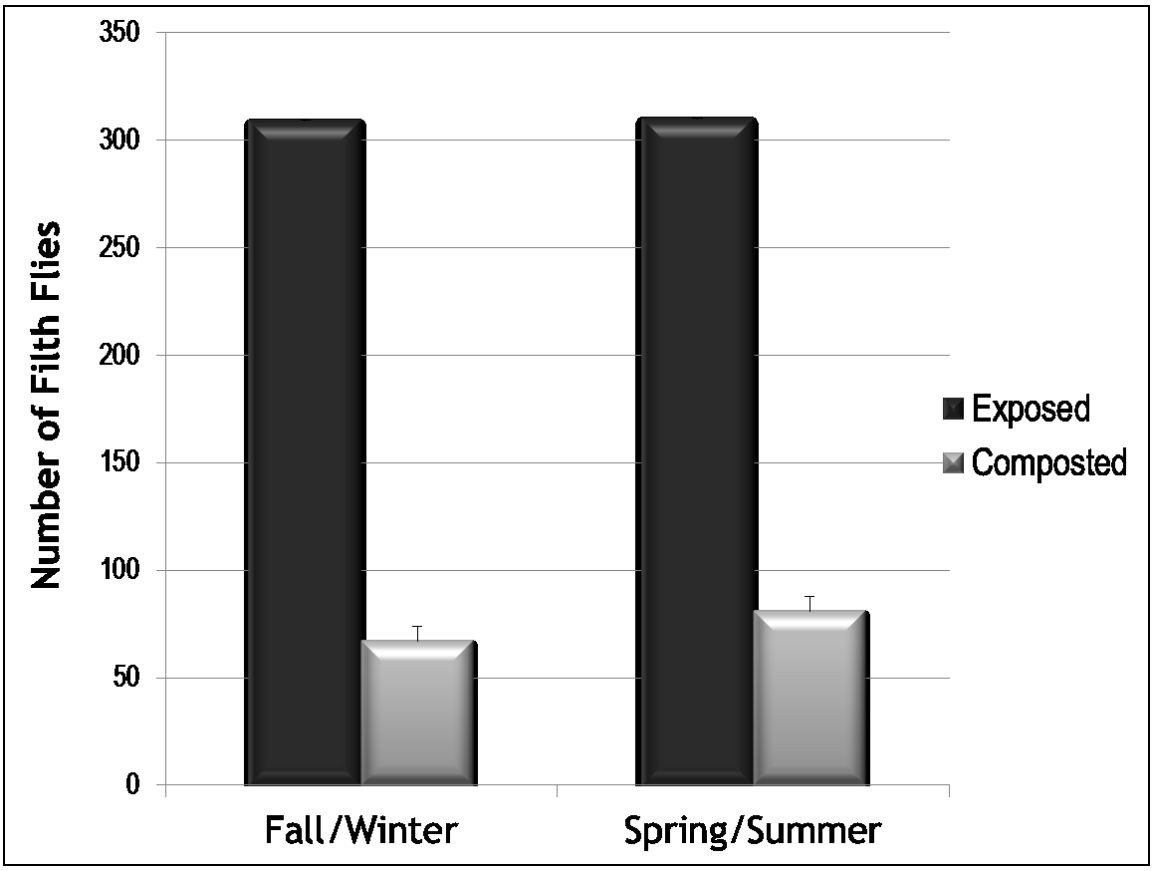


Figure 5.4 - Total abundance of filth flies typically associated with carrion trapped near composted and non-composted beef cadavers throughout the entire period of decomposition (n=4). The number of filth flies at the exposed animal site was significantly higher than the composted site (p=0.0009).

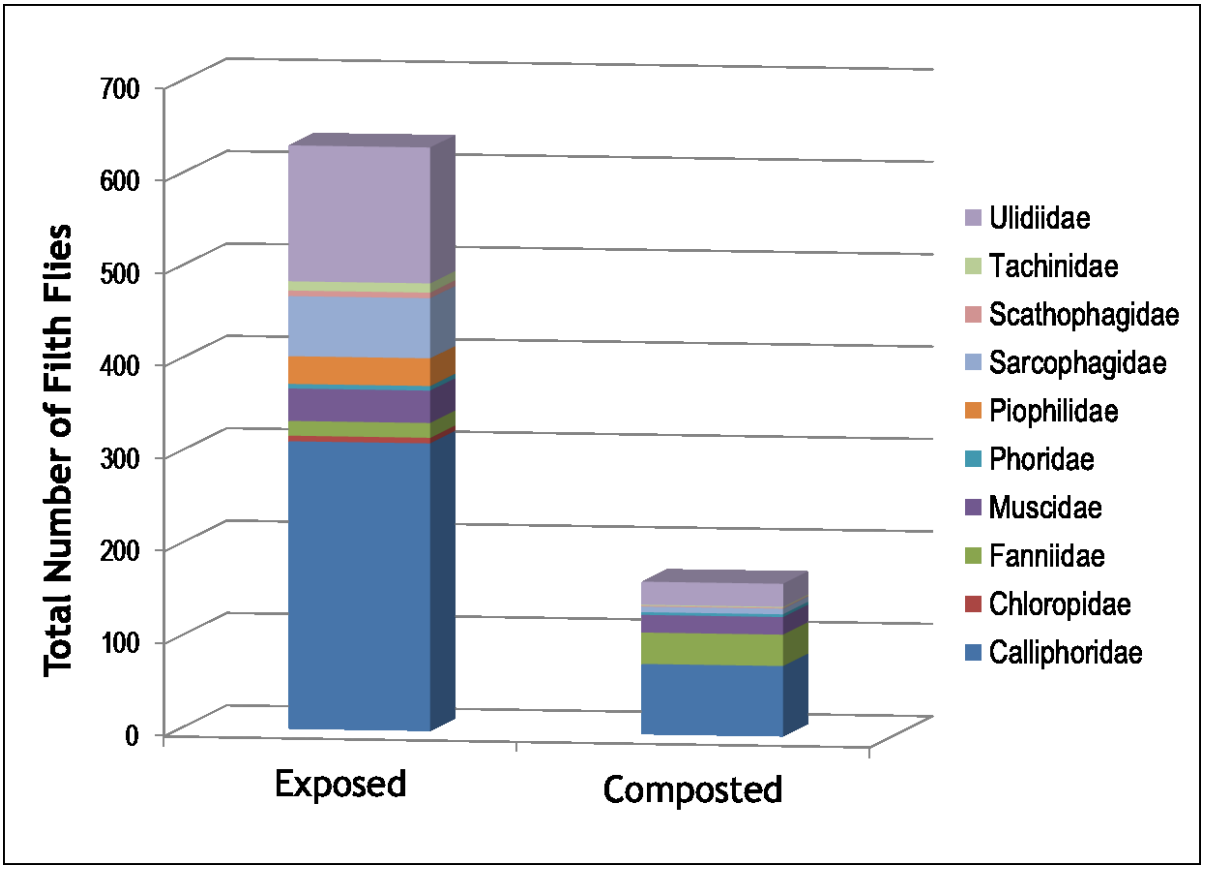


Figure 5.5 – Comparison of the proportions of filth fly families found at the composted and non-composted sites for both sampling periods (Fall/Winter and Spring/Summer)

