EVALUATION OF DIFFERENT COMMERCIAL LURES AND HORSE ODORS AS AN ATTRACTANT AND THEIR ABILITIES TO INCREASE MOSQUITO TRAP NUMBERS AT THE UNIVERSITY OF FLORIDA HORSE TEACHING UNIT

By

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To my mom and dad for all of their love and support

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Mosquitoes have a significant economic impact on the equine industry from nuisance biting and the potential for pathogen transmission resulting in debilitating diseases. Traps are effective tools for monitoring species composition but little is known about the efficiency of commercial traps and mosquito attractants in the presence of a horse. Horses readily attract mosquitoes, but the role of horse produced volatile chemicals for attraction is undefined. Studies were conducted at the University of Florida Horse Teaching Unit to determine if mosquito trap collections could be increased using commercial lures or odors collected from horse. A year long seasonality study was conducted using four Mosquito Magnet® Pro (MMPro) (American Biophysics, Corp., North Kingston, RI) traps and three commercially available lures: octenol, Lurex, and Lurex^3. Trap counts were taken every 3-4 d while rotating the lures through a 4 x 4 Latin square every fourteen days. Seasonal population trends and efficacy of the lures were evaluated. Temperature and rainfall were recorded throughout the study, which ran from September 2005 through September 2006. The species trapped in greatest numbers was *Mansonia* spp., followed by *Anopheles crucians* (Wiedemann), and *Coquillettidia perturbans* (Walker). The fall months of September, October, and November of 2005 yielded significantly

greater ($P \leq 0.05$) numbers of mosquitoes compared to the other seasons, including the summer months. Octenol baited traps caught significantly more mosquitoes compared to the traps baited with other commercial lures, which in some cases caught fewer mosquitoes compared to the control traps with CO_2 alone. Lurex and Lurex[3] did not differ at any time during any season in the study.

In a separate series of trials, horse skin, hair, and dander samples were tested as lures. Two Mosquito Magnet® Pro (MMPro) (American Biophysics, Corp., North Kingston, RI) traps were baited with binary combinations of carbon dioxide and samples collected from the skin of two different horses using cotton balls. Traps were operated for twenty-four hour intervals, with new samples added each interval. Duplicate samples were taken and analyzed for chemical composition using gas chromatography and mass spectrometry (GC/MS). Compounds found on the horse included cholesterol, nonanal, and decanal, and unexpectedly, 9-octadecenamide. This compound seems to be unique to the skin of the horse. Mosquito numbers and species composition were evaluated and compared between the two horses. Inclusion of emanations from one horse tended to decrease the number of mosquitoes in the traps while inclusion of emanations collected from the second horse tended to increase the number of mosquitoes caught in the trap compared to the control trap, which ran with CO_2 alone. However, these differences were not statistically significant ($P \leq 0.05$). A final study was conducted to determine if the two horses differed in their ability to attract mosquitoes. A vacuum aspirator was used to collect the mosquitoes that landed on the two horses on two consecutive evenings for thirty-minute intervals. Species composition and total mosquitoes were evaluated. No significant differences ($P \leq 0.05$) were found between the two horses and similar species composition was noted.

CHAPTER 1
LITERATURE REVIEW

Mosquitoes have been a menace throughout the ages. Because of their resilience, persistence, and ability to carry pathogens, these insects are a major entomological concern worldwide. In spite of attempts to find improved methods of surveillance and control to reduce disease transmission to humans and livestock, millions of people are infected with mosquito-borne diseases worldwide each year. The Centers for Disease Control and Prevention (CDC) estimates 300 to 500 million cases of malaria are reported annually, with over 2 million deaths per year (over 75% African children). Research is done worldwide in an attempt to prevent and control the spread of malaria.

The introduction of West Nile virus (WNV) in New York resulted in 62 human cases and 7 deaths (Rose, 2001). This Old World flavivirus is related to the encephalitides, such as Saint Louis encephalitis (SLE) and Japanese encephalitis (JE). Ninety-eight percent of WNV cases result in minor symptoms, including fever. However, 2% produce West Nile Neuroinvasive Disease, which includes meningoencephalitis that can be fatal. In 2003, there were 9,862 human cases and 5,181 equine cases reported in the United States (Stark and Kazanis, 2003). The number of human cases reported in 2005 declined to 3000 cases, but there were 170 fatalities (CDC, 2006). Death rates are even higher in horses. The average mortality rate of infected horses is around 30% (Porter et al., 2001). It is difficult to diagnosis and supportive care is expensive in horses.

Since the early 1900s, attempts to reduce Florida mosquito-borne diseases have included the use of surveillance and chemical control. Concerns with environmental impact, insecticide resistance in mosquitoes, and health concerns of the human population may limit the use of chemical control in the future. Therefore, researchers are seeking novel control methods for mosquitoes (Kline and Mann, 1998). New traps have been developed to provide more accurate surveillance, as well as to safely and effectively control and limit mosquito numbers in a local area. Accurate surveillance plays an integral role in predicting future epidemics and disease transmission in local populations. Mosquito traps are now found in backyards and at livestock facilities. It is not yet clear if traps can compete against and attract more mosquitoes than a preferred host that is in proximity to the trap. Despite research done with humans and other livestock species, little is known about horse and mosquito interactions.

Dilling (2004) found that when a horse was in close proximity trap counts went down and concluded that traps do not compete well at luring mosquitoes away from the natural host. Dilling attempted to capture the odors from horses to increase the trap catch of mosquitoes but was unsuccessful. Mboera and others (1997) baited tents with human odors to increase trap numbers of *Anopheles gambiae* Giles. In the 1950s, researchers explored the attraction of mosquitoes to humans by building robots that mimic humans, including CO_2 release and clothing the robots in fabric soaked with human sweat (Brown et al., 1951). Scientists have used gas chromatography - mass spectrometry to analyze human skin emanations and identify the compounds from the skin that may function as mosquito attractants (Bernier et al., 2000, 2003). Livestock species have been studied to identify chemical compounds, other than CO_2, that can be used to formulate an insect-attracting lure (Hall et. al., 1984). The volatile compound, 1-octen-3-ol, was initially identified in the breath of oxen. This compound has been shown to be a potent mosquito attractant, especially when combined with CO_2 (Takken and Kline, 1989). It is unknown whether horses have chemicals that are odor cues to the mosquito that would be useful in lures for commercial traps.

This chapter reviews the pertinent background research and details the foundation for the current exploration into kairomones used by mosquitoes to locate horses for blood meals. Through the discovery of new kairomones, the development of more efficient traps is possible, and may eventually allow better methodology to control mosquitoes.

Taxonomy

"Mosquito" is a Spanish word meaning "*little fly*" and has been used in English since the late 1500s. Mosquitoes belong to the order Diptera and family Culicidae. The family Culicidae consists of approximately 3,200 recognized species. There are three subfamilies: Anophelinae, Culicinae, and Toxorhynchitinae. Most of the differences between these subfamilies are morphologically apparent in the larval stage. In the larval stage, Anophelinae do not have a siphon on the eighth segment. This adaptation allows the larvae to be submerged under the water but still obtain air. Anophelinae lay eggs which float on the surface of the water. The other two subfamilies, Culicinae and Toxorhynchitinae, have siphons on the eighth segment during the larval stage and the adult females have palps that are significantly shorter than the proboscis. Toxorhynchitinae species separate themselves from the Culicinae easily because of their predaceous larvae and larger sized adults. In addition, they have a uniquely curved proboscis which has been adapted for feeding only on the nectar of plants (Woodbridge and Walker, 2002).

The three subfamilies separate into thirty-eight genera of mosquitoes worldwide. Thirteen of these encompass 77 species in Florida alone: *Aedes* (Meigen), *Anopheles* (Meigen), *Coquillettidia* (Dyar), *Culiseta* (Felt), *Culex* (Linnaeus), *Deinocerites* (Theobald), *Mansonia* (Blanchard), *Ochlerotatus* (Lynch Arribalzaga), *Orthopodomyia* (Theobald), *Psorophora* (Robineau-Desvoidy), *Toxorhynchites* (Theobald), *Uranotaenia* (Lynch Arribalzaga), and *Wyeomyia* (Theobald) (Public Health Entomology Research and Education Center, 2002).

Life Cycle and Morphology

The mosquito goes through four distinct life stages, similar to other holometabolus insects. This allows for ample development and separation from the adult female and the young. The four stages begin with egg, larva, pupa, and then adult. Most mosquitoes have eggs that are found in various shapes, including ovoid, spherical, rhomboid, elongate, and spindle. Adult female *Aedes*, *Anopheles*, *Haemagogus*, *Ochlerotatus*, *Psorophora*, *Toxorhynchites*, and *Wyeomyia* lay their eggs individually. Other genera, including *Culex*, *Coquillettidia*, and *Mansonia* species, lay their eggs together in a single clump, forming an egg raft or a submerged cluster which will float on or near the surface of the water (Darsie, 2006). An average of 75 eggs per ovary develops two to three days after an adult female mosquito has taken a blood meal. *Aedes* and *Ochlerotatus* species lay their eggs on damp soil that will be flooded by water, which is required for hatching. On the other hand, *Culex*, *Culiseta*, and *Anopheles* lay their eggs on the surface of the water. Most eggs will hatch within 48 hours; others have adapted to withstand subzero winter temperatures before hatching (Harwood and James, 1979).

The next stage in mosquito development is the larval stage, which is an active stage that requires an aquatic environment for sustaining the larvae. Mosquito larvae have three distinct body regions: the head, the thorax and the abdomen. Depending on the species, the head is broad and usually round, with lateral antenna. *Toxorhynchites* larvae are predaceous and will grasp their prey; however, most other species have mouthparts that consist of brushes and grinding structures that filter bacteria and microscopic plants. The next main region is the thorax, which is broader than the head and flattened. Identification of larval mosquito species is possible with the help of the number, location, and structure of tiny hair-like projections known as setae that are found on the different segments of the thorax. The last main distinct body region is known as the abdomen, which consists of nine segments. The abdomen is elongated and cylindrical, with the first seven segments similar to each other. The last two segments are modified for specific functions. The eighth segment, in most species except *Anopheles*, is the respiratory apparatus, known as the siphon. The shape, width, and length of this segment are useful tools in identifying species as well. The last segment is the anal segment. As the mosquitoes pass through the four different stages of larval development, known as instars, they complete each one by molting and increasing in size. Depending on environmental factors, including temperature, the average mosquito species requires 7 days to complete larval development. Larvae can survive in water temperatures between 17°C and 35°C in Florida for a period of one to four days. However, temperatures above or below these will cause an increase in mortality in the population (Nayar, 1968). Following the fourth and last instar, the larva will molt again and become the third developmental stage, known as the pupal stage (Darsie, 2006).

