INSECT TIMING AND SUCCESSION ON BURIED CARRION IN EAST LANSING, MICHIGAN

By

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ABSTRACT

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This study examined pig carcasses buried at two different depths, 30 and 60 cm, to determine if insects are able to colonize buried carcasses, when they arrive at each depth, and what fauna are present over seven sampling dates to establish an insect succession database on buried carrion in East Lansing, Michigan. Thirty-eight pigs were buried, 18 at 30 cm and 20 at 60 cm. Four control carcasses were placed on the soil surface. Three replicates at each depth were exhumed after 3 days, 7 days, 14 days, 21 days, 30 days, and 60 days. One pig was also exhumed from 60 cm after 90 days and another after 120 days. *Sarcophaga bullata* (Diptera: Sarcophagidae) and *Hydrotaea sp.* (Diptera: Muscidae) were found colonizing buried carrion 5 days after burial at 30 cm. Insect succession at 30 cm proceeded with flesh and muscid flies being the first to colonize, followed by blow flies. Insects were able to colonize carcasses at 60 cm and *Hydrotaea sp.* and *Megaselia scalaris* (Diptera: Phoridae), were collected 7 days after burial. Insect succession at 60 cm did not proceed similarly as predicted, instead muscid and coffin flies were the only larvae collected. Overall these results reveal post-burial interval (PBI) estimates for forensic investigations in mid-Michigan during the summer, depending on climatic and soil conditions. The importance of these findings with respect to estimating a PBI for forensic investigations in a mid-Michigan location during the summer are discussed.

DEDICATION

To my sister, for always being by my side and encouraging my love of insects.

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Insect Timing and Succession on Buried Carrion in East Lansing, Michigan

Abstract

The most common application of forensic entomology involves estimating a post-mortem interval (PMI) to aid investigators in determining time of death. A number of factors can influence PMI, altering insect colonization and decomposition. Burial is a popular physical barrier chosen by assailants who are looking to dispose of a body. Rarely are bodies buried very deep since digging requires a great amount of time and effort. Therefore, assailants usually dig shallow graves to dispose of their victims with depths ranging between 0 and 90 cm. This study examined pig carcasses buried at two different depths, 30 and 60 cm, to determine if insects are able to colonize carcasses buried at 60 cm, when they arrive at each depth, and what fauna are present at each depth over seven sampling dates to establish an insect succession database on buried carrion in East Lansing, Michigan. Temperature data collected from data loggers buried with carcasses were compared to ambient air temperature and it was determined that there was no significant difference between temperatures when calculating accumulated degree days. *Sarcophaga bullata* (Diptera: Sarcophagidae) and *Hydrotaea sp.* (Diptera: Muscidae) were found colonizing buried carrion 5 days after burial at 30 cm. At 60 cm, *Hydrotaea sp.* and *Megaselia scalaris* (Diptera: Phoridae), were collected 7 days after burial. Multivariate ordination demonstrated significant separation of insect community structure between depths and between sampling dates. Indicator species analyses identified *S. bullata* as the significant indicator of remains found at 30 cm and *M. scalaris* as the significant indicator of remains found at 60 cm. Overall these results revealed post-burial interval estimates for forensic investigations in mid-Michigan during the summer, depending on climatic and soil conditions. The importance of

these findings with respect to estimating a PBI for forensic investigations in a mid-Michigan location during the summer are discussed.

Introduction

Forensic entomology is the application of knowledge of insects and other arthropods in civil and criminal investigations (Byrd & Castner, 2010). Although forensic entomology is not a new science, its first use being recorded in China during the 13th century (Sung, 1981), it was not until the 1960s that the discipline began to be accepted in the United States when predictable development rates and successional patterns of carrion insects were recognized (Byrd & Castner, 2010). These predictable development rates, driven largely by temperature, aided entomologists in establishing a postmortem interval (PMI) by examining the oldest insects present (VanLaerhoven & Anderson, 1999). Because insects arrive within minutes after death and begin laying eggs, the time between insect colonization and corpse discovery is often very close to time of death (VanLaerhoven & Anderson, 1999). In order to establish a PMI, one needs to identify the insect species present, the stage of development reached by those insects, the rate at which those species develop, and temperatures at the crime scene (Merritt & Benbow, 2008). Because insects are exothermic, they develop faster at higher temperatures and slower at lower temperatures. At lower and upper thresholds, specific to a species, no insect development takes place and may even cause death (Byrd & Castner, 2010).

Once an insect's species and stage of development has been determined, Kamal's (1958) development tables based on constant rearing temperatures in the laboratory, allow for determination of the time and temperature required for the insect to reach the observed stage of development (Byrd & Casnter, 2010). By comparing the thermal units required for development (accumulated degree days or degree hours) to the thermal units at the time of collection working

backwards, forensic entomologists are able to establish a PMI. Because season, location, and species play a role in the rate of development, forensic entomologists use different base temperatures to adjust to the different minimum threshold values. Three different base temperatures are commonly used: B0, which assumes no lower threshold; B6, which is used in colder climates and for cool weather species (early spring and late fall in temperate regions); and B10, which is used in warmer climates and for warm weather species (late spring, summer, and early fall in temperate regions) (Merritt & Benbow, 2008). Based on these criteria, a base temperature is chosen and subtracted from the daily average temperature in $^{\circ}$C to calculate degree days. Accumulated degree days are then found by adding the degree days over a period of time.

Successional patterns of carrion insects also aid in establishing a PMI. The presence or absence of an insect species can prove helpful because insect communities typically move from a more complex organization to a simpler community and are attracted to different stages of decomposition (VanLaerhoven & Anderson, 1999). Megnin (1894) was the first to describe the different stages of human decomposition and to break them into eight stages. Over the years, the eight stages have been combined into five stages by Payne and King, (1968) and to as few as four by Rodriguez and Bass (1983). The five stages described by Payne and King (1968) using pigs (*Sus scrofa* L.) as human models, are referred to as fresh, bloat, active decay, post decay, and dry. Pigs are used as a human model because they decompose very similarly and tend not to stimulate public protest (Catts & Goff, 1992). Like humans, they have very little hair, their skin and physiology are similar, and they have comparable muscle to fat ratios. Today, forensic entomologists routinely use the five stage model (VanLaerhoven & Anderson, 1999). Payne and

King (1968) were the first to associate specific insects with these different stages of decomposition.

A number of factors can influence PMI estimates, such as physical, chemical, climatic, animal scavengers, and narcotics (Merritt & Benbow, 2008). Physical barriers can be intentional or unintentional but the end result often alters insect colonization and decomposition. Burial is a popular physical barrier chosen by assailants who choose to dispose of a body. A number of studies have found that buried bodies decompose at a much slower rate than those exposed to the air (Payne, 1965; Payne & King 1968; Lundt, 1964). Rarely are bodies buried very deep because digging requires time and effort. Also, the longer the assailants are in contact with the body, the more likely they are to be apprehended with it in their possession, or leave behind evidence connecting them to the crime. Therefore, assailants usually dig shallow graves for their victims. In a study conducted by Manhein (1997) looking at preservation of buried bodies, 79% of cases were found at depths of 30 to 90 cm.

Major Insects of Forensic Importance

Blow flies belong to the Calliphoridae family, in the order Diptera, and have a worldwide distribution of over 1,000 species (Shewell, 1987). Adults of this group are recognized by their metallic green, blue, or black coloration. They range from 4 to 16 mm in length and are characterized by three-segmented antennae between their compound eyes, with the last segment possessing a plumose hair (Shewell, 1987). Females have been recorded locating a carcass and laying eggs within minutes of death (Byrd & Castner, 2010). Larvae of blow flies are white or cream colored, cylindrical in shape, have a tapered anterior end, and a condensed posterior end. Their posterior spiracles are surrounded by six or more cone-shaped tubercles with their

spiracular slits slanting towards the center of the larvae and surrounded by a dark peritreme (Shewell, 1987).