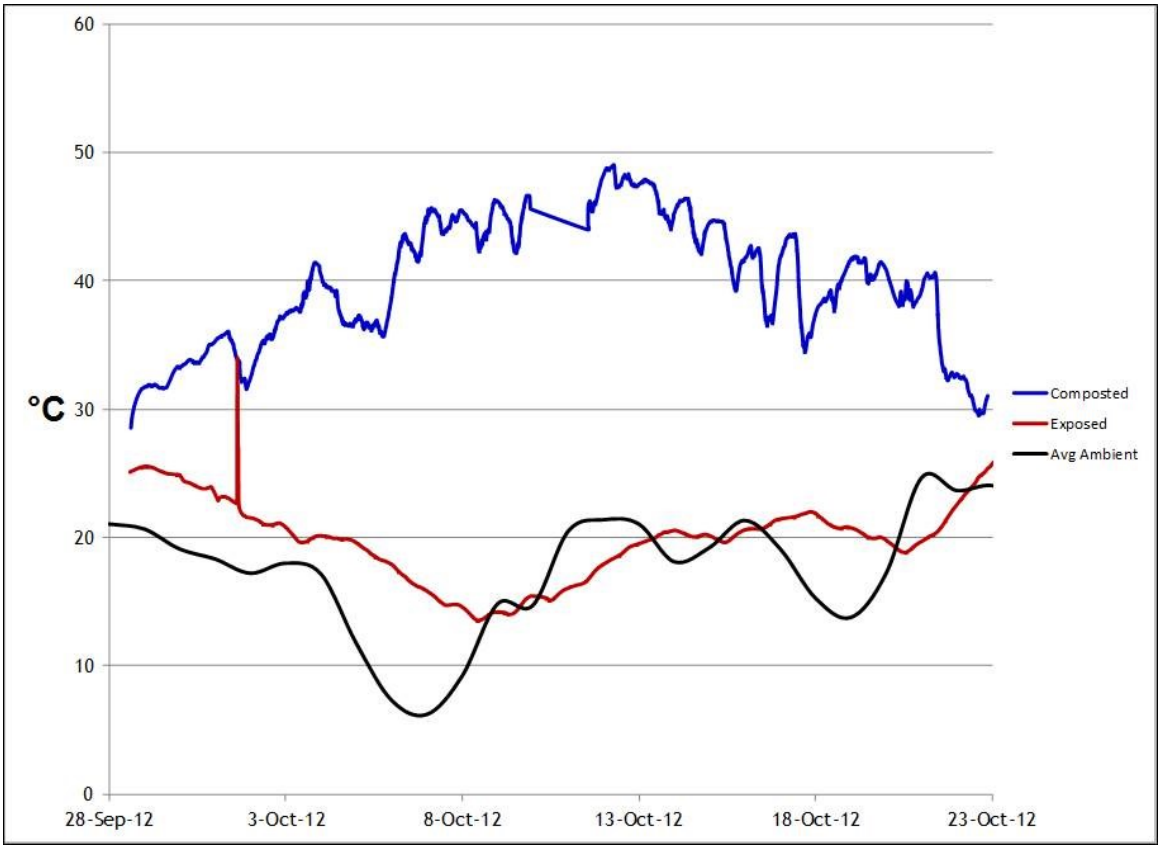


Figure 5.6 – Temperatures at both the composted and exposed animal sites throughout the period of decomposition during the fall/winter period. Note that the temperature within the compost pile was consistently higher than both the ambient temperature and the temperature of the exposed carcass.

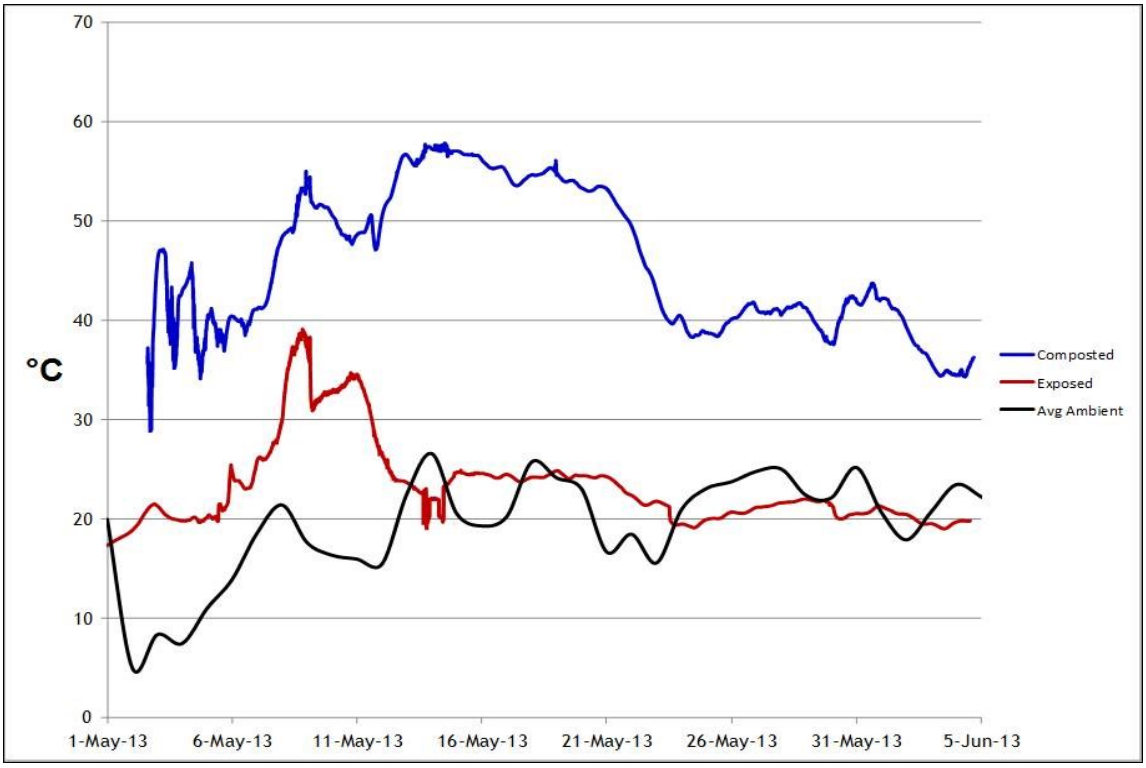


Figure 5.7 - Temperatures at both the composted and exposed animal sites throughout the period of decomposition during the fall/winter period. Note that the temperature within the compost pile was consistently higher than both the ambient temperature and the temperature of the exposed carcass.