The third stage in mosquito development is known as the pupal stage, where the juvenile does not feed and completes development. Pupae are mobile and are often called “tumblers” because of their jerky movements when they are disturbed. They tumble towards protection, usually deeper into their temporary home and then they float back up to the surface. The pupa is comma-shaped and has an outer shell of protection known as chitin. The pupa floats on the water because of its low density and receives oxygen through two breathing tubes called trumpets (Darsie, 2006). The sex can be determined by examining the overall size of the pupa along with the ninth segment of the pupa’s abdomen. The male mosquito has a more prominent ninth segment during this stage of development while the female is larger then the male. The maturation process into an adult mosquito is completed in the pupal case. When it is finished, the adult mosquito will split the pupal case and emerge to the surface of the water where it will rest until its body hardens and dries (Floore, 2003).

The last stage of mosquito development is the adult stage. The body of the adult mosquito is slender, with three distinct body regions: the head, thorax, and abdomen. Like other insects, they have six legs, which are thin and narrow. They have two wings, which are long and thin, with scales. The surface of the body is covered with setae and scales that allow for distinct markings and colorations, providing characteristics for identification. Females have long, filamentous antennae that are situated between the eyes on the head, whereas the male has larger, more hairy antennae which allow for distinction from the female. The proboscis is prominent and usually projects anteriorly at least two-thirds the length of the abdomen (Woodbridge and Walker, 2002). Adult mosquitoes of both sexes of most species feed regularly on plant sugars throughout their lives. Only female mosquitoes feed on hosts for a blood meal, which is essential for obtaining protein required for egg production. Females feed on cold and warm blooded animals and birds. Male mosquitoes do not bite, but feed on nectar of flowers or other suitable sugar sources. Females will also feed on nectar for flight energy. Females of some mosquito genera, such as *Toxorhynchites*, feed entirely on plant sugars and do not require a blood meal for egg development (Woodbridge and Walker, 2002). A combination of different stimuli influence biting and blood feeding such as carbon dioxide, temperature, moisture, smell, color, and movement (Floore, 2003). During the summer, adult mosquitoes have a short life span, usually lasting only a few weeks. However, it has been found that some species can survive through the winter as adults, therefore increasing their ability to have a longer life span of several months (Nasci et al., 2001).

Flight Behavior and Ecology

Once the mosquito has emerged from the pupal case, the adult will seek shelter for a rest period and to allow for hardening of the body. Normally a mosquito will take flight during one or two periods per day. This flight period depends on whether the specific species is characterized as being diurnal, nocturnal, and crepuscular. During these periods, both males and females will take flight without external cues (Woodbridge and Walker, 2002).

Generally, mosquitoes will not travel greater than two kilometers. Yet some mosquitoes require long distance flights in order to complete their egg-laying mission. The salt-marsh mosquito, *Ochlerotatus taeniorhynchus* (Wiedemann) requires long round trips to locate hosts for blood meals since they emerge in secluded areas where hosts are not readily available. They have been known to travel long distances with the help of the wind and may be carried hundreds of kilometers from where they first emerged. Eventually, they make it back to their original breeding sites for oviposition (Woodbridge and Walker, 2002).

It is possible to categorize mosquito flights into three main categories (Bidlingmayer, 1985). A one-way flight with no return, which usually lacks an objective and does not meet any need, is known as a migratory flight. Newly emerged mosquitoes will take these flights and rarely respond to stimuli. The destination is accidental as the mosquito relies on wind conditions at the time of departure. Direction of migration and the limits of the mosquito's energy bank reserves control the duration of the flight, as well as meteorological conditions during the flight (Bidlingmayer, 1985). When the mosquito undergoes a physical stimulus, it will usually respond by taking an appetential flight. The resting mosquito will begin a flight to satisfy a physical need, such as taking a blood meal, finding an oviposition site, or moving to a better resting place. The appropriate sensory organs will be alert during the appetential flight and will be seeking cues that indicate the presence of the target and the flight will conclude when the objective is located or until the mosquito's energy reserves are depleted (Bidlingmayer, 1985).

The final flight category is the consumatory flight, the subsequent flight after the female has located her goal (Haskell, 1966). The consumatory flight is usually direct and brief, since visual and biochemical cues are lost over long distances. If the cue encountered was olfactory a direct upwind flight is conducted until other cues, visual perception, movement or thermal, enable the female to locate her goal more precisely (Gillies and Wilkes, 1972). "Consumatory flights do not always have to follow appetential flights. An example would be the biting of a host that enters the daytime resting site of the female mosquito" (Bidlingmayer, 1985).

Other factors affecting the flight of mosquitoes usually involve the weather. The most influential meteorological factors are light, temperature, humidity, and wind (Day and Curtis, 1989). Nightly variations in wind, rainfall, and relative humidity influence mosquito patterns and maybe even feeding success. Daily rainfall patterns can potentially determine whether the mosquito population will continue to build, remain steady, or decline as it relates to feeding and oviposition behavior, mainly during the rainy season of late summer and early fall in Florida. Research indicates that most mosquito species possess a bimodal flight activity pattern during the night, with the larger peak occurring soon after sunset and the smaller peak just prior to dawn (Schmidt, 2003).

Mosquito activity can be forecasted using the four meteorological factors mentioned earlier. The Weather Channel has teamed up with the maker of the Mosquito Magnet Pro (MMPro), American Biophysics, to launch the first ever "Mosquito Activity Forecast" on the website, www.weather.com. The website link is managed by a team of meteorologists from the Weather Channel who provide hourly predictions of mosquito activity nationwide. People who want to participate in outdoor activities in areas inhabited by vector species may be able to better plan their activities using this valuable tool (Dilling, 2004).

Host Location Behavior

The female mosquito will look for a host from which she can obtain a blood meal one to three days following emergence. One study indicated host seeking was inhibited for a period of

40 hours following a blood meal for the mosquito *An. gambiae* (Takken et al., 2001). For over half the twentieth century, research has been conducted to determine why mosquitoes are attracted to certain hosts and what odors are responsible for the mosquito's attractive behavior to a specific host. The principle difference in the orientation of blood-sucking insects, compared with other insects, lies in the differences in behavioral responses to cues that may be distinct between long and short range. In 1942 it was demonstrated that unwashed naked children were preferred by *Anopheles* spp. over freshly washed naked children. This same group showed that the presence of dirty human clothing attracted more mosquitoes than an empty hut as the control (Haddow, 1942). The ability of humans to attract anthropophilic mosquitoes differs among individuals (Khan et al., 1965). The results of their studies demonstrated that one person was more attractive to *Ae. aegypti* than three other people (Khan et al., 1965). Based on these studies and others, host selection is based on host preference and availability, with a combination of visual, olfactory, and physical stimuli to help locate the host (Takken, 1991).

Although many mosquito species display visual response to distinct objects at a distance of up to 19 m (Bidlingmayer and Hem, 1980), olfactory stimuli from host odors are considered to be the strongest cues for location of hosts for blood meals (Allan et al., 1987 and Bowen, 1991). Olfactory cues which aid in host-seeking by orienting mosquitoes to a host are known as kairomones (Howse et al., 1998). Extensive work has been done to determine the mechanism of mosquito attraction to its host. There is ample evidence that host-seeking by mosquitoes is mediated by semiochemicals, chemicals which deliver a message, emanating from the host (Bernier et al., 2003 and Geier et al., 1999). Olfactory cues are detected through an intricate pathway, beginning with the sensilla located on the antennae and palpi which detect carbon dioxide (CO_2). Age and the physiological state of the mosquito determine whether the detection of olfactory cues results in a behavioral response (Takken, 1996). Volatile chemicals such as CO_2, octenol, and acetone and less volatile substances such as lactic acid and fatty acids are present on skin as a result of vertebrate metabolism (Sastry et al., 1980). CO_2 is universally attractive to mosquitoes and is probably the best understood of the volatile host cues (Gillies, 1980, Gibson and Torr, 1999). Many researchers believe that volatile compounds act as attractants that help orient the mosquito towards its host and that CO_2 can combine with other host odors to elicit a synergistic response (Smith et al., 1970, Gillies 1980, Bernier et al., 2003). Gillies (1980) also found that CO_2 and whole-body odors have an orienting effect when presented singly and an enhanced effect when presented together. Kline and Mann (1998) showed that traps baited with CO_2 capture 8-30 times more mosquitoes than traps without CO_2. Around the attractant plume any insect with a flight speed less than approximately 3.5 m/sec will be captured (Kline, 1999). High and low release rates (200 and 500 cc/min) of CO_2 are utilized during catches (Kline, 1994). It has been shown that CO_2 does not help mosquitoes discriminate different hosts (Mboera and Takken, 1997), but may actually function as a primer to activate mosquitoes and make them more receptive to other host odors. (Dekker et al., 2005).

In 1984, Hall et al., through studying the attractiveness of oxen to Tsetse flies in Africa, identified octenol from the ox. Field tests have demonstrated that octenol serves as a powerful attractant for certain species of mosquitoes and flies (Kline, 1994). Thus, 1-octen-3-ol (octenol) is another olfactory attractant documented as an effective mosquito attractant (Dilling, 2004). This volatile compound has been isolated from many natural sources, mainly plants and fungi (Dijkstra and Wiken, 1976). Chemically speaking, octenol is an 8-carbon mono-unsaturated alcohol that has an asymmetric center and therefore two optical isomers with a terminal double bond (Kline, 1994). This allows for different effects by the different isomers. At the present time, manufacturers of mosquito control equipment such as Mosquito Magnet Pro, (American Biophysics Corporation) recommend octenol as supplementary bait to mosquito traps. Kline (1994) suggested that even though octenol was effective at attracting some species, it is not correct to say that the compound will successfully attract all mosquito species (Kline, 1994). The natural release rate of octenol by oxen is 0.043 mg/h (Hall et al., 1984). In studies conducted in 1994, the release rate used was 4 or 40 mg/h (Kline, 1994). Octenol is now available in slow release packets, which have a release rate of 0.5 mg/hour (Kline, 1999).