Flesh flies belong to the Sarcophagidae family, in the order Diptera, and have a worldwide distribution of over 2,000 species, with approximately 327 species found in the United States and Canada (Shewell, 1987). Adults of this group are recognized by their non-metallic gray color accompanied by three to five black dorsal stripes on their thorax (Alrich, 1916). They range in length from 2 to 18 mm and are generally covered in bristles (Shewell, 1987). Species are identified by the highly noticeable genitalia located on the tip of the abdomen, but only experts can decipher the slight differences. Females do not lay eggs, but rather lay live larvae on decomposing tissues and tend to arrive concurrently or slightly after blow flies (Byrd & Castner, 2010). Too little is known about the larvae, so identifying them past family level is nearly impossible. Larvae of flesh flies are similar in appearance to larvae of blow flies. Their posterior spiracles are their distinguishing characteristic because they are located in a distinct depression surrounded by 12 tubercles with their spiracular slits being close to vertical in orientation (James, 1947).

Muscid flies belong to the family Muscidae, in the order Diptera, and have a worldwide distribution with more than 700 species in North America (Huckett & Vockeroth, 1987). Adults of this group are relatively diverse but tend to be dark and dull in color and range in length from 2 to 14 mm (Huckett & Vockeroth, 1987). Species are identified by varying taxonomic features of the wings and head (Huckett & Vockeroth, 1987). Females tend to arrive and lay eggs after the blow flies and flesh flies. Larvae of muscid flies are sub-cylindrical in shape, tapering

towards the head and condensed at the posterior end. They are white, yellow, or cream colored and are generally smooth (Byrd & Castner, 2010).

Coffin flies belong to the family Phoridae, in the order Diptera, and have a worldwide distribution of more than 2,500 species (Peterson, 1987). Half of these species belong to the genus *Megaselia*, and 226 species are found in the United States (Byrd & Castner, 2010). Adults of this group are black, brown, or yellow, range in length from 0.5 to 5.5 mm, and are known by their humpbacked appearance (Peterson, 1987). Larvae of coffin flies are similar in shape to those of muscid flies, are white in color, and are very small, making them difficult to identify. Rearing larvae to adults is the best way to get a positive identification.

Rove beetles belong to the family Staphylinidae, in in the order Coleoptera, and have a worldwide distribution with over 47,700 species found in North America (Byrd & Castner, 2010). Adults of this group are long and slender with tiny wing coverings. They vary in color from black to brown, with some species possessing patches of yellow or orange spots on their thorax (Byrd & Castner, 2010). Larvae of rove beetles are also long and slender but tend to be pale in color with a darker head (Byrd & Castner, 2010). Both adults and larvae commonly found with carrion are predators of maggots rather than the carrion itself.

Insects and Buried Remains

Insects are known to colonize buried remains. Blow flies, the dominant family on exposed carrion, are thought to be completely incapable of penetrating below a depth of 2.5 cm (Smith, 1986). Lundt (1964) found larvae of the genera *Muscina* and *Ophyra* (=*Hydrotaea*) (Muscidae) at depths of 2.5 to 10 cm, and larvae of *Conicera* and *Metopina* (Phoridae) at 25 to 50 cm (Byrd & Castner, 2010). Merritt et al. (2007) found *Conicera tibialis* (Schmitz)

(Phoridae) pupae in an unsealed casket, placed inside an unsealed cement vault, buried at 1.8 m for 28 years. When studying pigs buried at 30 cm, Payne and King (1968) found that of the 48 arthropod species present, 26 species were not associated with above-ground carrion. It is important to note that the pig carcasses were buried in coffins, which does not characterize an assailant's typical disposal technique. While Rodriguez and Bass (1985) were studying decomposition rates of buried human cadavers, they found insects, mainly blow fly and flesh fly larvae, on their 30 cm cadavers. They witnessed female flies laying their eggs on top of the soil after a heavy rain. By laying the eggs near soil cracks, the larvae could hatch and migrate down to the carcasses, explaining the presence of maggots (Rodriguez & Bass, 1985). The bodies buried at 60 and 120 cm showed no insect activity; thus depth plays a major role in species presence and abundance.

VanLaerhoven and Anderson (1999) established an insect succession database of pig carrion buried at 30 cm in British Columbia, Canada. Although flesh flies, blow flies, and coffin flies (Phoridae) were observed on the buried carcasses, when comparing insect colonization of the surface control carcasses to that of the buried, it was found that muscid larvae (Muscidae) were most common on the buried carcasses. Rove beetles (Staphylinidae) were common at all five stages of decomposition. Not only was insect colonization different on the buried carcasses, but the species that colonized both the control and buried carcasses did so at different time points. The time intervals examined were: 2 weeks; 6 weeks; 3 months; 11 months; and 16 months.

Calculating a PMI has been extremely useful for forensic entomologists in attempting to establish time of death based on insect colonization on surface carrion. Similar to a PMI, a post-

burial interval (PBI) estimate, first introduced by VanLaerhoven and Anderson (1999), applies temperature development data of the species found on buried carrion and comparing them to temperatures from where they were collected. With knowledge of predictable insect succession patterns on buried carrion, it could be possible to establish a PBI. Although VanLaerhoven and Anderson (1999) recorded a predictable insect succession database at 30 cm in British Columbia however, there are different species present, differences in insect behavior and developmental rates, and other factors that need to be taken into consideration before a PBI estimation can be established in other locations (Byrd & Castner, 2010). This PBI estimate operates under the assumption that after insects reach the carcass, they colonize, develop, and feed in a normal fashion, similar to an exposed carcass (VanLaerhoven & Anderson, 1999). No research, to my knowledge, has been conducted in attempting to establish or examine more closely PBI estimations in other locations.

Goal, Objective, and Hypotheses

The goal of this research was to establish an insect succession database on buried carrion in East Lansing, MI. The objective for this study was to observe insect succession and arrival time on buried pig carcasses at two different depths, 30 and 60 cm. It was hypothesized that:

1) Insects will be able to colonize carrion buried at 60 cm.
2) It will take insects less than two weeks to colonize a carcass buried at 30 cm. Because previous studies have found insects colonizing carcasses buried at 30 cm after two weeks, and did not sample prior to two weeks, it was hypothesized that insects would be able to colonize earlier.

3) It will take insects longer to colonize a carcass buried at 60 cm compared to 30 cm. Because of the increased depth, it will take insects longer to travel through the soil barrier and colonize a carcass buried at 60 cm.
4) Insect succession at both depths will proceed with blow and flesh flies being the first to colonize, followed by muscid flies.

Very little is known about when exactly insects arrive, or how far they are able to travel through the soil to colonize a carcass, because most experiments only found insects at 30 cm (Rodriguez & Bass, 1985; VanLaerhoven & Anderson, 1999). Two preliminary trials were conducted where two pigs were buried at 90 cm, one starting on September 15, 2010 and ending November 2, and another starting on May 9, 2011 and ending July 1. These preliminary trials were conducted to determine if 90 cm would be a depth that would yield insect activity. No insects were found during either trial at the end of the experiment. Therefore, the current study was designed to examine pig carcasses buried at 30 and 60 cm. A depth of 30 cm was chosen because other studies focused on this depth, and 60 cm was chosen because Manhein (1997) found 79% of buried bodies are found at depths between 30 and 90 cm. By increasing the frequency of sampling, insect arrival time at both of these depths could be determined. While examining these two depths at a number of time intervals, differences in fauna will be recorded to develop a distinct successional history of colonizing insects in a mid-Michigan climate.