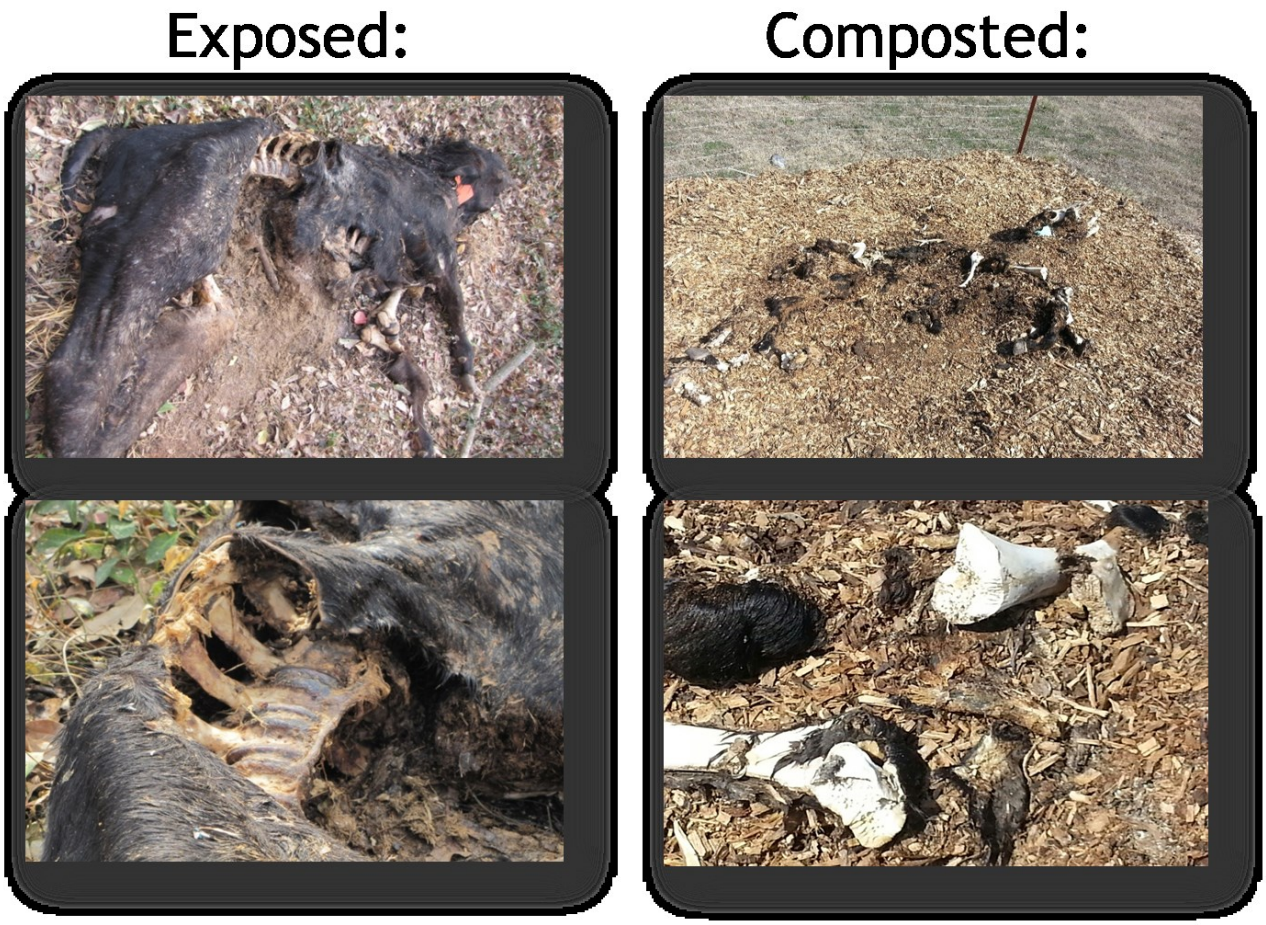


Figure 5.8 – Condition of each cadaver after 142 days of decomposition throughout the fall/winter seasons. The composted carcass was reduced to small amounts of hide and brittle bones. There was also no odor emitting from the composted carcass site. The exposed remains still included some soft tissue, gut contents, and hide along with significant odor.

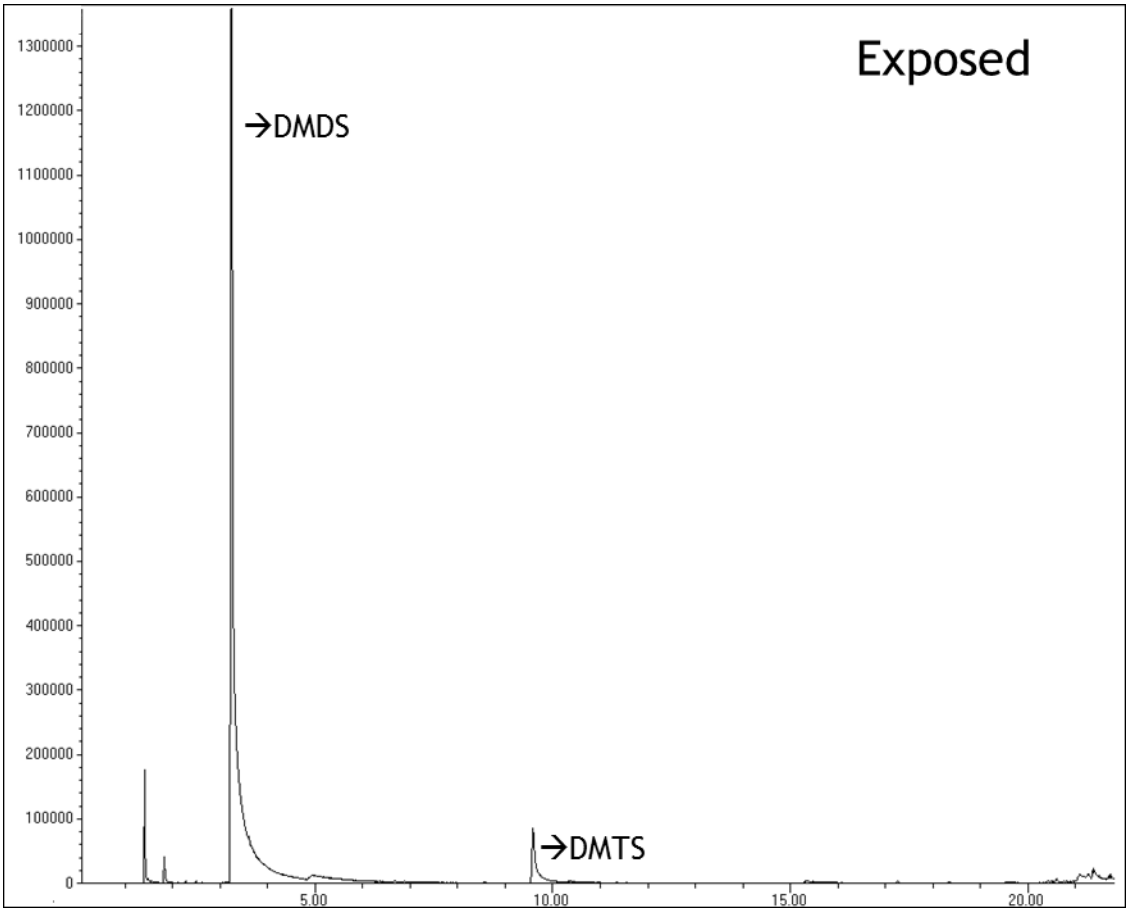


Figure 5.9 - Chromatogram of volatile organic compounds emitted from an exposed rat cadaver during the active decay period of decomposition. Dimethyl disulfide (DMDS) and dimethyl trisulfide (DMTS) were consistently present**.**

