UNIVERSITY OF CENTRAL OKLAHOMA

Edmond, Oklahoma

Jackson College of Graduate Studies

Necrophagy in Honey bees (*Apis mellifera*); A Forensic Application of Scent Foraging Behavior

A THESIS

SUBMITTED TO THE GRADUATE FACULTY

In partial fulfillment of the requirements

For the degree of

MASTER OF SCIENCE IN FORENSICS

By

Brian David Morice

Edmond, Oklahoma

2019

Necrophagy in Honey bees (*Apis mellifera*); A Forensic Application of Scent Foraging Behavior

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APPROVED FOR THE INSTITUTE OF FORENSIC SCIENCE

APRIL 2019

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ACKNOWLEDGMENTS

The completion of this research would not have been possible without the help and support from many people. I am thankful to my advisor, Dr. Wayne Lord, for his guidance, patience, knowledge and field experience. I have admired and appreciated his approach to teaching and instruction since his first arrival at UCO in the summer of 2008. This began when I met Dr. Lord during a summer field biology course as he was preparing to begin his first semester teaching at UCO. I was impressed by his demeanor and this led to my enrollment of his Entomology course my final semester before graduation as an undergraduate. His style of teaching impressed me again. When I had the idea of pursuing a graduate degree, it was no surprise that his name came to mind and was the first person I contacted. I would also like to thank the other members of my graduate committee, Dr. John Barthell and Dr. Thomas Jourdan for their advice and hands on assistance with my research. In addition, I would like to thank Dr. Tracy Morris for her expertise and assistance with the statistics for my research as well as suggestions for journal submissions. I'm grateful to the undergraduate students that helped in various capacities throughout my research and to my graduate student friends that assisted in field trials. This research would not have been possible without financial support from the UCO College of Mathematics & Sciences; Student Transformative Learning Record (STLR); Research, Creative, and Scholarly Activities (RCSA) grants; the UCO Center for Wildlife Forensic Science and Conservation Studies (C-FACS); and the W. Roger Webb Forensic Science Institute at UCO. Lastly, I express my gratitude for my family. I am thankful for my wife for allowing me to keep the bees on our property, collect dead animals on the side of the road and support me both financially and emotionally during

my research. My parents have been of great assistance and support throughout my graduate work and I appreciate them more than they will understand. I also express my appreciation to my oldest son Calen, now fifteen years old. My pursuit of a graduate degree started with a simple conversation about education and life with Calen as we drove to an extracurricular science program, he and my oldest daughter Jaime were in at the time. I was explaining why I was working a job that was unfulfilling and why it was important to finish as much school as you want because then life happens. The words he said next still feel as fresh as the moment he said them. “I think you should go back to school. We can be poor for a while so you can do a job that can help people and that you want to do.” To my surprise, my wife agreed, and my journey began.

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ABSTRACT OF THESIS
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TITLE OF THESIS: Necrophagy in Honey bees (*Apis mellifera*); A Forensic Application of Scent Foraging Behavior

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PAGES: 87

ABSTRACT: Scent training and field detection trials were conducted to determine if honey bee (*Apis mellifera*) foraging behavior could be employed as a practical application in the location of vertebrate carrion for use in interdiction and recovery efforts of illegally trafficked wildlife. Resource recognition and querying trials consisted of two components. Honey bees were trained to associate chemical compounds found in decaying tissues with a high-quality food source via introduction of the compounds into a sugar solution. Randomized scented and non-scented sugar solution choices were subsequently provided to hived bees at varying distances within a rural outdoor study area. Following initial forager recruitment by scouts, twice the number of bees were observed feeding at carrion-scented stations.

Additional field trials performed using wildlife carrion reinforced experimental results. Scent trained scout bees showed a marked interest in decomposing wildlife remains by aerially investigating and landing on the carrion. These findings demonstrate honey bee retention of carrion sensory recognition capabilities and support the cabronid wasp theory of honey bee evolutionary origins. Applications for forensic remains detection, wildlife trafficking interdiction, and endangered species conservation are indicated.

KEYWORDS*: Apis mellifera*, carrion foraging, forensic

GLOSSARY

Carrion - the decaying flesh of dead animals.

Clandestine - kept secret or done secretively, especially because illicit

Interdiction - the action of intercepting and preventing the movement of a prohibited commodity or person

Olfactory – relating to the sense of smell

Necrophagy - the eating of dead or decaying animal flesh

Chapter 1

THESIS INTRODUCTION

Statement of Problem

As criminals make changes to the tactics they employ in the commission of crimes of all types, so must those who are charged with the apprehension and prosecution of them. As forensic scientists, it is our responsibility to research and implement novel ideas to aid in the detection of crimes and the capture of those who engage in illegal activity. One of the areas in which advances in forensic science can be of assistance is in the interdiction and detection of illegal wildlife trafficking.

Honey bees

Honey bees (*Apis mellifera*) have been found to have extraordinary olfactory senses. They use these senses to forage naturally for rich food sources quickly and efficiently in their natural environment. It has also been found that bees are conditioned to forage for food sources through different means. Bees can locate a known food source through visual stimuli as well as olfactory or scent detection (Giurfa, 2007). Studies have been performed over the past few decades that show that bees can also be conditioned to detect and forage for odors introduced to them as a food source through reward-based conditioning. The success of this conditioning has led to further studies regarding the ability to train the bees to search for a variety of items. Research targeted at the honey bees' ability to find items of interest have included the following: Can bees be conditioned to find forensically significant items, for example, dead bodies? Is the training and usage of bees economically reasonable? Are bees reliable in their location of these targets? Results of this research indicate that the answer is yes (Bromenshenk et al., 2003; Carlsten et al., 2011; Couvillion et al., 2015; Matsumoto, Menzel, Sandoz & Giurfa, 2012; Leitch, Anderson, Kirkbride & Lennard, 2013).

Honey bee Associative Learning and Conditioning

As mentioned earlier, honey bees have a remarkable ability to locate food sources during their foraging. Food sources that are determined to be rich are then then communicated with the hive via waggle dances where more foragers are recruited and therefore more visitors come to these sources to exploit them (Seeley, 2010). Although bees are only equipped with a brain the size of grass seed, they have a great ability to learn, remember, and communicate information that is beneficial to their hive. Bees learn and remember this beneficial information not only through learned visual stimuli but also the use of olfactory sensors. Odor or scents have been shown to be part of the bees learning that indicate a good food source. It is through this knowledge that we as researchers can manipulate the bee's stimuli to condition them to go to "food sources" that may or may not be beneficial to them (Giurfa, 2007).

Several experiments have been conducted over the last 50 years that show the effectiveness of conditioning Honey bees to forage for odors of interest. The bees show classic Pavlovian conditioning through what is call the proboscis extension response or PER. This protocol was first introduced by Kimihisa Takeda in 1961 (Matsumoto, Menzel, Sandoz & Giurfa, 2012). This conditioning is done by exposing a bee that is hungry to odor that is near a sucrose reward. Bees then extend their proboscis to drink from the sucrose solution thus being rewarded. Then over continued exposure to the odor the bee then associates the odor with the sucrose reward and will seek it out, whether or not it is next to the sucrose solution (Matsumoto, Menzel, Sandoz & Giurfa, 2012).

Recently, researchers have found that introducing caffeine to the sucrose solution increases the foraging and recruitment behaviors in honey bees (Couvillon et al., 2015). The researchers added caffeine to sucrose solution that was in proportion to the sucrose naturally found in their nectar sources. Resulting number of foraging visits and frequency of the waggle dance for recruitment increased four times. It is believed by these researchers that caffeine may lead to efficient and effective foraging by aiding honeybee memory and suggests caffeine may enhance bee reward perception. Results of the increased recruitment of additional bees is a potential of a 14% increase in honey production. This research further reinforces the capability to manipulate and condition these animals (Couvillon et al., 2015).

The most closely related research to our question was done by Jerry J. Bromenshenk over the course of four years. The question he and the other researchers had was to determine if honey bees could be trained to find bombs and landmines, as well as other chemicals of interest, including drugs and decomposing bodies. Through multiple trials they found that "bees behaved like a very fine-tuned, nearly ideal detector at vapor levels higher than 10 parts per trillion, with a 1.0 – 2.5 percent probability of false positive and less than one percent probability of false negative (Bromenshenk et al., 2003). The bees were trained very similar to that of dogs. They used conditioning methods where food was the reward. Researchers showed that the bees would concentrate over vapor plumes associated with the target and then the concentrations would then be counted and compared with concentrations over the rest of the area. Higher concentrations were then compared to the known targets for accuracy. According to the researchers, there are several limitations for working with bees in this manner.

Bees do not fly at night, in the wind, in heavy rain, or when temperatures are too cold. However, there are some advantages as well. Bees don't need to bond with a handler, are inexpensive to use, and only take a couple of days to train (Bromenshenk et al., 2003).

Honey bees are not the only biological organisms that have been and that are being studied. Several different organisms such as canines, rats, wasps and moths have been studied and shown to be effective in locating volatile compounds. However, biological organisms all seem to have similar disadvantages, most of which revolve around environmental factors that may impact the effectiveness of the animal. One of the hardest issues to overcome for the widespread use of these animals, with exception of dogs, is the ability to track the animals in an uncontrolled environment (Leitch, Anderson, Kirkbride & Lennard, 2013).

African Elephants in Decline

African elephant populations have been in decline for several decades. A more rapid decline has been observed over the last 5-10 years. Central Africa's elephant population has declined in the Congo Basin by 76% since 2002 (Milliken, 2014). Similarly, Tanzania's Selous Game Reserve has reported decline in population from 100,000 animals in the mid-1970's, to a population of 13,000 in 2013 (Milliken, 2014). Remarkably, there was a population decline in the 2007-2013 period from 70,000-13,000, a drop of over 80% in only 6 years (Milliken, 2014). The total population of African elephants is decreasing by approximately 40,000 per year (Moyle, 2014). This population decline is not sustainable. If decline continues at the same pace, it is thought that the African elephant could become extinct in as little as 10 years (Blanc et al., 2007). Illegal killing for ivory is the driving force for the decline of the African elephant population

(Sukumar et al., 1998; Milliken, 2005). Overall, illegal wildlife trade has risen to a very high rate globally, causing substantial loss of biodiversity (Mondol et al 2014). Overharvesting is currently the second leading cause of biodiversity loss and local extinction. Overharvesting effects not only local populations of one species but the entire ecosystem in which they live (Wittemyer et al., 2014; Leimgruber et al., 2003; Sukumar, 2003; Hedges, 2006).