Materials and Methods

Study Site

The experimental area was located at the Entomology Research Farm of Michigan State University (MSU). This field lies directly east of a man-made pond, southeast of a horse pasture,

and is surrounded by other crop research plots (Fig. 1). Grass, wildflowers, and common weeds, cover the field. Soil type, determined by the MSU Soil and Plant Nutrient Laboratory, is classified as sandy clay loam on the east side and loam on the west side (Table 1), with the majority of soil content composed of sand, silt and clay, in that order. Measurements of the area approximated 50 m x 150 m. This size allowed for each carcass to be roughly 12 m apart in all directions in order to limit overlapping olfactory cues. Thirty-eight holes, (90 x 60 cm), were dug the day before burial using a backhoe. The hole's bottom measured the proper depth of 30 or 60 cm (Fig. 2).

Experimental Animals

Forty-two pigs, weighing approximately 14 to 27 kg, were provided by the MSU Swine Teaching and Research Center (STRC). Use of pigs was approved by the MSU Institutional Animal Care and Use Committee. All pigs, except one, were euthanized the morning of burial via lethal injection of FATAL-PLUS™ (5 mL/25 kg, Vortech Pharmaceuticals) directly into the heart by a campus veterinarian. One pig died of natural causes. It was stored in a freezer until July 6, 2011, when it was allowed to thaw in an empty room without fly access at the STRC. This pig was used as a control carcass atop the soil to prevent inconsistent results.

Carcasses were placed into two heavy duty trash bags to prevent colonization and transported from the STRC to the study site by enclosed pickup truck. Pigs were placed on top of, and covered by, a 60 x 90 cm piece of chicken wire, with 25 mm mesh hole size, for easy removal and to prevent animal scavenging (Fig. 3). Four control carcasses were placed on the soil surface under chicken wire exclosure boxes to prevent animal scavenging. Pigs were set on their right sides, heads pointing north, and covered within 20 min after death to prevent any

colonization before burial. Six Hobo© temperature loggers were buried with the carcasses, three remaining underground at 30 cm for 2 mo, two at 60 cm for 2 mo, and one at 60 cm for 4 mo (Fig. 4). They were placed directly across from the pig's midsection and were programmed to take a temperature reading every 10 min and average the temperature every hour. Once all pigs were buried, the soil filling the graves was raked smooth.

The depths and times at which the carcasses were exhumed were determined at random. Three replicates at each depth were exhumed after 3 d (Jul. 9), 7 d (Jul. 13), 14 d (Jul. 19), 21 d (Jul. 27), 30 d (Aug. 5), and 60 d (Sept. 4). One pig was also buried at 60 cm for 90 d (Oct. 6) and another at 60 cm for 120 d (Nov. 3), resulting in 38 buried pigs, 20 at 60 cm and 18 at 30 cm (Fig. 3). On the first exhumation day, a carcass was removed from each depth resulting in no insect activity. It was decided to exhume the four remaining pigs, two at each depth, after 5 d.

Insect Sampling and Processing

Soil above the buried carcasses was scanned for insects before digging began. While digging via shovel, soil was continually hand-sorted for insects. Only soil disrupted in the 60 x 90 cm volume of the grave was removed and sorted through for insects. Soil was removed until the entire carcass was exposed, allowing it to be lifted directly from the hole, aided by the chicken wire. Once the carcass was removed from the hole, insect collection started at the head and proceeded towards the rear for a time of 15 min. Insects were collected from the surface of excavated pigs and from the soil surface exposed at the bottom of the hole. Soil from the bottom of the holes was not sorted through, but was examined visually for any insect life stages. Larval specimens were collected via forceps and preserved in 70% ethanol.

Live fly larvae were also collected and reared to adulthood to aid in identification. Rearing containers were constructed according to Byrd and Tomberlin (2010). The live fly larvae were also collected for a time of 15 min, placed directly on beef liver, and transported on ice to the laboratory. Larvae were reared in a 25°C growth chamber with 65% humidity and constant light. Once all larvae had matured, they were stored in a freezer until identification. Adult, and larval flies if possible, were identified using Marshall et al. (2011), Marshall and Richards (1987), Huckett and Vockeroth (1987), Peterson (1987), Smith (1987), or sent to specialists. Adult and larval beetles were identified using Byrd and Castner (2010), or sent to a specialist. Only insects of forensic importance were included in the database and therefore those insects normally associated with soil were not included.

Insects also were collected from only the surface of control carcasses at each sampling date, stating at the head and proceeding towards the rear. Adult insects were collected by sweep net for approximately 10 min. Adult specimens were placed in jars with a plaster bottom saturated with ethyl acetate, frozen, and then pinned. Larvae were collected following the same methods as above but, no live larvae were collected from the controls.

Statistical Analysis

A student's t-test was used to compare soil temperatures at each depth and surface temperatures using a t-test to determine which temperature should be used in calculating a post-mortem interval. Surface temperatures were collected by MesoWest MSU Horticulture station, East Lansing, MI and located approximately 3 km from the experimental plot.

A Non-Metric Multi-Dimensional Scaling (NMDS) ordination was used to evaluate insect community structure differences between the two depths and time intervals (McCune

2002) using PC ORD (version 5; MJM software, Gleneden Beach, Oregon, USA). A total of 250 interactions for both real data and Monte Carlo analysis were run with a random seed start. A multiple response permutation procedure (MRPP) using Sørensen distances was performed to test for significant differences in community structure in response to depth and time. When significant differences were found in insect community structure, Indicator Species Analysis (ISA) was used to determine which insect taxa were significant indicators of the respective communities. Taxa were considered significant indicators when indicator values (% of perfect indication) were >55% with $p < 0.001$. Higher indicator values demonstrate better predictive power of that taxon for its assigned group as defined by the results of the MRPP analysis. Also when significant differences were found in insect community structure in response to depth and time, a permutation based MANOVA was used to determine if there was a significant interaction between depth and time. All insect taxa represented > 3% of all samples were used in the ordination procedures.