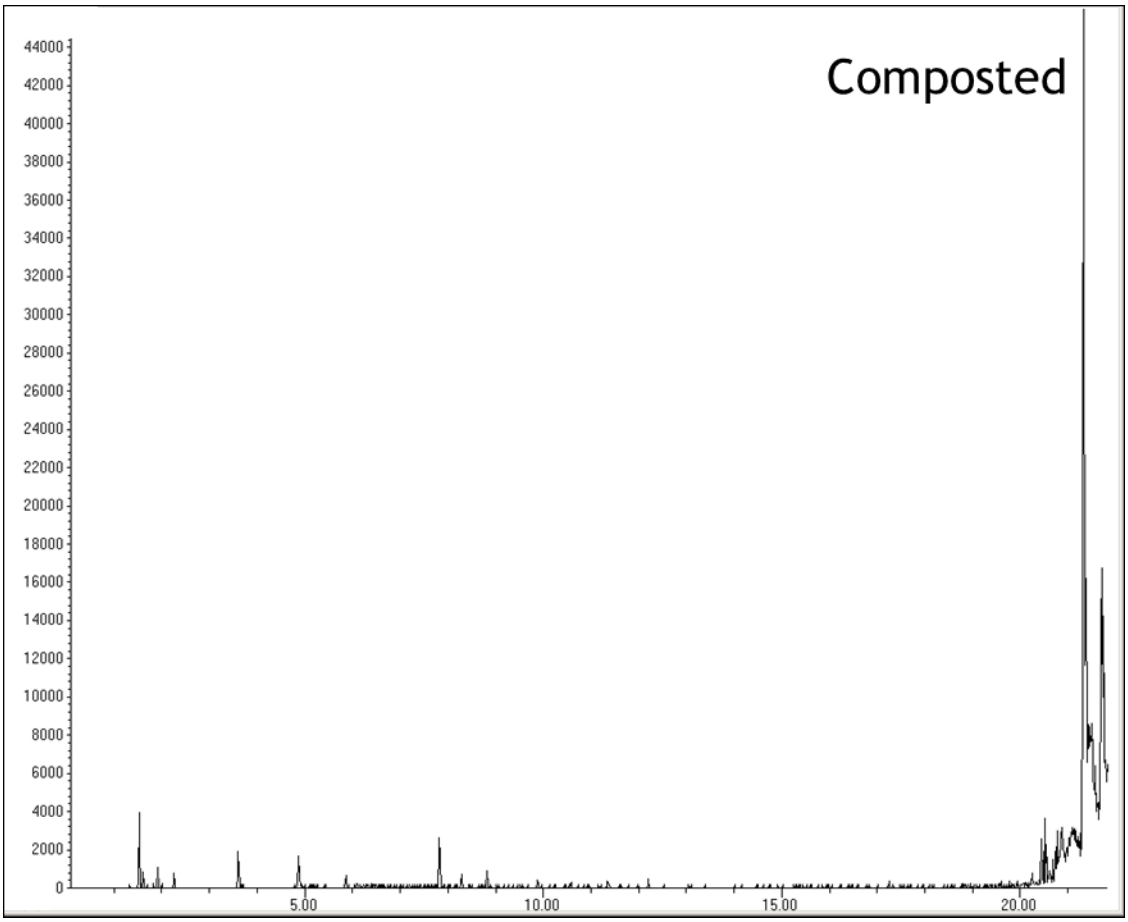


Figure 5.10 – Chromatogram of volatile organic compounds emitted from a composted rat cadaver during the active decay period of decomposition. No compounds known to attract filth flies were isolated from these samples with exception to DMDS early in the decomposition period. Note the difference in the abundance scale due to significantly lower amounts of odors emitted from the composted animals.

CHAPTER VI

ARTHROPODS ASSOCIATED WITH BOVINE CARRION IN CENTRAL OKLAHOMA

ABSTRACT

The purpose of this study was to qualitatively examine the major arthropod taxa visiting carrion in central Oklahoma. Adult and immature arthropods were collected from beef cadavers throughout the period of decomposition in the fall/winter and spring/summer seasons. The arthropods were identified to the lowest taxonomic level possible, and over 35 taxa of Diptera and Coleoptera commonly associated with carrion were confirmed. As expected, Calliphoridae were the most abundant Diptera present throughout both seasons followed by Sarcophagidae, Muscidae and Ulidiidae. The major species of blow flies in the fall/winter were *Phormia regina* and *Chrysomya rufifaces*. *Phormia regina* and *Cochliomyia macellaria* were the major species present in the spring/summer season. The majority of the Coleoptera collected were in the family Staphylinidae, *Creophilius maxillosus*, for both seasons. Beetles belonging to the families Trogidae and Dermestidae were also recovered.

INTRODUCTION

A decomposing animal left exposed in the environment becomes a veritable ecosystem in itself ensuing colonization and visitation by a wide variety of arthropod taxa. Some insect groups feed directly on dead tissue and others are attracted to the abundance of prey (Payne 1965). Since Megnin observed human decomposition and arthropod activity in 1894, various studies have documented from 27 to 522 different species associated with carrion (Goff 2010). The succession of arthropod groups at a decomposing carcass is generally predictable with slight variation (Payne 1968, Valdes-Perezgasga et al. 2010). The regional information obtained for carrion feeding species and patterns arthropod succession can be utilized in the field of forensic entomology (Catts and Goff 1992).

Arthropods can be valuable in legal investigations. There are three divisions of forensic entomology including urban/structural, stored product and the medicolegal or medicocriminal area that typically involves human remains. Insect groups visiting carrion can be useful in medicolegal forensic entomology in two main ways. The first involves primarily the blow fly groups and focuses on their development in relation to environmental factors such as temperature to determine a period of insect activity and postmortem interval associated with the dead body (Tabor et al. 2005). Flies are typically first to arrive at a dead body and can often locate the body and oviposit within minutes (Catts 1990). The timely arrival of flies along with the predictability of their life cycles allows forensic entomologists to calculate the amount of time the body

has been colonized by the flies that were recovered. Information regarding the development of specific species previously obtained in the laboratory as well as additional information obtained by rearing specimens collected at the body can be used by investigators (Tabor et al. 2004).

The second method involves establishing a timeline using the succession patterns of insect groups on the body. Throughout the period of decomposition, the body undergoes many changes and different insect groups are attracted to the condition of the body at certain stages. The insects groups that are present at certain stages are predictable, and information gathered from controlled studies can be used along with the insect evidence found at a crime scene to establish a postmortem interval (Goff 2010, Tabor 2010). Research focusing on the arthropod fauna associated with carrion in specific areas can help to improve predictions based on succession patterns.

Another area of forensic entomology that is often overlooked is livestock entomology. Many of the fly groups associated with carrion are also important livestock pests as well as mechanical vectors of zoonotic pathogens. These groups are referred to as filth flies because many can develop in various types of decaying organic matter together with some that feed on decomposing animal tissue. These include house flies and blow flies that can carry pathogens such as *Escherichia coli* 0157:H7, *Salmonella* spp., *Camplyobacter jejuni*, and strains that are resistant to antibiotics (Wright 1983, Graczyk et al. 2001, Zurek and Ghosh 2014). In addition to the large numbers of flies than can be generated and subsequently become nuisances for neighboring homes and

businesses, filth flies attracted to manure and dead animal waste from livestock facilities can contaminate the surrounding environment. Identifying the species associated with carrion in the area will also uncover the resident mechanical vectors.

