# Louisiana State University LSU Digital Commons

## LSU Master's Theses

**Graduate School** 

2011

# Decomposition rates in tobacco and hay barns in Kentucky

Valerie Kauffeld Louisiana State University and Agricultural and Mechanical College, stashadrian@gmail.com

Follow this and additional works at: https://digitalcommons.lsu.edu/gradschool\_theses Part of the <u>Social and Behavioral Sciences Commons</u>

## **Recommended** Citation

Kauffeld, Valerie, "Decomposition rates in tobacco and hay barns in Kentucky" (2011). *LSU Master's Theses*. 3699. https://digitalcommons.lsu.edu/gradschool\_theses/3699

This Thesis is brought to you for free and open access by the Graduate School at LSU Digital Commons. It has been accepted for inclusion in LSU Master's Theses by an authorized graduate school editor of LSU Digital Commons. For more information, please contact gradetd@lsu.edu.

# DECOMPOSITION RATES IN TOBACCO AND HAY BARNS IN KENTUCKY

A Thesis

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College

> in partial fulfillment of the requirements for the degree of Master of Arts

> > in

The Department of Geography and Anthropology

by Valerie Kauffeld B.A., East Tennessee State University, 2008 May 2011

## **DEDICATION**

I would like to dedicate this thesis to my grandfathers, Benjamin "Pappy" Baumgardner and Russell "PopPop" Kauffeld, both of whom have long since passed. They shared my passion for life and my thirst for knowledge. I have never known two kind hearted, selfless gentlemen such as these. During their time here, they taught me a great deal about compassion, family, and how to help others who could not help themselves. Your love and guidance mean more to me than you will ever know.

#### ACKNOWLEDGEMENTS

To the following individuals and agencies that have helped with this project in so many ways, I would like to offer with heartfelt sincerity my thanks: To Mr. Hoskins Carrol, Mr. Gary Sparks, and Mr. Charles McQuinn for allowing me to use their land and their barns for this project. To the people of Wolfe County, KY, for their kindness, hospitality, and curiosity while I conducted my study on their lands. To Charles, and Bonnie McQuinn, who generously opened their home to me and allowed to me stay with them for the duration of this study. You are truly wonderful people. To Charlana McQuinn, my peer, friend, and partner in crime. Our adventures in Louisiana and Kentucky are truly ones for the books! To Daniel Tallent, who helped construct the loft in the control barn. Daniel, that board never moved. I promise. To Lonnie "Squeaky" Sparks, whose used his spider-like ability to construct the loft in the tobacco barn. I would also like to thank Rebecca Lirette who oversees the Swine Unit at Ben Hur Farm for providing me with the nine young pigs that were used in the study. Lastly, to Norman E. Woodley who identified the Diptera specimens and Geoffrey White who identified the Coleoptera specimens, both of the Systematic Entomology Laboratory, Agriculture Research Service, US Department of Agriculture. Thank you for your patience, time, and commitment to this study.

To my advisor Ms. Mary Manhein of the LSU FACES, Geography and Anthropology Department. You were my primary reason for choosing to attend LSU. Ms. Manhein, your never ending quest for the truth and for doing what is right has inspired me beyond measure. Thank you for your guidance, understanding, supervision, and even the occasional kick in the pants. I am truly blessed to have had the pleasure of knowing you. To Dr. Tague, who has been not only a wonderful professor, but a great friend.

iii

To Amy Hernandez and Nina Huffstetler of the Agricultural Chemistry Laboratory at Louisiana State University for extracting my nicotine samples. You ladies are amazing and I would not have been able to complete this project without you! Thank you both for everything! Also, to Dr. James Geaghan, Department Chair of Statistics at Louisiana State University, and Chelsea Deroche, a graduate student in the same department. Your vast understanding of statistics and willingness to help with this project is appreciated beyond measure. Thank you both for you statistical wisdom and guidance.

To my committee members Ms. Mary Manhein, Dr. Robert Tague, and Dr. David Chicoine, who graciously devoted their time to reading and editing this work. Your constructive comments and criticisms have helped make this thesis into a more concise and readable document. Thank you all from the bottom of my heart.

I would like to extend a special thanks to Maria Allaire, Research Associate in the LSU FACES Lab. You willingness to help those who are just starting out in this field is astounding. Not many people would do this. Your expertise, guidance, and wisdom have impacted my life in so many ways. I am very lucky to have met you and even more blessed that I can call you my friend. I will never be able to thank you enough for all that you have done for me.

To my parents, Russell and Martha Kauffeld, who have had greater faith in me than I have had in myself during these past two years. You have always pushed me to be the best that I can be, and although I rebelled and, at times, questioned your actions, I truly believe that I would not be standing here today were it not for that constant pushing. Your love, support, and encouragement have meant the world to me. To my sister Tara, who has always been there to make me laugh, and who helped me in the dissection of my first *Sus scrofa* before the project even began, thank you from the bottom of my heart. I am truly blessed to have a sister like you.

iv

To my fiancé, Sean, whose unwavering confidence in me has kept me going through thick and thin. You were truly my rock these past couple of years and I am so very thankful. To my aunt and uncle, Mary and Brian Dugger, whose guidance and support throughout my life have, in many ways, brought me to where I am today. To my grandmother, Norma, whose has wisely taught me that life is nothing without laughter and family. To my grandmother, Patsy, whose courage and bravery will forever inspire me. Lastly, to all my family who are far too numerous to list here, thank you all for your support and words of encouragement. None of this would have been possible without you.

DEDICATIONii
ACKNOWLEDGEMENTS iii
LIST OF TABLESviii
LIST OF FIGURESx
ABSTRACTxi
CHAPTER 1: INTRODUCTION1
CHAPTER 2: LITTERATURE REVIEW
CHAPTER 3: METHODS AND MATERIALS19
CHAPTER 4: RESULTS.24Hay Barn (Site 1).24Control Barn (Site 2).31Tobacco Barn (Site 3).38Insects.46Weather.55Nicotine Analysis.57
CHAPTER 5: DISCUSSION59
CHAPTER 6: CONCLUSION65
REFERENCES CITED
APPENDIX A: HIGHS AND LOWS FOR SITE 1
APPENDIX B: HIGHS AND LOWS FOR SITE 270
APPENDIX C: HIGHS AND LOWS FOR SITE 371
APPENDIX D: RAINFALL FOR SITES 1, 2, AND 372

## **Table of Contents**

<b>APPENDIX E:</b>	<b>DECOMPOSITION I</b>	RATES FOR SI	TES 1, 2, AND 3	73
VITA				74

# List of Tables

1	Activity Chart for Sub-site 1A26
2	Activity Chart for Sub-site 1B28
3	Activity Chart for Sub-site 1C30
4	Activity Chart for Sub-site 2A32
5	Activity Chart for Sub-site 2B34
6	Activity Chart for Sub-site 2C38
7	Activity Chart for Sub-site 3A40
8	Activity Chart for Sub-site 3B42
9	Activity Chart for Sub-site 3C44
10	MANOVA of Decomposition Rates Between Barns and Local45
11	Diptera Taxa Collected at All Sub-sites47
12	Coleoptera Taxa Collected at All Sub-sites48
13	Insect Activity for Sub-site 1A48
14	Insect Activity for Sub-site 1B49
15	Insect Activity for Sub-site 1C49
16	Insect Activity for Sub-site 2A50
17	Insect Activity for Sub-site 2B51
18	Insect Activity for Sub-site 2C51
19	Insect Activity for Sub-site 3A52
20	Insect Activity for Sub-site 3B53
21	Insect Activity for Sub-site 3C53

22	Variation of Maximum and Minimum Temperatures for All Sub-sites56
23	Kestrel data for Weather Variables at Research Sites56
24	Summary of Nicotine Analysis58

# List of Figures

1	Preparation of Sub-site 1A	25
2	Visual Decomposition of Remains Located at Sub-site 1A	26
3	Visual Decomposition of Remains Located at Sub-site 1B	27
4	Visual Decomposition of Remains Located at Sub-site 1C	30
5	Visual Decomposition of Remains Located at Sub-site 2A	32
6	Visual Decomposition of Remains Located at Sub-site 2B	34
7	. Visual Decomposition of Remains Located at Sub-site 2C	37
8	Visual Decomposition of Remains Located at Sub-site 3A	40
9	Visual Decomposition of Remains Located at Sub-site 3B	42
10	Visual Decomposition of Remains Located at Sub-site 3C	44
11	Comparative View of Determinate Ions for Nicotine	57
12	Individual Ion Peaks for Nicotine	57

#### Abstract

Nine pig (*Sus scrofa*) carcasses were placed in three different microenvironments in eastern Kentucky in the summer of 2010 in order to aid in the determination of a Postmortem Interval (PMI) template for recently mummified remains. The three microenvironments were a tobacco barn, hay barn, and control barn. Three pigs were placed in specific locations in all of the barns – outside the barn on the ground, inside the barn on the ground, and in a loft placed within five feet of the roof. Little to no natural mummification occurred at each site. Residual nicotine was also analyzed in order to determine whether or not it had a significant impact on the decomposition rate of the remains located in the tobacco barn loft. While residual nicotine was present, it did not have a significant impact on the decomposition rate for the remains at this site.

**Key Words:** mummification, residual nicotine, PMI, decomposition, eastern Kentucky, insects, tobacco, hay, barns

#### **Chapter 1: Introduction**

In the field of Forensic Anthropology, investigators are primarily concerned with the identification of human remains. Once determined, the "how", "why", and "when" are assessed. In order to ascertain these crucial elements, in particular, the "when", or Postmortem Interval (PMI), must first be established. In most instances, such as fires, death by gunshot, etc., the PMI is readily available and has been agreed upon and accepted. In other instances, though, such as natural mummification, a PMI has never been determined. I am proposing to determine a PMI for natural mummification by looking at the rate of mummification (the drying out and shrinkage of tissue and internal organs) versus skeletonization in tobacco barns and regular barns in Eastern Kentucky. Instances of natural mummification have been found in settings such as hay barns and reported on by Dr. Emily Craig, Kentucky's former State Forensic Anthropologist (Kentucky State Police, 2002). The main causes of natural mummification are extreme temperatures, dry or very humid climates, and bogs. This research primarily examines two natural elements that have never been tested as mummifying agents: tobacco and hay. Hay is a natural dehumidifier. Tobacco will be employed due to its emission of nicotine, which is used in many insecticides and is considered a natural insect repellant. The impact of insect succession and residual nicotine will also be examined. I hypothesize that the remains in the tobacco barn loft will mummify due to location (25 feet from the ground) and the presence of residual nicotine, and that the remains in the hay barn on the ground will also mummify. This research could greatly benefit the forensic community by providing a "template" PMI for naturally-mummified remains and would also help others, such as Egyptologists, historians, and archaeologists in determining a more precise date for mummified remains.

#### **Chapter 2: Literature Review**

#### **Intentional Mummification**

To fully comprehend this project, a brief discussion of intentional and natural mummification is necessary for clarification and comparative purposes. I have chosen to discuss the Egyptian culture and its processes of intentional mummification largely due to the public's familiarity with this culture. Intentional (or deliberate) mummification was practiced by the Egyptians as early as BC 4500 during the Predynastic Period (David, 2008). Throughout that time, pharaohs and other prominent figures were embalmed with bitumen, a dark type of resin usually found in petroleum. The word "bitumen" actually comes from the Arabic and Persian word "mummiya" (Andrews, 1984; Harrell, 2002). This substance gives the remains their black, tar-like appearance. The exact location of the bitumen used by ancient Egyptians has been debated for several years. Samples of bitumen were collected from seeps found in the Dead Sea, Abu Durba, and Gebel Zeit. These were later analyzed and compared with the bitumen found on five sets of mummified human remains: Cleopatra (AD 100), Soter (AD 100), Djedoler (BC 200), Pasenhor (BC 900) and the remains of an unknown priest (BC 800). All of these "mummies" come from the western portion of Thebes, with the exception of Djedoler who was found at Akhmin (Harrell et al., 2002). The molecular-fossa indices from these samples were analyzed and then compared to those collected from the actual seeps. The bitumen from the oldest mummy, Pasenhor, was found to correlate with the Gebel Zeit seep, while the rest correlate with the Dead Sea seep. Even though the Gebel Zeit seep is closer to Egypt, it appears that the Egyptians preferred the consistency and quality of the Dead Sea bitumen.

Usually, before the Egyptians used bitumen or tree resin (from cedar trees, for example), they would first embalm the body. The term "embalm" is a Latin word meaning "[to put] into

aromatic resins" (Andrews, 1984:6). Embalming these remains required the removal of internal organs and placing them into canopic jars so they could accompany their owner in the afterlife. The Egyptians removed these organs not only for religious purposes but also for medicinal and scientific purposes. They realized that the body cavity could be treated more easily with oils after organ removal. Additional evidence suggests that they knew decomposition started in the internal organs. The removal of organs allowed for a more rapid dehydration process, in turn, quickening the mummification process (Andrews, 1984).

Natron is another chemical that was added to the body in order to make it more flexible. Natron is a natural compound of sodium carbonate and sodium bicarbonate with varying mixtures of sodium chloride and sodium sulfate (Andrews, 1984; Lucas, 1932). This compound is mentioned in the works of Herodotus as well (Halsall, 1999). In the text below, Herodotus describes the process of embalming (mummification) and the three levels at which it can be performed. This firsthand account written by the early Greek historian in his *Histories* has been used by several modern day scholars. He writes:

...they fill the cavity with the purest bruised myrrh, with cassia, and every other sort of spicery except frankincense, and sew up the opening. Then the body is placed in natrum for seventy days, and covered entirely over. After the expiration of that space of time, which must not be exceeded, the body is washed, and wrapped round, from head to foot, with bandages of fine linen cloth, smeared over with gum, which is used generally by the Egyptians in the place of glue, and in this state it is given back to the relations, who enclose it in a wooden case which they have had made for the purpose, shaped into the figure of a man. Then fastening the case, they place it in a sepulchral chamber, upright against the wall (Halsall, 1999, http://www.fordham.edu/halsall/ancient/herodotus-history.txt).

If a person cannot afford the aforementioned method, then this one is employed:

Syringes are filled with oil made from the cedar-tree, which is then, without any incision or disembowelling, injected into the abdomen. The passage by which it might be likely to return is stopped, and the body laid in natrum the prescribed number of days. At the end of the time the cedar-oil is allowed to make its escape; and such is its power that it brings with it the whole stomach and intestines in a liquid state. The natrum meanwhile has dissolved the flesh, and so nothing is left of the dead body but the skin and the bones. It is returned in this condition to the relatives, without any further trouble being bestowed upon it (Halsall, 1999, http://www.fordham.edu/halsall/ancient/herodotus-history.txt).