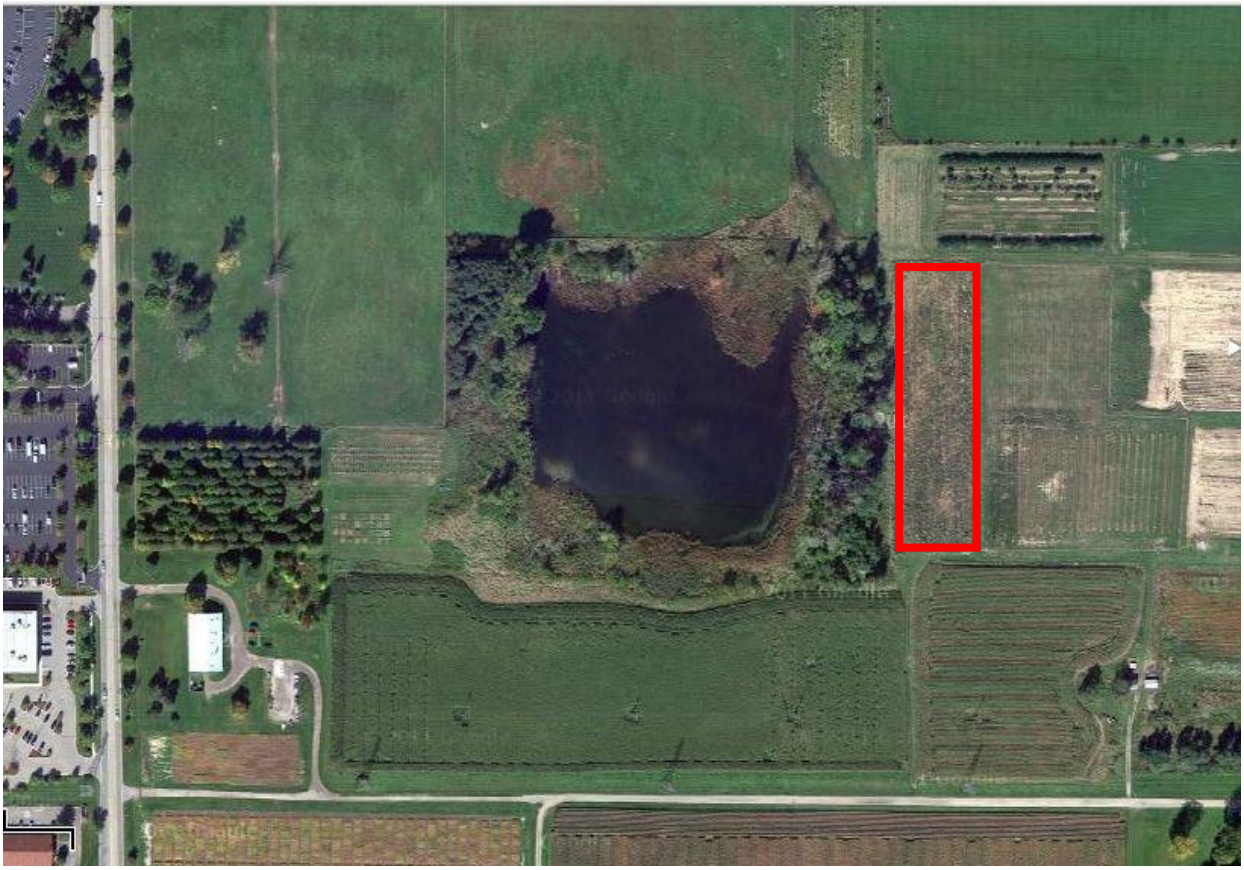


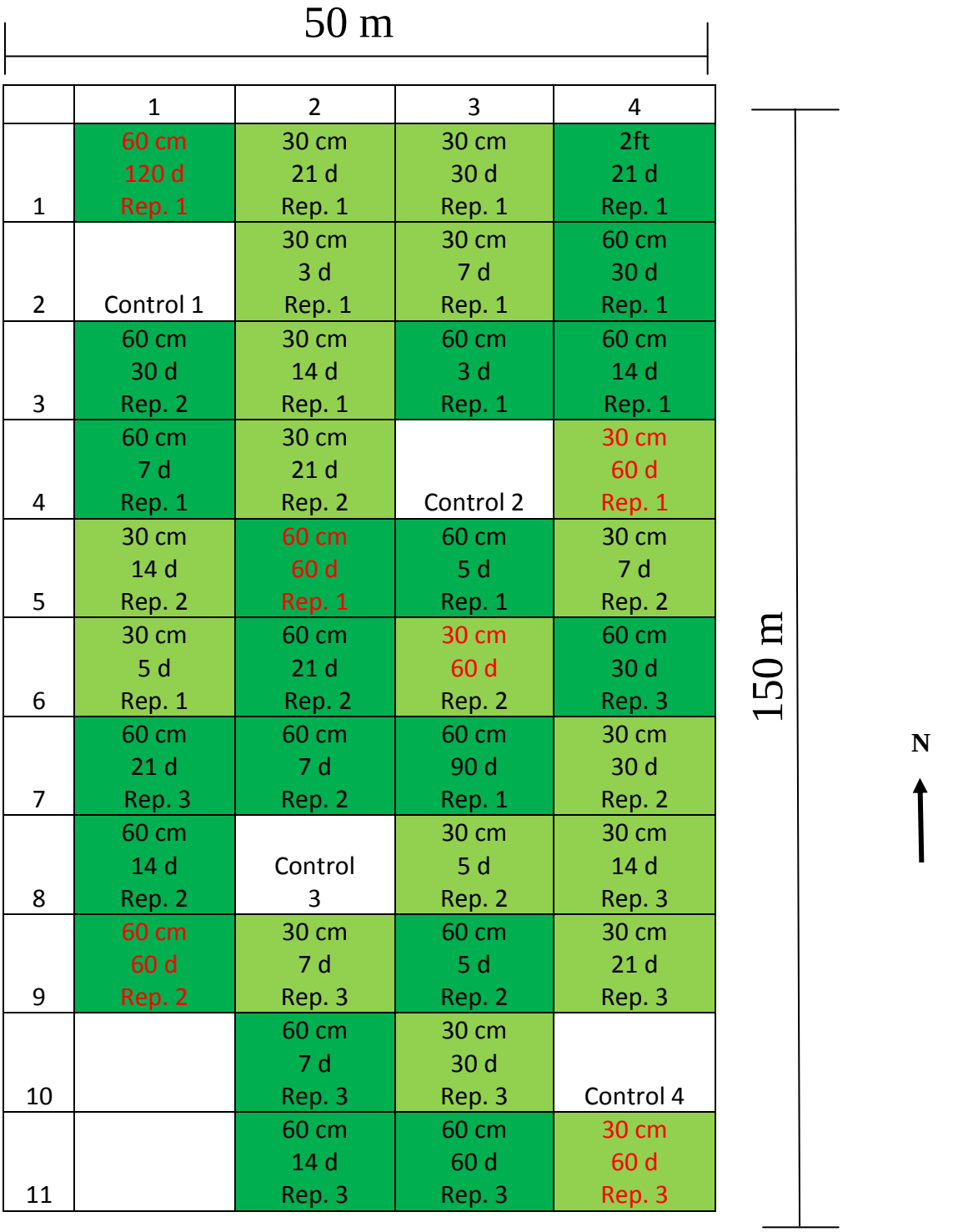
Figure 1**.** Test site (red rectangle) of pig burial at the Entomology Research Farm at MSU (Source: Google Map, 2012). For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this thesis.

Table 1. Soil test results as determined by the MSU Soil and Plant Nutrient Laboratory.

Sample ID	% Sand	% Silt	% Clay	Soil Type	% Organic Matter
Northeast corner	52.6	26.7	20.7	Sandy Clay Loam	2.5
Northwest corner	45.3	32.0	22.7	Loam	2.8
Center	57.3	18.0	24.7	Sandy Clay Loam	4.6
Southeast corner	53.3	24.0	22.7	Sandy Clay Loam	2.8
Southwest corner	51.3	28.0	20.7	Loam	3.0

Figure 2. Hole dug by backhoe with proper length, width, and depth measured.

Figure 3. Pig placed on, and covered by, a 60 x 90 cm piece of chicken wire with 25 mm mesh hole size before burial.