Another volatile chemical believed to play a role in host location is lactic acid, which is a by-product of anaerobic metabolism, common to all mammals. The acidity of freshly secreted sweat is due to the production and secretion of lactic acid by the eccrine sweat glands (Thurmon and Ottenstein, 1952). In one study, both fresh and incubated human sweat was used to catch the malaria mosquito, *Anopheles gambiae.* They found incubated sweat was more effective than fresh sweat in catching this particular species. They noted a decrease in lactic acid concentration associated with the two day old sweat, and concluded that lactic acid may not play a role in attracting *An. gambiae* (Braks and Takken, 1998). *An. gambiae* seem to be attracted to volatiles of Limburger cheese, which to a human, resemble human foot odor (De Jong and Knols, 1995). Skin emanations are important because odors from live hosts have been shown to be more attractive than any combination of these chemicals provided in a warm, humid airstream (Woodbridge and Walker, 2002). Some researchers believe continuous bacterial action in secretions on the human skin results in volatiles that function as kairomones for mosquitoes (Braks and Takken, 1998).

American Biophysics Corporation has been working on additional compounds that have proven useful in trapping mosquitoes. Lurex and Lurex [3], lactic acid based compounds, when combined with CO_2, are thought to be effective in increasing trap catches of *Aedes albopictus* (Skuse) (McKenzie et al., 2004).

Visual attraction is also a key component in host location. Both male and female adult mosquitoes have two compound eyes and two ocelli. Compound eyes are suited for navigation and sensing movement, patterns, contrast, and color, while ocelli are believed to sense light levels, and possibly polarized light (Allan et al., 1987). The compound eyes have relatively poor resolution but overall high light sensitivity (Muir et al., 1992). It has been reported that diurnal species respond to visual characteristics of hosts such as color, brightness, pattern, and movement (Allan et al., 1987). It may be concluded that in a human dwelling, when given the choice, the host seeking *Mansonia* mosquitoes are more attracted to the blue and red spectra than the white, yellow, green, and black (Bhuyan and Das, 1985). Movement may also play a role in host location by mosquitoes and a consistently small but positive attraction to movement has been affirmed (Wood and Wright, 1968). Within one meter of the host, convective heat and humidity become the main attractants rather than chemical or visual stimuli (Woodbridge and Walker, 2002).

Physical stimuli such as temperature and humidity are also attractive to mosquitoes. With the help of a clothed human robot, Brown (1951) was able to show that mosquitoes landed three times as often on the clothed robot when the “skin” temperature was body temperature (98°F) than when the surface temperature was lower, around 50-65°F (Brown, 1951). In addition, Brown noticed that moisture coming off of the robot’s clothing increased the landing numbers two to four times, but only at temperatures above 60°F. Despite extensive work completed thus far, it has yet to be determined what causes mosquitoes to locate and feed on a host. It has been concluded on several occasions that a combination of olfactory, visual and physical stimuli are attractive factors.

Host Preference

As with host-seeking behavior, host preference varies widely among different genera of mosquitoes and this preference may change within genera depending on a geographic location. Some species may feed almost entirely on one host while others who are more opportunitistic may feed on two or three different vertebrate classes. *Culex* genus prefers to feed on avian species, but if the population of birds is insufficient or unavailable, they will happily feed on mammals (Braverman et al., 1991). Species in Florida such as *Aedes, Anopheles, Coquillettidia*, *Mansonia*, and *Psorophora* prefer to feed on mammals. Mosquitoes can be found in high numbers most of the year in Florida on livestock facilities. Unfortunately, some mosquito species are competent disease vectors which threaten livestock industries year round.

A number of mosquito species are known to feed on horses. *Aedes* and *Ochlerotatus* species have been found to be readily attracted to horses (Loftin et al., 1997) as well as *Psorophora columbiae* (Dyar and Knab) (Kuntz et al., 1982). *Anopheles* spp., *Coquillettidia* spp., *Culex* spp., *Culiseta* spp., and *Mansonia* spp. are also equine feeders (Constantini et al., 1998, Kuntz et al., 1982, and Loftin et al., 1997). *Culex nigripalpus* Theobald and *Culiseta melanura* (Coquillett) have been shown to be vectors of WNV and Eastern Equine Encephalitis, respectively (Darsie, 2006). Concerned horse owners seek methods to minimize the exposure of animals to mosquitoes.

Humans as Attractants

Researchers are examining the attraction of the natural host as a way to draw the mosquitoes to traps and away from the host. Entomologists have used host-baited traps since the early 1900s for collecting Anopheline mosquitoes during malaria investigations (Mitchell et al., 1985). In the mid-1960s, researchers were able to determine that people had varying levels of attractiveness by using *Ae. aegypti* (Linnaeus) and measuring bloodfeeding and probing responses (Khan et al., 1965). Human bait catches have been reported as the standard and most useful method for collecting host-seeking anthropophilic mosquitoes (Service, 1993). Kline (1994) stated there is a need for new, safe and effective ways to kill and control pest and vector mosquito species and to deter blood seeking mosquitoes from feeding on animals and humans. The use of chemical insecticides and topical repellents faces increased restrictions due to environmental concerns and mosquito resistance.

Several researchers have stated that a difference exists between individuals (Haddow, 1942, Khan et al., 1965, Schreck et al., 1990, Canyon et al., 1998) Therefore, it has been established that human skin odor contains volatile chemical substances that increase mosquito attraction in the laboratory (Schreck et al., 1981, Eiras and Jepson 1991, 1994) and in the field (Gillies and Wilkes, 1972). Once it was determined that differences among people existed, attempts were made to identify those differences through biological and chemical methods. Samples collected from people in various locations were typically analyzed by mass spectrometric detection, e.g. GC/MS, whether the emphasis is on skin emanations, breath, urine, blood, oral cavity, or the total composite of emanations from an entire person (Bernier et al., 2006). Volatile substances produced by human skin have been shown to act as either attractants or repellents. Many of the volatiles responsible for these actions are found in sweat (Takken, 1991). Smallegange reported that carboxylic acids make up an important part of human sweat (Smallegange et al., 2005). Laboratory studies aimed at elucidating the compounds constituting human-produced odor blends that mosquitoes use for host location have yielded several active mixtures and individual substances (Smallegange et al., 2005). In their study, *An. gambiae* rely on the combination of ammonia, lactic acid, and carboxylic acids in its orientation to human hosts, different from the information reported concerning the *Ae. aegypti* (Smallegange et al., 2005). Humans seem to have uniquely high levels of lactic acid on their skin compared to other animals (Dekker, et al., 2002). Lactic acid is known to play an important role in the host-seeking behavior of another anthropophilic mosquito species, *Ae. aegypti* (Acree et al., 1968). Ammonia was also identified as an attractant for *Ae. aegypti*. It is not attractive when tested alone, but it enhances the attractiveness of lactic acid. Fatty acids of chain length C1-C3, C5-C8, or C-13-C-18 had the same effect when mixed with lactic acid. Experiments with *An. gambiae* females that were done in Y-tube olfactometers showed that the synergistic effect could also be achieved when combining ammonia and lactic acid with only one of the short-chain carboxylic acids that was present in their mixture: hexanoic acid (Smallegange et al., 2005). Others reported an attractiveness of an unsaturated carboxylic acid, 7-octenoic acid, which is a human-specific component, secreted from the aprocrine sweat glands in the axillary regions (Smallegange et al., 2005). Braks concluded that the kairomones to which *An. gambiae* responds were also present in fresh sweat but that the quantity or quality of the attractive volatiles was enhanced strongly during incubation. Skin microorganisms are presumed to break down sweat-borne compounds into smaller, more volatile components. Furthermore, they noted that the preference for the incubated sweat decreased after twenty minutes of exposure in the olfactometer. The combined results led them to suggest that the components responsible for the preference of *An. gambiae* for incubated sweat to fresh sweat are highly volatile (Braks et al., 2001).

Attractants from Other Hosts

In addition to human odors, samples from other mammals have been used to attract mosquitoes and have shown high success rates. Researchers found an increase in trap numbers when the attractant used was collected from mice using a closed-air system, without the help of other attractants (McCall et al., 1996). Another study in Israel, led by Braverman in 1991, used several different animals as bait in traps to catch *Culex pipiens* (Linnaeus). Sheep, chickens, calves, and turkeys were the most successful baits. The calf tended to increase the total trap number when compared to the other three animals.

Birds, such as sentinel chickens, are commonly used for attracting mosquitoes, especially for surveillance techniques and to predict disease outbreak (Day and Stark, 1996). Cotton swabs coated with crow uropygial gland secretions caused a significant increase in trap counts when compared to a clean cotton swab, leading researchers to continue work with other avian species (Russell and Hunter, 2005).

Previous experiments attempting to use horse odors have been conducted. Several studies were conducted using a vacuum aspirator to vacuum the odors directly from the horse's body, which were then fed into a trap in an attempt to increase trap counts. Very little difference was seen when horse odors were collected by this method. When a horse is present near a trap, trap counts decrease and the mosquitoes go to the horse (Dilling, 2004). Dekker and fellow researchers measured the amounts of L-Lactic acid in skin-rubbing extracts from humans compared with twelve other mammals and chickens, including horses. A greater amount was found on humans compared to horses (Dekker, 2002). Further research needs to be completed with the horse to determine if odors exist that have the capability to attract mosquitoes. Traps baited with horse odor could conceivably protect the horses nearby.

CHAPTER 2
SEASONALITY OF MOSQUITOES AT THE UNIVERSITY OF FLORIDA HORSE TEACHING UNIT IN NORTH CENTRAL FLORIDA USING TRAPS BAITED WITH THREE DIFFERENT LURES.