Corresponding with the decline in elephant populations, seizures of unworked or "raw" ivory has increased (Milikin, 2014). Government and independent agencies have increased regulations and restrictions on ivory over the past decade (CITES, 2014). Unfortunately, this has had an adverse effect on poaching cases and seizures. As a result of more stringent regulations, the demand for ivory has increased, especially on the black market. Most of the illegally trafficked ivory arrives to Asian countries with most going to China. Demand from Chinas' growing "middle class" seems to be a catalyst to the trafficking (Moyle, 2014).

Ivory is believed by some within Asian culture to have healing or medicinal powers. This only adds to the demand. In addition to middle class buyers in Asia, many purchasers are believed to be involved with organized crime groups throughout Asia and the Middle East (Wittemeyer et al., 2014). Terrorist groups are also believed to be involved in the illegal trade in wildlife. An investigation by the Elephant Action League indicated that al Qaeda generated between 200,000 and $600,000 per month from selling elephant tusks in 2011 alone (Elephant Action League, 2014). This accounts for just some of the millions of dollars of income these groups generate across all areas of illegal wildlife trafficking.

Illegal Wildlife Trafficking

With the demand for ivory increasing, there are questions concerning the transportation, destination, and origin of these items to help stop it (Moyle, 2014) Origination of the poached elephants has been researched by several methods. Most recently the use of genetics assignment has been used to identify major areas of poaching activity (Wasser et al., 2015). Scientists use DNA-based methods to assign origination of African elephant ivory. Large seizures are sampled and traced back to the native populations still left in the areas (Wasser et al, 2015). This is critical in the fight against poaching because once the origin is established, it helps local law enforcement place measures to deter the poachers (Wasser et al., 2015). Most of the elephant seizures over the last decade are from elephants that were poached from west central and eastern Africa. Most of the seizures were at sea ports in eastern Asia or destined for it. The figure below taken from TRAFFIC's report on illegal trade in ivory and rhino horn shows the increase in seizures over the last decade. (Miliken, 2014)

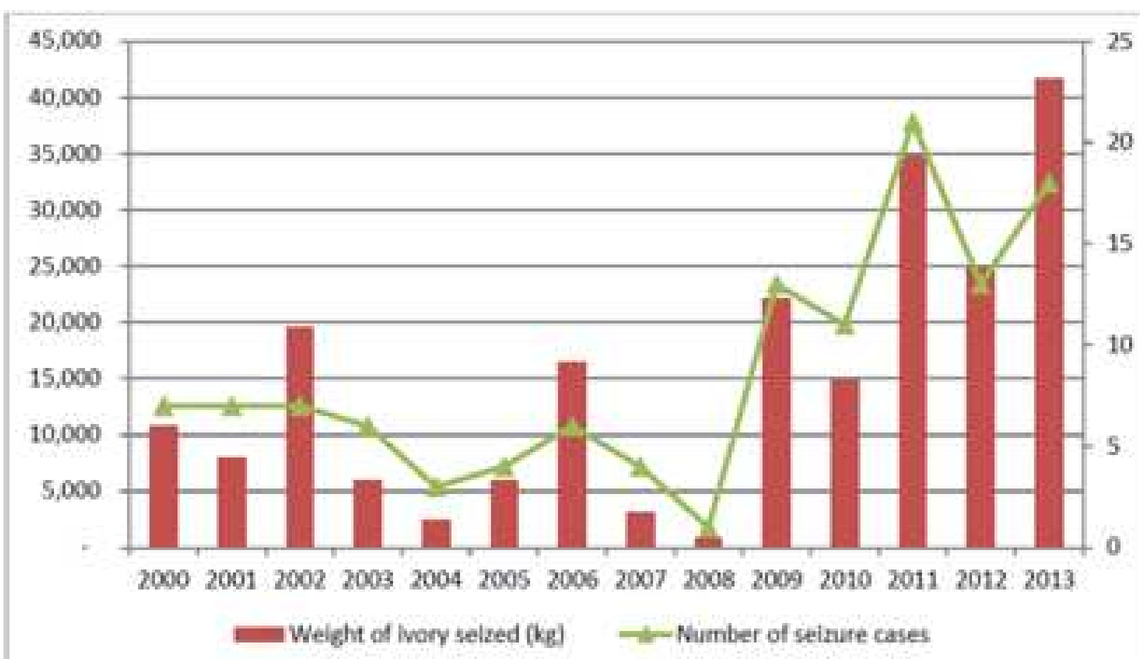

Figure 1: Estimated weight and number of large-scale (>500 kg) ivory seizures by year, 2000-2013 (ETIS 09 Janaury 2014)

While modes of transportation such as air and ground may be used for short-range distances, shipping provides a perfect avenue for the movement of large quantities of smuggled goods. Shipping allows for both mass quantities and a low detection rate of poached wildlife. Due largely to financial constraints, African governmental agencies are normally more concerned with shipments coming into the countries rather than what is going out. This allows for shipments to go undetected leaving and thus explains why so many of the seizures take place in Asian countries. A recent study shows that ivory is unusual compared to many other smuggled wildlife items in that it is almost exclusively transported in shipping containers. Two thirds of the seizures over the past decade have been found in shipping containers. The same study shows that the organizations shipping these goods are sensitive to the costs associated with their transportation. As costs go up, the number of seizures shows a decline. The opposite is also consistent (Moyle, 2014). The figure below taken from TRAFFIC's latest report on illegal trade in ivory and rhino horn shows trade routes for recent large quantity seizures (Miliken, 2014).

Figure 2: Trade routes for large-scale (>500kg) seizures of ivory, 2012-2013 (ETIS, 03 Novermber 2013)

Current Detection Methods of Smuggled Ivory

Most smuggled ivory is discovered in shipping containers at ports of entry. As such, it is important to look at how these discoveries are made. (Milliken, 2014). According to TRAFFIC's report, most discoveries are made through intelligence leads, followed by routine inspection, and targeting. Lesser amounts are discovered through investigation, x-ray, and sniffer dogs. In fact, at the time of the TRAFFIC publication, there had been no discoveries made by x-ray and only one from sniffer dogs. Sniffer dogs are generally only used in small areas and are more effective at finding smaller amounts of ivory in an environment such as an airport searching through luggage (Gazit and Terkel 2003). To date, sniffer dogs have not been employed in any kind of a large-scale search in a shipping yard. According to the report, x-ray equipment is hardly ever used in the ports in Africa due to its limited or absent availability (Milliken, 2014).

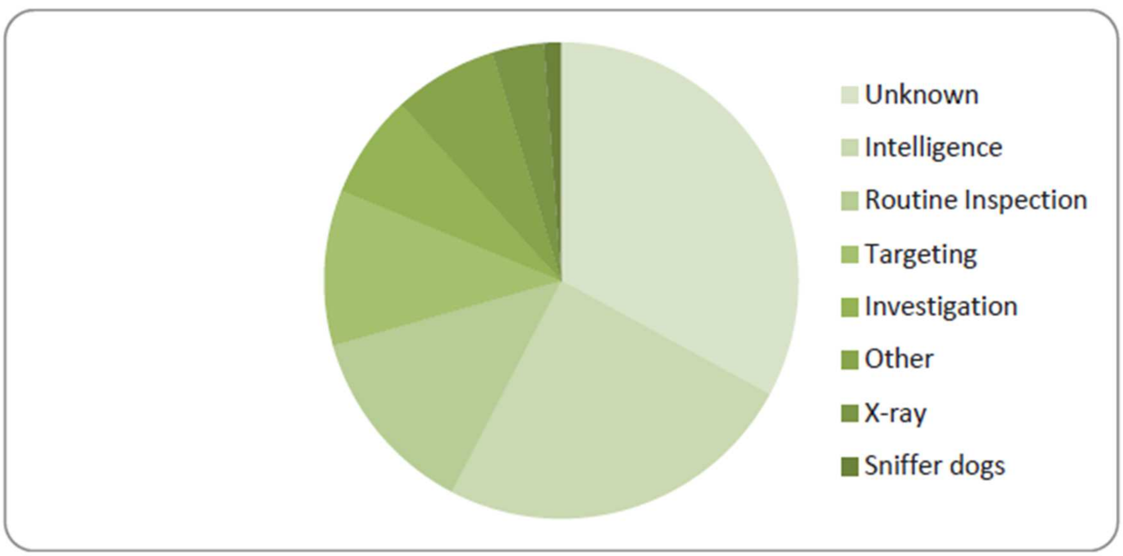

Figure 3: Reported method of detection of large-scale (>500 kg) ivory seizures, 2009-2013 (ETIS 09 January 2014)

Limitations of Current Detection Methods

As with most things, there are limitations with the current methods of detection. A major limitation for the initial detection from ports of exit is financing. African governments in the areas of the most trade lack the funding, manpower and technology to combat trafficking routes at their origin. Without adequate staff and technology, it is a perfect situation for smugglers to get their products out of the countries (Milliken, 2014)

As stated earlier, sniffer dogs are a good means of detection when it comes to finding small amounts of illegally trafficked wildlife remains and covering smaller areas. Limitations of this method include high cost to train, yearly upkeep, training of a handler, and other environmental conditions. A study published in *The Journal of Wildlife Management* indicates the cost in 2010 for to train a dog to a new scent is approximately eight thousand dollars (Duggan, Heske, Schooley, Hurt & Whitelaw, 2002). This does not include initial training estimated to cost between $20,000 and $30,000. In addition to training, the average cost to work a dog team with handler, salary, and per diem runs approximately $4,000 per week. This is not economically feasible considering the number of dogs and handlers that would be needed to cover a shipping yard containing hundreds or even thousands of containers. Due to the large area that sniffer dogs would need to cover in a shipping yard, it would be nearly impossible to get enough dogs or handlers to tackle the task either economically or logistically. Detection dogs also have limited working hours and their performance declines over time. (Gazit & Terkel, 2002).

X-ray scanners are almost non-existent in the African ports, especially with regards to outgoing containers. There have been advancements in X-ray scanning abilities in Asian ports of entry, but it’s not close to 100% effective. Even with the ability to scan these containers, the reliability is highly dependent on competence of the user. While x-ray scanning is a method of detection, it has not provided many, if any, seizures. Cost associated with equipment used for X-ray scanning can reach upwards of 200,000,000 USD. This high cost has proven to be prohibitive, especially in poorer African countries where most of the illegally trafficked ivory is coming out of (Milliken, 2014).

Research Questions

My main research questions are, will honey bees show preference to a scent of interest, or more specifically, the chemical compounds associated with carrion? Secondly, will honey bees then show interest in actual carrion in a field environment? Finally, if successful field trials are conducted, what are the potential practical applications and limitations associated with wildlife interdiction and recovery. The research presented in the next two chapters are each formatted as papers for publication in two different journals. Chapter two is formatted and accepted with revision for publication in the Kansas Entomological Society Journal. Chapter three will be submitted to Forensic Science International.