The reliability of insect evidence is dependent on the availability of information on specific insect groups, locality and ambient conditions. Studying the arthropod taxa in a region will benefit various areas of forensic entomology. The arthropod taxa associated with decomposing animal remains in central Oklahoma have not yet been studied. This study is a qualitative exploration of the local arthropod taxa in association with bovine carcasses left exposed to the environment throughout the fall/winter and spring/summer seasons in Oklahoma.

METHODOLOGY

Samples were collected from exposed beef cadavers. The animal mortalities were obtained from beef research facilities at Oklahoma State University in Stillwater, OK. Each carcass was kept within a 3 m x 3 m fence to keep out scavenger animals that would disturb the experimental site. Field sites were located at the Willard Sparks Beef Research Center as well as the Medical Veterinary Entomology building in Stillwater, OK. Sampling took place throughout the entire period of decomposition. Field sites were set up in the fall/winter season as well as the spring/summer. Flying insects were captured using modified malaise traps suspended above each carcass and anchored to

the fence on one side. The jars were changed every 2-4 days, and the insects were kept frozen until they were identified. Pitfall traps placed next to the carcass were also used to capture ground dwelling insects (Figure 6.1).

Larvae were also sampled throughout the active decay period when masses were present. One set of samples was taken for preservation, and another set was taken to rear in the laboratory. Larvae were fixed using KAA solution and transferred to 70% ethanol after approximately one hour. Live larvae were reared on beef liver in a room kept between 21°C and 26°C with 70-80% relative humidity.

Insects collected in malaise traps and pitfall traps were identified to the lowest taxonomic level possible and priority was given to groups commonly associated with carrion. The live larvae were reared to the adult stage and the adults subsequently identified. Preserved larvae were reserved for later identification.

RESULTS

This study focused on groups commonly associated with carrion, and 14 families, 26 genera and 15 species have been identified (Table 6.1). The majority of Diptera captured at each site were Calliphoridae. Blow fly activity was noticeable immediately while setting up the field sites. By 23 hours after setting up the carcasses for the fall/winter period of observation, larval activity was observed around the exposed stomach that had was opened by the feedlot staff to examine and take samples. The primary species in the

fall/winter observation period were *Chrysomya rufifacies* and *Phormia regina*. The primary blow fly species captured throughout the spring/summer observation period were *P. regina* and *Cochliomyia macellaria* (Fabricius). Live larval samples taken throughout both seasons that were successfully reared to adults in the laboratory contained only *P. regina*, *Ch. rufifacies and C. macellaria*. Other species of blow flies captured were *Calliphora coloradensis* (Hough), *Calliphora livida* (Hall), and other *Calliphora* spp. The species *Cynomya cadaverina* (Robineau-Desvoidy) as well as several flies in the genus *Lucilia* including *L. sericata* were also identified.

A substantial amount of picture winged flies in the family Ulidiidae were captured throughout both seasons. The majority of the specimens belong to the genus *Euxesta* including *Euxesta notata* (Wiedemann)). Flies belonging to the families Muscidae and Fanniidae included *Musca domestica L.*, *Stomoxys calcitrans* (Linnaeus), *Haematobia irritans* (Linnaeus), *Fannia canicularis* L., *Fannia scalaris* F. and other *Fannia* spp. Flesh flies belonging to the family Sarcophagidae were represented by several genera. These include *Boettcheria, Oxysarcodexia, Helicobia*, and *Sarcophaga*. Flies in the family Piohilidae were also collected. The majority of the specimens are known as waltzing flies, *Prochyliza xanthosoma* (Walker) with a small number of unknown species in the genus *Prochyliza*. Additional flies were recovered that could not be identified to species that belong to the families Phoridae, Chloropidae, Scathophagidae, and Tachinidae.

The majority of the Coleoptera collected in pitfall traps were Staphylinidae, primarily *Creophilus maxillosus* (Gravenhorst). Additional specimens in the genera *Platydracus* and *Coproporus* were found. Clown beetles in the family Histeridae were also recovered. The majority of specimens belong to the genus *Hister*. One representative of the beetle family Cleridae was found, *Necrobia rufipes* (DeGeer), the red-legged ham beetles. In the post-decay stages, large proportions of beetles in the family Trogidae were collected. Both genera of this family were found, *Trox* and *Omorgus*.

DISCUSSION

Studies examining arthropod succession in relation to carrion vary widely and can recover a multitude of different taxa. Some studies sample frequently using multiple methods to recover every type of arthropod in the vicinity and others target specific groups. Payne (1965) identified 522 species using baby pigs and included necrophagous, omnivorous, predaceous, parasitic and incidental groups. The animal model used can vary from birds and mice to pigs and humans, and the fauna associated remains generally consistent (Goff 2010). Experiments incorporating different animal taxa have shown that the insect fauna visiting larger mammal carcasses is relatively consistent although can be substantially different from other animals such as reptiles (Watson and Carlton 2005). There is a need for continued research in forensic entomology that integrates basic and applied studies focused on decomposition ecology (Tomberlin et al. 2011). The ecosystems in which bodies are discarded vary

greatly and research on the arthropods associated with carrion should make every effort to accommodate this diversity covering a range of habitats, seasons and other environmental conditions. The insect fauna inhabiting animal cadavers have not yet been studied in Oklahoma. This study focused primarily on the carrion feeding species, major predators and important filth fly groups commonly associated with carrion.

Beef carcasses that were obtained from the feedlot had to be opened and examined following facility protocols. Exposing the gut contents no doubt facilitated blow fly attraction. The abundance of these materials is also likely what attracted the different Fanniidae and Muscidae as they are usually more abundant when gut contents and excrement are present (Gennard 2012). Dung flies in the family Scathophagidae are usually present under these circumstances although very few were collected in this study. Blow flies were observed on the animal exposed in the fall before the site could be completely set up, and the following morning (approximately 23 hours) a larval mass was found in the gut region. Adults captured in the first sample included *P. regina*, *C. macellaria* and *Ch. rufifacies* (Fig. 6.2 and 6.3).

The primary species of blow fly captured as an adult in malaise traps and collected and reared to adult from the larval stage was *Ch. rufifacies*. This species is originally an Australasian and Oriental fly but was introduced to the United States in 1981 (Byrd and Castner 2010). Since then, it has quickly spread throughout most of the south and can be readily found developing on carrion. This species is also predaceous on other blow fly larvae (Baumgartner

1993). Also noted in a review of *Ch. rufifacies* was the benefit of late instar larvae as they are known to prey on other species that transmit pathogens and cause myiasis in the United States (Baumgartner 1993). This voracious appetite for carrion and other larvae may explain why the first sample of larvae reared in the laboratory contained only this species and not *P. regina* and *C. macellaria* although adults were captured on the same day. In their native regions, such as Thailand, this species can cause myiasis (Sukontason et al. 2005). These behaviors can complicate the use of *Ch. rufifacies* as a forensic indicator due to the possibility of perimortem colonization which would alter the calculation of the minimum postmortem interval (Sanford 2014). Larval colonization of a body before death by species such as *Ch. rufifacies* that can cause facultative myiasis will make the post mortem interval appear longer because the larvae were already developing before the person was actually deceased. Research on species that arrive early to a cadaver and can inhabit wounds before death will help forensic entomologists to more precisely differentiate between perimortem and postmortem colonization.