Introduction

Florida's equine industry is affected by mosquitoes which have a direct impact on the management of horse farms. Although nuisance biting, disease transmission and their economic effects have not been studied in horses, mosquitoes have been found to have a negative correlation in other livestock species, specifically dairy cattle. Reduced milk yield, lower weight gain, and a compromised immune system leading to an increase in disease from stress caused by insects have been noted (Steelman, 1979 and Byford et al., 1992). Since the arrival of the West Nile Virus (WNV) in the United States in 1999, Florida's equine industry has lost millions of dollars through disease prevention, health care, and overall morbidity of infected horses (Porter et al., 2003).

Mosquito trapping is an effective surveillance tool used to study local mosquito populations. Trapping allows for the determination of potential disease vectors, the prediction of disease transmission, and the study of behavior and other patterns. It is helpful to know the population seasonality trends, which results in more efficient mosquito control, subsequently paving the way for more accurate protective measures which could be implemented on horse farms. Because trapping mosquitoes has become so important to their control, extensive research has been performed to improve the ability of the traps to work more effectively (Dilling, 2004).

Unfortunately, traps may be less successful in many instances on horse farms because the horse becomes a competitor against the trap. In this case, the mosquito prefers the horse over the trap, causing a decrease in mosquitoes trapped and an increase of mosquitoes that are present on the horses (Dilling, 2004). Dilling determined that the traps commonly used to study populations of mosquitoes, namely the Center for Disease Control (CDC) trap model 1012 (John W. Hock Company, Gainesville, FL) and the Mosquito Magnet Pro (MMPro) (American Biophysics Corp., North Kingston, RI), shown in Figure 2-1, were effective at trapping mosquitoes as long as a natural host was not placed in a competitive situation. Many traps use stimuli to mimic host-preference qualities that attract mosquitoes, such as heat, carbon dioxide, kairomones, and moisture (Kline and Mann, 1998). It has been thought that adding odors that mimic the scent of the natural host directly to the trap would improve the efficacy of the trap. Octenol, discovered in 1984 by Hall et al., has been successful in the past at increasing numbers of certain species captured. Other odors have been isolated as attractants and are also used in mosquito traps. Lurex, a human-based lactic acid compound and Lurex^3, a lactic acid + ammonia - compound designed for use in the MMPro, are two such products that were used in this study as attractive baits that may potentially enable mosquito traps to out-compete the natural host.

There is a definite need for volatile baits (odors) that can make mosquito traps more competitive with or out-compete the natural host. Therefore, the main objectives of this study were 1) to conduct competitive trapping studies using three different lures, octenol, Lurex and Lurex^3; and 2) to evaluate the total mosquito population profiles caught when traps are baited with volatile odors (lures).

Materials and Methods

The lure trapping studies were conducted at the University of Florida Horse Teaching Unit (HTU) in Gainesville, Florida. The 60-acre facility houses approximately 45 quarter horses used for breeding, teaching, and training, from weanlings to retired age.

The MMPro trap was used for the study. It is a self-powered mosquito trap that uses propane as an energy source. Upon combustion, the propane catalytically converts to produce CO_2, heat and moisture, which act as attractants, and electricity to power the fans. MMPros may be used with or without an additional lure. When a mosquito nears the base of the outflow of CO_2 and heat, a fan which creates a counter flow current vacuums the mosquitoes into a collection net where they die of dehydration. The MMPro, with its patented technology, is made of stainless steel with a PVC outer covering and stands about 40 inches tall (Figure 2-1).

During the study, a weather station was utilized to measure the minimum and maximum air temperatures (°C) and total rainfall (cm). The station is located just north of the large pond in the west portion of the HTU. The wooden post used to hold the weather station stands 1.5 meters tall, with a permanent rain gauge mounted on the top. Located just below the rain gauge is a waterproof thermometer.

Experimental Design

The study began September 2, 2005, and was completed September 26, 2006. MMPro traps were placed in 4 predetermined locations at the HTU (Fig. 2-2). Traps remained at their designated locations for the entire project. Treatments in combination with carbon dioxide (CO_2) included three different baits: Octenol (Treatment #1), Lurex (Treatment #2) and Lurex^3 (Treatment #3), plus a control treatment of CO_2 alone (Treatment # 4). Using a predetermined schedule, treatments were rotated through the four traps in a 4x4 Latin square design and replicated 9 times (Table 2-1). Each Latin square was completed in 8 weeks. Treatments were rotated and the lures were replaced every 14 days. The MMPros' 9-kg propane tanks were changed approximately every 19 days. The nylon collection nets were changed two times a week, on Tuesday and Friday afternoons around 4 pm. The minimum and maximum air temperatures were recorded, as well as the total rainfall for the past 3- or 4-day trapping period. Mosquitoes were stored in a freezer at -25°C until counting and identification were completed. The species data was combined for the total study to analyze the seasonality trends at the HTU.

Data were analyzed by General Linear Model (GLM) after transformation by log (n + 1) and the means were separated by Duncan's Multiple Range Test (SAS 2006). The significance interval was set at $P < 0.05$. Standard error was calculated from the means using SSPS.

Results

Traps baited with treatment #1 (Octenol + CO_2) captured significantly more mosquitoes than traps baited with the other three treatments (Table 2-2). Mean numbers of mosquitoes captured by traps baited with treatments #2 (Lurex[3] + CO_2) and #3 (Lurex + CO_2) were not significantly different from each other, but were significantly lower than those captured by control traps CO_2 alone. Thus the addition of the two Lurex baits to CO_2 actually reduced the numbers of mosquitoes captured (Table 2-2). The mean number of mosquitoes captured by the trap at location 1 (Fig. 2-2) was significantly higher than that captured by the trap at location 4, no matter which attractant combination was used. Numbers of mosquitoes captured were greatest at location 1 and decreased from locations 2 through 4 (Table 2-3). There was no significant difference between the mean numbers of mosquitoes captured daily during the two collection intervals (nets were collected on Tuesdays and Fridays). Significantly higher numbers of mosquitoes were trapped during the month of September (2005) than during the other 12 months of the study (Table 2-4, range of means ± SE). The mean numbers of mosquitoes captured in October and November were significantly higher than the remaining ten months of the study. There was no significant difference in the mean numbers of mosquitoes captured between the remaining months (Table 2-4). The mean differences between the individual mosquito species to the four attractant combinations are shown in Table 2-5 and the mean differences of the individual mosquito species trapped in each of the four locations are shown in Table 2-6.

Figure 2-3 compares the monthly total numbers of mosquitoes trapped with each attractant combination. Octenol was the most attractive for the majority of the 13 months, except for February, April, and August, where the control was more effective.

A total of 71, 850 mosquitoes were trapped during this study combined (Table 2-7). Data were plotted to show the seasonality differences and mosquito population fluctuations that occurred at the HTU (Figure 2-4). The highest numbers of mosquitoes were caught during the months of October, November, and December of 2005, but populations decreased during the months of February, March, and June (Fig. 2-4). An increase in mosquito population numbers was seen approximately 2-3 weeks following large rainfalls in October and November (Fig. 2-5). But the same occurrence failed to happen during December of 2005. Mosquito populations were increasing during rain events in June and July, but populations did not peak until late July or early August (Figure 2-5). Average rainfall measured during the study was lower than the previous three years at the HTU (Figure 2-6). Minimum and maximum temperatures recorded at the HTU throughout the study (Figure 2-7) further help to explain the trends of mosquito populations.

Figure 2-8 illustrates the total mosquito species composition for the study. The most prominent species caught was *Mansonia* spp. females (50.6 %) *Ma. titillans*

(Walker); *Ma. dyari* (Belkin, Heinemann and Page), with *Mansonia* spp. males (4.7%) combined for a total *Mansonia* spp. trapped at 55.1%, followed by *Anopheles crucians* (Wiedemann) (19.6 %) and *Coquillettidia perturbans* (Walker) (14.5%). To a lesser extent, *Culex erraticus* (Dyar and Knab) (3.4%), *Cx. nigripalpus* (Theobald) (2.9 %), *Cx. salinarius* (2.2 %), *Anopheles quadrimaculatus* (Say) (1.7 %) were trapped during the study. *Psorophora columbiae* (Dyar and Knab), *Uranotaenia sapphirina* (Osten Sacken), and *Ochlerotatus infirmatus* (Dyar and Knab) combined for just over 1% of the total collection. *Ma*. spp. females, *An. crucians, Cq. perturbans, Cx. erraticus, Cx. nigripalpus, Cx. salinarius*, and *An. quadrimaculatus* were trapped in numbers >1,000 over the course of the 13 months and individual seasonality trends are shown in Figures 2-9 through 2-15.

Discussion

During this 3-Lure study, octenol combined with CO_2 was found to be significantly more effective than Lurex, Lurex[3], and CO_2 alone at increasing mosquito trap counts on a north central Florida horse farm. In past studies, octenol has proven to be a more effective bait at increasing mosquito trap numbers in the northern states, with Lurex[3] being more efficient at trapping *Aedes albopictus* (Skuse) in the southern states (American Biophysics Corp., 2004). Researchers from American Biophysics found that *Ae. albopictus*, which is very difficult to catch (Jensen, et al., 1994), was more attracted to MMPro traps when they were baited with Lurex[3] instead of octenol.[1] The reverse was found in this study with octenol out-trapping *Mansonia* spp. females, *An. crucians, Cq. perturbans,* and *Cx. nigripalpus* when compared to the other lures, including Lurex[3]. No

[1] McKenzie KE, Bedard SD. 2004. Article retrieved November 2006.

Aedes spp. were captured in this study, which may be a reason for the lower total catch for traps baited with Lurex^3. The human-skin-based lures, Lurex and Lurex^3, should be recommended for use in environments where Aedes spp., such as Ae. *albopictus* are abundant.