If this method is also too expensive, the family is left with one last option:

...to clear out the intestines with a clyster, and let the body lie in natrum the seventy days, after which it is at once given to those who come to fetch it away (Halsall, 1999, http://www.fordham.edu/halsall/ancient/herodotus-history.txt)

Here, one can see the importance of Natron (or "natrum" as it is referred to in the text). Perhaps the most important element of Natron is sodium chloride, or common table salt. Many translators and historians thought that mainly salt and not Natron was employed to dehydrate the remains. They now believe, after several years of analysis, that salt was used in Natron and not as a separate entity. In cases where high concentrations of salt have been found, historians suggest that it was used in a ritual context, such as sprinkling the remains with water from the sea (Lucas, 1932). An air of uncertainty exists regarding the many translations of the aforementioned text. Many translators disagree with those who interpret Herodotus' use of the word "bath" as meaning "soaked in" (Lucas, 1932). Historians and translators say that Herodotus never uses the word "bath" in his text and that the Egyptians used Natron in a crystallized form instead of as a solution. This has never been verified.

While Egyptian mummification is the most widely known form of intentional mummification, other cultures, such as those found in China and Chile, performed similar kinds of mummification (Pringle, 2001). The methods and tools for mummification vary slightly throughout these cultures, but the underlying principle remains the same. Though intentional mummification is the more widely recognized form of mummification, another form of mummification exists: natural mummification.

#### **Natural Mummification**

Natural mummification, the form with which I am primarily concerned, is the lesser known form. Although it appears commonly all over the world, many people are unaware of the ways in which it can occur. Natural mummification occurs when human remains are exposed to

cases of extreme heat or cold, habitats with an environment outside of that dictated by heat or cold (peat bogs, for example), and spaces sealed from the elements. Egypt, with its hot, dry climate, was an ideal place for natural mummification to occur. The Egyptians buried their dead in shallow graves. The sand absorbed the body fluids, which stopped the decomposition process and caused mummification to occur. David (2008) suggests that these instances of natural mummification may have inspired intentional mummification. Several different forms of natural mummification exist, but the most well-known of these are discussed below.

#### **Natural Mummification Due to Cold and Dry Climates**

Natural mummification can occur due to cold and dry climates. Currently, the most famous case is that of Ötzi, the iceman from the Alps. In September, 1991, two hikers, Erika and Helmut Simon, decided to climb Similaun and Finail, two mountains that tower over 12,000 feet near the Austrian Border (Deem, 2008). The Niederjoch Glacier, which shares its immense body with both the Similaun and Finail, had been thawing since the late 1800s. Hikers must cross part of this glacier if they wish to reach the summit. By the time the Simons undertook the climb, several deep crevasses scarred the Niederjoch Glacier.

After completing the Similaun climb, the two hikers proceeded on to Finail. When they were descending the Finail summit, by way of the Niederjoch Glacier, they came upon a body lying face down in one of the newly visible gullies. Erika and Helmut Simon had found the body of a man who had lived 5,200 years ago (Deem, 2008; Stone, 2000). Ötzi's body was perfectly preserved due to the naturally-freezing climate and altitude. His 51 tattoos were also perfectly preserved as were what are believed to be acupuncture marks (Stone, 2000).

A more widely known example of natural mummification comes from the Andes and the Inca culture. The Inca culture flourished during the early  $13^{\text{th}} - 17^{\text{th}}$  centuries in the central

Andes. The Incas are mostly known for their vast empire, tales of golden cities, elaborate ceremonies, and sacrifices. Less known are the mummies that these sacrifices produced. Children (boys and girls) and young adolescent girls were sacrificed to the "high mountain gods" (Bahn, 2002). They would usually be given *chicha*, a type of beer, or coca leaves before they were dropped into shafts below the sacrificial platforms (Deem, 2008). This would serve to ease their fears and often make them sleep. The frigid air and unforgiving mountain climate would do the rest.

In 1995, anthropologist Johan Reinhard, while climbing Ampato, discovered a bundle with the preserved body of a 14-year-old girl. Tests would later reveal that she had died almost 500 years earlier. They named her Juanita. She was not, however, one of the sacrificial victims. She had been killed by a blow, possibly from a club, to the right side of her head.

Reinhard's story does not end there. He later uncovered 14 sacrificial burials. His most notable find, however, came from the world's highest archaeological site: Mount Llullaillaco (Deem, 2008). The Incas built a road to this volcano, which in itself tells of its importance. The summit of this volcano rests at 22,057 feet above sea level (Bahn, 2002). Here, in 1999, Reinhard and his expedition found what would be known as the "Frozen Children of the Andes" (Deem, 2008). Uncovered were three bodies wrapped in bundles. One was of a six year-old girl. Her clothes, hair, and artifacts were equally as well preserved. A seven year-old boy was the next to be found. He was dressed in a red wool tunic and his feet were bound with rope. Lastly was an adolescent girl who was about 15 years old. She is known as La Doncella (the Maiden) and is dressed in a man's gray tunic and the same type of dress as the younger girl (Bahn, 2002; Deem, 2008). These children, 18 in total, had been perfectly preserved by the snow and ice.

In recent studies conducted by the University of Montana at Missoula, McKeown et al. (2011) found that the arid and cool climate of the Rockies results in the mummification of the external tissue at an early stage in the decomposition process. They reviewed 12 cases and scored their degree of mummification based on the percentage of the body that retained mummified tissue and also scored the degree of scavenging according to the percentage of the skeleton that exhibited evidence of carnivorous activity. McKeown et al. (2011:387) found that 75% of the reviewed cases retained mummified tissue on over 50% of the body. Where scavenging was present, the mummified tissue covered less than 50% of the body. The PMIs for these cases ranged from three weeks to two years. They concluded that remains with less carnivorous activity retained a greater amount of mummified tissue than those with a high amount of carnivorous activity (McKeown et al., 2011:387). Cold, arid regions are not the only climatic zones that cause remains to mummify. Hot, dry climates are also cause for mummification.

### **Natural Mummification Due to Hot and Dry Climates**

The Chinchorro people lived on the northern coast of Arica, Chile, in early half of 7000 BC (Arriaza, 1995). This culture included hunters, gatherers, and fisherman. They were also people who were steeped in a tradition of mortuary practices. The Chinchorros not only practiced intentional mummification, but also natural mummification. In the town of Acha, Chinchorro burials were found wrapped in reed mats, camelid furs, and pelican skins. Some of the legs of these mummies were tightly wrapped (Arriaza, 1995). They had been naturally mummified by the intense dryness and heat of the Atacama Desert (Bahn, 2002).

In 2002, Maira Allaire (Allaire, 2002) also observed two instances of natural mummification during her study in Southwestern Colorado. While looking at PMI through decomposition, three of the pigs used in her study (*Sus scrofa*) mummified. Her first was at

8,700 feet above sea level in the northern region of La Plata County, Colorado, in a shaded Aspen grove forest in June. The maximum mean temperature was 91.2°F and a minimum temperature of 50.9°F. Mummification began on June 24, eight days after she had originally set out the pig. Several of the orifices were dried and hard and the dermis had begun to "darken and dry out" (Allaire, 2002:41). Mummification continued into days eight and nine, and the derma took on a leathery appearance. On day 13, the remains were fully mummified.

The other was at 11,100 feet above sea level in the northern region of San Juan County, Colorado, in a "treeless opening that was surrounded by a subalpine pine forest where the site received only four hours of direct sunlight" in August (Allaire, 2002:22). The mean maximum temperature was 62.9°F while the mean minimum temperature was 41.8°F. On August 14 (day 15 at this site), mummification was recorded at the ground level. The derma lying on the ground was "darkened, devoid of hair and leathery in appearance" (Allaire, 2002:51). The onset of mummification was probably brought on by the "season of initial exposure, isolation from extreme moisture (high or low humidity), and isolation from arthropod access" (Allaire, 2002:20). Temperatures play a major role in the mummification of human remains, but other environments, such as peat bogs, can also contribute to mummification.

#### **Natural Mummification Due to Peat Bogs**

Temperature is not the only cause for natural mummification, although it is the one for which we have the most evidence. Peat bogs can also mummify both animal and human remains. A peat bog is commonly known as an acidic wetland. Plants there often grow and die faster than they can decay (Bahn, 2002). These remains accumulate over time and form peat. Many people today use peat as a form of soil enrichment for a garden. Remains in peat are preserved due to

the lack of oxygen. This lack of oxygen negatively affects the organisms that normally break down the remains.

Most bogs consist of bog moss which produces a substance called *sphagnan*. *Sphagnan* has two main effects when it comes in contact with human remains: 1) it extracts calcium from the body, and 2) it "simultaneously binds nitrogen" (Bahn, 2002:99). The effects of *sphagnan* make it hard for bacteria to feed on human remains present in the bog. This results in the bodies having a dark appearance and the bones being decalcified or, in some cases, completely dissolved.

Many of the "bog bodies" found have been dated to about 2,000 years ago. In 1897, peat bog workers in the Netherlands found the remains of a young girl who became known as Yde Girl (Fischer, 1998). Her hair, clothes, and the woolen belt that she had been strangled with were all perfectly preserved. A stab wound was also present at the base of her neck. In 1938, the remains of a woman were recovered from a peat bog in Bjaeldskov Valley in Denmark (Bahn, 2002). Her hair (over one yard long and braided), sheepskin wrapping, and leather cloak were all preserved. A deep groove was present around her neck, which has led many to believe that she was either hanged or strangled. Another body was found in the same valley 12 years later. He is known as Tollund Man. Like the woman found before him, he had been hanged. The noose was still perfectly intact around his neck. His leather hat and belt, again perfectly preserved, were all the clothing he was wearing. These remains date back to the Iron Age (Glob, 2004). Perhaps the most famous "bog body" to date is the Lindow Man whose remains were found in a bog near Manchester, England (Bahn, 2002). All that was recovered was a foot, a torso with a head, and an arm. His short hair and beard were both perfectly preserved as was the fox-fur band which

adorned his arm. He had been strangled, had his throat cut, and had multiple blunt force trauma wounds to his skull.

Several factors can result in natural mummification. Extreme temperatures and acidic bogs are only two examples from a vast number of natural elements that can cause this type of mummification to occur. The primary goal of this study is to look at two natural elements in particular: tobacco and hay. No prior research has been undertaken on these two elements as factors in mummification. The next section will serve to discuss the growth and maintenance of tobacco as well as the insects that commonly call its leaves their home.

#### <u>Tobacco</u>

Tobacco comes in a variety of species, but the most common are Dark (*Nicotiana solanaceae*) and Burley (*Nicotiana tabacum L.*). Burley tobacco will be discussed to a greater extent because the current research will be dealing with it. In Kentucky, Burley tobacco is usually planted during late May, tended throughout the summer, and hung to dry in late August. Hoskins Carroll (the owner of the tobacco barn for this project) uses the KC204LC Burley tobacco, which is a low converter, meaning it produces a lower amount of nicotine. All information about the growth and tending of tobacco were gained during a telephone interview with Mr. Carroll (H. Carroll, personal communication, March 23, 2010). When tending tobacco, the seeds are first planted in float beds. These are trays made of polystyrene designed to aid in the early stages of growth. These trays essentially ensure growth. Each one is made up of a specific number of cells, usually chosen according to the amount of tobacco one wishes to produce. These are monitored for a month and then the plants are set in the field.

Mr. Carroll monitors his trays from the first of May until the first of June, at which time he transplants them in the field. He will then continue to monitor his field, three acres total

(which will yield roughly 6,000 pounds of Burley tobacco) for 100 days, constantly checking insect levels, water levels, and leaf quality. He will also infuse the soil with Nitrogen 3400, Fertilizer 9/18/27 and Lime. This helps to replenish nutrients that have been taken out by the tobacco. At the end of 100 days, the tobacco will be harvested. He separates the stalk from the leaves by hand. He then hangs the stalks and leaves on a stick and hangs them in the barn to dry. During the initial tending and hanging processes, Mr. Carroll dusts his crop with insecticide in order to deter hornworms, wireworms, budworms, etc.

#### **Tobacco Insects**

My primary concern is with the insects that infect tobacco leaves. Using the 2009-2010 Kentucky & Tennessee Tobacco Production Guide as my main reference, I found that several insects use tobacco leaves for food and as habitats in general (Seebold, 2009-2010). Tobacco aphids (*Myzus nicotianae*) are common in the fields. Aphid eggs are laid by winged adult flies. They reside on the underside of the leaves and, if not dealt with quickly, can cause infestations. Tobacco budworms (*Helicoverpa virescens*) are one of the most destructive insects. They chew round holes in the upper leaves of the plants. Budworms are essentially young moths. They look like small, black droppings on leaves. These insects must be properly dealt with or the crop will be ruined (Seebold, 2009-2010).

Hornworms (*Manduca sexta*) follow budworms in the destructive category. These worms thrive on tobacco leaves. While infestations are common in June, if not dealt with properly, a larger infestation can occur in July, thus destroying the crop. These worms like the upper leaves of the plant as well, although they usually reside on the leaves' underbelly. Flea beetles (*Epitrix hirtipennis*) are the last common category of tobacco insects. They survive by chewing small round holes, called shot holes, in the leaves. Again, if not dealt with properly, the damage can be

catastrophic. Some pests must be dealt with only occasionally and are usually found, to one extent or another, in every tobacco field. These are the common armyworms and cutworms (Spodoptera litura), grasshoppers (Melanoplus differentialis), Japanese beetles (Popillia *japonica*), stink bugs (*Halyomorpha halys*), and thrips (*Frankliniella fusca*). Cutworms can eat through the stalk, often cutting it off right above the ground. Wireworms burrow into the soil and attack the underground stalk and roots, which can cause the plant to die. All of these insects can cause extensive damage and all must be monitored and dealt with in case they become too numerous. When Mr. Carroll hangs the tobacco to dry, his primary concern is tobacco aphids. These insects are not as harmful as they are pests. They do not eat holes in the stalks or leaves, but they feed on the sap from the shucked surfaces. They secrete a sticky substance with a sweet smell which is often referred to as "honeydew" and are known to carry disease that will cause the plant to die (Meyer, 2003). Carroll also has minor problems with ladybugs (Coccinella novemnotata). They do no harm to the tobacco and often eat the aphids. Some years there are hornworms present on the leaves when drying, although they are not as numerous at this stage and can usually be dealt with fairly easily.

Hoskins Carroll's farm mainly has a problem with flea beetles, cutworms, wireworms, and hornworms. The flea beetles, cutworms, and wireworms are seen during the early growth stage for the crop while the hornworms are seen in both the early and late growth stages. The insecticides that he uses are *Admire* for the flea beetles, wireworms and cutworms and *Orthene* for hornworms. If crops are damaged in any way, a growth retardant is applied. This helps to slow the plant's growth, giving it more time to heal itself and lessen the current amount of damage. Mr. Carroll uses sprayers to apply all insecticides and keeps records of which insecticides he used, how much was used, and how often they were used.