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Chapter 2

Necrophagy in Honey bees (*Apis mellifera*); A Forensic Application of Scent Foraging Behavior

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ABSTRACT: Scent training and field detection trials were conducted to determine if honey bee (*Apis mellifera*) foraging behavior could be employed as a practical application in the location of vertebrate carrion. Resource recognition and querying trials consisted of two components. Honey bees were trained to associate chemical compounds found in decaying tissues with a high-quality food source via introduction of the compounds into a sugar solution. Randomized scented and non-scented sugar solution choices were subsequently provided to hived bees at varying distances within a rural outdoor study area. Following initial forager recruitment by scouts, twice the number of bees were observed feeding at carrion-scented stations. Additional field trials performed using wildlife carrion reinforced experimental results. Scent trained scout bees showed a marked interest in decomposing wildlife remains by aerially investigating and landing on the carrion. These findings demonstrate honey bee retention of carrion sensory recognition capabilities and support the crabronid wasp theory of honey bee evolutionary origins. Applications for forensic remains detection, wildlife trafficking interdiction, and endangered species conservation are indicated.

KEYWORDS*: Apis mellifera*, carrion foraging, forensic

INTRODUCTION

Honey bees (*Apis mellifera*) play an important role in the pollination of plants worldwide (Conrad, 2018). In addition, honey bees avidly seek, recruit, and collect nectar and pollen as resource precursors to honey and beeswax (Bohart and Nye, 1956). They selectively forage for nectar and pollen by discerning food source quality (Seeley and Visscher, 1988). High quality being defined as maximal sucrose concentration per foraging cost (Seeley, 1995). Honey bees are also descendants of predatory wasps and potentially retain carrion scent recognition capabilities from their cabronid ancestors (Harris and Oliver, 1993; O'Donnell, 1995). I performed an experimental forensic application of honey bee foraging using scent training techniques to capacitate bees to the scent of vertebrate carrion (Kalinova et al., 2009). I successfully trained the bees to associate vertebrate carcass scent with a high-quality food source. I subsequently observed bees seeking and investigating actual carrion. These behaviors suggest potential use of induced honey bee carrion foraging in a variety of forensic, wildlife protection, and endangered species conservation scenarios.

As awareness of the honey bees' sentinel role in agriculture continues to grow, so does the need for additional research into the origins, and capabilities of foraging behaviors (Corn and Johnson, 2015). Over the years, there has been substantial research completed concerning the chemosensory abilities of the honey bee, due in a large part to an interest in the structure, function and origins of complex chemoreceptors (Robertson et al., 2010; Martin et al., 2011). Honey bees have been observed to learn quickly and to respond to a broad spectrum of chemical cues (Wenner and Wells, 1990). Honey bees have been demonstrated to be capable of scent recognition at compound concentrations of parts per trillion (Wenner et al., 1969, Wenner and Wells 1990). I investigated the potential to apply this ability forensically. More specifically, I attempted to determine if scent-induced the honey bees would recognize and preferentially seek carrion odor producing compounds in the field.

MATERIALS AND METHODS

Honey bee Conditioning and Scent Foraging Trials

The initial phase of my study conditioned hived honey bee colonies *(Apis mellifera)* using Pavlovian behavioral inducement by rewarding the honey bees with a 2.0M sugar syrup solution (Abramson et al, 2007, Seeley, 2010). The honey bees were trained by placing feeders filled with scented 2.0M sucrose solution at the entrance of three active hives. The solution was made by first preparing a 2.0M sucrose solution and then adding a scent "cocktail" to the solution. The cocktail consisted of equal parts S-Methyl Thioacetate, Dimethyl Sulfide, Dimethyl Disulfide and Dimethyl Trisulfide. These chemical compounds were used because they have been demonstrated to characterize decomposing vertebrate tissues (Kalinova et al. 2009). Two µL of the "cocktail" was added per liter of sucrose solution for the prepared scented sucrose solution. A feeder was left at the entrance of each hive for two days. Subsequently, the feeders were removed from the hive entrances and newly prepared scented solution was placed into each feeder and placed 1 m from the hive entrances. The feeders were left at this distance for two days. The process was repeated for the following five days, each day the feeders being moved greater distances from the hives to 2.5 m, 5 m, 10 m, 15 m and 25 m respectively.

Fig.1. Hives with entrance feeders containing scented solution

Fig.2. Feeder with scented solution placed at 10 meters from hives

Following the conditioning of the honey bees, field trials were conducted by adapting the olfactory experimental design of Wenner (1969). The evening prior to the trials, the scented sugar solution was removed from the field. On the first day of field trials, the feeders were placed 25m from the hives and spaced 12m from one another. One of the feeders was scented and the other two feeders were non-scented and used as the control for the trials. Position of the scented feeder was chosen using a random number generator for numbers 1 through 3 with 1 representing the left position, 2 representing the center position and 3 representing the right position (Fig:3). Honey bees were counted as visitors if they landed on and started feeding from the feeder. The bees were counted at each position for specified periods of time. After the first replicate, the feeders were moved to different positions based once again on the random numbers generator. Additionally, the same process was repeated at 50m. The length of time honey bees counted shortened in later replicates as the number of honey bees coming to the feeders increased dramatically.

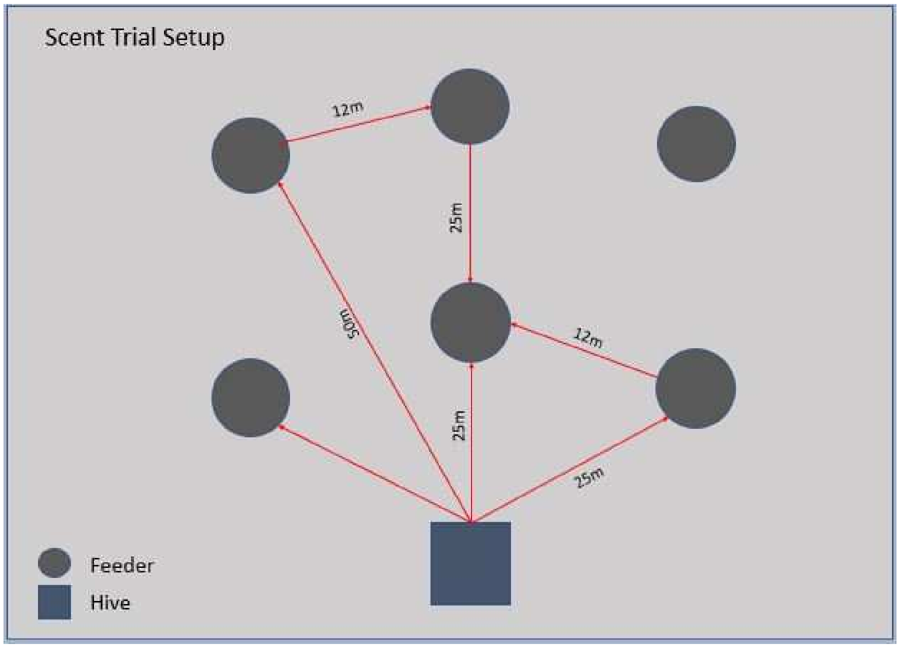

Fig.3. Feeder station positioning layout

Carrion Foraging Trial

The second phase of my foraging study consisted of the placement of locally obtained wildlife carcasses, in varying stages of decomposition, in proximity to the study hives to determine if the now "scent trained" honey bees would show interest in actual carrion. Prior to the day of the carrion experiment, the same rural roadside route was traveled in the morning and afternoon to locate suitable roadkill carcass samples and evaluate their approximate time since death. On the morning of the trial, carcasses were collected and placed in pre-determined study locations. Species collected for the trial were a bobcat (*Lynx rufus*), racoon (*Procyon lotor*) and opossum (*Didelphis virginiana*). Study carcasses were placed at distances of 10m, 12.5 and 25m from the hives and honey bee activity on and around the carrion was counted; carrion at each distance was observed for honey bee activity for ten minutes. I counted only honey bees that, after aerially investigating the carcasses showing interest, landed on the carrion.

Trials took place on 9/23/2017 and 9/30/2017 with each trial consisting of 6 replicates. Location of conditioning and trials was a rural area in Piedmont, OK approximately 22 km from the University of Central Oklahoma in Edmond, OK.

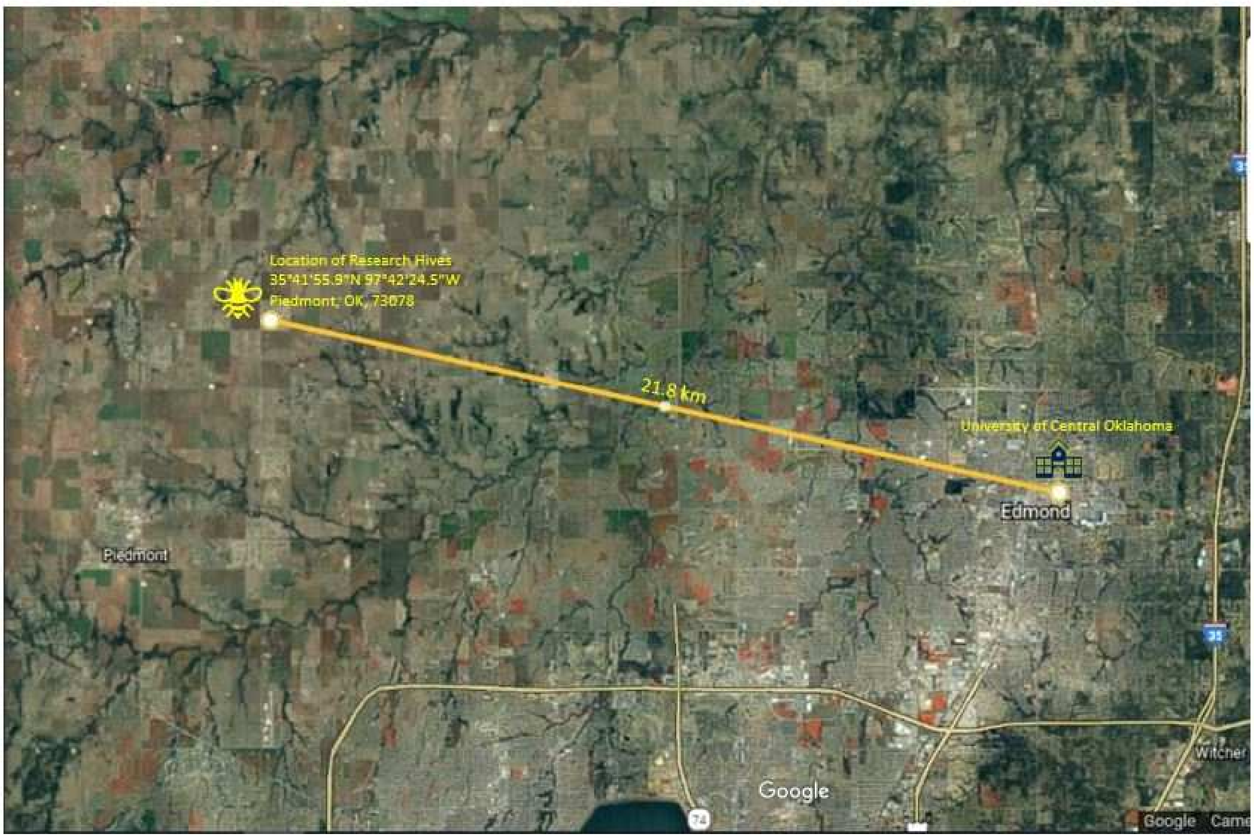

Fig.4. Location of research hives in proximity to University of Central Oklahoma

RESULTS

For each conditioning and scent trial, a two-factor (Scent & Replicate) repeated measures ANOVA, on the log-transformed data, assuming a variance components covariance structure was performed. This was done using proc mixed in SAS v. 9.4.