Kline et al. (1990) found that there was a highly significant positive response to CO_2 by all species except *Cx. erraticus* and *An. quadrimaculatus* while working in the phosphate mine pits in central Florida. Both *Cq. perturbans* and *Ma.* spp. showed a significant synergistic enhancement in catch with octenol- supplemented CO_2 when compared with CO_2 alone. This disagreed with findings by Kline and Mann in 1998, where *Cq. perturbans* was equally attracted to octenol + CO_2 and CO_2 alone. Kline (1994) found that the addition of octenol to CO_2-baited traps caused a decrease in *Culex* species trapped, which disagrees with my study. At the HTU, traps baited with the combination of octenol and CO_2 captured significantly more *Cx. nigripalpus* than traps baited with other lures. However, with *Cx. erraticus* there were no significant differences between the numbers captured by traps baited with any of the 4 attractant combinations (Table 2-5). With *Cx. salinarius,* there was no difference between traps baited with octenol or with CO_2 alone. *An. quadrimaculatus* was more attracted to Lurex and Octenol when combined with CO_2 compared to CO_2 alone and Lurex^3, where there was no difference (Table 2-5). Both of the aforementioned studies by Kline et al. made use of traps different than the MMPros, which may have a direct correlation to the differences in performance with the different lures.

For some reason, the *Mansonia* spp. males were attracted to and captured in the MMPro traps. There was no significant difference between octenol baited traps, CO_2

alone, or Lurex[3] baited traps. Perhaps *Ma*. males related the other 3 lures with areas frequented by *Ma*. spp. females. Instances of *Mansonia* males frequenting traps and being captured in the MMPros have not been reported in the literature.

Octenol combined with CO_2 was found to be the most effective at trapping several key species, including *Mansonia* spp., *An. crucians, Cq. perturbans*, and *Cx. nigripalpus. Cq. perturbans* and *Cx. nigripalpus* are known vectors for equine diseases (Woodbridge and Walker, 2002), while *Mansonia* has not yet been ruled out as a competent vector for WNV (Darsie, 2006). Under conditions similar to those at the HTU, octenol + CO_2 could be used in MMPro traps to increase trap counts of these species.

A significant difference was noted in the four different trap locations for the study (Table 2-3). Trap #1 trapped significantly more mosquitoes than the other three traps used in the study. Trap #1 was located on the southern most part of the farm, near the covered riding arena and a wet, marshy area that always contained water and downed trees. Trap #2, just south of the large swamp, caught the most following trap #1, as the swamp provided a consistent breeding ground throughout the study. Trap #3 was just north of the swamp, however, was most near the small paddocks that always contained horses, thus presenting a constant natural host for the trap to compete against throughout the study. Therefore, trap #3 location trapped the third most mosquitoes. Finally, trap #4 was at the northern most part of the farm, near the feeding barn. This presented natural competitors as well as a constant supply of dust, which caused the trap to clog and malfunction several times. Subsequently, trap #4 was down more than any other trap during the study. A 4 x 4 Latin square was instituted in order to control for location differences. This step allowed the four attractant combinations to be rotated randomly throughout the farm, eliminating a constant location for a single lure. Despite the trap randomization, trap #1 was closest to the marsh, where *Ma*. spp. and *Cq*. *perturbans*, both tree hole breeders. This would allow a constant supply of mosquitoes, especially since these species were trapped the most abundantly during the study. Trap location differences during my study disagreed from what Dilling (2004) discovered, who's traps were in similar locations. She found that trap #2 and trap #3 caught significantly more mosquitoes than did trap #1 and trap #4.

Campbell (2003) stated that mosquitoes are found in north central Florida 12 months a year, but they are present in much more significant numbers during the warm, wet seasons of summer and fall; this is similar to the results in my study. Mosquito populations differed throughout the entire study, but were at their highest during the rainy fall months of September, October, and November, 2005, and again in the late summer months of July, August, and September of 2006 (Figure 2-4). During these peak periods of production, the temperature was also ideal for larval development, rarely dropping below 55°F (Figure 2-7). Lower populations were seen during the cool, dry months of February and March, as well as the dry, late spring months of May and June. The latter half of 2005 experienced normal rainfall, resulting in peak mosquito population numbers 2-3 weeks following a major rain event, observed in October and November. According to a 30 year study conducted by the University of Utah's Department of Meteorology, Gainesville's normal yearly rainfall is 51.81 inches, which is close to the rainfall measured during my study. However, the time of year when the rainfall should have been collected (the summer months of June, July, and August) resulted in decreased numbers for the second half of the study. This agrees with several other studies conducted at the

UF HTU, including those of Campbell (2003) and Dilling (2004). Both stated that temperature and rainfall appear to be major factors affecting mosquito seasonality trends. This trend continued until the nights stayed fairly cool, below 55°F, around mid-December. As a result of low temperatures and lower rainfall, the mosquito population numbers did not reach a peak for the remainder of the study. A major rain event occurred in late July, but the mosquito numbers never again reached the previous numbers from earlier in the study. The temperature in Gainesville, Florida, does fluctuate in the winter months, and becomes steady during the summer months, as noted by other research conducted in Florida by Campbell (2003) and Dilling (2004). Because of the differences in temperature during the cool season months, the mosquito populations are never able to rise until the spring time due to constant change during the night time, with sudden drops below 55°F. During my study, Gainesville experienced lower rainfall than in previous years (Figure 2-6), resulting in decreased mosquito populations. In addition, the decreased rainfall caused certain mosquito species breeding sites to go dry, causing a shift in mosquito species complex. Dilling (2004) found the *Culex* species to be in greater numbers through the majority of her study, because numerous hurricanes made landfall and caused flooded conditions. *Culex* spp. were not as prevalent in my study (Figure 2-12, 2-13, 2-14) because of less rainfall (Figure 2-5).

Similar population trends were found throughout this study, closely following Dilling (2004) despite differences in trap and baits. During the seasonality study *An. crucians* and *Cx. erraticus* were trapped all 13 months. *An. crucians* and *Cx. erraticus* peaked during the cool months and remained steady for the remainder of the trial. This closely followed previous work by Dilling (2004). *Mansonia* spp. was present the entire study except for February. *Cq. perturbans,* was not present until mid-March, where it began to rise steadily and remained in high numbers for the rest of the study. The *Mansonia* species and *Cq. perturbans* have the ability to pierce and attach to the roots of aquatic plants because of the presence of their attenuated siphon which allows them to withstand longer periods of drought and develop in pools with less water (Darsie, 2006). This adaptation allowed both species to maintain higher population numbers through the drought experienced during my study. One species, *Ps. columbiae,* was not noticed in large numbers until June, along with *An. quadrimaculatus*, which disagrees with Dilling (2004), who found *Ps. columbiae* in greater numbers during October and *An. quadrimaculatus* during November and December. Probably because of climatic differences such as lower rainfall compared to previous years, several species including *Cx. nigripalpus, Ps. columbiae,* and *An. quadrimaculatus* were not collected in all 12 months or in such high numbers as with Dilling in 2004.

In the future, attempts would be made to keep the MMPro traps in an operational state by tending to them with regular maintenance, and keeping them clean and running properly. Several times the traps, especially the trap at position #4, stopped working because of dust. Furthermore, I would recommend adjusting the CO_2 output of the MMPro trap to a level that more closely matches that of the horse, its main competition at the HTU. Horses expire approximately 2000 cc/min, (Pelletier and Leith, 1995) or > 4 times the amount that the MMPro trap releases. This fact could increase the chances that the trap could be beneficial on a horse farm, if in fact olfactory cues are the main attractant for mosquitoes.

It is important to incorporate mosquito surveillance with effective trapping methods in order to achieve maximum control of disease. It is crucial to combine the technique of surveillance with effective lures, such as octenol combined with CO_2 and to monitor the environmental and meteorological factors which could potentially influence the mosquito populations.

Conclusion

This study further supported the fact that CO_2 is an effective lure for increasing trap counts. In addition, when combined with octenol, the trap counts can be increased even further. Lurex and Lurex^3 significantly suppressed trap counts when combined with CO_2. Under similar metrological conditions, octenol + CO_2 are the most effective attractant combination for trapping certain species of mosquitoes in the MMPro, especially the *Mansonia* spp. If this species becomes a competent WNV vector, octenol + CO_2 could be used to trap this species. This attractant combination could be useful on livestock facilities to lower the total mosquito population that would have access to the animals, especially the *Mansonia* spp. and *Cq. perturbans*. Under different meteorological conditions, such as a cooler climate, a different mosquito species complex trend might be found and a different lure might be required if trapping is to be effective.

During this study conducted in Gainesville, Florida, 71, 850 mosquitoes were trapped, comprising a total of 10 different species. The most prominent genus trapped was *Mansonia,* followed by *An. crucians* and *Cq. perturbans*. The *Mansonia* genus has not yet been ruled out as a competent WNV in Florida. Possibly due to the lower rainfall, *Cx. nigripalpus* was in lower than expected numbers, indicating that the threat of the WNV virus at the HTU was low during the study. Because of the lack of rainfall in the warm summer months, smaller population peaks were seen in July and August, contrary to previous studies conducted at the HTU. Instead, the large peaks were seen in the late fall month of November, with relatively stable trap counts in December. Mosquitoes were trapped all thirteen months, with the lowest numbers occurring in the cold winter months of January and February. Data from the study suggests that rainfall has a huge impact on mosquito populations by pausing larval development and preventing them from continuing into the pupal stage. Furthermore, low nighttime temperatures also had an adverse effect on population numbers, inhibiting larval development. Generally, 2-3 weeks following a heavy rainfall, mosquito populations increased. In my study temperature and rainfall appear to play major roles in the production of mosquito populations.

Figure 2-1. Mosquito Magnet Pro (American Biophysics Corporation, East Greenwich, RI) mosquito trap.

Figure 2-2. Aerial Photograph of the UF HTU showing the location of the 4 MMPro traps used in the 3-Lure Seasonality study conducted from September 2005 – September 2006.