In order to deal with the aforementioned pests, several different insecticides are employed. Altogether, 24 different insecticides are used in varying degrees for different pests. Each insecticide amount is determined by the number of insects, usually measured in ounces, pints, and pounds. All of these insecticides are applied using four different methods: through the use of power spray equipment, drop lines, backpack/hand sprayers, and jugs. The insecticides must be measured accordingly. Overuse may cause harm not only to workers, but to the plant itself (Seebold, 2010). Additional insecticides may be used once the tobacco is hung. Most insecticides contain nicotine, a chemical found in the tobacco leaf. In recent literature, Early Woodland pipes have been tested for nicotine in order to determine the type of plant smoked throughout that time period.

#### Nicotine and Archaeobotanical Importance

In 2002, Sean M. Rafferty published on the use of nicotine during the Early Woodland period in order to identify a "Smoking Complex" (Rafferty, 2002:897). Rafferty used the residual nicotine found in the tubular pipes that had been excavated in 1958 at the Cresap Mound Site in West Virginia, an area which is home to the Adena culture (as is the entire Ohio Valley) to further the archaeobotanical knowledge of the temporal depth of the *Nicotiana species*. The radiocarbon dates taken at this site range from 2506+/-175 BP to 2020+/- 150 BP. The pipe used for the analysis was taken from the grave goods of burial 48 associated with this site.

The extraction process used by Rafferty was first defined by Gager et al. (1960:1021) and Zahlsen et al. (1994) and involved the use of methylene chloride, a soxhlet extractor, nitrogen, and a GC/MS HP5890. A methylene chloride solvent was added to the sample for extraction purposes. The soxhlet extractor served to ensure the sample absorbed the solvent. The nitrogen was employed to evaporate the methylene chloride and concentrate the sample. It was then run

through the GCSM which determines the chemicals present. Both a SCAN mode (a mode which scans all ions from 60m/z to 600m/z) and SIM mode (a mode which allows the researcher to concentrate on specific ion peaks) were employed. The retention time for nicotine was 12.9 minutes. At this time, three ions that are considered the "fingerprint" for nicotine were identified: 84, 133, and 161.

The first archaeological sample tested by the aforementioned process was a 19<sup>th</sup> century clay tobacco pipe from West Africa. The pipe tested positive for nicotine. The results helped to solidify the extraction and identification process. These results also show that tobacco has a "shelf-life" of at least a century (Rafferty, 2002:902). These results also coincided with the results from the Adena pipe. The identification of nicotine in the Adena pipe serves several functions. Firstly, it signified the use of tobacco prior to 400 BC at the Adena site, which is earlier than previously thought. Secondly, it is the first archaeobotanical evidence that the people of Adena culture used tobacco (Rafferty, 2002:905, 906).

This same method was also employed by Rafferty in his 2006 publication in order to identify the use of tobacco in northeastern North America. Rafferty used a sample obtained from the Boucher site in Vermont. The Boucher site is a prehistoric cemetery characteristic of the Middlesex complex, an Early Woodland complex primarily found in New York dating from 885+/-35 BC to 115+/-25 BC (Rafferty, 2006). Again, Rafferty sought to expand the archaeobotanical knowledge of plants not only in this area, but also for this time period. While the actual pipes could not be analyzed, the "preliminary site reports were available, and pipe residue samples were retained for analysis" (Rafferty, 2006:254). The residue used for analysis was obtained from a pipe situated with a flexed burial. This residue exhibited the same ion peaks as the Adena pipe and the Cresap Mound pipe. An AMS radiocarbon date for this context dates

to approximately 300 BC. This signifies that this pipe, along with its residue, "predates the earliest known archeobotanical evidence for tobacco in eastern North American by at least 500 years" (Rafferty, 2006:455).

The identification of residual nicotine will play a part in this project as well. The residual nicotine in the loft of the tobacco barn will serve to supplement a PMI template for this type of microenvironment.

#### **Taphonomy and PMI**

Taphonomy is a wide area of study and, therefore, I will only discuss that which pertains to my research. The PMI can be estimated by looking at the state of the remains and the "decompositional timetable" for the area in which they are found (Rhine et al., 1998). Five main stages of decomposition – fresh, bloated/early, active, advanced, and dry/remains – are generally used when estimating PMI (Anderson et al., 1996; Galloway et al., 1989; Rodriguez et al., 1983; Shean et al., 1993). These stages will be used to compare the rate of decomposition between the remains in the control barn and the remains in the tobacco and hay barns. Each of these stages has a different time frame in which it occurs. This time frame depends on the location and climate, i.e. whether the remains are in an arid environment, in a house or other enclosed location, or in July or December. Under exposed conditions in the summer, the fresh stage usually occurs at the time of death and lasts into the early stages of bloat.

The fresh stage is characterized by no odor and minimal insect activity. The second stage, or bloat stage, is characterized by a swelling of the abdomen due to the rising of internal gases. Oftentimes, the remains will roll over if they were originally positioned on their side. The skin has a marbled appearance and looks blackish-green. Insect activity begins to increase and several genera of flies start to lay eggs in open orifices. The end of this stage comes with the eruption of

the abdomen and the release of internal gases. In the active stage, a strong odor appears and insect activity is at its greatest point. The presence of first and second instars can be seen in all orifices. In the advanced stage of decomposition, the odor has receded and third and fourth instars appear and mature. The skin starts to lose its moisture and stretches across the bone. The last stage of decomposition, the dry/remains stage, sees no insect activity, little to no odor, and bones with tissue/cartilage still attached in some places (mostly at the ends of long bones or down the spine) (Anderson et al., 1996; Galloway et al., 1989; Shean et al., 1993).

Several different elements affect PMI, such as temperature, elevation, humidity, location (enclosed or exposed), environment (arid, shaded, exposed, etc.), and climate. All of these must be taken into account when determining a PMI. I have chosen to place all of the specimens in the shade, mainly because two of the three pigs in each setting will be in shaded areas. The temperature will vary in each location (outside on the ground, inside on the ground, and in the loft) due to the rising of heat, coverage, and wind. In a study done by Shean et al. (1993), the authors found that remains (pigs) decompose faster when left in an exposed area than when shaded. Elevation and temperature also play an essential role in the decomposition of remains. For example, when remains are left in dry areas at a high elevation and extreme heat, they tend to mummify faster. Decreased humidity also serves to mummify remains (Mann et al., 1990).

The taphonomic stages of natural mummification vary greatly with regard to all other stages. In these stages, the researcher is not looking at decomposition rates or bloating stages, but desiccation and preservation. Seventy-five percent of the human body is made up of water, which accounts for most of our weight. At the end of the desiccation process, that weight will be reduced by 70%-77%, thus resulting in a lighter set of remains (Quigley, 1998:17). A body must go through several stages in order to reach complete mummification.

Some people tend to think that the putrefaction process does not take place when remains naturally mummify. Putrefaction does take place. Bacteria infect the openings of the body and make their way to the inner organs. These organs begin to liquefy and escape through various openings, most notably through the mouth, nose, and anus. The brain liquefies and escapes through the foramen magnum (Dzierzykray-Rogalski, 1986; Quigley, 1998).

In the early stages of dehydration, Quigley (1998:17) states that a body will have "sunken eyes, sunken temporal areas, and shallow cheeks." The epithelium is the first layer to mummify, followed by the dermis, subcutaneous muscles, fasciae and muscles (Dzierzykray-Rogalski, 1986). The desiccation process begins around the scalp, eyelids, fingers, toes (i.e. places that have little water/fluid). Dzierzykray-Rogalski supports this statement by stating that the skin on the 'hard bony or chondral bedding" will be the first to mummify. Most of the features are preserved here (Dzierzykray-Rogalski, 1986:103).

After the organs have, for the most part, leeched from the body, the trunk will begin to mummify and stretch across the ribs. The skin and muscle of the limbs will quickly desiccate. When the flexor muscles of the hand desiccate quickly, the fingers are pulled toward the body, resulting in curled hand (Dzierzykray-Rogalski, 1986). A fully mummified body will be completely dry, hard to the touch, and light in weight (Quigley, 1998). The only reference to time and naturally mummified remains comes from a study done by Mann et al. (1990). They found that, after death, the skin of naturally mummified remains would be present for two to six years. They provided PMI for these types of remain. Although no PMI template exists for mummified remains, there are ways that these remains can be identified.

#### **Identification and Dating of Naturally Mummified Remains**

Identification of naturally mummified remains is simple. The fingers from a naturally mummified hand can be rehydrated by using Ruffer's solution, and fingerprints can be obtained (Haglund et al., 2002; Quigley, 1998). DNA samples can be taken from the remaining tissue and virgin teeth (teeth that have not been damaged in any way). If the tissue of the skull is well preserved, a sketch can be made. Throughout the years, dozens of naturally mummified remains have been identified through sketches (Dzierzykray-Rogalski, 1986; Haglund et al., 2002; Quigley, 1998). The usual method of dating ancient mummified remains, either natural or intended, is by radiocarbon dating (C14). Currently, no other dating technique has been used to date these types of remains.

#### **Chapter 3: Methods and Materials**

For this study, I used nine fetal pigs (*Sus scrofa*) in three different settings. Pigs were used because their anatomy closely resembles that of a human. All pigs were provided by the Swine Unit, a division of the Louisiana State University (LSU) Agricultural Center. These pigs died as a result of being still born or from being suffocated as a result of the mother rolling on top of them. They were wrapped in plastic grocery bags and frozen immediately after they died. They were then taken out of their original bags, double bagged in fresh grocery store bags, and placed in a Whirlpool freezer. After two weeks, they were transported to the McQuinn residence in Kentucky in two 45-quart Styrofoam ice chests. Dry ice was used in order to maintain the core temperature. Upon arrival in Kentucky, they were stored in a deep freezer where they remained, undisturbed, until May 30, 2010. They were kept in plastic bags and placed in lukewarm water. This brought the core temperature to room temperature. Once the pigs were thawed, they were refrigerated until the morning of June 1, 2010, after which they were transported to their respective sites.

All of the pigs were weighed and placed in barns located in Rogers, Kentucky (elevation 1217 feet above sea level), on June 1. None of the pigs was cleaned. There were three pigs per barn and each barn and pig had a different designation. The hay barn was designated Site 1, the control barn Site 2, and the tobacco barn Site 3. For each barn, there were three additional designations. The ground inside of the barns was designated A, the lofts B, and the ground outside the barns C. All pigs were enclosed in cages constructed from steel shelving and chicken wire in order to keep them safe from ravens, crows, rats, and outdoor animals such as coyotes. A hinged top was also constructed for easy access and the cages were secured to the ground with a stick of rebar. Each cage was 20 inches in length, 20 inches in width, and 12 inches in height.

Over the course of my research, I worked in three separate microenvironments, with each barn representing a different microenvironment. All barns were within two miles of one another. Mr. Hoskins Carroll's tobacco barn was built in the mid to late 1960s by a barn builder who lived in the community. The barn is made of hemlock wood which naturally deters insects. It is 108 feet in length, 40 feet in width, and 30 feet in height. Every summer for over 30 years, the Carroll's have tended and hung tobacco in this barn. Tobacco will not be harvested and hung in the barn until late August, 2010. The barn is less that 300 yards from the tobacco field; therefore, the residual nicotine in the barn as well as the nicotine currently being produced by the tobacco should have the same effect on the remains that the presence of hanging tobacco would have. Enough residual nicotine may be present in the barn, in addition to what is growing in the field, to result in naturally mummified remains. This barn has no stalls and is only used for tobacco. For the purposes of my research, a loft was constructed five feet from the roof (25 feet off the ground) using four wooden planks. It measured 48.5 inches in length and 35.5 inches in width.

Mr. Gary Sparks owns the hay barn that I used for this project. It was constructed of yellow pine in the mid-1990s. It is 34 feet high and 40 feet in length. Interiorly, there are seven stalls and one tack room. All of the stalls are ten feet in both length and width. The lofts fall approximately 12 feet from the ground and from there is another 24 feet to the roof, which is made of tin and has two sky lights. The loft used for this project was located toward the back of the barn where the roof came in at an angle. Five feet were measured from the roof to the loft at this angle in order to determine the placement of the remains.

Mr. Charles McQuinn owns the control barn. It was constructed in 1970 and is 36 feet wide, 54 feet in length, and 18 feet high. It is made from oak, white and yellow pine, and poplar lumber. This barn has five stalls, a front shed, a feed storage room, hay feeding room and tractor

and equipment store room. Horses are also kept in one stall. A loft was constructed five feet from the roof for this project. It measured 71 inches in length and 25 in inches in width.

Each site was visited three times a day and the locations and times rotated with every visit. An hour was spent at each site. For example, if I visited the tobacco barn at 6:00am, 12:00pm, and 6:00pm on the first day, then on the second day I visited it at 7:00am, 1:00pm, and 7:00pm the next. This rotation was maintained throughout the course of my research. Internal temperatures were recorded using an internal thermometer. During each visit, I collected flies, larvae, maggots, and beetles. The flies were caught with a standard trapping net, transferred into freezer bags, and placed in the freezer. The maggots and larvae were collected with a pair of soft tweezers and were placed in vials with a solution of 70% alcohol and 30% water. Each vial was labeled by site, date, and content. The beetles were also collected with a pair of soft tweezers and were placed in plastic containers and labeled in the same manner as the previous collections. All beetle and flies were placed in special packaging boxes and sent to the Maryland Agricultural Research Service – Plant Sciences Institute for Identification Systematic Entomology Laboratory - Taxonomic Services Unit for identification. These were compared with the insects normally associated with tobacco and hay to see if they were the same or different. In order to measure the rate of mummification, I looked at the amount of dehydration that occurred. Pictures of each stage were taken and cataloged daily. Remains were labeled as mummified if desiccated skin was present at the end of the study. Decomposition stages were assigned using the criteria set forth by Anderson et al. (1996), Galloway et al. (1989), and Shean et al.(1993). A multivariate analysis of variance (MANOVA) was performed in order to determine a significance between the rate of decomposition, barn, and local.

Weather data were also collected using a handheld Kestrel and standard mL rain gauges

placed within 20 feet of both the hay and tobacco barns. The rainfall for the control barn was recorded by an electronic rain gauge located on the McQuinn's property. The maximum and minimum temperatures recorded for these sites were taken with magnesium-filled magnetic thermometers placed in shaded areas at each sub-site. All collected data were analyzed using SAS® and a basic *proc means* procedure.