For conditioning and scent trial 1, there was no significant Scent*Replicate interaction ($F = 2.79$, p-value = 0.1215), but both main effects were significant (Scent: $F = 28.96$, p-value = 0.0017, Replicate: $F = 140.38$, p-value < 0.0001). Specifically, there were significantly more bees per minute at the scented station (mean = 4.59, se = 0.08) than at the unscented stations (mean = 2.42, se = 0.05). Also, there were significantly more bees per minute at the stations during replicate 4, than during replicates 1, 2, and 3 (p-value < 0.003); and during replicates 5 and 6, than during replicates 1 through 4 (p-value < 0.004). There were no other significant differences among the replicates during trial 1.

For conditioning and scent trial 2, there was no significant Scent*Replicate interaction ($F = 0.85$, p-value = 0.5600), but both main effects were significant (Scent: $F = 7.74$, p-value = 0.0319, Replicate: $F = 6.13$, p-value = 0.0236). Specifically, there were significantly more bees per minute at the scented station (mean = 25.16, se = 0.23) than at the unscented stations (mean = 11.76, se = 0.16). Also, there were significantly more bees per minute at the stations during replicates 4, 5, and 6, than during replicate 1 (p-value < 0.05). There were no other significant differences among the replicates during trial 2.

No statistical analyses were conducted on the actual carrion foraging trials as the results were solely observational.

Fig.5. Raw bee visitations per minute trial 1

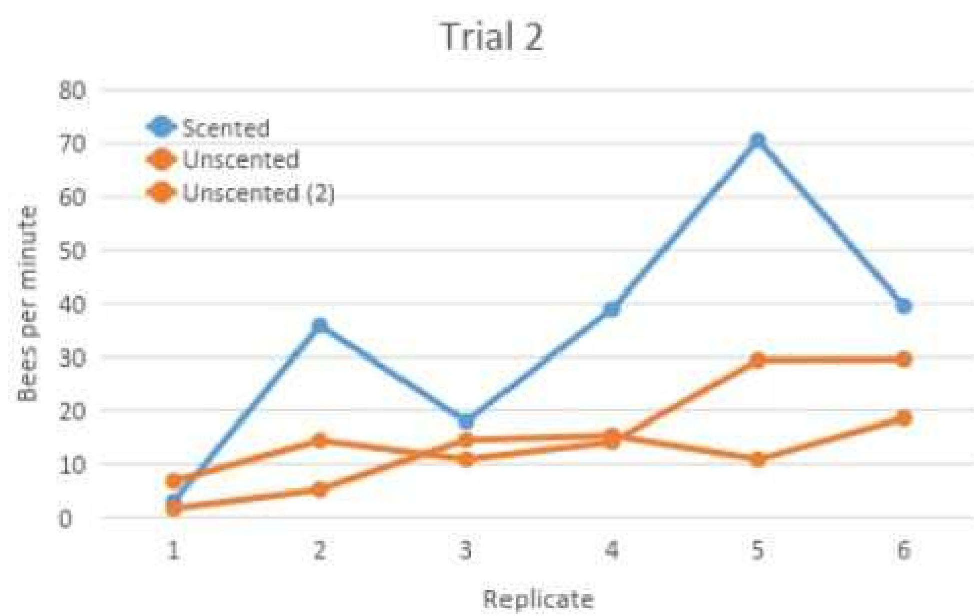

Fig.6. Raw bee visitations per minute trial 2

Distance from hive	Scouts flying around carrion	Landing on carrion
25m	4	1
25m	3	2
25m	3	0
12.5m	4	2
12.5m	5	2
12.5m	4	1
10m	5	2
10m	4	2

Table1. Honey bee activity on or around carrion left in the field

Figure.7. Honey bee (Apis mellifera) on entrails of carrion opossum (Didelphis virginiana)

Conditioning was shown to be successful as the number of honey bees foraging at the scented solutions was consistently significantly higher than that of the unscented solutions. There was also a significant difference in the number of honey bees frequenting the scented solutions following the first replicates and throughout later replicates. During the first replicates there was no statistical difference in the number of honey bees visiting each station. Later replicates consistently showed almost double the number of honey bees at the scented stations. This demonstrates successful recruitment behaviors on the part of scout bees and foragers via communication of the availability and location of the scented high-quality food source.

During the carrion foraging trials, multiple scout bees were observed flying around and landing on the supplied vertebrate carcasses. Since actual carrion would not be considered a high-quality food source by scout bees, there was not an expectation that the bees would recruit significant numbers of additional hive members, thus the number of visiting bees would be restricted. The majority of honey bee activity seen was on and around the opossum *(Didelphis virginiana),* which was the carrion that displayed the longest post-mortem interval and was the most odorous. Multiple species of necrophagous insects commonly associated with local carrion, including flies, beetles and wasps were observed on all carcass samples regardless of stage of decomposition and on a test plot of the experiment scent solution cocktail.

DISCUSSION

These studies reveal, in multiple contexts, that honey bees can easily learn and track the odor of dead animals. This ability to discern such odors in the environment is consistent with the phylogenetic history of the Apoidea, including current taxonomic conclusions that bees arose from cabronid wasps (Sann et al. 2018). This necrophagous trait remains apparent in the regular feeding activity of at least one eusocial Neotropical social bee species, *Trigona hypogea*, and its behavior can actually extend to predation (Mateus and Noll, 2004; Roubik, 1984). Indeed, carrion traps in a Brazilian rainforest yielded several genera of social bees (including *Apis mellifera*) further suggesting that necrophagy may have a genetic basis in olfactory behavior found in social bee species (Tobias Silveira et al., 2005).

As previously noted, scent trained scout bees showed a marked interest in decomposing wildlife remains by aerially investigating and landing on the carrion. These findings demonstrate honey bee retention of carrion sensory recognition capabilities and support the cabronid wasp theory of honey bee evolutionary origins. My observations provide additional evidence for the potential utilization of honey bees as agents of vertebrate carrion scent location. Honey bees can be trained quickly when compared to other animals normally used in forensic and investigative scent location scenarios, such as canines. The cost of training and maintaining scent induced bees is minimal and their employment does not require a specialized handler (Duggan et al., 2011). Additionally, hive conditions and stressors do not appear to significantly impact bee scent learning ability and activity (Matilla and Smith 2008: Gazit and Terkel 2003).

While these observations demonstrate the ability of honey bees to recognize, seek, and detect vertebrate carrion under field conditions, there are still areas of research that need to be completed prior to the practical implementation of this ability. In the journal article *Bees as Biosensors: Chemosensory ability, Honey bee monitoring systems, and Emergent sensor technologies derived from the pollinator syndrome* (Bromenshank et al., 2015) advances in honey bee biosensor abilities and monitoring capabilities are summarized. These types of advanced tracking technologies may prove central to the practical application of the experiments we have performed. Future research also needs to be completed to determine if vertebrate carrion recognition can be species specific. Additionally, as most honey bee foraging activity was observed around the opossum, which was in the latest stage of decomposition, studies targeted at determining the role of decomposition in optimal honey bee carrion detection is needed.

My findings clearly demonstrate conservation of carrion recognition by honey bees and the contemporary inducement of these sensory discrimination capabilities. Numerous. applications for forensic remains detection, wildlife trafficking interdiction, and endangered species conservation are indicated.

Acknowledgments

A am grateful to the University of Central Oklahoma (UCO) Forensic Science Institute for their logistical support and funding. Additional funding was provided by UCO's Research, Creative, & Scholarly Activities grant program (Office of High-Impact Practices), Student Transformative Learning Record (Center for Transformative Teaching and Learning), Center for Undergraduate Research and Education in STEM (UCO College of Mathematics and Science), and the Research Experiences for Undergraduates (REU) program funded by the National Science Foundation (DBI 1560389). I thank undergraduate students L.S. Henning, J.C. Latham, D.R. Williams, B.A. Savoy, R.L. Parham, J.K. Lord and graduate student Thu Ngo for their assistance with this project. With great appreciation we thank Calen A. Morice for his continued assistance maintaining the research hives and assisting with all field trials.

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Chapter 3

Necrophagy in Honey bees (*Apis mellifera*); A Forensic Application of Scent Foraging Behavior for the Conservation of Illegally Trafficked Wildlife

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ABSTRACT: Scent training and field detection trials were conducted to determine if honey bee (*Apis mellifera*) foraging behavior could be employed as a practical application in the location of vertebrate carrion for the purpose of interdiction and recovery of illegally trafficked wildlife. Resource recognition and querying trials consisted of three components. Proboscis Extension Response (PER) experiments were completed to confirm the ability of honey bees to recognize scents associated with decaying biological material. Honey bees were trained to associate chemical compounds found in decaying tissues with a high-quality food source via introduction of the compounds into a sugar solution. Randomized scented and non-scented sugar solution choices were subsequently provided to hived bees at varying distances within a rural outdoor study area. Following initial forager recruitment by scouts, twice the number of bees were observed feeding at carrion-scented stations. Additional field trials performed using wildlife carrion reinforced experimental results. Scent trained scout bees showed a marked interest in decomposing wildlife remains by aerially investigating and landing on the carrion. These findings demonstrate honey bee retention of carrion sensory recognition capabilities and support the cabronid wasp theory of honey bee evolutionary origins. Applications for forensic remains detection, wildlife trafficking interdiction, and endangered species conservation are indicated.