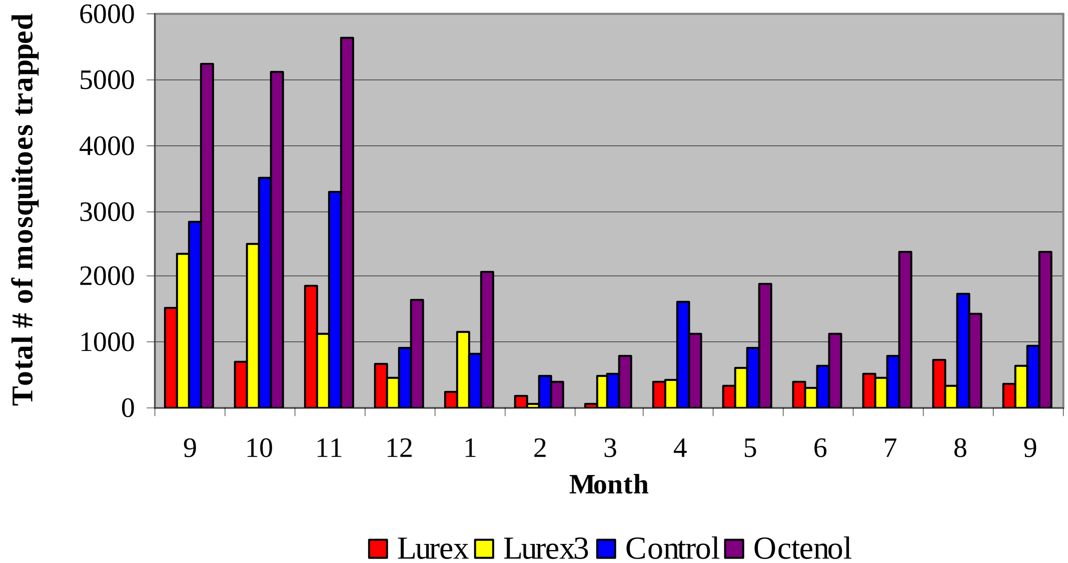

Figure 2-3. Comparison of all four lure combinations and total mosquito composition trapped during the 3-lure seasonality study conducted from September 2005 until September 2006. Note: Month number equals corresponding calendar date (i.e. 1 = January).

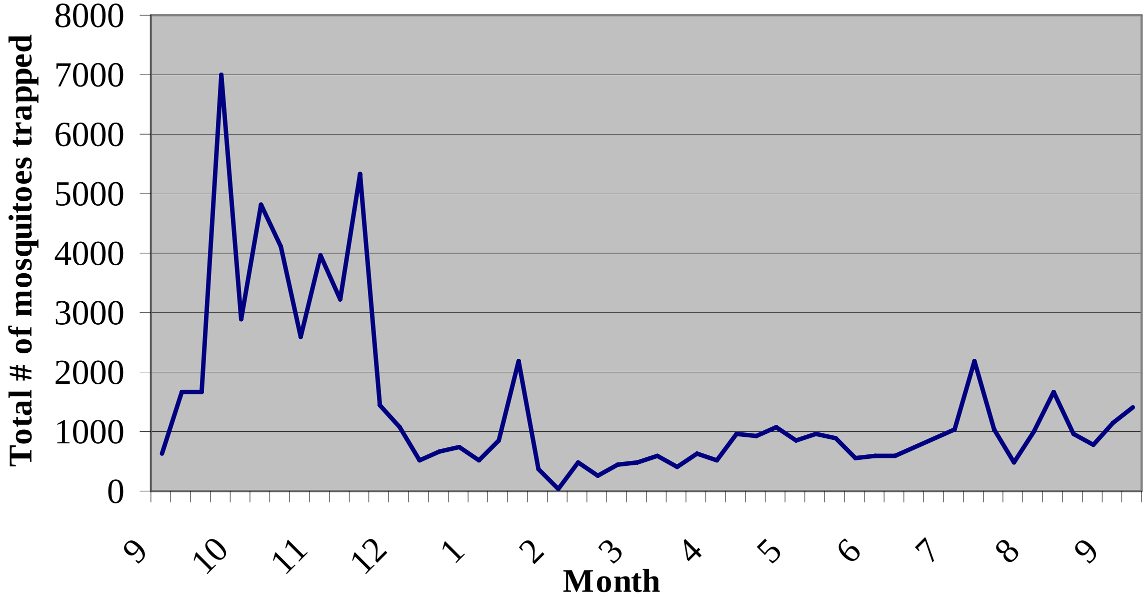

Figure 2-4. Total Mosquito counts as related to months during the 3-Lure seasonality study conducted at the UF HTU from September 2005 – September 2006. Note: Month number equals corresponding calendar date (i.e. 1 = January).

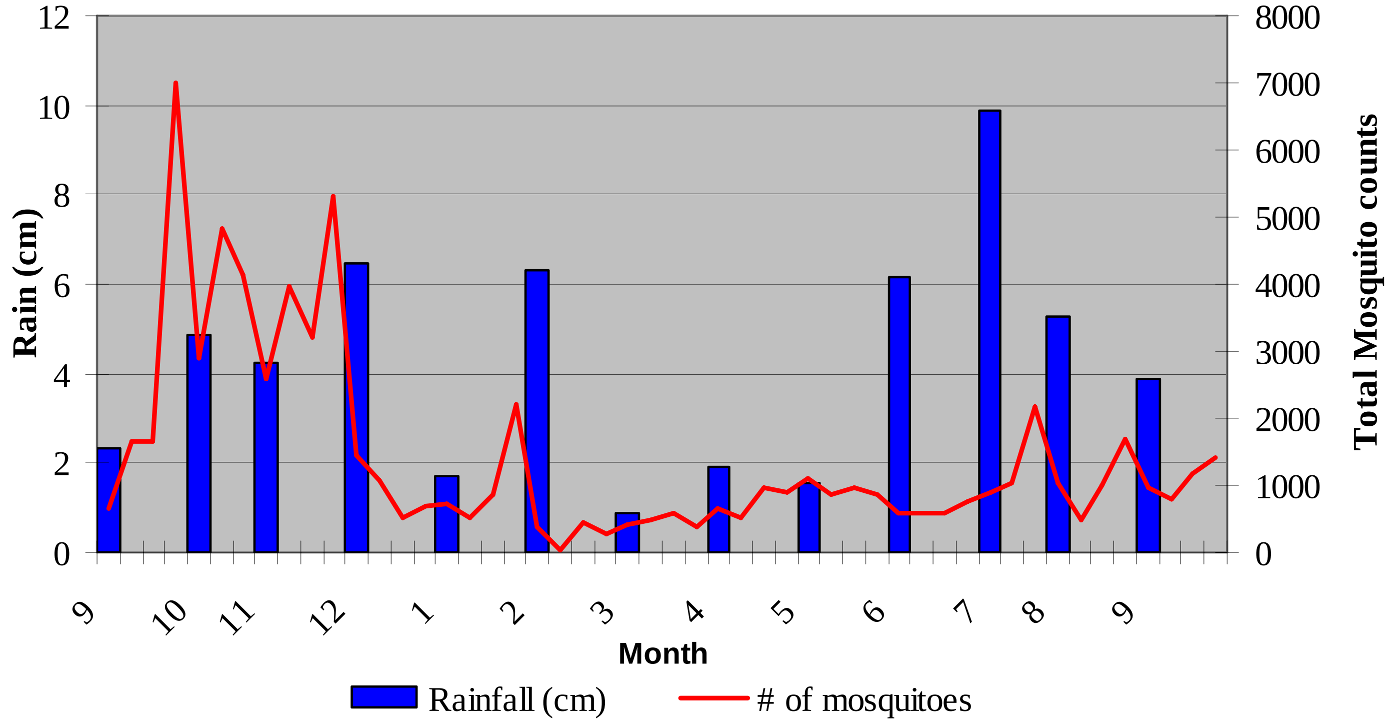

Figure 2-5. Total mosquito count from the MMPro traps related to rainfall in centimeters in the 3-Lure seasonality study conducted at the UF HTU from September 2005 through September 2006. Note: Month number equals corresponding calendar date (i.e. 1 = January).

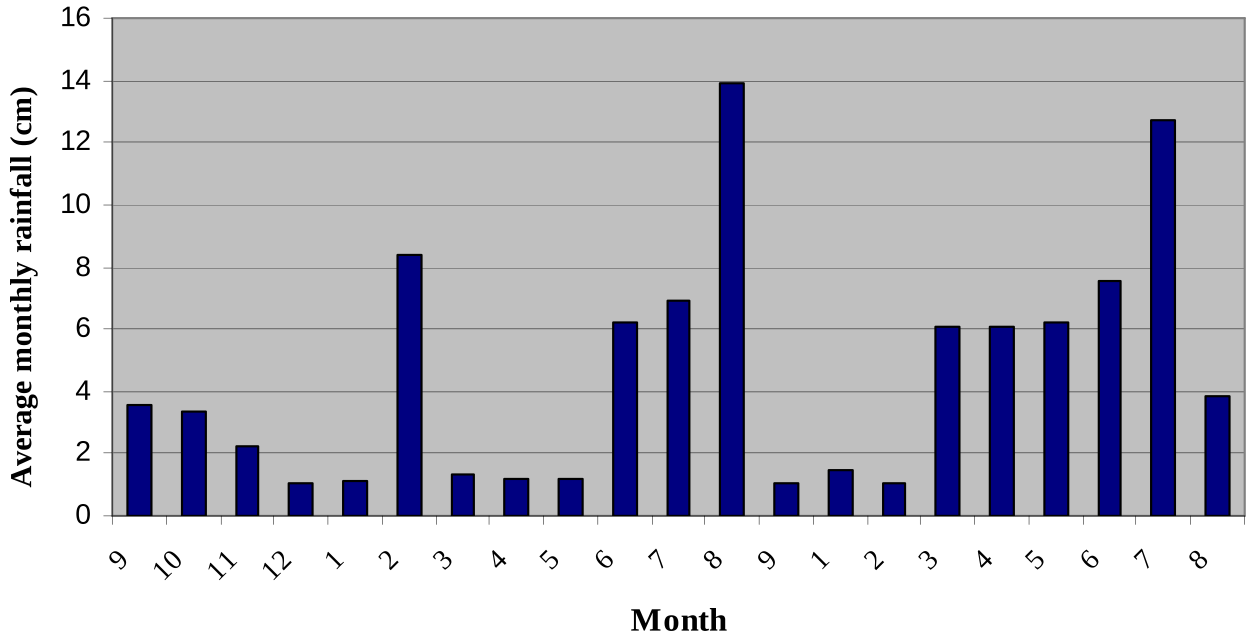

Figure 2-6. Total rainfall in centimeters measured before the 3-Lure seasonality study at the UF HTU from September 2003 through August 2005. Note: Month number equals corresponding calendar date (i.e. 1 = January).