	1	2	3	4
1	60 cm 120 d Rep. 1	30 cm 21 d Rep. 1	30 cm 30 d Rep. 1	2ft 21 d Rep. 1
2	Control 1	30 cm 3 d Rep. 1	30 cm 7 d Rep. 1	60 cm 30 d Rep. 1
3	60 cm 30 d Rep. 2	30 cm 14 d Rep. 1	60 cm 3 d Rep. 1	60 cm 14 d Rep. 1
4	60 cm 7 d Rep. 1	30 cm 21 d Rep. 2	Control 2	30 cm 60 d Rep. 1
5	30 cm 14 d Rep. 2	60 cm 60 d Rep. 1	60 cm 5 d Rep. 1	30 cm 7 d Rep. 2
6	30 cm 5 d Rep. 1	60 cm 21 d Rep. 2	30 cm 60 d Rep. 2	60 cm 30 d Rep. 3
7	60 cm 21 d Rep. 3	60 cm 7 d Rep. 2	60 cm 90 d Rep. 1	30 cm 30 d Rep. 2
8	60 cm 14 d Rep. 2	Control 3	30 cm 5 d Rep. 2	30 cm 14 d Rep. 3
9	60 cm 60 d Rep. 2	30 cm 7 d Rep. 3	60 cm 5 d Rep. 2	30 cm 21 d Rep. 3
10		60 cm 7 d Rep. 3	30 cm 30 d Rep. 3	Control 4
11		60 cm 14 d Rep. 3	60 cm 60 d Rep. 3	30 cm 60 d Rep. 3

Figure 4. Experimental field design showing depths and burial time of carcasses pigs at the Entomology Research Farm at MSU. Light green indicates 30 cm and dark green indicates 60 cm depth. Replicates highlighted in red contain temperature loggers.

Results

Temperature

Differences between grave soil temperatures at both depths and surface (ambient air) temperatures were observed. At both depths, soil temperature did not fluctuate as much as the ambient air temperature (Fig. 5). However, after calculating accumulated degree days (ADD) using a base value of 10°C for all three depths, temperatures were not significantly different (ambient air vs. 30 cm p=0.20, ambient air vs. 60 cm p=0.15).

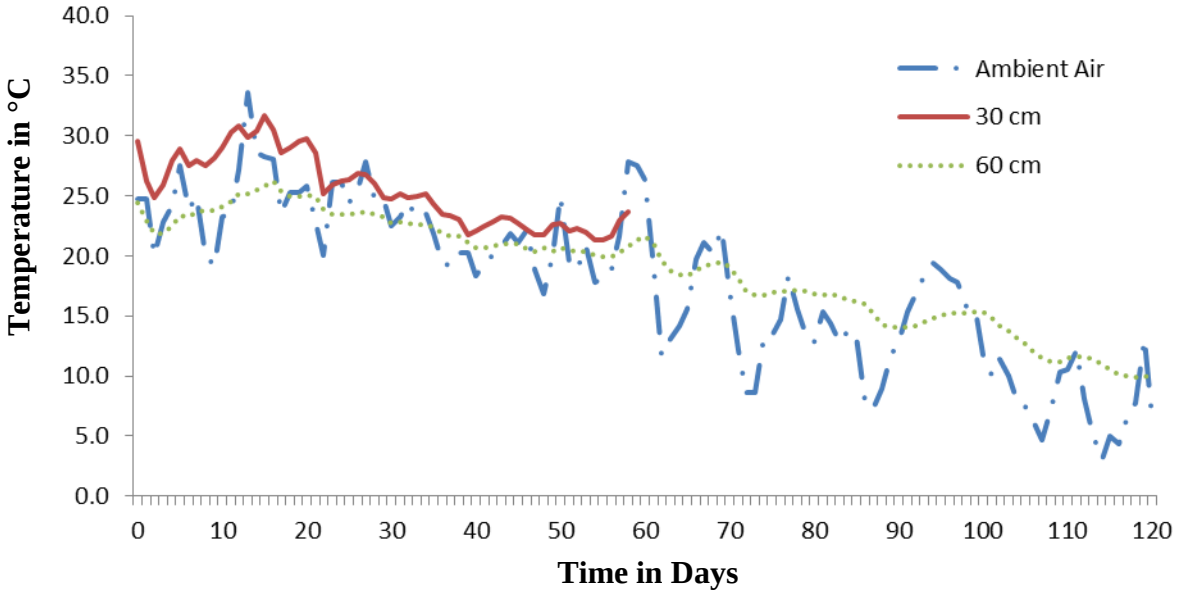


Figure 5. Mean daily temperature of ambient air (dashed) and soil at 60 cm (dotted) for 120 days and soil at 30 cm (solid) for 60 days.

Fly Succession at 30 cm

At 30 cm, insect succession proceeded similarly to hypothesized (4), with multiple Diptera species being the most predictable and common inhabitants (Fig. 6, Table 2). Flesh flies (Sarcophagidae), *S. bullata*, and muscid flies, *Hydrotaea sp.*, were the first to colonize the buried corpse, followed by blow flies (Calliphoridae), *Phormia regina* (Meigen), and another muscid

species, *Hydrotaea ignava* (Wiedemann). *Hydrotaea sp.* and *S. bullata* larvae were collected 5 days after burial, supporting the hypothesis that insect colonization would occur earlier than two weeks (2). *Sarcophaga bullata* was the dominate species at this depth over time, found on five of the six sampling dates.

Fly Succession at 60 cm

Insects were found colonizing buried carrion at 60 cm, supporting the hypothesis that insects would be able to colonize carrion buried at 60 cm (1), with two Diptera species being the most predictable and common inhabitants (Fig. 6, Table 2). At this depth, insect succession did not proceed similarly as hypothesized (4), with blow and flesh flies being the first to colonize, followed by muscid flies. At 60 cm *Hydroteaea sp.* (Muscidae) and *Megaselia scalaris* (Loew) (Phroidae) were the only fly larvae collected from corpses at this depth. Both of these species were collected 7 days after burial, supporting the hypothesis that it would take insects longer to colonize a carcass buried at 60 cm compared to 30 cm (3). *Megaselia scalaris* remained present on days 14, 21, and 30, whereas *Hydroteaea sp.* larvae were only found on days 7 and 90. *Megaselia scalaris* was the dominant species at this depth over time, collected on four of the seven sampling dates.

Beetles

At both depths, beetles from the family Staphylinidae were most often collected, starting on day 5 and remaining up to day 7 at 60 cm and up to day 60 at 30 cm (Table 2). Beetles from the family Histeridae were also collected at both depths. Within this family, *Phelister subrotundatus* (Say) was only collected at 60 cm on day 5, whereas at 30 cm, *P. subrotundatus* was collected only on day7 and *Euspilotus assimilis* (Paykull) on days 7, 14, and 21. Other

beetles collected at 30 cm include: *Necrobia rufipes* (DeGeer) (Cleridae), *Dermestes caninus* (Germar) (Dermestidae), and *Omosita colon* (L.) (Nitidulidae).

Table 2. Timing and succession of insect species collected from pig carcasses buried at depths of 30 and 60 cm. L = larvae and A = adult. Number of insects collected in parenthesis.