KEYWORDS: *Apis mellifera*, carrion foraging, forensic, wildlife conservation

INTRODUCTION

As criminals make changes to the tactics they employ in the commission of crimes of all types, so must those who are charged with the apprehension and prosecution of them. As forensic scientists, it is our responsibility to research and implement novel ideas to aid in the detection of crimes and the capture of those who engage in illegal activity. A potential ally in our efforts is the honey bee.

Role of Honey bees

Honey bees (*Apis mellifera*) play an important role in the pollination of plants worldwide (Conrad, 2018). Honey bees avidly seek, recruit, and collect nectar and pollen as resource precursors to honey and beeswax (Bohart and Nye, 1956). They selectively forage for nectar and pollen by discerning food source quality (Seeley and Visscher, 1988). High quality being defined as maximal sucrose concentration per foraging cost (Seeley, 1995). Honey bees are also descendants of predatory wasps and potentially retain carrion scent recognition capabilities from their crabronid ancestors (Harris and Oliver, 1993; O'Donnell, 1995).

Recently, honey bees have been found to have an important new role in agriculture. Researchers have found that African elephants fear honey bees. Although an elephants' hide is too thick for the honey bees' stinger to penetrate, the honey bees can sting them on more sensitive areas such as their eyes, trunks and mouths. Researchers have used this fear to help keep elephants out of agricultural fields where they have commonly foraged. By placing bee hives at regular intervals around fields, the decrease in elephant activity has decreased by 80 percent. This strategy has served a dual purpose for farmers in Africa. First, it discourages elephants from foraging their crops and secondly, it discourages farmers from cutting down trees to expand their farms (King et al., 2007, 2011)

As awareness of the honey bees' sentinel role in agriculture continues to grow, so does the need for additional research into the origins, and capabilities of foraging behaviors (Corn and Johnson, 2015). Over the years, there has been substantial research completed concerning the chemosensory abilities of the honey bee, due in a large part to an interest in the structure, function and origins of complex chemoreceptors (Robertson et al., 2010; Martin et al., 2011). Honey bees have been observed to learn quickly and to respond to a broad spectrum of chemical cues (Wenner and Wells, 1990). Honey bees have been demonstrated to be capable of scent recognition at compound concentrations of parts per trillion (Wenner et al., 1969, Wenner and Wells 1990). We investigated the potential to apply this ability forensically. More specifically, we attempted to determine if scent-induced the honey bees would recognize and preferentially seek carrion odor producing compounds in the field.

We performed an experimental forensic application of honey bee foraging using scent training techniques to capacitate bees to the scent of vertebrate carrion (Kalinova et al., 2009). We performed preliminary proboscis extension response (PER) experiments to confirm the honey bees' ability to recognize and respond to the scents we would use in the next phases of our research. We then successfully trained the bees to associate vertebrate carcass scent with a high-quality food source. We subsequently observed bees seeking and investigating actual carrion. These behaviors suggest potential use of induced honey bee carrion foraging in a variety of forensic, wildlife protection, and endangered species conservation scenarios.

African Elephants in Decline

African elephant populations have been in decline for several decades. A more rapid decline has been observed over the last 5-10 years. Central Africa's elephant population has declined in the Congo Basin by 76% since 2002. Similarly, Tanzania's Selous Game Reserve has reported decline in population from 100,000 animals in the mid-1970's, to a population of 13,000 in 2013. Remarkably, there was a population decline in the 2007-2013 period from 70,000-13,000, a drop of over 80% in only 6 years (Milliken, 2014). The total population of African elephants is decreasing by approximately 40,000 per year (Moyle, 2014). This population decline is not sustainable. If decline continues at the same pace, it is thought that the African elephant could become extinct in as little as 10 years. Illegal killing for ivory is the driving force for the decline of the African elephant population. Overall, illegal wildlife trade has risen to a very high rate globally, causing substantial loss of biodiversity.

Corresponding with the decline in elephant populations, seizures of unworked or "raw" ivory has increased (see Fig.1.). Government and independent agencies have increased regulations and restrictions on ivory over the past decade. Unfortunately, this has had an adverse effect on poaching cases and seizures. As a result of more stringent regulations, the demand for ivory has increased, especially on the black market. Most of the illegally trafficked ivory arrives to Asian countries with most going to China. Demand from Chinas' growing middle class seems to be a catalyst to the trafficking. Ivory is believed by some within Asian culture to have healing or medicinal powers. This only adds to the demand. In addition to middle class buyers in Asia, many purchasers are believed to be involved with organized crime groups throughout Asia and the Middle

East. Terrorist groups are also believed to be involved in the illegal trade in wildlife. An investigation by the Elephant Action League indicated that al Qaeda generated between 200,000 and $600,000 per month from selling elephant tusks in 2011 alone (Elephant Action League, 2014). This accounts for just some of the millions of dollars of income these groups generate across all areas of illegal wildlife trafficking.

Illegal Wildlife Trafficking

With the demand for ivory increasing, there are questions concerning the transportation, destination, and origin of these items to help combat it. Origination of the poached elephants has been researched by several methods. Most recently the use of genetics assignment has been used to identify major areas of poaching activity. Scientists use DNA-based methods to assign origination of African elephant ivory. Large seizures are sampled and traced back to the native populations still left in the areas. This is critical in the fight against poaching because once the origin is established, it helps local law enforcement place measures to deter the poachers (Wasser et al., 2015). Most of the elephant seizures over the last decade are from elephants that were poached from west central and eastern Africa. Most of the seizures were at sea ports in eastern Asia or destined for it. The figure below taken from TRAFFIC's report on illegal trade in ivory and rhino horn shows the increase in seizures over the last decade. (Miliken, 2014)

While modes of transportation such as air and ground may be used for short-range distances, shipping provides a perfect avenue for the movement of large quantities of smuggled goods. Shipping allows for both mass quantities and a low detection rate of poached wildlife. Due largely to financial constraints, African governmental agencies are normally more concerned with shipments coming into the countries rather than what is going out. This allows for shipments to go undetected leaving and thus explains why so many of the seizures take place in Asian countries. A recent study shows that ivory is unusual compared to many other smuggled wildlife items in that it is almost exclusively transported in shipping containers. Two thirds of the seizures over the past decade have been found in shipping containers. The same study shows that the organizations shipping these goods are sensitive to the costs associated with their transportation. As costs go up, the number of seizures shows a decline. The opposite is also consistent (Moyle, 2014). The figure below taken from TRAFFIC's latest report on illegal trade in ivory and rhino horn shows trade routes for recent large quantity seizures (see Fig.2.).

Current Detection Methods of Smuggled Ivory

Most smuggled ivory is discovered in shipping containers at ports of entry. As such, it is important to look at how these discoveries are made. According to TRAFFIC's report, most discoveries are made through intelligence leads, followed by routine inspection, and targeting. Lesser amounts are discovered through investigation, x-ray, and sniffer dogs. In fact, at the time of the TRAFFIC publication, there had been no discoveries made by x-ray and only one from sniffer dogs. Sniffer dogs are generally only used in small areas and are more effective at finding smaller amounts of ivory in an environment such as an airport searching through luggage. To date, sniffer dogs have not been employed in any kind of a large-scale search in a shipping yard. According to the report, x-ray equipment is hardly ever used in the ports in Africa due to its limited or absent availability (see Fig.3.).

Limitations of Current Detection Methods

As with most things, there are limitations with the current methods of detection. A major limitation for the initial detection from ports of exit is financing. African governments in the areas of the most trade lack the funding, manpower and technology to combat trafficking routes at their origin. Without adequate staff and technology, it is a perfect situation for smugglers to get their products out of the countries (Milliken, 2014)

As stated earlier, sniffer dogs are a good means of detection when it comes to finding small amounts of illegally trafficked wildlife remains and covering smaller areas. Limitations of this method include high cost to train, yearly upkeep, training of a handler, and other environmental conditions. A study published in *The Journal of Wildlife Management* indicates the cost in 2010 for to train a dog to a new scent is approximately eight thousand dollars (Duggan, Heske, Schooley, Hurt & Whitelaw, 2002). This does not include initial training estimated to cost between $20,000 and $30,000. In addition to training, the average cost to work a dog team with handler, salary, and per diem runs approximately $4,000 per week. This is not economically feasible considering the number of dogs and handlers that would be needed to cover a shipping yard containing hundreds or even thousands of containers. Due to the large area that sniffer dogs would need to cover in a shipping yard, it would be nearly impossible to get enough dogs or handlers to tackle the task either economically or logistically. Detection dogs also have limited working hours and their performance declines over time. (Gazit & Terkel, 2002).

X-ray scanners are almost non-existent in the African ports, especially with regards to outgoing containers. There have been advancements in X-ray scanning abilities in Asian ports of entry but is not close to 100% effective. Even with the ability to scan these containers, the reliability is highly dependent on competence of the user. While x-ray scanning is a method of detection, it has not provided many, if any, seizures. Cost associated with equipment used for X-ray scanning can reach upwards of 200,000,000 USD. This high cost has proven to be prohibitive, especially in poorer African countries where most of the illegally trafficked ivory is coming out of (Milliken, 2014).

MATERIALS AND METHODS

Proboscis Extension Response Experimentation

Proboscis Extension Response or (PER) is a process used to classically condition honey bees using Pavlovian food-based reward in a controlled environment (Abramson 2007). Honey bees are collected, starved, satiated and conditioned. Honey bees were fed until satiated once individually secured. They were then starved for twenty-four hours. This was done to encourage them to condition easier during the experiment the following day (Redd 2014). A scented sucrose reward was given to the honey bees to condition them to associate this reward with scents of interest. The success of this conditioning was made evident by the extension of the honey bee proboscis in the presence of the target scents. Scents of interest included S-Methyl Thioacetate, Dimethyl Sulfide, Dimethyl Disulfide and Dimethyl Trisulfide. These scents have been shown to be associated with decomposing biological material or carrion and are of significance to subsequent field experiments. (citation)

Honey bee Conditioning and Scent Foraging Trials

The initial phase of our field study conditioned hived honey bee colonies *(Apis mellifera)* using Pavlovian behavioral inducement by rewarding the honey bees with a 2.0M sugar syrup solution (Abramson et al, 2007, Seeley, 2010). The honey bees were trained by placing feeders filled with scented 2.0 molar sucrose solution at the entrance of three active hives. The solution was made by first preparing a 2.0 molar sucrose solution and then adding a scent "cocktail" to the solution. The cocktail consisted of equal parts s-methyl thioacetate, dimethyl sulfide, dimethyl disulfide and dimethyl trisulfide. These chemical compounds were used because they have been demonstrated to characterize decomposing vertebrate tissues (Kalinova et al. 2009). Two µL of the “cocktail” was added per liter of sucrose solution for the prepared scented sucrose solution (Appendices 1-4). A feeder was left at the entrance of each hive for two days. Subsequently, the feeders were removed from the hive entrances and newly prepared scented solution was placed into each feeder and placed 1 meter from the hive entrances. The feeders were left at this distance for two days. The process was repeated for the following five days, each day the feeders being moved greater distances from the hives to 2.5m, 5m, 10 m, 15m and 25m respectively (see Figs.4,5.).