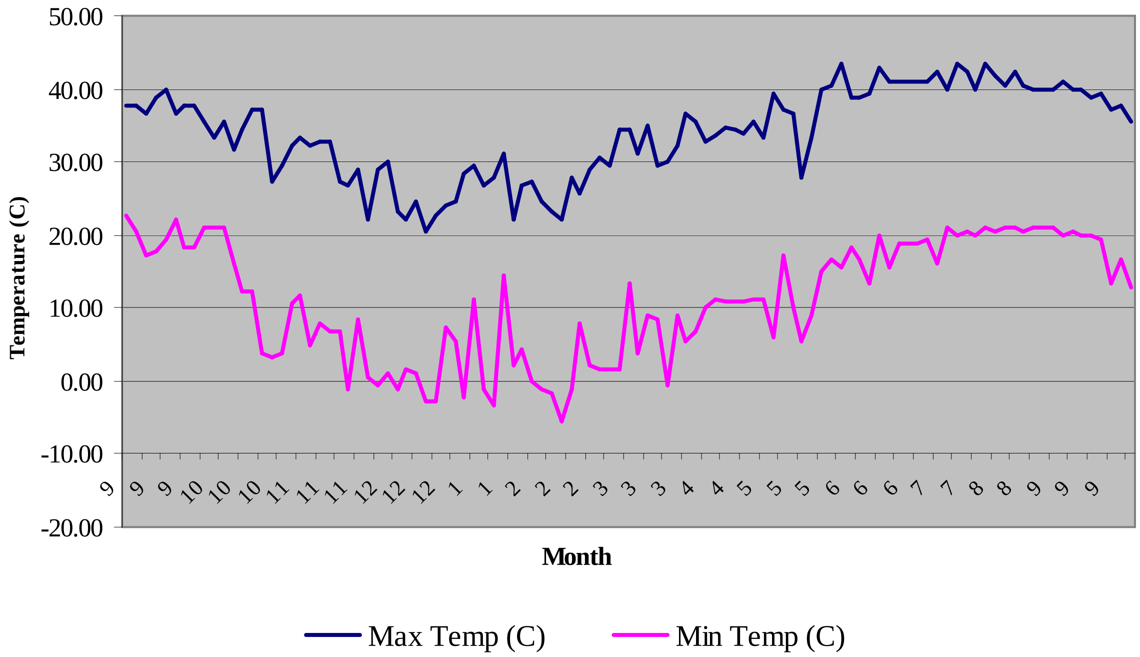

Figure 2-7. Minimum and Maximum temperatures recorded using the meteorological station during the 3-lure seasonality study conducted at the UF HTU from September 2005 through September 2006. Note: Month number equals corresponding calendar date (i.e. 1 = January).

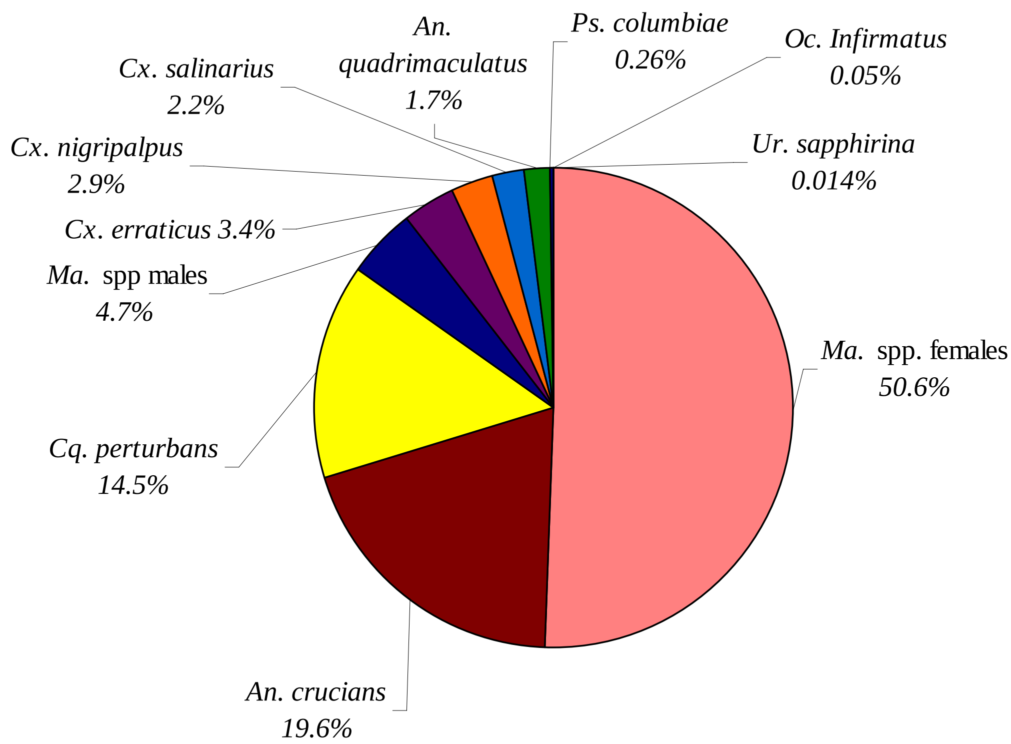

Figure 2-8. Total Mosquito Species composition of the 3-Lure seasonality study conducted at the UF HTU conducted from September 2005 through September 2006.

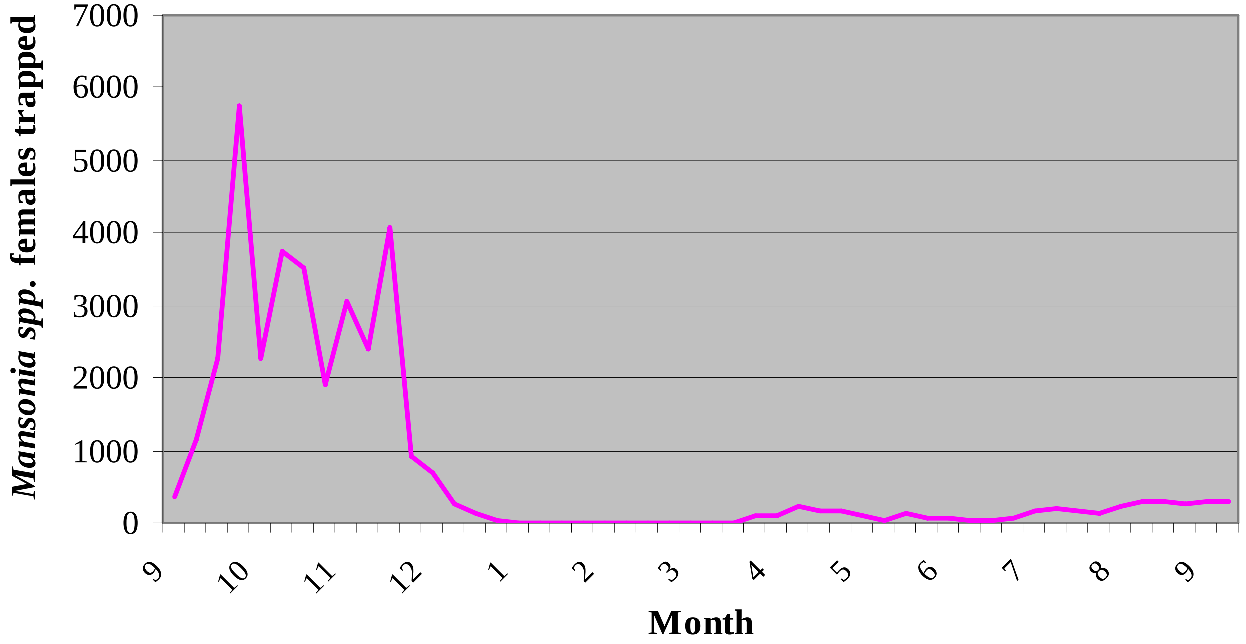

Figure 2-9. Total numbers of *Mansonia spp*. females trapped by the MMPro traps during the 3-Lure seasonality study conducted at the UF HTU through September 2005 through September 2006. Note: Month number equals corresponding calendar date (i.e. 1 = January).

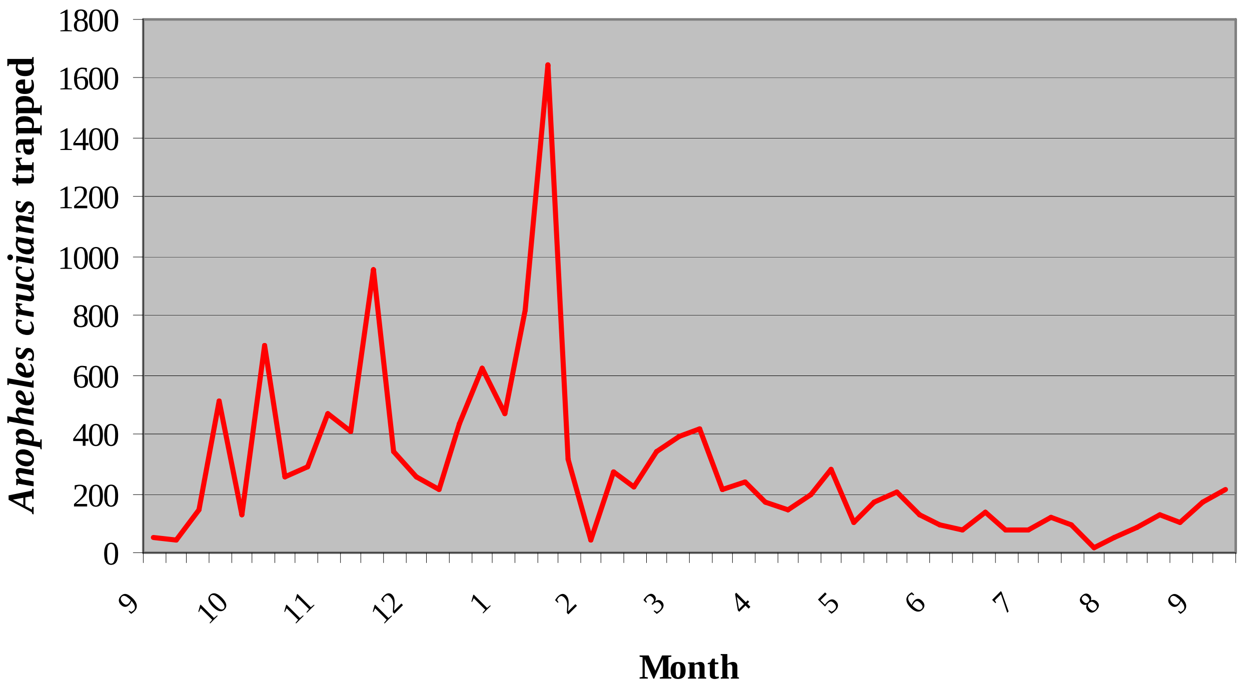

Figure 2-10. Total numbers of *Anopheles crucians* trapped by the MMPro traps during the 3-Lure seasonality study conducted at the UF HTU through September 2005 through September 2006. Note: Month number equals corresponding calendar date (i.e. 1 = January).