Days	Order and Family		Genus and Species	30cm	60cm
5	Diptera:	Muscidae	Hydrotaea spp.	L (18)	-
		Sarcophagidae	Sarcophaga bullata (Parker)	L (23)	-
	Coleoptera:	Histeridae	Phelister subrotundatus (Say)	-	A(1)
		Staphylinidae	Aleochara curtula (Goeze)	A (1)	-
			Aleocharinae (sub-family)	L (1), A (3)	A (4)
7	Diptera:	Calliphoridae	Phormia regina (Meigen)	L (79)	-
		Muscidae	Hydrotaea sp.	L (4)	L (37)
		Phoridae	Megaselia scalaris (Loew)	-	L (344)
		Sarcophagidae	Sarcophaga bullata (Parker)	L (166)	-
	Coleoptera:	Histeridae	Euspilotus assimilis (Paykull)	A (1)	-
			Phelister subrotundatus (Say)	A (4)	-
		Staphylinidae	Aleocharinae (sub-family)	A (2)	L (1), A (2)
			Platydracus sp.	A (1)	-
14	Diptera:	Calliphoridae	Phormia regina (Meigen)	L (367)	-
		Muscidae	Hydrotaea ignava (Wiedemann)	L (16)	-
		Phoridae	Megaselia scalaris (Loew)	-	L (856)
		Sarcophagidae	Sarcophaga bullata (Parker)	L (65)	-
	Coleoptera:	Cleridae	Necrobia rufipes (DeGeer)	A (1)	-
		Dermestidae	Dermestes caninus (Germar)	A (1)	-
		Histeridae	Euspilotus assimilis (Paykull)	A (3)	-
		Nitidulidae	Omosita colon (L.)	A (1)	-
		Staphylinidae	Aleocharinae (sub-family)	A (3)	-
			Creophilus maxillosus (Gravenhorst)	L (14)	-
21	Diptera:	Calliphoridae	Lucilia sericata (Meigen)	L (1)	-
			Phormia regina (Meigen)	L (121)	-
		Muscidae	Hydrotaea ignava (Wiedemann)	L (85)	-
		Phoridae	Megaselia scalaris (Loew)	-	L (249)
		Sarcophagidae	Sarcophaga bullata (Parker)	L (113)	-
	Coleoptera:	Cleridae	Necrobia rufipes (DeGeer)	A (7)	-
		Histeridae	Euspilotus assimilis (Paykull)	A (9)	-
		Staphylinidae	Aleocharinae (sub-family)	A (3)	-
			Creophilus maxillosus (Gravenhorst)	A (3)	-
			Philonthus politus (L.)	A (1)	-

Table 2 Continued.

Days	Order and Family		Genus and Species	30cm	60cm
30	Diptera:	Muscidae	Hydrotaea ignava (Wiedemann)	L (14)	-
			Hydrotaea sp.	L (1)	-
		Phoridae	Megaselia scalaris (Loew)	-	L (6)
		Sarcophagidae	Sarcophaga bullata (Parker)	L (5)	-
	Coleoptera:	Cleridae	Necrobia rufipes (DeGeer)	A (1)	-
60	Diptera:	Muscidae	Hydrotaea sp.	L (26)	-
		Phoridae	Megaselia scalaris (Loew)	L (2)	-
		Sphaeroceridae	Leptocera sp.	L (3)	-
	Coleoptera:	Staphylinidae	Aleocharinae (sub-family)	A (1)	-
90	Diptera:	Muscidae	Hydrotaea sp.	-	L (2)

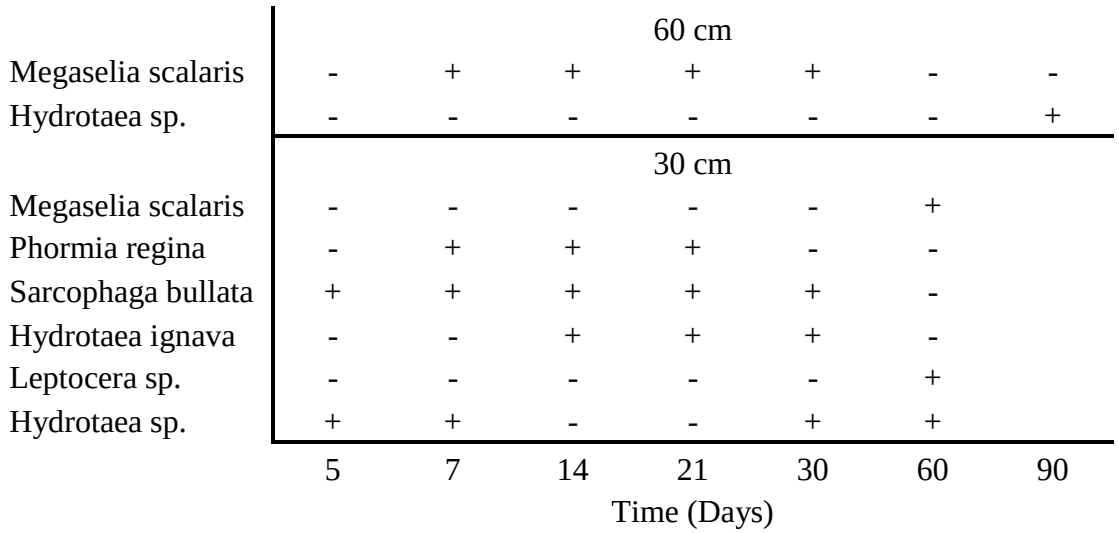


Figure 6. Fly species presence (+) or absence (-) over time at 30 and 60 cm.

Insect Community Structure

The NMDS ordination and MRPP revealed a significant ($T=-7.89$; $A=0.093$; $p < 0.001$) difference in insect community structure between 30 cm and 60 cm depths (Fig. 7), and a significant ($T=-2.04$; $A=0.058$; $p = 0.035$) difference between sampling dates (Fig. 8). A total of 35.4% of the variation in the insect community structure was explained by a three axis solution: 1^{st} = 12.6%, 2^{nd} = 13.6%, and 3^{rd} = 30.5%. Mean stress was 18.9 for the ordination and 22.6 for the Monte Carlo solution. Because both depth and time revealed a significant difference, a

permutation based MANOVA was run and determined a significant interaction between depth and time ($p<0.001$). Species at each depth were considered a significant indicator of insect communities at that depth, *S. bullata* (Indicator value = 100%) and *H. ignava* (99.8%) at 30 cm and *M. scalaris* (99.9%) at 60 cm.

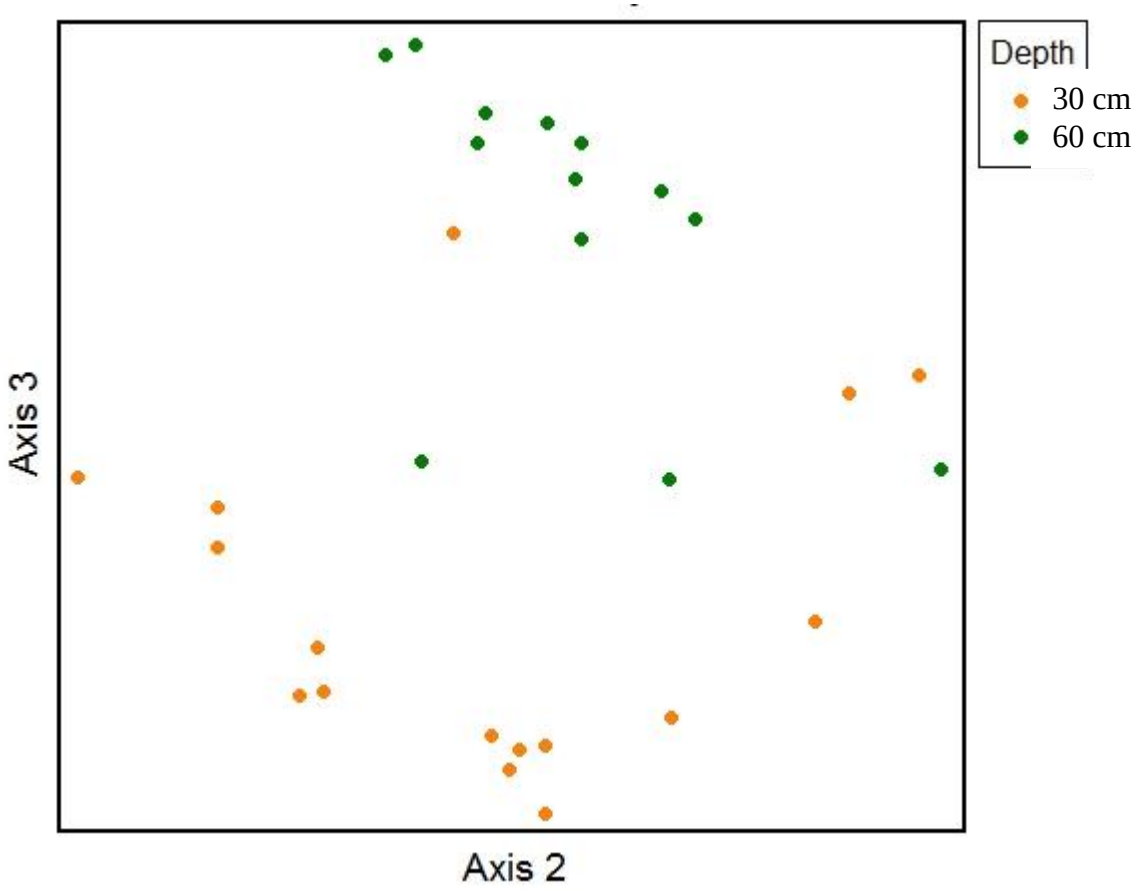


Figure 7. Non-Metric Multi Dimensional Scaling ordination showing the separation of insect community structure at the two different depths (orange = 30 cm, green = 60 cm).