Following the conditioning of the honey bees, field trials were conducted by adapting the olfactory experimental design of Wenner (1969). The evening prior to the trials, the scented sugar solution was removed from the field. On the first day of field trials, the feeders were placed 25m from the hives and spaced 12m from one another (see Fig.6.). One of the feeders was scented and the other two feeders were non-scented and used as the control for the trials. Position of the scented feeder was chosen using a random number generator for numbers 1 through 3 with 1 representing the left position, 2 representing the center position and 3 representing the right position Honey bees were counted as visitors if they landed on and started feeding from the feeder. The bees were counted at each position for specified periods of time. After the first replicate, the feeders were moved to different positions based once again on the random numbers generator. Additionally, the same process was repeated at 50 m. Trials took place on 9/23/2017 and 9/30/2017 with each trial consisting of 6 replicates. The length of time honey bees counted shortened in later replicates as the number of honey bees coming to the feeders increased dramatically.

Carrion Foraging Trial

The second phase of our foraging study consisted of the placement of locally obtained wildlife carcasses, in varying stages of decomposition, in proximity to the study hives to determine if the now “scent trained” honey bees would show interest in actual carrion. Prior to the day of the carrion experiment, the same rural roadside route was traveled in the morning and afternoon to locate suitable roadkill carcass samples and evaluate their approximate time since death. On the morning of the trial, carcasses were collected and placed in pre-determined study locations. Species collected for the trial were a bobcat (*Lynx rufus*), racoon (*Procyon lotor*) and opossum (*Didelphis virginiana*). Study carcasses were placed at distances of 10 m, 12.5 m and 25 m from the hives and honey bee activity on and around the carrion was counted; carrion at each distance was observed for honey bee activity for ten minutes. We counted only honey bees that, after aerially investigating the carcasses showing interest, landed on the carrion (see Fig.9.).

RESULTS

For each conditioning and scent trial, a two-factor (Scent & Replicate) repeated measures ANOVA, on the log-transformed data, assuming a variance components covariance structure, was performed. A mixed model ANOVA was performed with a fixed effect for Scent and a repeated effect for Replicate. This mixed model was coded in SAS v. 9.4 using proc mixed.

For conditioning and scent trial 1, there was no significant Scent*Replicate interaction ($F = 2.79$, p-value = 0.1215), but both main effects were significant (Scent: $F = 28.96$, p-value = 0.0017, Replicate: $F = 140.38$, p-value < 0.0001). Specifically, there were significantly more bees per minute at the scented station (mean = 4.59, se = 0.08) than at the unscented stations (mean = 2.42, se = 0.05). Also, there were significantly more bees per minute at the stations during replicate 4, than during replicates 1, 2, and 3 (p-value < 0.003); and during replicates 5 and 6, than during replicates 1 through 4 (p-value < 0.004). There were no other significant differences among the replicates during trial 1 (see Fig.7.).

For conditioning and scent trial 2, there was no significant Scent*Replicate interaction ($F = 0.85$, p-value = 0.5600), but both main effects were significant (Scent: $F = 7.74$, p-value = 0.0319, Replicate: $F = 6.13$, p-value = 0.0236). Specifically, there were significantly more bees per minute at the scented station (mean = 25.16, se = 0.23) than at the unscented stations (mean = 11.76, se = 0.16). Also, there were significantly more bees per minute at the stations during replicates 4, 5, and 6, than during replicate 1 (p-value < 0.05). There were no other significant differences among the replicates during trial 2. (see Fig.8.).

No statistical analyses were conducted on the actual carrion foraging trials as the results were solely observational (see Table 1.).

DISCUSSION

Conditioning was shown to be successful as the number of honey bees foraging at the scented solutions was consistently significantly higher than that of the unscented solutions. During the carrion foraging trials, multiple scout bees were observed flying around and landing on the supplied vertebrate carcasses. Since actual carrion would not be considered a high-quality food source by scout bees, there was not an expectation that the bees would recruit significant numbers of additional hive members, thus the number of visiting bees would be restricted. The majority of honey bee activity seen was on and around the opossum *(Didelphis virginiana),* which was the carrion that displayed the longest post-mortem interval and was the most odorous. Multiple species of necrophagous insects commonly associated with local carrion, including flies, beetles and wasps were observed on all carcass samples regardless of stage of decomposition and on a test plot of the experiment scent solution cocktail.

These studies reveal, in multiple contexts, that honey bees can easily learn and track the odor of dead animals. This ability to discern such odors in the environment is consistent with the evolutionary crabronid wasp theory that honey bees are the evolutionary descendants of wasps (Sann et al. 2018). Wasps are predatory and feed on carrion. This necrophagous behavior remains in the regular feeding activity of at least one eusocial Neotropical social bee species, *Trigona hypogea*, and its behavior can extend to predation (Mateus and Noll, 2004; Roubik, 1984). Indeed, carrion traps in a Brazilian rainforest yielded several genera of social bees (including *Apis mellifera*) further suggesting that necrophagy may have a genetic basis in olfactory behavior found in social bee species (Tobias Silveira et al., 2005). Our observations provide evidence for potential to utilize the honey bees in the detection of human remains as well as illegally trafficked wildlife.

Several advantages are apparent when comparing honey bees to current animal scent detection methods such as canines. Honey bees are non-invasive, this would allow for crime scenes involving human remains to be located without foot traffic or other activities to interfere with the scene. This would be of great benefit for remains detection of clandestine graves. In addition, honey bees have to ability to search in directions and over distances that sniffer dogs either cannot or would be time prohibitive. Honey bees regularly forage up to several miles to find food. This could be done in a short period by a hive a scent trained honey bees. Also, honey bees can search better in three dimensional spaces, meaning they can fly higher and get to areas not easily accessed by canines. This would include small cracks and crevices that canines cannot fit in.

Olfactory abilities in honey bees is said to be one hundred times greater than that of a human and canines are believed to be around 40 times that of a human. This ability makes them valuable tools in detection of odors. Honey bees can also be trained quickly when compared to other animals normally used in forensic and investigative scent location scenarios, such as canines. Honey bees can be trained in as little as one day, as opposed to months of training for a canine. This speed can become crucial if the honey bees were to be deployed at a scene. Cost of training and maintaining scent induced bees is minimal and their employment does not require a specialized handler (Duggan et al., 2011). Honey bees and the initial hive setup may cost only a few hundred dollars versus the cost to train a sniffer dog that may reach upwards of one hundred thousand dollars. This cost difference would allow areas in poverty to have access to tracking capabilities that are previously cost prohibitive. Since honey bees are inexpensive and have a short lifespan when compared to other tracking animals, their expendability is greater making it easier to start over in the case of a loss.

While our observations demonstrate the ability of honey bees to recognize, seek, and detect vertebrate carrion under field conditions, there are still areas of research that need to be completed prior to the practical implementation of this ability. In the journal article *Bees as Biosensors: Chemosensory ability, Honey bee monitoring systems, and Emergent sensor technologies derived from the pollinator syndrome* (Bromenshank et al., 2015) advances in honey bee biosensor abilities and monitoring capabilities are summarized. Tracking technologies such as LIDAR may prove central to the practical application of the experiments we have performed. Potential tracking technologies such as radar-reflective paints, transponder tags and drones may also be alternative routes to tracking the scent induced foraging honey bees.

Our findings clearly demonstrate conservation of carrion recognition by honey bees and the contemporary inducement of these sensory discrimination capabilities. Numerous applications for forensic remains detection, wildlife trafficking interdiction, and endangered species conservation are potential beneficiaries of this research. Honey bees are already in use in parts of Africa to deter and redirect elephants from farmland. African elephants are naturally afraid of honey bees and stay away from them. Conditioning the honey bees to actively seek out elephants may increase the effectiveness of their deterrent. Additionally, the honey bees can provide farmers additional sources of income from beeswax products and honey.

ACKNOWLEDGEMENTS

We are grateful to the University of Central Oklahoma (UCO) Forensic Science Institute for their logistical support and funding. Additional funding was provided by UCO's Research, Creative, & Scholarly Activities grant program (Office of High-Impact Practices), Student Transformative Learning Record (Center for Transformative Teaching and Learning), Center for Undergraduate Research and Education in STEM (UCO College of Mathematics and Science), and the Research Experiences for Undergraduates (REU) program funded by the National Science Foundation (DBI 1560389). We thank undergraduate students L.S. Henning, J.C. Latham, D.R. Williams, B.A. Savoy, R.L. Parham, J.K. Lord and graduate student Thu Ngo for their assistance with this project. With great appreciation we thank Calen A. Morice for his continued assistance maintaining the research hives and assisting with all field trials.

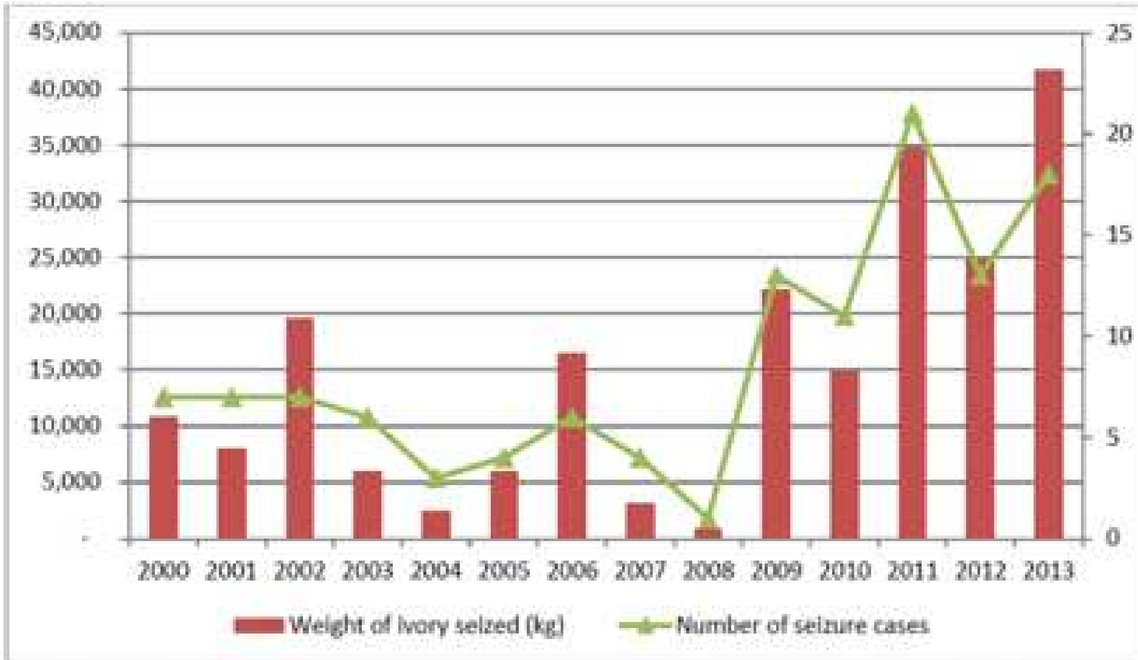

Figure 2: Estimated weight and number of large-scale (>500 kg) ivory seizures by year, 2000-2013 (ETIS 09 Janaury 2014)