At the end of this project, five samples of wood were taken from the central supporting beam closest to the constructed loft. Each sample was taken in five-foot intervals. The samples were then taken to the Agriculture Chemistry Department at LSU for analysis. The goal was to determine the amount of nicotine present on the surface level of the wood. Each sample was weighed and then placed in individual beakers; approximately 40mL of Ethyl Acetate solvent was added for extraction purposes. The Ehtyl Acetate solution was placed in a Sonicator for two hours. The Sonicator is used to extract the nicotine. It causes a vibrating action which facilitates the extraction of the nicotine from the wood. A funnel, lined with a filter with sodium sulfate added, is placed in the top portion of a 15 mL test tube. Soduim sulfate is used to remove moisture from the sample. After this is done, the sample is then taken to an N-Evap (nitrogen evaporator) machine. Essentially, this is a water bath at 35 degrees Celsius with a steady stream of nitrogen which serves to concentrate the sample. The sample was concentrated to 1mL. After this process is finished, the liquid sample is then removed and placed into an Autosampler Vial, capped, and placed on the Gas Chromatographer Mass Spectrometer Detector (GCMSD). This is an Agilent 5975 Mass Spectrometer Detector (MSD) equipped with an HP 5890 Gas Chromatographer (GC) system.

Once the sample is placed into the GC, it undergoes vaporization. The liquid is injected into the GC where it separated on an RTX-35SilMS Column (30m x .25mm x .25uL) silica

capillary column in a GC oven. The temperature program of the oven is as follows: set at 60 °C. Hold for one minute. Increase the temperature ramp at 12 °C per minute up to a final temperature of 340°C. Hold for 2.67 minutes. In the end, the total run time is 27 minutes. The inlet temperature was held at 280 °C. The injected sample is separated by its affinity between the gas phase and the solid phase of the column and elutes at a retention time of 10 minutes and 46 seconds. From there, it moves into the connected MSD. An MSD contains an ion chamber and magnetic field. Here, electrons are released into the nicotine compounds, breaking them apart and making fragments, or ions.

Approximately 10 minutes and 46 seconds passed before the ion peaks for nicotine were obtained. The MSD was run in a SCAN mode (a mode which scans all ions from 60m/z to 600m/z) and a Select Ion Monitoring (SIM) mode (a mode which allows the researcher to concentrate on specific ion peaks). During SIM mode, a quantitation ion (an ion with the highest resolution) is chosen, as is/are qualifier ion(s) (an ion which holds less resolution than the quantitation ion, but falls within a certain percentage ratio to the quantitation ion). Three criteria must be met in order to identify nicotine: correct retention time of 10.46, ions 84, 133, 162, 161 must be present, and the ions must have the correct ratio to the quantitation ion. These three ions -84, 133 and 161 – will appear only if nicotine is present. After the ions were identified, the amount of nicotine present was determined by using the following formula provided by Nina Huffstetler of the Agriculture Chemistry Laboratory at Louisiana State University:

All of the samples were analyzed in this manner.

#### **Chapter 4: Results**

This chapter is comprised of three sections. The first section includes a basic description of the main site and a brief synopsis of what transpired at each sub-site. The tables included provide a comprehensive timeline of the activity at each sub-site. The second section contains a comparative analysis of the insects and insect activity found at each sub-site along with the weather data collected. The third section includes the nicotine analysis performed on the wood samples taken from the tobacco barn. All analyses are discussed in greater detail in Chapter Five.

#### Hay Barn (Site 1)

The hay barn for this project belonged to Mr. Gary Sparks, Wolfe County's local embalmer. It is approximately 20 feet high. The barn door faces south. The sun rises on the east side of the barn and sets on the west side. The barn sits on a geographical shelf. A steep incline makes up the area to the east side of the barn while a steep decline makes up the west side. The north side of the barn is relatively flat, eventually sloping down into a field. Horses are primarily kept in this area. The inside of the barn consisted of two lofts and six stalls. Horses were housed in three of the stalls during rainy weather. Although hay was always present in the barn, bales of hay were not stored in the barn until half-way through the project. The bales were placed in a loft opposite the loft containing one set of remains.

#### **Remains on the Ground Inside the Hay Barn (1A)**

This site was located inside the barn in a stall. The stall, which was originally a horse stall, was modified by the addition of hay (Figure 1). This was the only sub-site that required preparation. After much speculation, a decision was made to plasce the hay underneath the remains instead of on top of them, due primarily to the fact that insect observation and collection would be easier without the obstruction of hay.


Figure 1. Preparation of Sub-site 1A

The distance between sub-sites located inside the barn on the ground (1A), in the loft (1B), and outside the barn on the ground (1C) was great enough so as to not cause an overlap of insect activity. After a small section of the floor was covered in hay, the cage with the 2.125 pound pig inside was placed in the stall. The stall was completely closed and locked when no one was present. Figure 2 provides a look at the progression of decomposition, while Table 1 summarizes the stages of decomposition for this sub-site.

By the second day, the remains were in the bloat stage and lingered in this stage until the fourth evening. The epidermis also remained intact until the evening of the fourth. At this time, the chest to the mandible had split, and instars had invaded the chest cavity, mouth, and the area posterior to the exposed ear.

On the morning of the fifth day, the epidermis on the head appeared to have lost moisture. I assumed that this could be the early stages of mummification, but later, on the morning of the seventh, the skin had sloughed off. On the 13<sup>th</sup> day, the skeletal remains were completely void of decomposing tissue. Instar activity continued into the evening of the eighth, at which time the remains were classified as dry.



**Figure 2.** Visual decomposition of remains located inside the hay barn on the ground (1A). (1) Fresh – Day 1; (2-3) Bloat – Days 2-4; (4) Active – Days 4-6; (5) Advanced – Days 7-8; (6) Dry – Days 9-30

Table 1. Activity	chart for r	emains lo	ocated i	inside t	he hav	barn on the	e ground (	1A)

Day	Stage		Eggs/Instars	Insects	Odor
1	Fre	esh	-	-	-
2	Blo	oat	Present	Minimal Fly	None
3	Blo	oat	Present	Minimal Fly	None
4	Bloat	Active	Present & Burrowing	Minimal Fly	None
5	Act	ive	Present & Burrowing	Minimal Fly	Slight
6	Act	ive	Present & Burrowing	Minimal Fly	Slight
7	Adva	nced	Present & Burrowing	Minimal Fly	Slight
8	Adva	nced	Present & Burrowing	Minimal Fly, Gnats	Slight
9	D	ry	Burrowing	None	None

# Remains in the Loft of the Hay Barn (1B)

The second site was located in the loft of the barn approximately 54 inches from the roof. The pig for this site weighed 2.875 pounds. Figure 3 provides a look at the progression of decomposition, while Table 2 summarizes the stages of decomposition for this sub-site.





**Figure 3.** Visual decomposition of remains in the hay loft (1B). (1) Fresh – Day 1;(2) Bloat – Days 2-3; (3) Active – Days 4-5;(4) Advanced – Days 6-7; (5) Dry – Days 8-30

Day	Stage	Eggs/Instars	Insects	Odor
1	Fresh	-	-	-
2	Bloat	Present	Minimal Fly	None
3	Bloat	Present	Minimal Fly	Slight-
				Strong
4	Active	Present	Minimal Fly, Black Beetle	Strong
5	Active	Present & Burrowing	Maximum Fly	Strong
6	Advanced	Present & Burrowing	Minimal Fly	Slight
7	Advanced	Present & Burrowing	Minimal Fly	Slight
8	Dry	Burrowing	Minimal Fly	None

**Table 2.** Activity chart for remains located in the loft of the hay barn (1B)

The remains had progressed from fresh to bloat by the second morning. Marbling was still present and a slight odor could be detected as I neared the cage on the third day. The skin had split between the hindlimbs, although the epidermis was the only layer that had been penetrated. By the end of the third day, the skin on the chest had started to slough off and active decomposition had begun.

The morning of the fourth day saw significant changes. The top layer of the facial epidermis was gone. The remaining layers of epidermis had turned black, as had the abdomen, and rear area. The skin on the exposed portion of the remains had turned grey. The first layer of the epidermis was still present on the abdomen. The area between the forelimbs had been taken down to the muscle. That evening, the abdomen and stomach had split toward the hindlimbs. The epidermis surrounding the rear had also started to peel back from the tail toward the hindlimbs. By the fifth morning, all bones with the exception of the hindlimbs were exposed. The epidermis had split and was peeling back from the abdomen. Skin was still present on the skull. The remains had started to progress into the dry stage by the morning of the seventh. Skin could be found in small patches on the exposed head as well as around the tail. A ligament was still present attaching the head to the tail. On the eighth day, the remains were classified as dry.

### **Remains on the Ground Outside the Hay Barn (1C)**

This site was located to the west of the barn outside on the ground. A downgrade made up this side of the barn leading into a fenced-in field. Five horses and one colt were present at the time of this study. Along with the horses, deer were sighted several times. Copperheads were also present throughout the study, although none was spotted around the pig.

A ledge had formed over the years and runs along the edge of the barn. Horse manure made up the first 10 feet of the ledge. In order to avoid extra variables, a decision was made to place the cage at minimum of five feet behind the manure and four feet from the left, rear corner of the barn. A hi/lo thermometer was placed on the lower portion a fence post behind the cage, so as to avoid sunlight. The *Sus scrofa* weighed approximately 2.215 pounds. After the initial weighing, it was placed in the cage and left until the second day. Figure 4 provides a look at the progression of decomposition, while Table 3 summarizes the stages of decomposition for this sub-site.

This site was visited only once on the second day due to time constraints. That morning the pig was well into the bloat stage and going into active. By early afternoon on the third day, the pig had progressed well into the active stage. This activity carried over into the fourth day. By the fifth day, advanced decomposition had taken place. The epidermis was only present around the upper portion of the face, fore and hind limbs, rear area, neck, and chin.

On the sixth day, the moist epidermis was present around the hooves, head, rear, and shoulders. On the morning of the eight day, the remains were classified as "dry". Skin was still present around the rear, hooves, and head, but insect activity had diminished significantly and almost no instars were present. The skin remained on the rear for the next three days, after which it was subsequently washed from the bones due to rainfall. The skin remained around the head



5

Figure 4. Visual decompositon of remains located outside the hay barn on the ground (1C). (1) Fresh – Day 1; (2) Bloat – Day 2; (3) Active – Days 3-4; (4) Advanced – Days 5-7 (5) Dry – Days 8-30

Table 3. Activity chart for remains located outside the hay barn on the ground (1C)

D	<u>a</u> ,		<b>•</b> • • • • • • • • • • • • • • • • • •	01
Day	Stage	Eggs/Instars	Insects	Odor
1	Fresh	-	_	-
2	Bloat	Present	Minimal-Moderate Fly, Ants	None
3	Active	Present	Moderate Fly, Carrion Beetles,	Slight
			Ants, Spiders	
4	Active	Present	Maximum Fly, Carrion Beetles,	None
			Black Ants	
5	Advanced	Present	Maximum Fly, Carrion/Rove	Slight
			Beetles, Butterflies, Ants	
6	Advanced	Present	Minimal Fly, Black Ants, Black	Slight
			Beetles, Bees	
7	Advanced	Present & Burrowing	Minimal Fly, Ants, Gnats, Rove	None
			Beetles	
8	Dry	Burrowing	Minimal Fly	None

until the evening of the 13<sup>th</sup>. By the morning of the 15<sup>th</sup>, the remains had completely skeletonized.

## **Control Barn (Site 2)**

Mr. Charles McQuinn owned the barn in which I set up the variables used for the control barn. It is approximately 18 feet high. The barn door faces north. The sun rises on the east side of the barn and sets on the west side. The barn is located in the middle of a field occupied by two horses. The east side of the barn slopes downward into the open field. The ground immediately on either side of the barn is level. This particular barn was split into several sections. The northeast side of the barn contained two stalls, neither of which was in use during this experiment. The west side of the barn was comprised of a tackle room, a fair size stall for the two horses, and a stall for hay. The southwest side consisted of an open area used for the storage of hay and the southeast side was made up of another stall used primarily for storage. The southeast stall, the northwest corner, and the northwest stall were all employed during this project. The southeast stall did contain a bale of hay, although it never came into contact with the remains and it was situated far enough from the remains so as to not affect the study.

## **Remains on the Ground Inside the Control Barn (2A)**

The remains for this sub-site were placed in the southeast stall of the barn on the ground. The remains weighed three pounds. No adjustment was made to the sub-site. The cage and remains were located 25 inches from the east side of the wall. The dirt underneath the remains was dry at this time. This stall also had a gate so that the horses and other large animals could not enter at any time. Figure 5 provides a look at the progression of decomposition, while Table 4 summarizes the stages of decomposition for this sub-site.



**Figure 5.** Visual decompositon of remains located inside the control barn on the ground (2A). (1) Fresh; (2-3) Bloat; (4) Active; (5) Advanced; (6) Dry

Iubic	Tuble "Theirity chart for femalis focuted inside the control ball of the ground (217)				
Day	Stage	Eggs/Instars	Insects	Odor	
1	Fresh	-	-	-	
2	Bloat	Present	Minimal Fly	None	
3	Bloat	Present	Minimal Fly	None	
4	Bloat	Present	Minimal Fly, Ants	Slight	
5	Active	Present	Maximum Fly, Black Ants	None	
6	Active	Present & Burrowing	Minimal Fly, Black Ants,	None	
			Carrion Beetles		
7	Active	Present & Burrowing	Minimal Fly, Carrion Beetles,	None	
			Spider, Black Ants		
8	Advanced	Present & Burrowing	Minimal Fly, Carrion Beetles	None	
9	Dry	Burrowing	Minimal Fly	None	

Table 4. Activity chart for remains located inside the control barn on the ground (2A)

The remains were well into bloat by the second day, and minimal fly activity was observed. This continued until day five when the remains reached the active stage of decomposition. At this time, maximum fly activity was recorded. On the sixth day, the remains rolled to the back of the cage and the skin on the abdomen split. Carrion beetles started to arrive and were present until the remains reached the advanced stage of decomposition on the eighth day. On the ninth day the remains were classified as dry.

Skin from the dorsal area of the *Sus scrofa* was the only tissue that remained at this time. The skin around the hooves and the skin located at the back of the cage were still moist, as was the ground behind the cage. The skin on the bottom of the cage, however, was dry. This location was visited every other day in order to take pictures and observe the skin left in the cage. No significant changes were noted throughout the duration of this observation. The remaining skin never decomposed, although it retained or lost moisture in conjunction with weather conditions.

## **<u>Remains in the Loft of the Control Barn (2B)</u>**

The remains for this sub-site were placed in a loft fashioned from two planks that were lying in the nearby stall. These planks were placed on top of the stall wall and extended the entire length of the stall. A two-inch gap lay between the two planks. The loft was 54 inches from the room and 64 inches off the ground. The remains weighed three pounds. Figure 6 provides a look at the progression of decomposition, while Table 5 summarizes the stages of decomposition for this sub-site. Although fresh on the first day, the remains had proceeded into the bloat stage by the second day. The umbilical cord was present and, although moist on the first day, was completely dry by the second day. Bloat extended in to the third day. The skin at the exposed neck as well as the skin between the hind limbs had started to split.