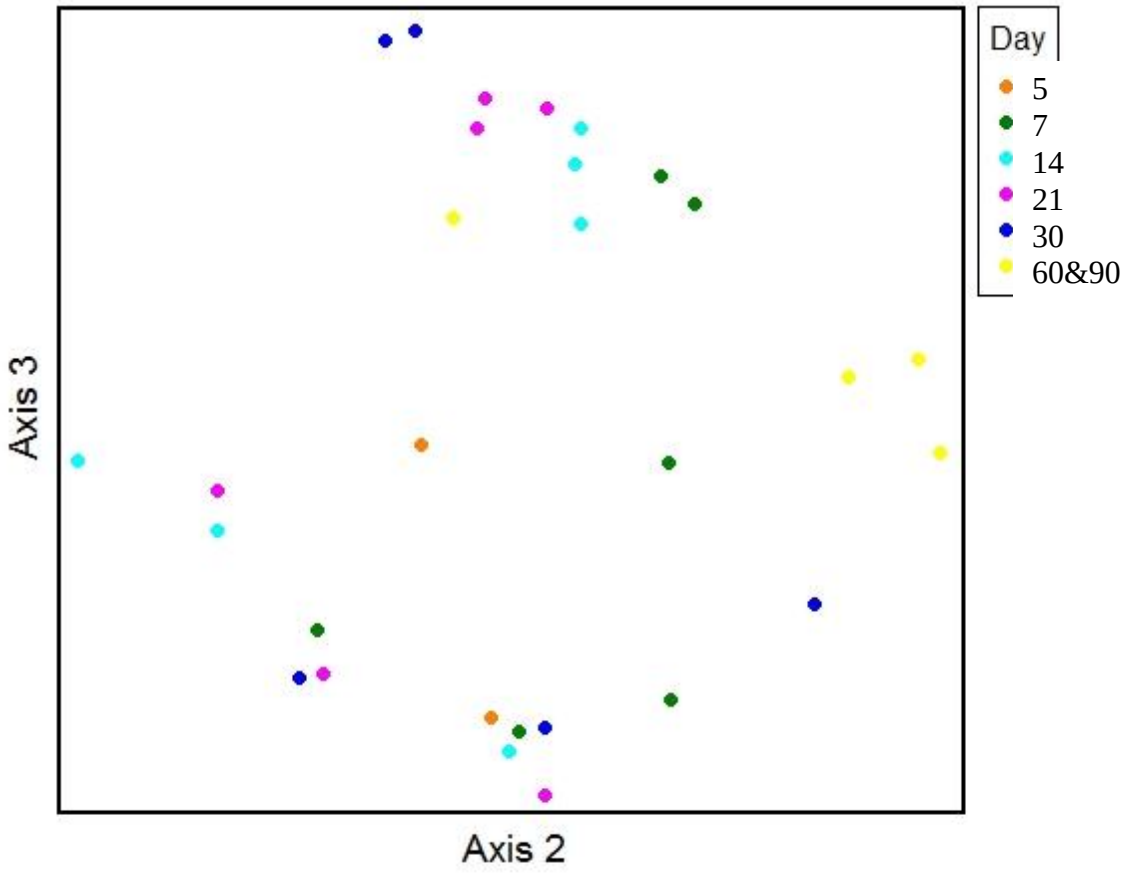


Figure 8. Non-Metric Multi Dimensional Scaling ordination showing the separation of insect community structure on the different sampling dates (orange = 5 d, green = 7 d, light blue = 14 d, purple = 21 d, dark blue = 30 d, yellow = 60 & 90 d).

Discussion

When estimating a PMI from a crime scene based on the insect evidence present, temperature from the scene is generally compared to a nearby weather station during the same time period. The weather station temperatures can be used in calculating a PMI if there is no significant difference between the weather station and the scene. Physical barriers, such as soil, affect temperature data and could skew a PMI estimate. In this study, it was found that there was no significant difference between ADD-B10 estimates using ambient air temperatures and soil temperatures at 30 or 60 cm. If obtaining soil temperature from a scene is not possible, it would

have been acceptable in this situation to use ambient air temperatures from a nearby weather station when calculating a post-burial interval estimate (PBI). These results are consistent with the findings of VanLaerhoven and Anderson (1999) in British Columbia.

Large numbers of adult blow flies were observed on the vegetation covering the field two weeks after burial. It is thought that these adult flies were meeting on the vegetation to mate and lay eggs on the soil above the carcasses. This observation could direct forensic investigators to the location of buried remains and aid in search and recovery.

Because flesh flies, (*Sarcophaga bullata)*, and muscid flies, (*Hydrotaea sp.*) were the first to colonize carcasses at 30 cm, followed by blow flies, *Phormia regina*, and another muscid species, *Hydrotaea ignava*, insect succession proceeded similarly to what was hypothesized (4). This successional pattern of muscid flies occurring earlier on buried carrion follows a similar pattern found in previous studies (Megnin, 1894; Motter, 1898; Anderson, 1995; and VanLaerhoven & Anderson, 1999). Flesh flies and muscid flies were collected on day 5, supporting the hypothesis that it would take insects less than 2 weeks to colonize a carcass buried at 30 cm (2). Because decomposition of buried carrion occurs at a much slower rate than exposed, (Payne, 1965, Payne & King, 1968, Lundt, 1964), it appears logical that fly larvae associated with the earlier stages of decomposition were collected up to day 30. It was observed two days prior to the 30 day sampling date that there was 2 cm of rain. Insects collected on this date were few in number and had drowned or had been washed away from the carcass, considering the sandy loam soil condition they were exposed to. This could explain the shift in insect species presence on day 30, *S. bullata*, *H. ignava*, and *Hydrotaea sp.* compared to the collection of species *M. scalaris*, *Leptocera sp.* (Sphaeroceridae), and *Hydrotaea sp.* on day 60.

Rather than finding the larvae on mucous membranes of the carcasses, such as eyes, nose, mouth, and anus, larvae were most often collected from the folds of the legs, inside the ear facing the soil, between the hooves, and throughout the ventral torso. Rain could be an influential factor as to the colonizing location of insects since all of those areas of the carcass provided some form of protection.