The black blow fly, *P. regina*, is widely distributed throughout the Holarctic region. It is typically known as a colder weather species and not commonly found in the southern U.S. throughout the summer months (Byrd and Caster 2010). These flies were found in both the fall/winter and spring/summer samples in this study although the warmer season replicates concluded in June, and temperatures in central Oklahoma at the time were just beginning to increase (average approximately 23°C). Multiple samples of live larvae reared

to adults contained this species as well as some that had developed along with *Ch. rufifacies and C. macellaria*.

The secondary screwworm fly, *C. macellaria*, was historically widespread throughout the Americas but is now found less often in some areas of the U.S. due to the spread of competitive *Chrysomya* species (Byrd and Castner 2010). These flies were found throughout both fall/winter and spring/summer sampling periods. They are considered an important species in forensic entomology that arrive early to carrion, and they have been included with other important species in very recent research associated with the timing of oviposition as well as behaviors such as diel patterns in order to better understand early colonization of carcasses and what may take place pre-colonization (Boatright and Tomberlin 2010, Mohr and Tomberlin 2014). They can also cause facultative myiasis and may be present on a person perimortem in wounds and remain on the body postmortem. Currently, very little research has been devoted to the time immediately preceding colonization of a body although some researchers have recently been focusing on factors influencing the pre-colonization interval with blow flies such as *C. macellaria* (Tomberlin et al. 2011).

Additional blow fly species found in smaller amounts include *C. coloradensis, C. livida* and other flies in the genus *Calliphora*. Several species in this genus are considered cold tolerant, and not commonly found in the southern states throughout the warmer summer months (Byrd and Castner 2010). This is consistent with the specimens recovered in this study as they

were only found during the fall/winter observation period. A particularly interesting fly that was observed in this study is the waltzing fly, *P. xanthosoma*. These flies are in the family Piophilidae and are usually associated with the post-decay or late stages of decomposition (Byrd and Castner 2010). In this study, these flies were found throughout the early, active decay and post decay stages. This group of flies as well as some other species of Piophilidae feed on carrion as larvae in the post decay stages but can be observed as adults early in decomposition because of their elaborate behaviors associated with courtship and male competition (Byrd and Castner 2010). The male *Prochyliza* spp. will encounter a carrion source, choose an area on the body or nearby, and then defend this area from other males as well as bout with another male if a female is encountered (Bonduriansky 2003). They also have interesting courtship behaviors. When a male encounters a female, he will proceed to "dance" in a zig-zag pattern for the female to get her attention and subsequently copulate with her (Bounduriansky 2003). They are also sexually dimorphic. The males have a uniquely shaped head that is elongated and antennae that may be involved in sexual selection as well as male-male combat (Bonduriansky and Rowe 2003) (Fig. 6.4).

Large numbers of flies belonging to the families Muscidae and Fanniidae were also captured in malaise traps. The most important of these flies is *Musca domestica* as it is a well-known mechanical vector of several zoonotic pathogens associated with livestock (Sasaki et al. 2000, Nazni et al. 2005). Other flies belonging to the genus *Fannia* such as *F. canicularis* L., the little

house fly, have been implicated in the carriage of harmful human pathogens as well (Forster et al. 2007). Most of the research on this fly group has been done outside of the U.S. in other areas where some *Fannia* spp. are more problematic. House flies (*M. domestica)* remain the major species of concern in this region although the large numbers found in this study may suggest that there may be a need for further research involving *F. canicularis* and other *Fannia* species.

CONCLUSIONS

Overall, research that has focused on arthropods associated with dead animals provides useful information for many areas of forensic entomology as well as expands knowledge of the biology and ecology of these groups. This qualitative study revealed a tremendous amount of information on the native insects visiting carrion that had not been previously studied in central Oklahoma. These findings included important filth flies whose interactions with livestock waste and ability to transmit important pathogens is currently in need of further investigation. Gathering regional information on taxa that can inhabit carrion is essential to increasing the proficiency of medicolegal forensic entomology. Continued research on the native carrion feeding species as well as other filth flies attracted to the area will benefit both medical/veterinary and forensic entomology.

TABLES

Table 6.1 – Arthropod taxa recovered from bovine carcasses throughout the fall/winter and spring/summer seasons in Oklahoma.

			Season	
Order	**Family**	**Genus species**	**fall/winter**	**spring/summer**
Diptera	Calliphoridae	*Phormia regina*	*	*
		Cohliomyia macellaria	*	*
		Chrysomya rufifaces	*	*
		Lucilia sericata	*	
		Lucilia spp.	*	*
		Callphora coloradensis	*	
		Calliphora livida	*	
		Calliphora spp.	*	
		Cynomya cadaverina	*	
	Sarcophagidae	*Boettcheria* spp.	*	
		Oxysarcodexia spp.	*	*
		Ravinia spp.	*	*
		Sarcophaga spp.		*
		Helicobia spp.	*	
		Unknown	*	*
	Muscidae	*Musca domestica*	*	*
		Unknown	*	*
	Fanniidae	*Fannia canicularis*	*	
		Fannia scalaris	*	
		Fannia spp.	*	*
		Unknown	*	*
	Ulidiidae	*Euxesta notata*	*	*
		Euxesta spp.	*	*
		Unknown	*	*
	Chloropidae	*Hippelates* spp.	*	
	Piophilidae	*Prochyliza xanthosoma*	*	*
		Prochyliza spp.	*	*
		Unknown	*	*
	Phoridae	Unknown	*	*
Coleoptera	Staphylinidae	*Creophilius maxillosus*	*	*
		Platydrcus spp.	*	*
		Coproporus spp.		
		Unknown	*	*
	Silphidae	*Necrophilia americana*	*	*
		Necrodes surinamensis	*	
		Unknown	*	
	Cleridae	*Necrobia rufipes*	*	*
	Histeridae	*Hister* spp.	*	*
		Unknown		
	Dermestidae	*Dermestes* spp.	*	*
	Trogidae	*Trox* spp.	*	*
		Omorgus spp.	*	

FIGURES

Figure 6.1 - Traps placed at each field site A) Modified malaise trap built to be suspended over the cadaver B) Pitfall traps placed on the ground near the animals

Figure 6.2 - Lateral view of *Chrysomya rufifacies*, easily recognized by the dark coloration of the posterior margins of abdominal tergites 3 and 4 as well as the pale genal dilation and setae surrounding the anterior spiracle. Picture courtesy of bugguide.net ©Alan Chin-Lee