**Figure 6.** Visual decomposition of remains located in the loft of the control barn (2B). (1) Fresh – Day 1; (2) Bloat – Days 2-3; (3-4) Active – Days 4-6; (5) Advanced – Day 7; (6) Dry – Days 8-30

Table 5. Activit	y chart for rema	ins located in the	e loft of the contro	ol barn (2B)
------------------	------------------	--------------------	----------------------	--------------

Day	Stage	Eggs/Instars	Insects	Odor
1	Fresh	-	-	-
2	Bloat	Present	Minimal Fly	None
3	Bloat	Present & Burrowing	Minimal Fly	Slight-
				Strong
4	Active	Present & Burrowing	Minimal Fly	Strong
5	Active	Present & Burrowing	Minimal Fly, Carrion Beetles,	Strong
			Black Ants, Granddaddy	
			Longlegs	
6	Active	Present & Burrowing	Minimal Fly, Carrion Beetles	Slight
7	Advanced	Present & Burrowing	Minimal Fly, Black Ants	Slight
8	Dry	Burrowing	Minimal Fly, Black Ants	Slight

By that evening, active decomposition had begun. At this time, instars were observed slipping through the planks to the ground below.

Activity reached its peak on days four and five. On the fourth, seepage was present at the abdomen, hind limbs, forelimbs, and both ground contact and facial region. The active stage of decomposition continued into that evening. The fifth day saw the arrival of exposed bone. The ribs, shoulder blades, upper arms, and vertebrae were all exposed. The abdomen had completely split from the rear to the mouth, although the remains did not change in their positioning.

The activity level had greatly decreased by the morning of the sixth. The remains were very moist. The only intact piece of skin ran from the rear to the posterior portion of the head. Ninety percent of the bones were exposed at this time. At this point, the remains had reached the advanced stage of decomposition and were rapidly proceeding into the dry stage. By that evening, the skin, with the exception of the skin around the hooves, was dry.

All bones were exposed on the morning of the seventh, but a minimal amount of instar activity was still present in the skin around the hooves. The remains were classified as dry on the eighth. Minimal fly activity was observed from the bloat stage until the dry stage of decomposition. Carrion beetles were only present on the fifth and sixth days in the stall below the loft. No carrion beetles were observed in the loft.

#### <u>Remains on the Ground Outside the Control Barn (2C)</u>

The remains for this sub-site were located outside the northwest corner of the barn and weighed two-and-a-half pounds. The hi/low thermometer was placed in the shade under the overhang of the barn above the cage. The cage was placed in a corner created by the barn wall and wooden loading ramp. This placement served to deter the horses from disturbing the remains. Figure 7 provides a look at the progression of decomposition, while Table 6

summarizes the stages of decomposition for this sub-site. One fly, possibly a Muscidae, landed on the remains as soon as they were placed in the cage.

Bloat had occurred by the second day. Marbling was evident around the abdomen and hind limbs. Bloat continued into the third morning. The marbling had spread across the abdomen to the exposed shoulders and face. By that evening, the remains had entered the active stage of decomposition. The skin had taken on a moist sheen, and the bones comprising the snout and mouth were becoming visible.

The active stage of decomposition continued into the fourth day. The skin was still moist and ranged in color from black to purple to blue. Some of the maxillary bones and teeth were exposed and lay on the bottom of the cage. That evening, the skin on the remains had turned a molted green and pink, but the black around the abdomen remained unchanged. The skin had split from the exposed shoulder to the ground contact rear portion of the remains. The mandible and most of the teeth were exposed at this time. Pieces of the skull and pelvis were also visible.

A vulture was roosting on the roof of the barn directly above the remains on the fifth morning. The remains and cage appeared to be untouched. Skin was still present on the skull, fore and hind limbs, and the exposed portion of the proximal abdomen and shoulder. The majority of the skin present during that morning's observation was still in a good state of preservation that evening. The remains had progressed into the advanced stage of decomposition on the sixth evening and were classified as dry on the seventh. Skin remained intact around the rib cage, cranium, fore and hind limbs for the next six days.



Figure 7. Visual decomposition of remains located outside the control barn on the ground (2C). (1) Fresh – Day 1; (2) Bloat – Days 2-3; (3) Active Days 3-5; (4) Advanced – Day 6; (5-8) Dry – Days 7-30

Day	Stage		Eggs/Instars	Insects	Odor
1	Fre	esh	-	-	-
2	Blo	oat	Present	Minimal Fly	None
3	Bloat	Active	Present	Minimal Fly	Slight-
					Strong
4	Act	ive	Present	Moderate Fly, Carrion Beetles,	Strong
				Gnats, Ants	
5	Act	ive	Present	Maximum Fly, Butterfly	Strong
6	Adva	nced	Present & Burrowing	Minimal Fly, Ants, Rove Beetles	Slight
7	D	ry	Burrowing	Minimal Fly, Rove Beetles	Slight

**Table 6.** Activity chart for remains located outside the control barn on the ground (2C)

After the 13<sup>th</sup>, only hair remained on some of the bones, most notably the ribs. By the morning of the 17<sup>th</sup>, virtually no hair and skin remained.

## **Tobacco Barn (Site 3)**

For my third sub-site, I employed Hoskins Carroll's tobacco barn. The barn stands approximately 30 feet high and has no natural lofts. Mr. Carroll graciously allowed a loft to be built in his barn specifically for this project. The loft was constructed by Lonnie Sparks. It was 25 feet from the ground and five feet from the barn roof. The platform was 35.5 inches long and 48.5 inches wide. The north doors remained open throughout much of this project. For this reason, the remains located in the loft and inside the barn on the ground (3B and 3C) were placed toward the south end of the barn. The barn for this site also had slats that could be opened in order for the tobacco to dry out. The base of the barn fell six inches short of connecting to the dirt floor. This also allows the tobacco to dry quicker. The remains located inside the barn on the ground (3A) were placed in the southwest corner of the barn less than 1.5 feet from this opening.

## Remains on the Ground Inside the Tobacco Barn (3A)

This sub-site was located in the southeast corner of the barn on the ground. The remains, which weighed two pounds, were placed out on June 1, 2010. Figure 8 provides a look at the progression of decomposition, while Table 7 summarizes the stages of decomposition for this

sub-site. Within a half hour of setting out the remains, 13 flies had lighted on the pig and were laying eggs. The remains were swarming with flies within the hour. The remains were in the bloat stage of decomposition by the second day. The skin around the abdomen was tight and had taken on a bluish hue in some areas. By the third morning, the first layer of epidermis between the forelimbs had split. That evening, the skin had split in the abdominal region in a line that ran toward the pelvis.

The morning of the fourth brought some drastic changes. The skin around the facial region was almost gone. The mandible and palate were exposed. The remains were also emitting an odor that could be sensed outside of the barn wall. That evening, the skin on the remains started to decompose quickly. The palate was now completely exposed, although it was still being held together by a thin ligament stretching from the anterior portion of the skull to the underside of the chin. The exposed bones were mostly dry. The skin stretching from the skull to the shoulder was translucent.

The greatest amount of activity occurred on the fifth morning. The skin on the face had stretched tight around the skull and appeared to be in the process of drying. This piece of skin, aside from the minute amount on the hooves, was the only skin left on the remains. By that evening, the skin on the skull had completely dried and formed a helmet around the cranial bones. Dry skin was also found where the rear of the *Sus scrofa* once lay. The remains were classified as dry on the seventh. Dried skin was located around the skull, rear area, and around the vertebral area of the remains. This remained unchanged for the duration of the project.





**Figure 8.** Visual decomposition of remains located inside the tobacco barn on the ground (3A). (1) Fresh – Day 1; (2) Bloat – Days 2-3; (3) Active – Days 4-5; (4) Advanced – Day 6; (5) Dry – Days 7-30

<b>Table 7.</b> Activity chart for remains located inside the tobacco barn on the ground	(3A)
--	------

Day	Stage	Eggs/Instars	Insects	Odor
1	Fresh	-	-	-
2	Bloat	Present	Minimal Fly, Rove Beetles	None
3	Bloat	Present	Minimal Fly, Carrion Beetles,	None
			Ants	
4	Active	Present	Moderate-Maximum Fly, Ants,	Strong
			Rove & Carrion Beetles,	
5	Active	Present & Burrowing	Minimal Fly, Carrion & Black	None
			Beetles, Ants	
6	Advanced	Present & Burrowing	Minimal Fly, Carrion and Black	None
			Beetles	
7	Dry	Burrowing	Minimal Fly, Carrion Beetles	None

## **Remains in the Loft of the Tobacco Barn (3B)**

This sub-site was located in a loft approximately 25 feet off of the ground. A wooden ladder located at the sub-site connected each rafter and was built as part of the original construction of the barn. The remains weighed three pounds and were placed in a cage and situated in the loft on June 1, 2010. A portable, electric lantern was placed in the loft as well. Whenever pictures were taken, the lantern was placed in the cage with the remains so as to provide enough light for the camera to focus and capture the images. On the fifth day, the instars moved away from the light the lantern emitted. No reason exists for this. Figure 9 provides a look at the progression of decomposition, while Table 8 summarizes the stages of decomposition for this sub-site.

The activity that was occurring during day two was unexpected. The skin had pulled tight around the abdomen and was turning shades of purple and black not only there, but also around the shoulder and chest. The skin on the inferior portion of the pig was moist and sticky, but in some areas it appeared to be losing moisture and drying out. The umbilical cord was also sticky. The remains looked as if they were shedding in certain areas, such the exposed abdomen, shoulder, and ground contact abdomen. The first layer of the epidermis was drying out in areas, giving it the appearance of snake skin. While looking for evidence of fly activity (i.e. egg masses), I rolled the *Sus scrofa* carefully onto its back. When I did this, the first layer of the epidermis split, exposing the second layer of the epidermis in the aforementioned areas. This did not occur on or around the face and was only minimally visible around the hind limbs.



**Figure 9.** Visual decomposition of remains located in the loft of the tobacco barn (3B). (1) Fresh – Day 1; (2) Bloat – Days 2-3; (3-4) Active – Days 4-6; (5) Advanced – Days 7-8; (6) Dry – Days 9-30

Day	Stage	Eggs/Instars	Insects	Odor
1	Fresh	-	-	-
2	Bloat	Present	Moderate Fly	None
3	Bloat	Present	Minimal Fly	Slight
4	Active	Present	Moderate Fly, Granddaddy	Strong
			Longlegs	
5	Active	Present & Burrowing	Minimal Fly	Strong
6	Active	Present & Burrowing	Minimal Fly	Strong
7	Advanced	Present & Burrowing	Minimal Fly	Slight
8	Advanced	Present & Burrowing	Minimal Fly	Slight
9	Dry	Burrowing	Minimal Fly	None

Table 8. Activity chart for remains located in the loft of the tobacco barn (3B)

Bloat continued into the third day. The abdomen was tight, but not distended. A slight odor could be detected when leaning over the remains. The first layer of epidermis that had split the day before was dry in some places. That evening, the abdomen was more swollen, and the fore and hind limbs were no longer contacting one another. The remains looked as if they would roll, but this did not happen.

The *Sus scrofa* was rapidly progressing into the active stage of decomposition by the fourth day, although the abdomen was still swollen. The amount of instar activity increased throughout the day. During this time, instars began to leave the remains and drop to the ground 25 feet below. Active decomposition continued into the fifth day. Aside from the hooves, no flesh could be seen. All bones were now exposed, and many of them had been moved to the outside of the cage by the instars. This activity continued into the sixth day.

On the seventh day, the remains entered the advanced stage of decomposition. The remains were not classified as dry until the ninth morning because instars were still present on the boards under the remains and in the hooves.

## Remains on the Ground Outside the Tobacco Barn (3C)

This sub-site was located outside on the west side of the barn. The cage was situated eight feet away from the corner of the barn. A compost pile for tobacco stalks lay three feet behind the remains. The remains weighed approximately one-and-a-half pounds. Figure 9 provides a look at the progression of decomposition, while Table 8 summarizes the stages of decomposition for this sub-site. Flies were present within 15 minutes of placing the remains in the cage.



**Figure 10.** Visual decomposition of remains located outside the tobacco barn on the ground. (1) Fresh – Day 1; (2) Active – Days 2-3; (3) Advanced – Day 4; (4) Dry – Days 5-30

	2		U V	
Day	Stage	Eggs/Instars	Insects	Odor
1	Fresh	-	-	-
2	Active	Present	Maximum Fly, Bees, Ants	None
3	Active	Present	Maximum Fly, Bees, Ants,	None
			Rove/Carrion Beetles, Stink	
			Bug, Yellow Jacket, Granddaddy	
			Longlegs	
4	Advanced	Present & Burrowing	Minimal Fly, Bees,	Strong
			Carrion/Black/Rove Beetles	
5	Drv	Burrowing	Minimal Fly, Ants	None

Table 9. Activity chart for remains located outside the tobacco barn on the ground (3C)

The *Sus scrofa* was the only one in which bloat was not observed. On the second day, the remains were covered with insects. The greatest amount of decomposition had taken place around the head. The bones of the mandible and palate were visible at this time. By the third morning, all of the bones in the facial region were visible and the remaining skin on the head was holding them in place.

By the fourth morning, no skin was left, with the exception of the hooves and forelimbs. A strong odor was present 10 feet away from the cage. The remains appeared to have rolled over sometime during the night as the exposed fore and hind limbs were now located at the back of the cage. The remains were classified as dry on the fifth morning. Some of the bones had been moved outside of the cage, presumably by instar activity.

A multivariate analysis of variance (MANOVA) was used to test the significance of the decomposition rates between the barns and locations of each sub-site. This analysis was performed in SAS® and a type III sum of squares, or explained sum of squares, model was used. A type III sum of squares model measures how much variation exists in the modeled values and then compares that to the total sum of squares model, which measures the variation in the observed values. The table below shows the statistical analysis of the bloat, active, and advanced stages of decomposition (Table 10). The data used for these variables consisted of the total number of days of each stage. The fresh and dry stages were not included because each fresh stage lasted only one day and the dry stage signified the end of the decomposition stages.