Figure 2: Trade routes for large-scale (>500kg) seizures of ivory, 2012-2013 (ETIS, 03 Novermber 2013)

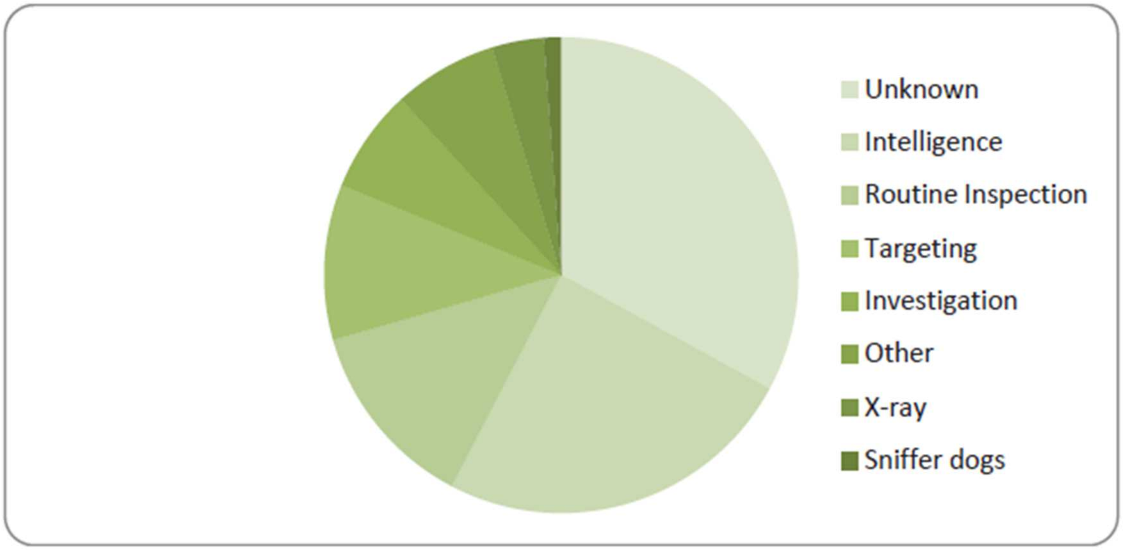

Figure 3: Reported method of detection of large-scale (>500 kg) ivory seizures, 2009-2013 (ETIS 09 January 2014)

Fig.4. Hives with entrance feeders containing scented solution

Fig.5. Feeder with scented solution placed at 10 meters from hives

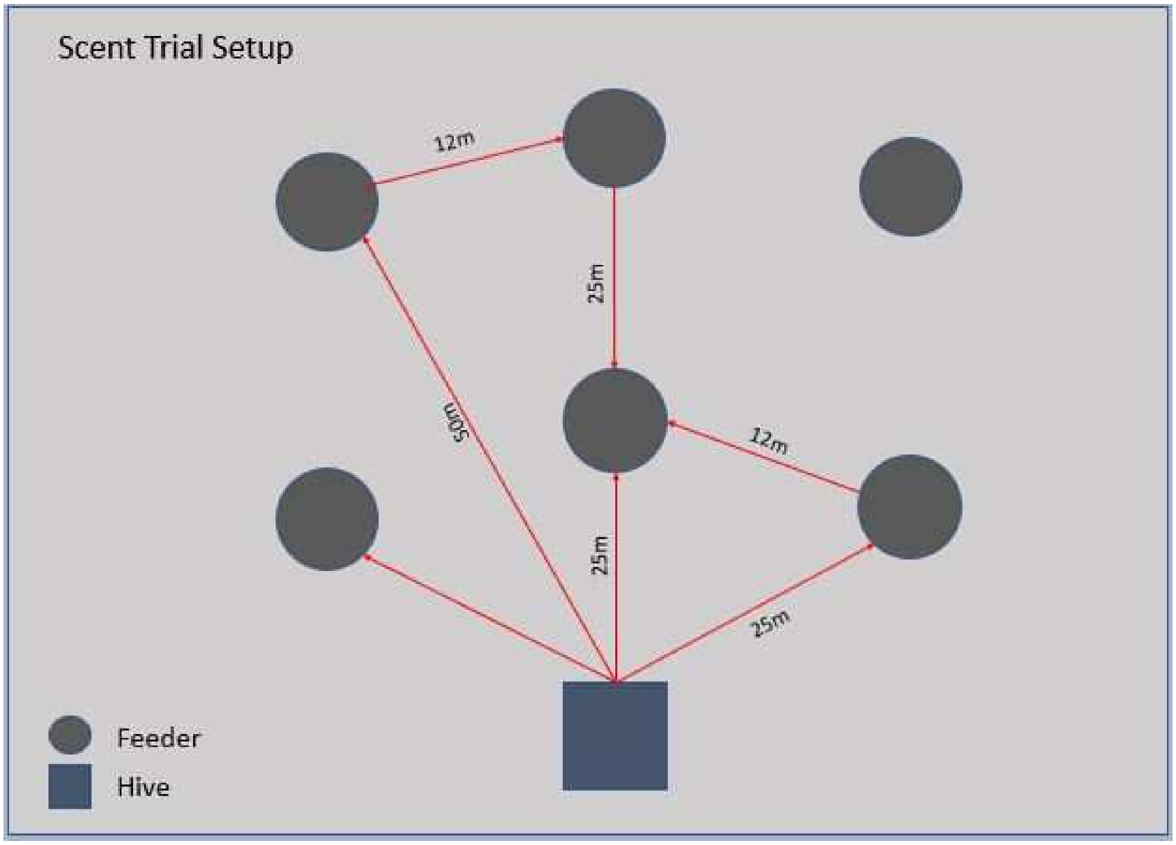

Fig.6. Feeder station positioning layout

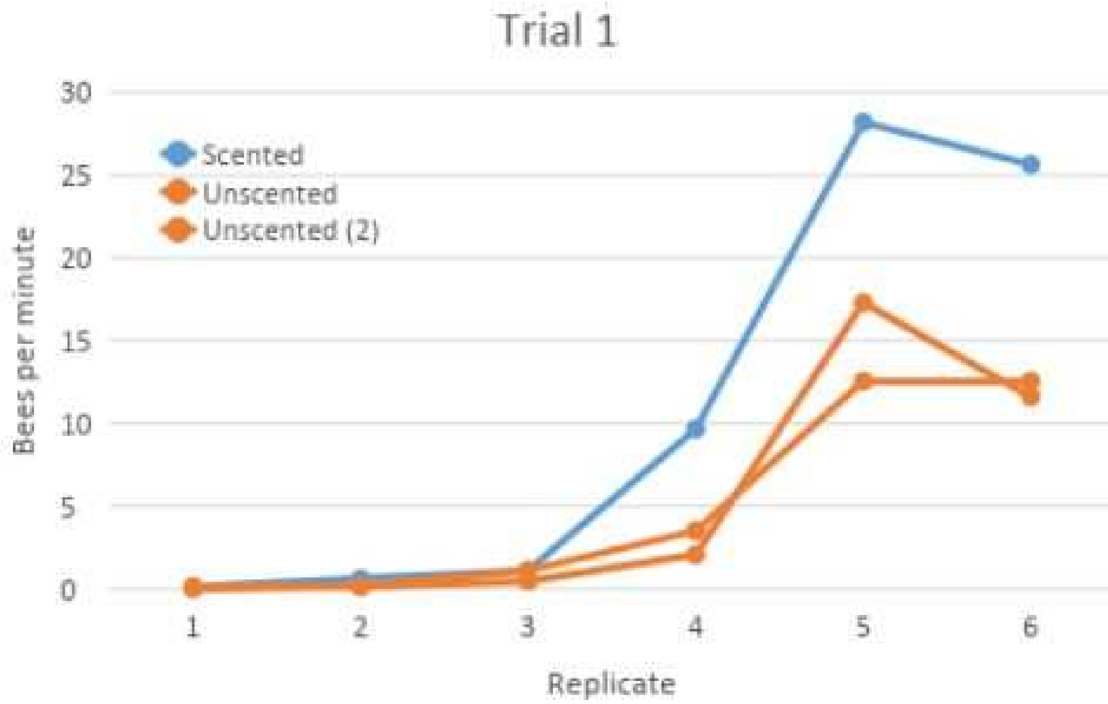

Fig.7. Raw bee visitations per minute trial 1

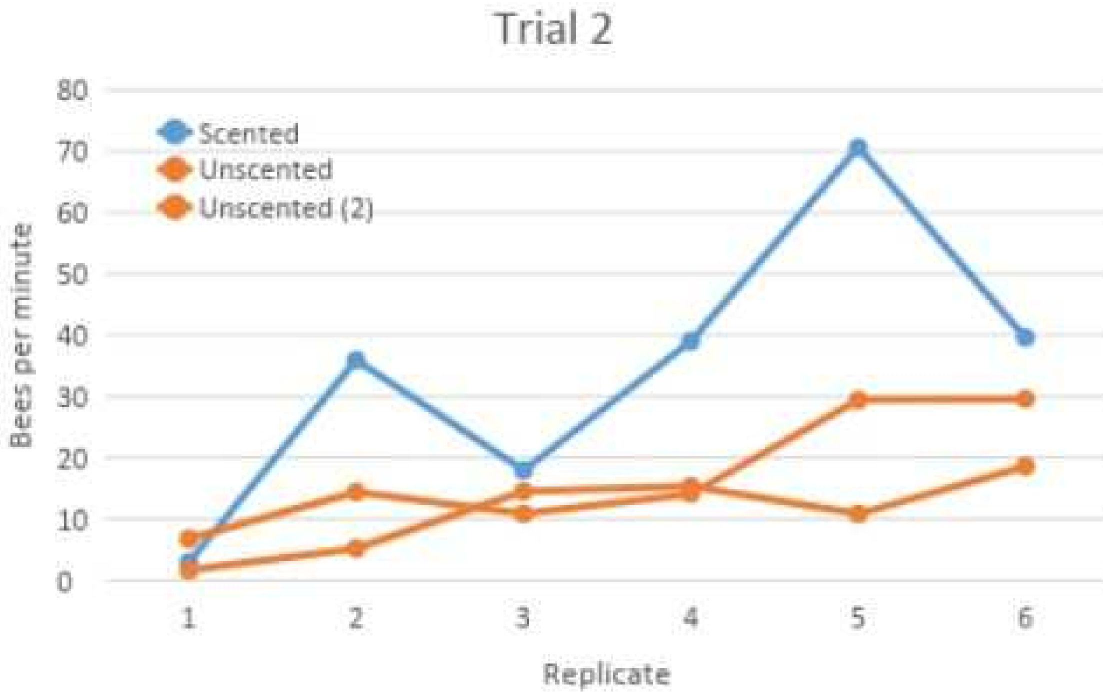

Fig.8. Raw bee visitations per minute trial 2

Distance from hive	Scouts flying around carrion	Landing on carrion
25m	4	1
25m	3	2
25m	3	0
12.5m	4	2
12.5m	5	2
12.5m	4	1
10m	5	2
10m	4	2