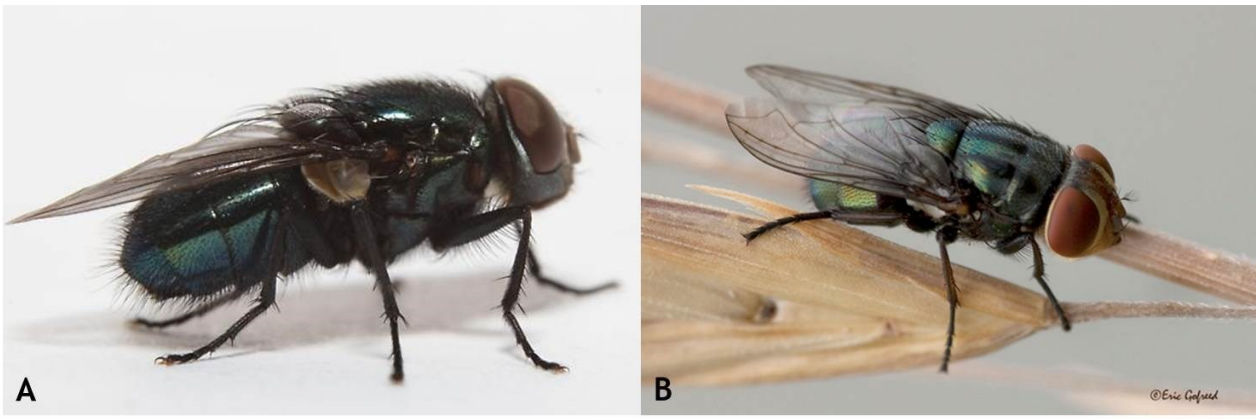


Figure 6.3 – A) *Phormia regina* ©Ben Coulter B) *Cochliomyia macellaria* ©Eric Godfreed. Both pictures courtesy of bugguide.net

Figure 6.4 – Waltzing fly, *Prochyliza xanthosoma* male. Picture courtesy of bugguide.net ©Steve Pelikan.

CHAPTER VII

SUMMARY

Livestock production is faced with the constant challenge of controlling filth fly populations. Despite the information already gained through investigations focused on filth fly pests, problems with pesticide resistance as well as a substantial encroachment of the urban landscape continue to elevate the need for research and development of novel pest control strategies. The primary goals of this research were to examine a variety of factors surrounding filth fly activity associated with livestock operations. These included the utilization of corridors and edges within the landscape by house flies as well as the abundance and types of filth flies attracted to composted cattle carcasses. Additional experiments evaluated the effects of composting on fly olfactory cues by examining volatile organic compounds released from composted and exposed animal cadavers. Lastly, all of the carrion feeding insects and other insects associated with the animal remains used for this research were identified to the lowest taxonomic level possible and recorded. Our overall understanding of filth fly activity and the risk of mechanical pathogen

transmission is dependent on continued research in these areas. The information obtained through these studies on filth flies will help to influence future research as well as waste and pest management programs for livestock production.

A diverse group of filth fly species have been implicated in the mechanical transmission of microorganisms, many of which are zoonotic pathogens important in food safety. Doctors and other scientists associated flies with disease centuries before the identification of the various modes of transmission that are examined by modern researchers. Filth flies that have been shown to contain pathogens include many blow fly species, dump flies, stable flies and most importantly house flies, *Musca domestica*. Most of these flies have a tremendous impact on public health and with the proliferation of fly breeding sites within animal production facilities it is important to understand the risk these flies might pose both for the animal facility as well as surrounding environments in close proximity. House flies, in particular have repeatedly been shown to effectively transmit pathogens and are the most prevalent mechanical vector species found in livestock production systems. These flies are readily found in both rural and urban environments worldwide. These flies are also very important in the spread of antibiotic resistant bacteria throughout the landscape. Research specifically targeting house flies that harbor antibiotic resistant strains of enterococci highlighted their ability to transfer these harmful microorganisms between rural and urban environments (Chakrabarti et al. 2010). Zurek and Ghosh (2014) recently highlighted the role

that house flies have in the spread of antibiotic resistant bacteria from rural areas where livestock operations are commonly located to urban areas where they can contaminate homes and businesses such as food production facilities and restaurants. The evidence for continued research on filth flies as mechanical vectors of zoonotic pathogens, including those that are drug resistant, is tremendous and remains a vital component of both pest control and food safety programs.

The dispersal of house flies from animal farms remains an important component of understanding the ecology of house flies and overall risk as mechanical vectors. Knowledge of how house flies interact with the surrounding landscape are not well understood. Limited research has shown that house flies can exhibit edge-following behavior when they encounter rows of vegetation such as lines of trees or shrubs. This was shown in only one other study conducted in a forest setting along with corridor use by house flies (Freid et al. 2005). Results of this research examining the use of artificial corridors substantiate these conclusions. House flies were shown to interact with corridors constructed of 3 meter camouflage walls significantly more readily than 1.5m meter walls and were captured consistently more within the corridor than at the control sites with no artificial corridor. Smaller scale laboratory studies have also shown that house flies are alerted to boundaries with high contrast such as light and dark, and they are more likely to remain within the boundary if the area contains a resource such as sucrose (Conlon and Bell 1991). Collectively, research investigating the distances that house flies will

disperse from livestock production facilities has not determined a predictable distance for the majority of the emigrating population. However, they have all shown that their dispersal capabilities are substantial. This information allows us to assume that homes, businesses, fresh produce farms could be at risk of contamination at even moderate distances from livestock operations. Continued research will lend insight into what distance can be considered safe for food production such as leafy greens and other fresh goods. Results of these experiments show that manipulation of the landscape within the ground of feedlots could influence house fly populations and perhaps their dispersal from the facilities. Although the information available for house fly dispersal and foraging behavior is somewhat limited, the information on corridor use, edge following behavior and response to high contrast boundaries all present abundant opportunity for additional field studies. Future research should certainly explore alteration of vegetation at livestock facilities as well as the availability of resources such as nectar or sucrose. Although livestock waste is an ideal source for house fly oviposition and larval development, the adults must forage for sugary foods as well as protein before they mate and lay eggs (Adams et al. 1990). Presenting these resources within the grounds may prohibit house fly dispersal from the facilities to the surrounding area. This could be done in conjunction with traps, pesticides or another type of control method to enhance the efficiency of integrated pest management for house flies overall. Currently, the relationship between filth flies and insects producing sugary excreta known as honeydew is being explored after

preliminary field examination of filth flies in leafy greens fields resulted in the recovery of several filth fly species, some of which tested positive for *E. coli* 0157:H7 (Talley et al 2009). This may present additional opportunities for field experiments utilizing phytophagous insects that produce honeydew and potentially attract house flies. Providing sugar containing resources to adult flies within boundaries created by tree and/or shrub lines could inhibit house fly dispersal from livestock facilities. Future research should utilize the data obtained from these projects as well as those previously mentioned to further address the use of vegetation in feedlot pest management.

Field experiments that monitor insect movement require efficient marking techniques. This research has shown that immunomarking techniques using chicken egg albumin could be used for monitoring house fly movement in feedlot systems. House flies retained large amounts of the 1:1 fluorescent with egg white marking powder after seven days in the field, and 100% of flies positive for fluorescent marker yielded positive optical density values with ELISA. The chicken ovalbumin assay has been reported to be detectable at levels as low as 1.9 ppb (Jones et al. 2006). Marked house flies in this study consistently yielded optical density values above values resulting from known dilutions of antigen and buffer at concentrations of 10 ppm. These results suggest that the protein marking technique could be used to study long distance dispersal patterns for house flies emigrating from feedlots. The sensitivity of the assay could prove to be useful in the detection of markers on house flies that have dispersed to great distances from the original facility.