Variable	Source	DF	SS	Type III SS	MS	F Value	Pr>F
Bloat	Model	4	4.4444		1.1111	4.00	0.1040
	Error	4	1.1111		0.2778		
	Barn	2		1.5556	0.7778	2.80	0.1736
	Local	2		2.8889	1.4444	5.20	0.0772
Active	Model	4	1.1111		0.2778	1.00	0.5000
	Error	4	1.1111		0.2778		
	Barn	2		0.2222	0.1111	0.40	0.6944
	Local	2		0.8889	0.4444	1.60	0.3086
Advanced	Model	4	3.1111		0.7778	2.80	0.1713
	Error	4	1.1111		0.2778		
	Barn	2		2.8889	1.4444	5.20	0.0772
	Local	2		0.2222	0.1111	0.40	0.6944
DF = Degrees of Freedom $SS = Sum of Squares$ $MS = Mean Square$							uare

 Table 10. MANOVA of Decomposition Rates Between Barns and Local

The two sources that were measured were barn, which represents the tobacco barn, control barn, and hay barn, and local, which represents all sub-sites. In order for the rate of decomposition for these sources to be significant, the p-value had to be less than 0.05. The values in blue show no statistical significance. Only two p-values were close to being statistically significant (yellow): 1) the duration of the bloat stage of decomposition and the location of the remains within the barn (p = 0.0772), and 2) the duration of the advanced stage of decomposition and the barns in which the remains were placed (p = 0.0772). These p-values suggest that location and placement of the remains for this study had no impact on the rate of decomposition. This is the only study that has been performed using these setting and conditions. Perhaps significance would exist if more studies were performed using the same conditions and settings and the data from those studies, in conjunction with the data from the present study, were compared using a MANOVA analysis.

#### **Insects**

Insects were collected on the second day of the project for all sites. Although all barns had a considerable amount of insect activity, the amount of activity varied from site to site and from day to day. Three hundred and three flies were submitted for identification. Each identification listed is represented by its species and/or genus name. All are from the order Diptera and the families are Calliphorida, Muscidae, and Sarcophagidae. The genus of the Muscidae species listed could not be identified because no specialist was available for that species. The Sarcophagidae listed required dissection or slide mounting, and only one of the seven was identified as a Ravinia.

Of the 11 Diptera present, only *Phormia regina* was found at all sub-sites (green), with the exception of the loft in the tobacco barn (3B) (Table 11). *Ravina* was only present at sub-site

1A (yellow), while *Pollenia pediculate* was only found at sub-site 2C (red) and *Lucilia cluvia* at sub-site 3A (blue). *Lucilia coeruleiviridis* was present at all loft sub-sites (1B, 2B, 3B) (gold) as well as at the tobacco barn (Site 3). *Lucilia sericata* was present at all sub-sites inside the barns on the ground (1A, 2A, 3A) (pink) and at the control barn (Site 2).

Тахопоту	1A	1B	1C	2A	2B	<b>2</b> C	3A	3B	<b>3</b> C
Ravinia	х								
Calliphora vicinia Robineau-Desvoidy	x		х	x	x				
Lucilia illustris Meigen	х		х		x				х
Lucilia coeruleiviridis Macquart	x	х			x	x	x	x	x
Phormia regina Meigen	x	x	х	x	x	x	x		x
Sarcophagidae - Dissection/Slide Mount. Req.			х				х		х
Lucilia sericata Meigen	х		х	х	х	х	х	х	
Muscidae - No Specialist Available	х	х	х	х	x	х			
Calliphora vomitoria Linnaeus				х		х	х		х
Pollenia pediculate Macquart						x			
Lucilia cluvia Walker							х		

**Table 11.** Diptera taxa collected at all sub-sites.

Diptera identifications by Norman E. Woodley, Systematic Entomology Laboratory, Agriculture Research Service, US Department of Agriculture.

All of the Coleoptera collected overlapped with the exception of Staphylinidae

*Creophillus maxillosus* (yellow) (Table 12). Silphidae *Silpha americana* was most prevalent at Site 2 (pink), but also overlapped with Site 1 and 3. The same can be said for Histerdae (blue). Beetle larva (green) was present at all sub-sites with the exception of sub-site 3B (the loft in the tobacco barn). The main reason for this is the make-up of the floor beneath the loft. In the tobacco barn, the floor consists of dry, hard-packed dirt. When the instars fell from the remains to the floor of the barn, they tried to migrate to an area that provided them with a chance to pupate. Unfortunately, most of the instars "dried-out" and died before they could pupate. The same can be said with the seepage from the remains. In this case, the beetles were not attracted to the seepage because it dried too quickly. With the other lofts, seepage from the remains fell to

the moist dirt beneath it, attracting several families of beetles. The beetle eggs laid in these areas

survived.

Taxonomy	1A	1B	1C	2A	2B	2C	3A	3B	<b>3</b> C
Beetle Larvae	X	x	х	X	X	X	x		x
*Silphidae Silpha americana			х	х	x	х	х		х
*Silphidae Oiceoptoma				x		х	x		х
*Staphylinidae Creophillus maxillosus			x						
*Scarabeidae						х			х
*Necrodes surinamensis		x		x			x		
*Histerdae			x	х	X	х	x		
*Dermestidae		x	x				x		х
*Carabidae		x		x					x
*Platydracus					х				х
*Hemiptera Heteroptera			х					x	X
*Halyomorpha halys				x					х

 Table 12. Coleoptera taxa collected at sub-sites.

Coleoptera identifications by Geoffrey White, Systematic Entomology Laboratory, Agriculture Research Service, US Department of Agriculture. \*Tentative Identification

Sub-site 1A held the lowest amount of fly activity for this site, and it varied throughout

the duration of this project (Table 13). Lucilia comprised the majority of the flies at this site.

This sub-site entered the active stage on day four, but at no time were more than five flies

present. The majority of fly activity took place during the morning.

				0 1
Date Collected	Number	AM	PM	Taxonomy
6/3/2010	1	х		Ravinia
6/3/2010	1	х		Calliphora vicinia Robineau-Desvoidy
6/3/2010	1	х		Lucilia illustris Meigen
6/3/2010	1	х		Lucilia coeruleiviridis Macquart
6/16/2010	1		Х	Phormia regina Meigen
6/21/2010	1	х		Lucilia coeruleiviridis Macquart
6/21/2010	1	х		Lucilia sericata Meigen
6/25/2010	1		X	Muscidae – No Specialist Available
6/27/2010	2	Х		Lucilia coeruleiviridis Macquart

**Table 13.** Insect activity for inside the hay barn on the ground (1A)

*Phormia regina* comprised the majority of flies for the loft in the hay barn (1B) - 87.5%

(Table 14). The majority of the flies was collected on days four and five when the remains were

in the active stage of decomposition. Carabidae was the most prevalent of the beetle species.

Again, the greatest amount of all fly activity took place during the morning.

<b>Lusic Lin</b> Insect weiting for the forth the hag such (1D)									
Date Collected	Number	AM	PM	Taxonomy					
6/4/2010	12	Х		Phormia regina Meigen					
6/5/2010	1	Х		Carabidae					
6/5/2010	4	Х		Phormia regina Meigen					
6/5/2010	5		Х	Phormia regina Meigen					
6/5/2010	1		Х	Muscidae – No Specialist Available					
6/7/2010	1	Х		Dermestidae					
6/13/2010	1	Х		Carabidae					
6/23/2010	2	X		Lucilia coeruleiviridis Macquart					

**Table 14.** Insect activity for the loft in the hay barn (1B)

The sub-site located outside of the hay barn on the ground (1C) had the greatest amount of fly activity (Table 15). This is most likely due to its outside placement. This sub-site had a large influx of *Phormia regina* - 53 in all. This species makes up 75.7% of the flies at this sub-site. Fly activity reached its peak during days three and four, which were the active stages of decomposition for this sub-site. Histerdae and *S. americana* were both present at this site as was *C. maxillosus*. The greatest amount of all fly activity took place during the morning.

Date Collected	Number	AM	PM	Taxonomy
6/2/2010	2	х		Sarcophagidae – Dissection/Slide Mount. Req.
6/2/2010	1	х		Calliphora vicinia Robineau-Desvoidy
6/2/2010	3	х		Lucilia illustris Meigen
6/2/2010	3	х		Phormia regina Meigen
6/3/2010	1	х		Sarcophagidae – Dissection/Slide Mount. Req.
6/3/2010	1	х		Muscidae – No Specialist Available
6/3/2010	13	х		Phormia regina Meigen
6/3/2010	1	х		Silphidae Silpha americana
6/3/2010	1	х		Dermestidae
6/4/2010	1	х		Muscidae – No Specialist Available
6/4/2010	18	х		Phormia regina Meigen
6/4/2010	2		х	Phormia regina Meigen
6/4/2010	1		х	Silphidae Silpha americana
6/4/2010	5	х		Histerade
6/4/2010	1	х		Staphylinidae Creophilus maxillosus
6/5/2010	3	х		Muscidae - No Specialist Available
6/5/2010	12	х		Phormia regina Meigen
6/6/2010	1		х	Sarcophagidae – Dissection/Slide Mount. Req.
6/6/2010	4		Х	Phormia regina Meigen
6/6/2010	1	Х		Histerdae
6/8/2010	1	Х		Hemiptera Heteroptera
6/13/2010	X	х		Platydracus

**Table 15.** Insect activity for outside the hay barn on the ground (1C)

6/23/2010	1	Х	Muscidae - No Specialist Available
6/23/2010	1	х	Lucilia sericata Meigen
6/23/2010	2	х	Calliphora vicinia Robineau-Desvoidy
6/23/2010	1	х	Phormia regina Meigen

Sub-site 2A exhibited the greatest amount of fly activity on days five and six, both active

days of decomposition (Table 16). Phormia regina comprises 59.5% of all flies present. S.

americana as well as Histerdae were seen at this site, as well as Oiceoptoma. Activity, again,

was at its height during the morning hours.

Date Collected	Number	AM	PM	Taxonomy
6/2/2010	2	х		Calliphora vicina Robineau-Desvoidy
6/2/2010	1	х		Lucilia sericata Meigen
6/4/2010	1	х		Lucilia sericata Meigen
6/4/2010	1		X	Calliphora vicina Robineau-Desvoidy
6/5/2010	11	х		Phormia regina Meigen
6/5/2010	1	х		Lucilia sericata Meigen
6/5/2010	1	х		Calliphora vomitoria Linnaeus
6/5/2010	1		X	Lucilia sericata Meigen
6/5/2010	2	х		Silphidae Silpha americana
6/5/2010	1	х		Histerdae
6/5/2010	1	х		Halyomorpha halys
6/6/2010	11	х		Phormia regina Meigen
6/6/2010	1	х		Muscidae – No Specialist Available
6/6/2010	1	х		Lucilia sericata Meigen
6/7/2010	1		Х	Necrodes surinamensis
6/8/2010	3	х		Silphidae Oiceoptoma
6/8/2010	1	х		Silphidae Siplha americana
6/8/2010	1	х		Necrodes surinamensis
6/9/2010	3	х		Silphidae Oiceoptoma
6/9/2010	1		X	Silphidae Siplha americana
6/11/2010	1	X		Platydracus
6/23/2010	5	х		Phormia regina Meigen

Table 16. Insect activity for outside the control barn on the ground (2A)

The remains located in the loft of the control barn (2B) held the lowest amount of fly activity for this site (Table 17). The majority of flies present was of the *Lucilia* genus – approximately 52.3%. At no time did this sub-site exhibit a moderate or maximum amount of fly activity when compared to the other sub-sites, and the presence of flies was noted throughout the study, as is consistent with the above table (Table 16). Sub-site 2B is significantly different from

the rest of the sub-sites at this location due to the absence of *Phormia regina*. No reason for this exists. *S. americana*, Histerade, and *P. comes* were all present at this sub-site.

Date Collected	Number	AM	PM	Taxonomy
6/2/2010	1	Х		Calliphora vicina Robineau-Desvoidy
6/5/2010	4		Х	Phormia regina Meigen
6/5/2010	2	Х		Silphidae silpha Americana
6/7/2010	1			Histerade
6/11/2010	1	Х		Platydracus
6/19/2010	3	Х		Calliphora vicina Robineau-Desvoidy
6/19/2010	1	Х		Lucilia illustris Meigen
6/19/2010	2	Х		Lucilia sericata Meigen
6/23/2010	2	Х		Lucilia coeruleiviridis Macquart
6/23/2010	2	Х		Lucilia sericata Meigen
6/27/2010	2		х	Lucilia sericata Meigen
6/29/2010	1	Х		Calliphora vicina Robineau-Desvoidy
6/29/2010	1	Х		Lucilia coeruleiviridis Macquart
6/29/2010	2	X		Lucilia sericata Meigen
6/29/2010	2	Х		Muscidae – No Specialist Available

**Table 17.** Insect activity for the loft in the control barn (2B)

Sub-site 2C, located outside the control barn on the ground, showed the greatest amount of fly activity (Table 18). *Phormia regina* was the most prevalent species of fly at this sub-site. Of the 71 flies caught, 76% of those were *Phormia regina*. The greatest amount of fly activity was recorded on days four and five. Active decomposition was occurring at this time. This sub-site also had the greatest dispersal of fly activity throughout the day, with 70% of the activity occurring during the morning and 30% occurring in the evening. The greatest amount of beetle activity occurred in the morning. *S. americana*, Histerdae, *Oiceoptoma*, and Scarabidae were all present at this site.

Date Collected	Number	AM	PM	Taxonomy
6/2/2010	1	Х		Lucilia coeruleiviridis Macquart
6/2/2010	3	Х		Lucilia sericata Meigen
6/3/2010	1	Х		Lucilia sericata Meigen
6/3/2010	2	Х		Muscidae – No Specialist Available
6/3/2010	2		Х	Silphidae Silpha americana
6/4/2010	10	Х		Phormia regina Meigen
6/4/2010	1		Х	Muscidae – No Specialist Available
6/4/2010	2		Х	Phormia regina Meigen
6/5/2010	26	Х		Phormia regina Meigen
6/5/2010	1	Х		Calliphoria vomitoria Linnaeus

**Table 18.** Insect activity for outside the control barn on the ground (2C)

6/5/2010	1	Х		Muscidae – No Specialist Available
6/5/2010	13		Х	Phormia regina Meigen
6/5/2010	1		Х	Muscidae – No Specialist Available
6/5/2010	1		Х	Histerdae
6/6/2010	1		Х	Muscidae – No Specialist Available
6/6/2010	1		Х	Phormia regina Meigen
6/6/2010	1		Х	Lucilia sericata Meigen
6/6/2010	2	Х		Histerdae
6/6/2010	1	Х		Silphidae Oiceoptoma
6/7/2010	2	Х		Phormia regina Meigen
6/11/2010	1	Х		Muscidae – No Specialist Available
6/11/2010	1	Х		Scarabidae
6/13/2010	1	Х		Pollenia pediculate Macquart
6/13/2010	1	X		Muscidae – No Specialist Available
6/19/2010	1		Х	Lucilia sericata Meigen

Sub-site 3A, located inside the tobacco barn on the ground, only exhibited fly activity until the sixth day (Table 19). After this, no flies visited the remains. Again, the most fly activity occurred earlier in the day, and *Phormia regina* comprised the majority of the flies caught. The greatest amount of activity occurred during the fourth when the remains reached the active stage of decomposition. During this time, fly activity ranged from moderate to maximum. The beetles seen at this site were *Oiceoptoma*, Histerade, *N. surinamensis*, and *S. americana*.