At a depth of 60 cm, two Diptera species, *Hydroteaea sp.* and *Megaselia scalaris* were collected 7 days after burial, supporting the hypotheses that insects will be able to colonize carrion buried at 60 cm (1) and that it would take insects longer to colonize carcasses buried at 60 cm compared to 30 cm (3). Like insects colonizing the carcasses at 30 cm, larvae were not found on the mucous membranes of the carcasses, but rather on the same areas as mentioned above. Since *Hydrotaea sp.* and *M. scalaris* were the only fly larvae collected, insect succession did not proceed as hypothesized (4), with blow and flesh flies being the first to colonize, followed by muscid flies. In comparison to blow and flesh fly larvae, these two former species are much smaller in size, making it easier for them to maneuver through the cracks in the soil to reach a carcass. Because blow and flesh flies were not present at 60 cm, they were no longer out-competing other species for those resources, allowing secondary species on exposed carrion, such as *Hydrotaea sp.* and *M. scalaris*, to become dominant on the buried carrion. Coffin flies have been documented colonizing remains at depths up to 1.8 m (Merritt et al., 2007), so it is not surprising they are capable of colonizing a carcass at 60 cm. Other studies have found coffin fly larvae on carrion buried at 30 cm during the active stage of decay (Payne & King, 1968), 2 months after death (Motter, 1898), and a year after death (Megnin, 1894). Lundt (1964) and Nuorteva (1977) also noted the presence of coffin fly larvae on buried carrion, but time of

colonization was not determined. The presence of *Hydrotaea sp.* larvae on day 90 could be the start of another insect succession cycle, however no insects were found on day 120, again possibly due to rain and only having one replicate at this sampling date.

At both depths, Staphylinidae beetles (rove beetles) were most often collected. These beetles are commonly found on buried carrion, but tend to be predaceous, feeding on fly eggs and larvae rather than the carrion itself (Rodriguez & Bass, 1985; Lundt, 1964; VanLaerhoven & Anderson, 1999). Other studies have collected rove beetles from buried remains at times ranging from 5 days to 5 months (VanLaerhoven & Anderson, 1999). Beetles from the family Histeridae also were collected at both depths. These beetles are also common predators of fly larvae, but are also known to feed on carrion ranging in decomposition from bloat to dry stages (Byrd & Castner, 2010).

Three other beetle species were collected at 30 cm: *Necrobia rufipes*, *Dermestes caninus*, and *Omosita colon*. *Necrobia rufipes*, commonly known as red-legged ham beetles, tend to be associated with drier stages of remains (Byrd & Castner, 2010), so it is unusual that they appeared so early on in the sampling dates: 14, 21, and 30 days. Similar to the beetles above, they tend to feed on fly larvae and sometimes carrion (Byrd & Castner, 2010). This is the first study to record these beetles on buried remains. *Dermestes caninus* (Germar) are attracted to all types of carrion but prefer drier remains (Byrd & Castner, 2010). Only one specimen was found on day 14. One *Omosita colon* (L.), commonly referred to as sap beetles, also was only found on day 14. This species of sap beetle is commonly associated with more advanced decay and co-occurs with Dermestidae beetles (Byrd & Castner, 2010). Other Coleoptera found in the soil included Japanese beetle larvae (Scarabaeidae), June beetle larvae (Scarabaeidae), and adult

ground beetles (Carabidae). It was determined that their presence near the carcasses was associated with the soil, and they were not of forensic importance. Even though beetles of forensic importance were collected at both depths, their timing and succession were not as predictable as fly larvae, therefore they are not as useful in determining a PMI.

Multivariate ordination demonstrated significant separation of insect communities at 30 and 60 cm, as well as over time, with *S. bullata* and *H. ignava* being significant indicators at 30 cm and *M. scalaris* at 60 cm. With the identification of these indicator species, how quickly they arrive, and how long they are present, forensic entomologists are one step closer to establishing a PBI estimate for buried carrion in mid-Michigan. It is likely however, that this separation of insect communities may be influenced by soil type and microbial presence, rather than by only depth and time together. More studies examining the effects of soil type and microbial influence on insect arrival and succession are needed, along with the developmental rates of these new indicator species. Precipitation also seemed to play a role in species presence over time. Again, more studies examining the effects of precipitation on insects colonizing buried remains are required. Once the developmental rates of these indicator species are better understood, when attempting to establish a PBI, soil temperature data should be compared to ambient air temperature from the nearest weather station. It would be most accurate if the weather station recorded soil temperature, but if not, ambient air temperature has been found to be sufficient in this instance. It is also important to remember that insect succession varies based on a regional climate specific location, so an insect successional database for the location should be established before other studies are conducted.

Conclusions

In accessing the importance of these findings with respect to estimating a PBI for forensic investigations in mid-Michigan during the summer, I concluded that if a body is found at 30 cm with no insect colonization, the body has most likely been dead less than 5 days (84.5 ADD-B10). If flesh, blow, and/or muscid fly larvae are present, the body could have been dead between 5 to 30 days (84.5 to 526.1 ADD-B10), depending on climatic and soil conditions. Once muscid, coffin, and dung fly larvae are found on a body buried at 30 cm, the body could have been dead over a month (526.1+ ADD-B10). Second, if a body is found at 60 cm with no insect colonization, the body could have been dead less than 7 days (90.3 ADD-B10). If the body was colonized by coffin and muscid fly larvae, the body could have been dead between 7 to 30 days (90.3 to 408.8 ADD-B10), depending on climatic and soil conditions. Investigators should search for larvae in the folds of the legs, inside the ear facing the soil, between the hooves, and throughout the ventral torso, rather focusing only on mucous membranes.

APPENDIX

Appendix 1

RECORD OF DEPOSITION OF VOUCHER SPECIMENS

The specimens listed on the following page have been deposited in the named museum as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the voucher number have been attached or included in the fluid-preserved specimens.
Voucher Number: 2012-01
Title of thesis:

Insect Timing and Succession on Buried Carrion in East Lansing, Michigan

Museum where deposited:
The Michigan State University (MSU) Albert J. Cook Arthropod Research Collection
Name: Emily Pastula Date: April 17, 2012
Table 3. Voucher specimens

Family		**Life Stage**	**Quantity**	**Preservation**
Calliphoridae	*Phormia regina*	Adult	10	Pinned
Calliphoridae	*Phormia regina*	Larvae	1 Vial	Ethanol
Carabidae		Adult	1 Vial	Ethanol
Cleridae	*Necrobia rufipes*	Adult	7	Ethanol
Dermestidae	*Dermestes caninus*	Adult	1	Ethanol
Histeridae	*Euspilotus assimilis*	Adult	7	Ethanol
Histeridae	*Phelister subrotundatus*	Adult	4	Pinned
Muscidae	*Hydrotaea ignava*	Adult	10	Pinned
Muscidae	*Hydrotaea ignava*	Larvae	6	Ethanol
Muscidae	*Hydrotaea sp.*	Larvae	10	Ethanol
Nitidulidae	*Omosita colon*	Adult	1	Pinned
Phoridae	*Megaselia scalaris*	Adult	2	Ethanol
Phoridae	*Megaselia scalaris*	Adult	2	Ethanol
Phoridae	*Megaselia scalaris*	Larvae	1 Vial	Ethanol
Sarcophagidae	*Sarcophaga bullata*	Adult	10	Pinned
Sarcophagidae	*Sarcophaga bullata*	Larvae	9	Ethanol
Scarabaeidae		Larvae	1	Ethanol
Scarabaeidae		Larvae	1	Ethanol
Scarabaeidae		Larvae	1	Ethanol
Sphaeroceridae	*Leptocera sp.*	Larvae	1	Ethanol
Staphylinidae	*Aleochara curtula*	Adult	1	Pinned
Staphylinidae	Aleocharinae	Adult	4	Ethanol
Staphylinidae	Aleocharinae	Larvae	1	Ethanol
Staphylinidae	*Creophilus maxillosus*	Adult	1	Ethanol
Staphylinidae	*Creophilus maxillosus*	Larvae	10	Ethanol
Staphylinidae	*Philonthus politus*	Adult	1	Pinned

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