Table1. Honey bee activity on or around carrion left in the field

Figure.8. Honey bee (Apis mellifera) on entrails of carrion opossum (Didelphis virginiana)

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Chapter 4

GENERAL SUMMARY

Illegal trade of endangered wildlife continues to be a problem throughout the world and does not show signs of slowing down at this point. From origination to destination, there are few deterrents that have had a large impact on stopping the trade. Some methods, such as the genetic assignment appear to be a breakthrough in helping locate the origins of the animals being poached. This should help law enforcement and wildlife advocates a better starting point to slow or stop the poaching in these areas. However, this is only helpful after a point at which it may be too late to catch the poachers as they may have moved on from the time the seizure takes place (Wasser et al., 2015; Patel et al., 2015).

There are few, if any, detection methods in place for ivory leaving the African countries on their way to Asia. As discussed earlier, this is likely because government is more concerned about what is coming in to their countries rather than what is leaving it. It may be possible that if there was a detection method that was inexpensive and proved well, that it could be put in place for departures of shipping containers. If the smuggled items could be found prior to leaving the country of origin it may be easier to get back to the poachers quicker, thus deterring their operations (Patel et al., 2015)

There are also few detection methods currently in ports of entrance in Asia. According to the data reviewed, few ivory shipments have been seized due to x-ray. Recently there have been several news articles stating seized ivory because of routine x-ray searches but they were unable to be verified and even if verified would account for a small percentage of overall seizures. Sniffer dogs are a good detector of smuggled wildlife, however, they have quite a few limitations, most notably, time and money (Duggan, Heske, Schooley, Hurt & Whitelaw, 2011; Milliken, 2014).

Could Honey bees be a tool to be employed in this type of fight against wildlife smuggling and poaching? Possibly. As discussed previously, honey bees have remarkable olfactory senses that can be used to locate items of interest. Additionally, bees are inexpensive, easy to take care of, can be trained quickly, and can cover large areas in a short amount of time. Bees also do not require a handler with significant training or have a need to bond with any outside person to do their job. While bees do have limitations, this is consistent with using other current methods of detection. These qualities make the honey bee an interesting candidate to research with their huge potential (Carlsten et al., 2011).

Honey bees, one of mankind's greatest friends for survival, could be one of the declining elephant population as well. Research of interest regarding the ability of honey bees to be trained to search out biological material from the ivory located in shipping containers in large shipping yards, either prior to departing African ports or after arrival in Asian ports. If the bees are successful in locating this ivory, or the biological material associated with it, then they may be likely to be able to cover large shipping yard full of containers in a fraction of the time required now and for a fraction of the cost.

APPENDICES

Appendix A1:

Sucrose Solution Protocol – Scent Study

Materials Needed

2 Liter Beaker or Container

Water

White sugar

Scale

Prepare 1.5M sucrose solution as follows:

1. Pour one liter of warm water into beaker.
2. Weigh out 513g of sugar.
3. Pour sugar into warm water slowly, while stirring, until sugar is dissolved.
4. Warm beaker on hot plate if necessary to completely dissolve sugar.
5. Allow to cool to ambient temperature before using in feeders.

Prepare 2.0M sucrose solution as follows:

1. Pour one liter of warm water into beaker.
2. Weigh out 684g of sugar.
3. Pour sugar into warm water slowly, while stirring, until sugar is dissolved.
4. Warm beaker on hot plate if necessary to completely dissolve sugar.
5. Allow to cool to ambient temperature before using in feeders.

All quantities may be scaled to size dependent on quantity needed.

Appendix A2:

Scented Sucrose Solution Protocol (Carrion)

Materials Needed

1.5L beaker or larger

Micropipette

Prepared Sucrose Solution (see sucrose solution protocol)

Carrion chemical scent mimics

-methyl thioacetate, dimethyl sulfide, dimethyl disulfide, dimethyl trisulfide,

Methanethiol, carrion cocktail (see bottom of page)

Prepare scented sucrose solution

1. Pour 1L sucrose solution into beaker
2. Add 2μL of chosen carrion scent mimic
3. Place in feeder

(note: prepare no more than 24 hours in advance of trials)

Carrion Cocktail

Prepare cocktail as follows:

1. Obtain small test tube for mixing
2. Place 2μL of each of the 5 scents into test tube using micropipette (use clean pipette tip for each chemical)
3. Mix solution thoroughly

Appendix A3:

Scent Study Foraging Protocol

1. Prepare scented solution. (see scented sucrose solution protocol)
2. Introduce scent to entrance feeder, allow 24-72 hours for acclimation to new scent.
3. Remove entrance feeder from hive and introduce scent in chick feeder at distance of 3m. Gradually move feeder further from hive at distances of 3 m, 5m, 10 m, 15 m and 25 m sequentially. (move feeders once bees have recognized scented solution and are feeding at station)
4. Begin scent study with 3 feeding stations.
    a. Begin study at 25 m for each container. Place 3 feeding stations equidistance from each other with 12 m between.
    b. Feeding stations consist of 1 scented sucrose solution station and 2 sucrose solution only stations.
    c. Count number of bees visiting (feeding at) each feeding station for period of 10 minutes. Time may be adjusted if needed.
    d. Move scented solution feeder position in exchange for unscented feeder and repeat count. Repeat this until scented solution has been at all three original feeding station locations.
    e. Repeat steps a-d at 50 meters

Appendix A4:

Scent Study Protocol -Modified (use of carrion)

1. Prepare carrion cocktail scented solution. (see scented sucrose solution protocol)
2. Introduce scent to entrance feeder, allow 24-72 hours for acclimation to new scent.
3. Remove entrance feeder from hive and introduce scent in chick feeder at distance of 3 m. Gradually move feeder further from hive at distances of 3 m, 5 m, 10 m, 15 m and 25 m sequentially. (move feeders once bees have recognized scented solution and are feeding at station)
4. Begin scent study with carrion.

   f. Remove scented feeder station.

   g. Allow 30 minutes before next step to allow for dissipation of odor where feeders were placed.

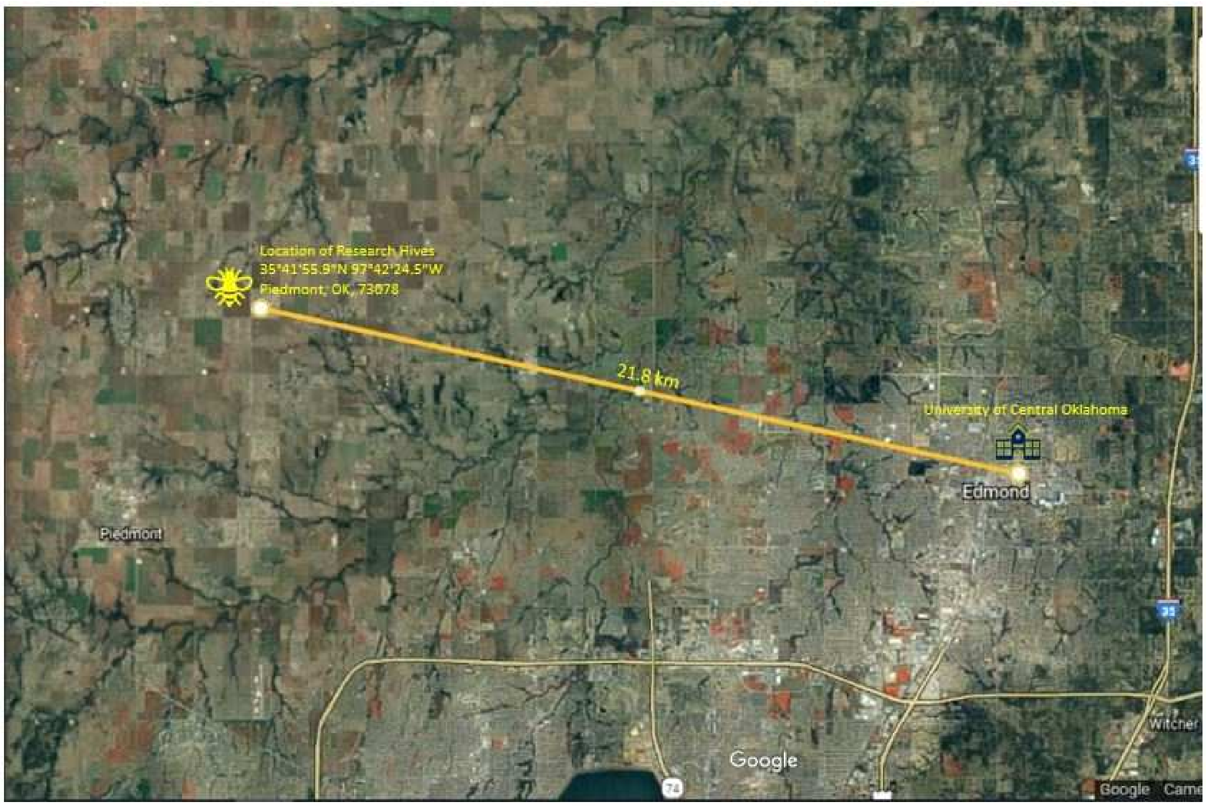
   h. Place carrion 25 meters from hive entrance.

   i. Count number of bees visiting carrion for period of 20 minutes.

   j. Move carrion to varying distances. Repeat bee count.

Appendix A5:

Location of Research Hives and Proximity to University of Central Oklahoma



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