Date Collected	Number	AM	PM	Туре
6/3/2010	1	Х		Lucilia sericata Meigen
6/3/2010	2	Х		Phormia regina Meigen
6/3/2010	3	Х		Silphidae Silpha americana
6/3/2010	1	Х		Histerdae
6/4/2010	1	Х		Silphidae Silpha americana
6/4/2010	14	Х		Phormia regina Meigen
6/4/2010	1	Х		Calliphora vomitoria Linnaeus
6/4/2010	1	Х		Lucilia coeruleiviridis Macquart
6/4/2010	5		Х	Phormia regina Meigen
6/5/2010	1	Х		Silphidae Oiceoptoma
6/5/2010	1		Х	Silphidae Oiceoptoma
6/5/2010	2	Х		Phormia regina Meigen
6/5/2010	1	Х		Lucilia cluvia Walker
6/5/2010	2	Х		Lucilia coeruleiviridis Macquart
6/5/2010	1	Х		Sarcophagidae – Dissection/Slide Mount. Req.
6/6/2010	2		Х	Silphidae Oiceoptoma
6/6/2010	3	Х		Phormia regina Meigen
6/7/2010	3	Х		Silphidae Oiceoptoma
6/7/2010	1		Х	Necrodes surinamensis
6/7/2010	1	Х		Silphidae Silpha americana
6/9/2010	3	Х		Silphidae Oiceoptoma

**Table 19.** Insect activity for inside the tobacco barn on the ground (3A)

6/11/2010	1	Х	Histerdae
6/13/2010	1	Х	Silphidae Silpha americana

The remains located in the loft of the tobacco barn (3B) show the least amount of fly activity of any of the recorded sub-sites (Table 20). Only two flies were caught during this project at 3B, both of which were *Lucilia* family. Moderate fly activity (greater than five flies) occurred on the second and the fourth, respectively. No flies were caught on the fourth. The only beetle present was the *Halyomorpha halys*.

**Table 20.** Insect activity for the loft in the tobacco barn (3B)

Date Collected	Number	AM	PM	Туре
6/2/2010	1	Х		Lucilia sericata Meigen
6/3/2010	1	Х		Lucilia coeruleiviridis Macquart
6/8/2010	1		Х	Halyomorpha halys

Sub-site 3C, located outside of the tobacco barn on the ground, exhibited a vast amount of fly activity early on, in particular on the third day, but the activity tapered thereafter (Table 21). Seventy-nine percent of the flies captured were *Phormia regina*. The remains were well into the active stage of decomposition on the third day and were classified as advanced on the fourth, which supports the amount of fly activity listed in the table. This sub-site held the greatest amount of beetle overlap. Carabidae, Dermestidae, *S. americana*, *P. comes*, *H. halys*, *Oiceoptoma*, and Scarabidae were all present at this site.

**Table 21.** Insect activity for outside the tobacco barn on the ground (3C)

Date Collected	Number	AM	PM	Туре		
6/2/2010	1	X		Calliphoria vomitoria Linnaeus		
6/2/2010	1	Х		Lucilia illustris Meigen		
6/2/2010	1	Х		Lucilia coeruleiviridis Macquart		
6/2/2010	1	X		Phormia regina Meigen		
6/3/2010	22	Х		Phormia regina Meigen		
6/3/2010	1	Х		Platydracus		
6/3/2010	1	Х		Halyomorpha halys		
6/3/2010	1	Х		Silphidae Silpha americana		
6/4/2010	1	Х		Silphidae Silpha americana		
6/4/2010	1	Х		Dermestidae		
6/4/2010	1	Х		Silphidae Oiceoptoma		
6/6/2010	1		Х	Dermestidae		
6/6/2010	1		Х	Carabidae		
6/7/2010	1	Х		Carabidae		

6/9/2010	2		Х	Dermestidae		
6/9/2010	1	Х		Platydracus		
6/9/2010	2	Х		Hemiptera heteroptera		
6/11/2010	1	Х		Dermestidae		
6/11/2010	1	Х		Silphidae Oiceptoma		
6/11/2010	1		Х	Scarabidae		
6/18/2010	2	Х		Lucilia coeruleiviridis Macquart		
6/21/2010	1		Х	Lucilia coeruleiviridis Macquart		
6/23/2010	1	Х		Sarcophagidae – Dissection/Slide Mount. Req.		

It was originally hypothesized that no flies would be attracted to sub-site 3B (tobacco loft) due to the both the height of the loft from the ground (25 feet) and the residual nicotine present in the beams of the barn. This was not the case. While no more than five flies visited the remains at any time, the rapid decomposition of these remains can, in part, be attributed to the heat in the loft (Appendix C).

All sites had a considerable amount of insect activity. The sub-sites located inside the barn on the ground collectively held the lowest amount of fly activity. The lofts held the second highest level of activity, while the remains located outside the barns held the greatest amount. This amount of activity coincides with the aforementioned decomposition rates.

The weather played an additional factor in insect arrival. It rained in the evening on day three, which is why there is a decrease in fly activity for that time. The same is true for the fourth and the sixth. It rained on the fifth morning, but only at Site 2 and the amount was less than 0.1mL. On the thirteenth, it rained less than 0.3mL at Site 3 during the morning, while it rained less than 0.2mL in the evening at Sites 1 and 2. Again, this, in conjunction with the decomposition rates for these sites, supports the recorded insect activity. The same can be said for the morning and evening of day 15, where 0.45mL fell in the morning at Site 1 and 0.4mL at Site 2, while only 0.125mL fell during the evening at Site 3 (Appendix D).

#### <u>Weather</u>

The number of observations varied due to the breakage and replacement of some of the thermometers. The placement of the thermometer for the remains located outside of the control barn on the ground (2.3 for this table) caused some anomalies with the recorded temperatures (i.e. maximum temperatures above the recorded heat index). The heat from the tin roof impacted the thermometer readings.

All collected data were analyzed using SAS® and a basic *proc means* procedure (Table 22). The means for the minimum temperatures fall within five degrees of one another. The means for the maximum temperatures fall within 5 degrees of one another when site 2.3 (outside the control barn on the ground) is excluded. Since the average temperatures did not vary to a great degree, it can be said that neither the cold nor the heat had more of an impact on the rate of decomposition for one site versus another.

The means for the minimum temperatures fall within 7.6°F of one another. The means for the maximum temperatures fall within 8.2°F of one another when site 2.3 (outside the control barn on the ground) is excluded. Since the average temperatures did not vary to a great degree, it can be said that neither the cold nor the heat had more of an impact on the rate of decomposition for one site versus another.

Table 23 shows a comparison of the variables taken by the Kestrel and rain gauges. Again, all data were analyzed by SAS® using a basic *proc means* procedure. The maximum heat index varied by no more than ten degrees at each site. While Site 3 had a maximum heat index of 110 degrees, the remains located outside the barn at this site (3C) decomposed the quickest (four days) (Appendix E). While the rain may have affected insect arrival, it did not have a great

impact on the decomposition rates for these sites. The maximum amount of rain for these sites was never greater than 0.7 inches.

Site	Observations (N)	Variable	Ν	Mean	Std Dev	Min	Max
1.1	17	Min	17	74.4	6.6	63.0	84.0
1.1	17	Max	17	82.9	4.0	72.0	89.0
1.2	10	Min	17	69.5	4.1	61.0	75.0
1.2	19	Max	17	91.1	5.3	80.0	100.0
1.2	10	Min	19	67.6	4.2	60.0	76.0
1.5	19	Max	19	86.3	4.7	77.0	96.0
2.1	10	Min	19	69.3	3.8	62.0	75.0
2.1	19	Max	19	86.2	4.1	78.0	92.0
2.2	18	Min	18	69.6	3.6	63.0	75.0
2.2	10	Max	18	90.2	5.5	78.0	99.0
2.2	2.2 10	Min	19	69.0	8.8	57.0	90.0
2.5	19	Max	19	99.0	7.8	82.0	115.0
3.1	10	Min	19	71.2	4.3	64.0	83.0
5.1	19	Max	19	82.6	5.1	70.0	94.0
3.2	10	Min	19	71.5	6.2	62.0	92.0
5.2	19	Max	19	89.5	7.5	66.0	99.0
2.2	19	Min	19	66.9	4.4	56.0	73.0
5.5	10	Max	19	85.3	7.6	63.0	94.0

**Table 22.** Variation of maximum and minimum temperatures for all sub-sites

**Table 23.** Kestrel Data for Weather Variables at Research Sites

Site	Observations (N)	Variable*	N	Mean	Std Dev	Min	Max
		WSC	35	0.7	1.7	0.0	10.0
		WSMa	35	3.3	2.0	0.8	9.7
1	25	WSA	35	1.0	0.8	0.0	3.9
1	55	Hum	35	74.0	18.7	39.0	100.0
		HI	35	91.0	10.2	69.8	110.6
		Rain	35	0.04	0.1	0.0	0.7
		WSC	35	0.3	0.8	0.0	4.0
		WSMa	35	3.6	1.9	0.9	10.4
2	25	WSA	35	0.8	0.9	0.0	4.5
2	55	Hum	35	77.5	21.0	10.0	100.0
		HI	35	88.5	11.6	68.7	117.4
		Rain	35	0.03	0.1	0.0	0.5
		WSC	35	0.4	0.6	0.0	2.1
		WSMa	35	2.3	1.1	0.0	4.4
2	35	WSA	35	0.6	0.6	0.0	2.8
3		Hum	35	79.9	17.0	49.0	100.0
		HI	35	86.2	10.1	64.8	107.1
		Rain	35	0.03	0.1	0.0	0.5
			·				

*WSC = Wind Speed Current	WSA = Wind Speed Average	HI = Heat Index
WSMa = Wind Speed Maximum	Hum = Humidity	Rain = Rain

## **Nicotine Analysis**

During the SCAN mode, three ion peaks were identified and isolated. The samples were then re-analyzed in SIM mode, which concentrated specifically on the three ion peaks: ion 84, ion 162, and ion 133 (Figures 11 and 12). These were the most concentrated ions and all of them occurred at 10 minutes and 46 seconds, which is the amount of time necessary for the identification of nicotine. Since ion 82 held the greatest resolution, it was used as the quantitation ion while the others were the qualifier ions.



Figure 11. Comparative view of determinant ions for nicotine



Figure 12. Individual ion peaks for nicotine

Table 24 provides a look at the nicotine analysis for five levels of the barn: ground level

(0-5ft), first level (5-10ft), second level (10-15ft), third level (15-20ft), and fourth level (20-25ft).

Also, it is crucial to note that this barn has hung tobacco consecutively for 30 years.

Sample Description	Sample Weight (g)	Analyte	Conc	Units					
Hoskins 0-5ft	0.53	Nicotine	2.4	ppm					
Hoskins 5-10ft	0.83	Nicotine	1.78	ppm					
Hoskins 10-15ft	0.69	Nicotine	23.5	ppm					
Hoskins 15-20ft	1.34	Nicotine	7.24	ppm					
Hoskins 20-25	0.73	Nicotine	26.8	ppm					

 Table 24. Summary of Nicotine Analysis

\*A fortified blank sample and a fortified wood sample of .313ug/mil of nicotine were added to determine a percent recovery of nicotine. A 98% method recovery was calculated and a 90% recovery of the nicotine was calculated from a wood sample.

The loft was located on the fourth level. At the surface level of the sample analyzed on the fourth level, only 26.8ppm held nicotine. The weight of the sample was 0.73g, which is a small sample weight. However, one level below that, a sample weighing 1.34g retained only 7.24ppm while the ground level sample weighing 0.53 contained 2.4ppm. There does not seem to be a correlation between weight and ppm based on the above results. For the loft (20-25ft), the minute

presence of nicotine did not impact the decomposition rate of the remains.

#### **Chapter 5: Discussion**

The goal of this project was to create guidelines to help determine a PMI for mummified human remains. Three sites were utilized in this project: a hay barn (Site 1), a control barn (Site 2), and a tobacco barn (Site 3). Each contained three sub-sites: inside the barns on the ground held a designation of "A", inside the barns in a loft held the designation of "B", and outside the barns on the ground held the designation of "C".

The stages of decomposition for each sub-site were classified according to the criteria defined by Anderson et al. (1996), Galloway et al. (1989), and Shean et al. (1993). The only discrepancy came when identifying the presence of an odor. While each sub-site retained a strong-to-slight odor during the active stage, the lofts held more of an odor than any other sub-site. This was due primarily to the proximity of the remains to the roof as well as the high temperatures for these sites (Appendices A, B, C). Also, the odors found at the locations outside of the barns were masked by the presence of horses, horse manure, and a compost heap. The only outside sub-site that exhibited a prolonged duration of odor was located at the tobacco barn (2C). Importantly, due to naturally occurring elements in certain regions, the researcher/investigator may not always be able to identify a decompositional odor. This does not mean that human remains are not present.

Each sub-site, while yielding different times for each decomposition stage, reflected the same results – little to no mummification (Appendix E). While the weather aided the decomposition processes at the sites, it did not play a large role in the different rates of decomposition obtained for each sub-site. The humidity, heat index, and rain varied little for each site. The maximum and minimum temperatures recorded for each site varied slightly, but not enough to influence the rate of decomposition.

Shean et al. (1993) stated that remains decompose faster in exposed areas. This proved true during this project. The remains located outside of the barns (1C, 2C, 3C) decomposed faster than those located inside the barns (1A, 1B, 2A, 2B, 3A, 3B), with the exception of sub-site 1C which was classified as dry on the same day as sub-site 1B. The remains at sub-site 3C (outside the tobacco barn on the ground) decomposed in five days. Upon discussing the area with the owner, I learned that a "compost heap" was located adjacent to the remains at 3C. This "compost heap" was composed of discarded tobacco sticks and possibly other organic refuse. The owner informed me that the refuse area had always been "hot," with organic turnover occurring more rapidly than other places on the farm. The remains at sub-sites 3A (inside the tobacco barn on the ground) and 2C (outside the control barn on the ground) spent seven days decomposing. The remains sub-sites 1B (the loft in the control barn), 1C (outside the hay barn on the ground), and 2B (the loft in the control barn) took approximately eight days to decompose. Sub-site 1A (inside the hay barn on the ground) took approximately nine days to decompose. This was also the case with sub-sites 2A (inside the control barn on the ground) and 3B (the loft in the tobacco barn). This researcher does not have an explanation for this. A MANOVA was performed in order to determine whether or not the location of the remains in and around the barns, as well as the barns themselves, had an impact on the individual rates of decomposition. The results showed no significant association between the location and placement of the remains and the individual rates of decomposition obtained.