Fluorescent markers can be cost efficient, but may not be detectable on the flies after extended periods of flight in the field even with the aid of a microscope and ultraviolet lamp. Protein markers extracted from house flies in this study that were left in laboratory observation boxes with food and water yielded positive optical density values after periods of two to three weeks. Any system should be properly evaluated before implementing an immunomarking technique as the proteins available for markers may be encountered in the environment rendering the technique inadequate. The native population should be trapped and assayed before marked insects are released. Several wild insects must be captured prior to the study to use as negative controls as well. These negative controls will be used on each ELISA plate to determine a positive threshold value for marked individuals, and if the protein is encountered by the native population this threshold will not be low enough to distinguish the marked insects from the unmarked insects. This research has shown that the anti-chicken ovalbumin assay can be an efficient technique for use in house fly studies conducted in beef feedlot systems.

In addition to investigations of house fly dispersal, it is important to consider all of the filth flies coming in to livestock production facilities as well as the possible sources of attraction to the animals. Waste management plays a significant role in the odors attracting flies as well as the availability of adequate resources such as oviposition and larval developments sites. Regular as well as catastrophic animal losses create additional challenges for feedlot waste management. The methods of disposal that have historically been

popular and environmentally sound are no longer cost efficient, and alternative means such as composting are being explored. Composting is an economical environmentally sustainable means of disposing of dead animal carcasses, and this study examined the associated insect activity as well as volatile organic compounds emitted from the compost pile. The composting treatment in this study clearly reduced filth fly activity and prevented larval development. Significantly fewer flying insects were trapped above composted beef carcasses concealed within a compost pile using a mixture of wood chips, sawdust and leaf litter than those trapped above completely exposed cadavers. This disposal method not only conceals the animal inhibiting the typical visual cues used by filth flies, increased temperatures and accelerated decomposition also change the animal tissue in such a manner that it is no longer suitable for fly development.

The small number of flies attracted to the area prompted experiments examining the olfactory cues that attract carrion feeding flies to dead animals. This research showed the composting process does inhibit the release of specific olfactory cues known to attract filth flies after the beginning of the active decay period of decomposition. Compounds including dimethyl disulfide were found in samples taken at the beginning of the active decay period and not in other samples. This was significantly different from the large amounts of dimethyl disulfide and dimethyl trisulfide detected in the headspace of completely exposed animals throughout the majority of the decomposition period. Some research has shown that specific compounds could be used as

indicators of the composting process (Akdeniz et al 2010). These compounds are the same as those detected in this study and known to elicit a response in blow flies including dimethyl disulfide and dimethyl trisulfide. Although there have been close to 500 volatile organic compounds isolated from decomposing animal remains, limited research has explored the specific apneumones that attract filth flies (Vass 2012). Continued research exploring electroantennogram analysis of decomposition odors could be informative and contribute to filth fly suppression methods in the future.

House flies are the most abundant filth fly species typically found in feedlots although several species captured in the area of livestock operations have been shown to carry pathogenic microorganisms. These include blow flies, *Calliphora grahami, C. vomitoria* and *Lucilia* spp., little house flies, *Fannia canicularis*, flesh flies, *Sarcophaga carnaria*, *S. peregrima*, and *S. cruenta*, flies in the Muscidae family such as dump flies, *Hydrotaea aenescens*, stable flies, *Stomoxys calcitrans*, and false stable flies, *Muscina stabluans* (Brazil et al. 2007, Forster et al. 2007, Baldacchino et al. 2013, Liu et al. 2013, Zurek and Ghosh 2014). Most of this work has been done in Europe and Asia therefore some of these groups are not as common or not found in the United States. Research examining filth flies in addition to house flies found in the U.S. that carry zoonotic pathogens would be informative. All of these species can be attracted to dead animal tissue. Cataloguing the species of filth flies in an area will identify important mechanical vectors as well as the carrion feeding fly species useful in forensic entomology. An opportunity to identify the insect

species visiting beef carrion in this study was exploited. Filth fly samples were not tested for pathogenic microorganisms; however, several species captured throughout these studies have been previously shown to carry pathogens. In addition to house flies these include multiple species of blow flies in the genus *Lucilia,* additional blow flies in the genus *Calliphora*, and filth flies formerly in the Muscidae family but now belonging to Fanniidae such as *Fannia canicularis*. Many other insect species commonly associated with animal remains captured throughout these studies that are potentially useful in forensic entomology include beetles belonging to the families Staphylinidae, Cleridae and Silphidae. Continued research involving these species as well as others can be useful in calculating the period of insect activity on a corpse to establish a post-mortem interval as well as a timeline using the succession of insect groups visiting the corpse (Midgely et al. 2010, Tomberlin et al. 2011).

The results of these experiments illustrate that information on the ecology and behavior of house flies and other filth flies will benefit the development of more efficient pest management programs in livestock systems. Manipulation of the landscape could prove to be a useful tool in integrated house fly management programs and continued research is needed to substantiate the data obtained from these experiments. Continued research focusing on dispersal may be more successful with the aid of immunomarking techniques. Additionally, waste management procedures are an essential component of filth fly suppression, and this research has shown that composting animal mortalities can in fact reduce the attraction and

proliferation of filth flies. Comprehensively, this research inspires confidence that more sustainable filth fly management techniques are possible through continued work on filth fly behavior and ecology. Successful suppression of filth flies through more efficient techniques employed in livestock systems will result in increased food safety and overall public health.

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VITA

Trisha Rose Dubie

Candidate for the Degree of

Doctor of Philosophy

Thesis: INFLUENCE OF VEGETATION HEIGHT ON THE DISPERSAL OF HOUSE FLIES (MUSCA DOMESTICA) FROM LIVESTOCK FACILTIES AND FILTH FLY ACTIVITY ASSOCIATED WITH COMPOSTED AND NON-COMPOSTED BEEF CADAVERS

Major Field: Entomology

Biographical:

Education:

Completed the requirements for the Doctor of Philosophy in Entomology at Oklahoma State University, Stillwater, Oklahoma in July, 2014.

Completed the requirements for the Master of Science in Entomology and Plant Pathology at Oklahoma State University, Stillwater, Oklahoma in 2010.

Completed the requirements for the Bachelor of Science in Forensic Chemistry at Lake Superior State University, Sault Ste. Marie, Michigan in 2006.

Completed the requirements for the Bachelor of Science in Criminal Justice - Criminalistics at Lake Superior State University, Sault Ste. Marie, Michigan in 2006.

Experience:

Teaching assistant, Lake Superior State University, Fall 2005 Biology II
Teaching assistant, Oklahoma State University
Courses: ENTO 2003 Insects and Society: 2007,2008,2009,2010,2011
ENTO 4483/5483 Aquatic Entomology: 2009
ENTO 3003 Livestock Entomology: 2007,2012,2013,2014
ENTO 4400/5020 Introduction to Forensic Entomology: 2013
Research assistant:2007,2008,2009,2010,2011,2012,2013,2014

Professional Memberships:
Entomological Society of America, Society of Southwestern Entomologists, Oklahoma Academy of Sciences