The insects, both flies and beetles, showed considerable overlap at all sites. *Phormia regina* overlapped all sites with the exception of 3B (loft in the tobacco barn). The only zone specific flies were *Ravia* at 1A (inside the hay barn on the ground), *Pollenia pediculate* at 2C (outside the control barn on the ground), and *Lucilia cluvia* at 3A (inside the tobacco barn on the
ground). Sub-site 3C (outside the tobacco barn on the ground) exhibited the greatest amount of insect activity in the shortest amount of time. Sub-site 1C yielded the most insect activity of the three sub-sites with *Phormia regina* being the most prevalent fly and Histerdae and *S. americana* being the most prevalent beetles. While sub-site 2C exhibited a greater amount of fly activity with *Phormia regina* again being the most numerous fly, sub-site 3A yielded a greater amount of beetles, with *Oiceoptoma* being the most frequent. The only zone-specific beetle was the Staphylinidae *Creophillus maxillosus*, which was only found at 1C (outside the hay barn on the ground). *S. americana* (present at six sub-sites) and Histerdae (present at five sub-sites) showed the greatest amount of overlap. While the greatest number of flies arrived more in conjunction with active decomposition, the greatest number of beetles appeared during advanced decomposition, when mostly skin was present.

Mann et al. (1990) found that remains left in dry areas at a higher elevation mummified faster. The study site, located in Rogers, Kentucky, sits at 1217 feet above sea level, which is not necessarily a high elevation. Therefore, it is unlikely that elevation played a significant role in this study. The presence of the skin after 30 days varied greatly between each sub-site. Sub-site 1A (inside the hay barn on the ground) had little skin left after the 30-day time allotment. The only skin present was found around one of the detached rear hooves. The remains were placed on top of hay so as not to obstruct observation and insect collection. Perhaps the complete immersion of the remains in hay would have had a more positve effect. This is cause for further study. The color of the bones ranged from light to dark brown. Sub-site 1B (the loft in the hay barn) saw a slightly different outcome. Skin, along with hair, was found on the exposed surface of the head around the eye, cheek area, and on the bottom of the cage. Sub-site 1C (outside the

hay barn on the ground) was completely devoid of skin by the end of the project. The bones had been bleached by the sun.

Site 2 was the control barn. Sub-site 2A (inside the control barn on the ground) retained the greatest amount of skin after 30 days. I had originally hypothesized that the remains located here would mummify. Although no skin was left on the bones themselves, virtually all of the skin located on the ground contact side remained. The skin held varying degrees of moisture according to the weather, but the majority of it remained dry and felt like hide that had been allowed to dry after it was cleaned. Mummification also occurred with sub-site 2B (the loft in the control barn). Dry skin was present under the forelimbs and around the fore and hind hooves. Dried skin was also found encompassing the head and stretching from the posterior portion of the skull to the area where the tail was once located. Skin could be found around the proximal and distal ends of several of the long bones. Sub-site 2C (outside the hay barn on the ground) retained no skin at the end of 30 days.

Site 3 was the tobacco barn. Sub-site 3A (inside the tobacco barn on the ground) retained varying amounts of skin around the head, back (where the vertebral column was once located), around the rear area and hooves. I hypothesized that site 3B (the loft in the tobacco barn) would be the site that would mummify since it was higher off the ground than the other lofts; however, virtually no mummification occurred at this sub-site. On the second day of decomposition, it was thought that the remains were entering the putrefaction stages of mummification. Seepage was occurring from the mouth and, on the third day, from the anus. These findings are consistent with the putrefaction stages described by Dzierzykray-Rogalski (1986) and Quigley (1998). Dzierzykray-Rogalski (1986) also states that the epithelium is the first layer to mummify. The dehydration of the epithelium was seen on the second day before the remains were lifted. Once

lifted, this layer started to peel away from the underlying dermis. Perhaps partial or even complete mummification would have occurred had the *Sus scrofa* remained undisturbed for the entirety of the project. Due to the high volume of instar activity, the majority of the skin had decomposed during the first few days of this project. Only fibrils of skin were left on the bottom of the cage. Sub-site 3C (outside the tobacco barn on the ground) was completely devoid of skin by the end of the project.

Residual nicotine was analyzed at this site in order to determine its role in the decomposition/mummification of the remains located in the loft. I hypothesized that, after 30 years of hanging tobacco in this barn, a significant amount of residual nicotine would be present and would have a substantial impact on the rate of decomposition and/or mummification with sub-site 3B (the loft in the tobacco barn). The results obtained from the samples were unexpected. It was found that, in this instance, nicotine had an identification time of 10:46 minutes. This differs slightly from Rafferty's time of 12.94 minutes (2000, 2006). The standards were also different. Rafferty (2000, 2006) used Methylene Chloride while the standard used for this study was Ethyl Chloride. This is, in large part, due to the affinity of the material for the standard. In this case, the wood from the tobacco barn had more of an affinity for Ethyl Chloride. It was found that this sub-site only contained 26.8ppm (parts per million) of residual nicotine for a sample weighing 0.73g. While this was one of the smallest sample weights of the five samples submitted, this one held the greatest concentration of residual nicotine. Theoretically, if larger samples were analyzed, the outcome would still yield a fairly insignificant amount of residual nicotine. This minute trace of residual nicotine had no effect on the preservation or decomposition rate of the remains at this sub-site. Thus, one can surmise that residual nicotine

has no impact on the decomposition of remains during the summer months in a barn loft located in Eastern Kentucky.

While no remains mummified completely, a noticeable amount of mummified skin was recorded at several of the sites. I believe that the overall weight of the remains played a significant role in this. No Sus scrofa weighed over three pounds. Perhaps if the Sus scrofa were larger, the outcome would be different. A study concentrating specifically on obtaining a PMI template for recently mummified remains has not been previously performed. This study not only reinforces the decomposition stages detailed by Anderson et al. (1996), Galloway et al. (1989), and Shean et al. (1993), but it also lends credence to the early stages of putrefaction defined by Dzierzykray-Rogalski (1986) and Quigley (1998). This research provides more information about insect succession for Eastern Kentucky and can potentially be used to create a PMI template for infant remains (due to the weight of the pigs) found in barns for this region. This research also has important implications for future research involving the use of a GCMS in the field of Forensic Anthropology. In other words, while the placement of the remains was known and wood samples located in close proximity to the remains were tested for residual nicotine, one must wonder if it would be possible to test desiccated tissue found in naturally occurring environments or in enclosed environments for chemical residue using a GCMS. This would aid in two aspects -1) providing the investigator with more knowledge of the environment in which the remains were found and 2) determining whether or not the remains mummified naturally or if they were intentionally mummified.

# **Chapter 6: Conclusion**

Residual nicotine had no impact on the decomposition of the remains located in the loft (3B) of Hoskins' barn. The hay, while prolonging the bloat stage for site 1A, also did not seem to have a significant impact on the rate of decomposition/mummification. At the end of this study, one can surmise that infants placed in or around barns in the summer in Eastern Kentucky are unlikely to exhibit great degrees of mummification. Based on the minute traces of desiccated skin, and the results from the wood samples, it is, at this time, difficult to construct a template for the PMI of mummified remains.

## References Cited

## Allaire, M.

(2000). *Postmortem Interval (PMI) Determination at Three Biogeoclimatic Zones in Southwest Colorado*. Master's Thesis, Louisiana State University, Baton Rouge.

## Anderson, G. S. and VanLaerhoven, S. L.

(1996). Initial Studies on Insect Succession on Carrion in Southwestern British Columbia. *Journal of Forensic Sciences*, 41(4), 617-621.

#### Andrews, C.

(1984). Egyptian Mummies. Harvard University Press, Cambridge, Massachusetts.

#### Arriaza, B. T.

(1995). Chinchorro Bioarchaeology: Chronology and Mummy Seriation. *Latin American Antiquity*, 6(1), 35-55.

#### Bahn, P.

(2002). Written in Bones – How Human Remains Unlock the Secrets of the Dead. Firefly Books, Buffalo.

# David, R.

(2008). *Egyptian Mummies and Modern Science*. Cambridge University Press, Cambridge, New York.

## Deem, J.

(2008). *Bodies from the Ice – Melting Glaciers and the Recovery of the Past*. Houghton Mifflin Company, Boston.

## Dzierzykray-Rogalski T.

(1986). *Natural Mummification in Egypt*. In Ann Rosalie David (Ed.), *Science in Egyptology* (101-104). Manchester University Press, Manchester.

## Fischer, Christian.

(1998). Bog bodies of Demark and northwestern Europe. In *Mummies, Disease & Ancient Cultures* (2nd ed.) Cockburn, A., Cockburn, E., C., and Reyman, T. A. (Eds.). Cambridge: Cambridge University Press.

#### Gager, F., Johnson, V. and Holmes, J.C.

(1960) Ancient Tobacco Smokers. Science, 132:1021.

Galloway, A., Birkby, W. H., Jones, A. M., Henry, T. E. and Parks, B. O. (1989). Decay Rates of Human Remains in an Arid Environment. *Journal of Forensic Sciences*, 34(3), 608-611.

#### Glob, P.V.

(1965). The Bog People: Iron-Age Man Preserved. Farber and Farber Ltd., New York.

## Haglund, W. D. and Sorg, M. A.

(2002). Advances in Forensic Taphonomy - Method, Theory, and Archaeological *Perspectives*. CRC Press, Boca Raton, Florida.

## Halsall, P.

(1999). *Internet Ancient History Sourcebook*. Retrieved March 16, 2010. <a href="http://www.fordham.edu/halsall/ancient/herodotus-history.txt">http://www.fordham.edu/halsall/ancient/herodotus-history.txt</a>>.

# Harrell, J. A., and Lewan, M. D.

(2002). Sources of Mummy Bitumen in Ancient Egypt and Palestine. *Archaeometry*, 44(2), 285-293.

## Kentucky State Police.

(2002). *Cold Case of Bobby Dethridge*. Frankfort, KY: Author. Retrieved February 10, 2010. <a href="http://www.kentuckystatepolice.org/cold\_case/post3coldcase10.php">http://www.kentuckystatepolice.org/cold\_case/post3coldcase10.php</a>.

## Lucas, A.

(1932). The Use of Natron in Mummification . *Journal of Egyptian Archaeology*, 18(3/4), 125-140.

## Mann, R., Bass, W., and Meadows, L.

(1990). Time Since Death and Decomposition of the Human Body: Variables and Observations in Case and Experimental Field Studies. *Journal of Forensic Sciences*, 35(1), 105.

## McKeown, A. H., Kemp, W. L., Dudzik, B., and Parsons, H.R.

(2011). Scavenging and Its Relationship to Decomposition in the Northern Rockies [Abstract]. *Proceedings American Academy of Forensic Sciences*, 17(1), 387.

#### Meyer, J.

(2003). *Tobacco Hornworm*. Retrieved March 23, 2010. <http://www.cals.ncsu.edu/course/ent010/tobacco\_pests/tob\_aphid.html>

#### Pringle, H.

(2001). *The Mummy Congress – Science, Obsession, and the Everlasting Dead.* Thia, New York.

#### Quigley, C.

(1998). Modern Mummies – The Preservation of the Human Body in the Twentieth Century. McFarland & Company, Inc, Jefferson, North Carolina.

# Rafferty, S. M.

(2002). Identification of Nicotine by Gas Chromatography/Mass Spectroscopy Analysis of Smoking Pipe Residue. *Journal of Archaeological Science*, 29(8), 897-907.

#### Rafferty, S. M.

(2006). Evidence of Early Tobacco in Northeaster North America? *Journal of Archaeological Science*, 33(4) 453-458.

## Rhine, S. and Dawson, J.E.

(1998). Estimation of Time Since Death in the Southwestern United States. In *Forensic Osteology* (2nd ed.).K. J. Reichs (ed). Springfield, Illinois.

## Rodriguez, W. C. and Bass, W. M.

(1983). Insect Activity and its Relationship to Decay Rates of Human Cadavers in East Tennessee. *Journal of Forensic Sciences*, 38(2), 425-428.

# Seebold, K.

(2009-2010). *Kentucky & Tennessee Tobacco Production Guide*. Retrieved March 10, 2010. <a href="http://tobaccoinfo.utk.edu/PDFs/2009-2010KYTNProducitonGuide/KY-TNProductionGuide2009-2010-ID160.pdf">http://tobaccoinfo.utk.edu/PDFs/2009-2010KYTNProducitonGuide/KY-TNProductionGuide2009-2010-ID160.pdf</a>>.

## Shean, B. S., Messinger, L. and Papworth, M.

(1993). Observations of Differential Decomposition on Sun Exposed v. Shaded Pig Carrion in Coastal Washington State. *Journal of Forensic Sciences*, 38(4), 939-944.

#### Stone, R.

(2000). Ice Man Warms up for European Scientists. Science, 289(5488), 2253-2254.

## Zahlsen, K. and Nilsen, O.G.

(1994). Nicotine in Hair of Smokers and Non-Smokers: Sampling Procedure and Gas Chromatographic/Mass Spectrometric Analysis. *Pharmacology and Toxicology*, 75(3-4) 143-149.



Appendix A – Highs and Lows for Site 1







**Appendix B – Highs and Lows for Site 2** 







Appendix C – Highs and Lows for Site 3







Appendix D – Rainfall for Sites 1, 2, and 3



# Appendix E – Decomposition Rates for Sites 1, 2, and 3

Site 1 is the Hay Barn. A, B, and C are used to denote sub-sites. Sub-site 1A is outside on the ground, 1B is in the loft, and 1C is inside on the ground.

# **Decomposition Rates for Site 2**



Site 2 is the Control Barn. A, B, and C are used to denote sub-sites. Sub-site 2A is outside on the ground, 2B is in the loft, and 2C is inside on the ground.



Site 3 is the Tobacco Barn. A, B, and C are used to denote sub-sites. Sub-site 3A is outside on the ground, 3B is in the loft, and 3C is inside on the ground.

Vita

Valerie R. Kauffeld was born on December 23, 1985, in Darby, Pennsylvania. She has spent most of her life living in a small town nestled in the Appalachian mountains of northeast Tennessee. She has traveled extensively and studied in Paris, France, and Santa Clara, Guatemala, while attending East Tennessee State University (ESTU) during her undergraduate career. She sought to combine her passion for languages and cultures, and was graduated with a major in both anthropology and foreign languages in December of 2008 from ETSU. She was introduced to forensic anthropology while at ETSU, and in the fall of 2009 entered the master's program for anthropology at Louisiana State University. Valerie's future plans involve a career in law enforcement, and she hopes one day to use her love of languages and her knowledge of forensic anthropology to help those close to home and abroad who have lost loved ones find closure.