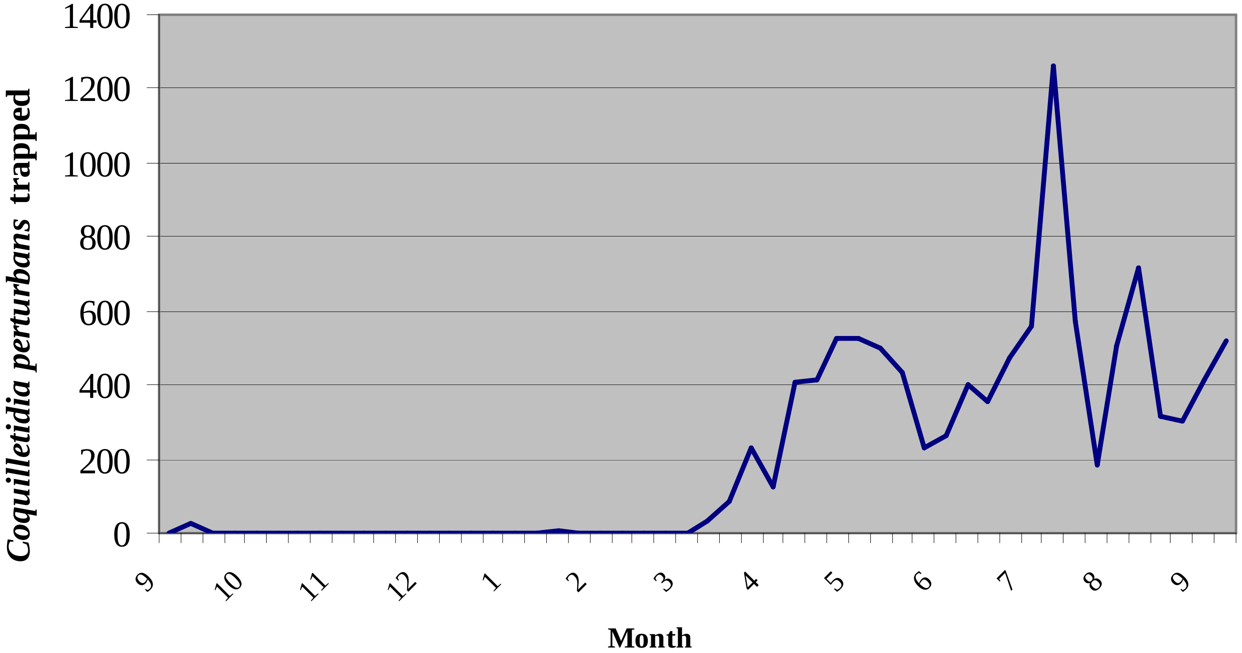

Figure 2-11. Total numbers of *Coquillettidia perturbans* trapped by the MMPro traps during the 3-Lure seasonality study conducted at the UF HTU through September 2005 through September 2006. Note: Month number equals corresponding calendar date (i.e. 1 = January).

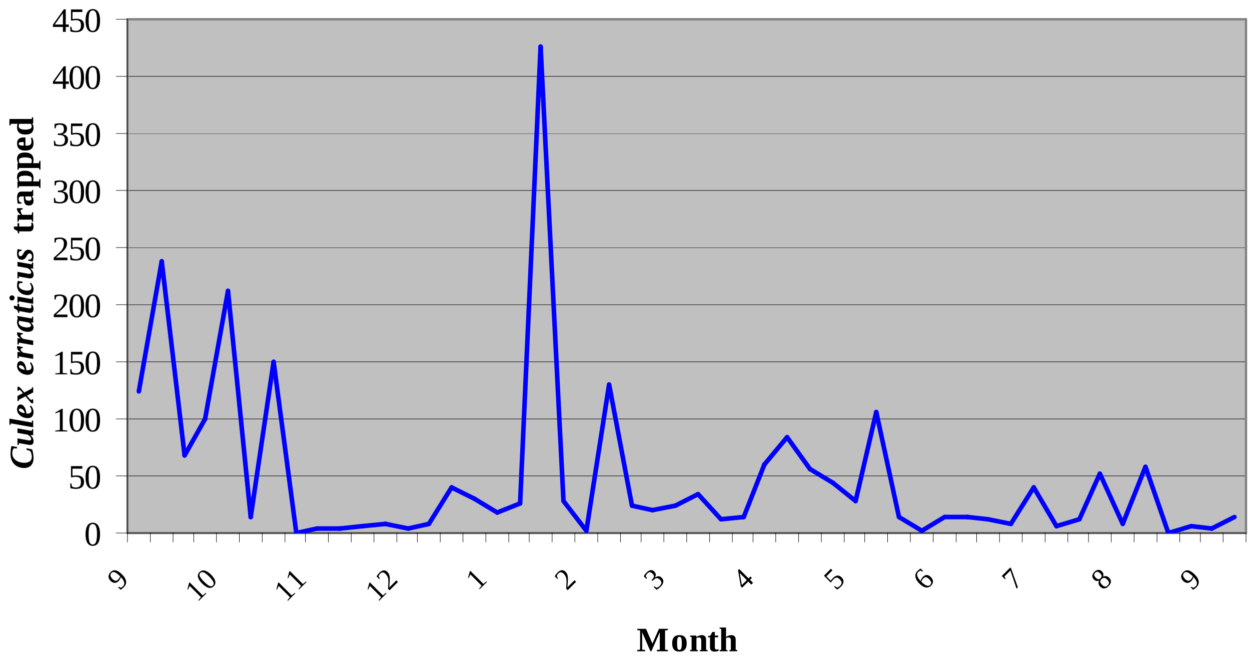

Figure 2-12. Total numbers of *Culex erraticus* trapped by the MMPro traps during the 3-Lure seasonality study conducted at the UF HTU through September 2005 through September 2006. Note: Month number equals corresponding calendar date (i.e. 1 = January).

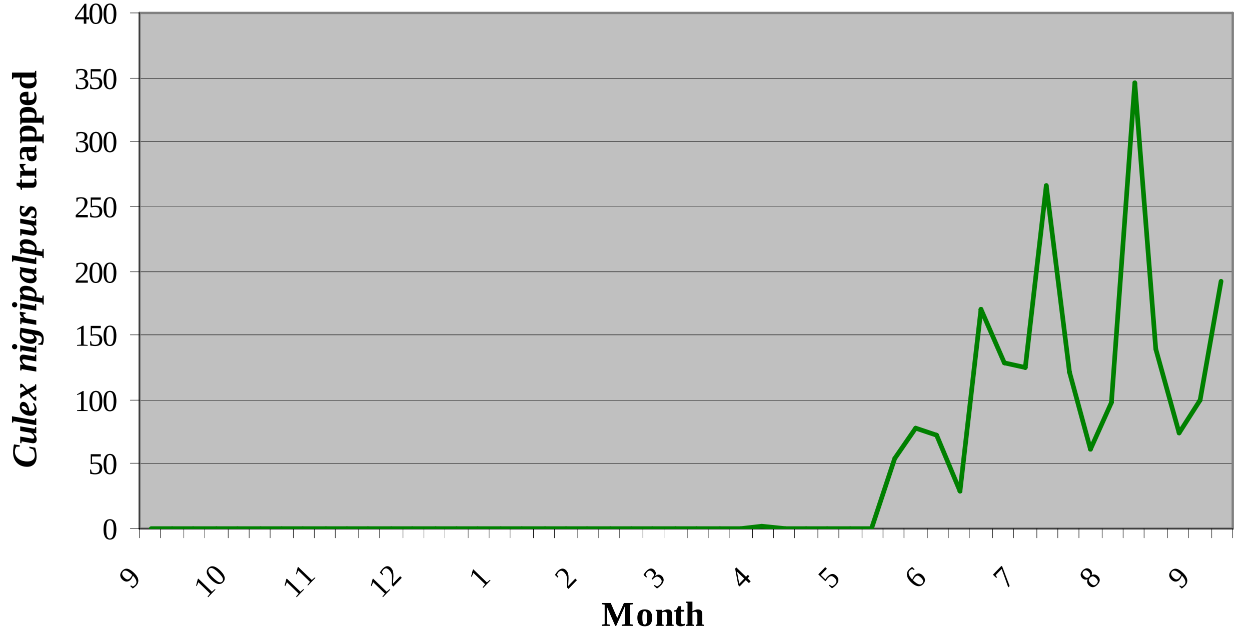

Figure 2-13. Total numbers of *Culex nigripalpus* trapped by the MMPro traps during the 3-Lure seasonality study conducted at the UF HTU through September 2005 through September 2006. Note: Month number equals corresponding calendar date (i.e. 1 = January).

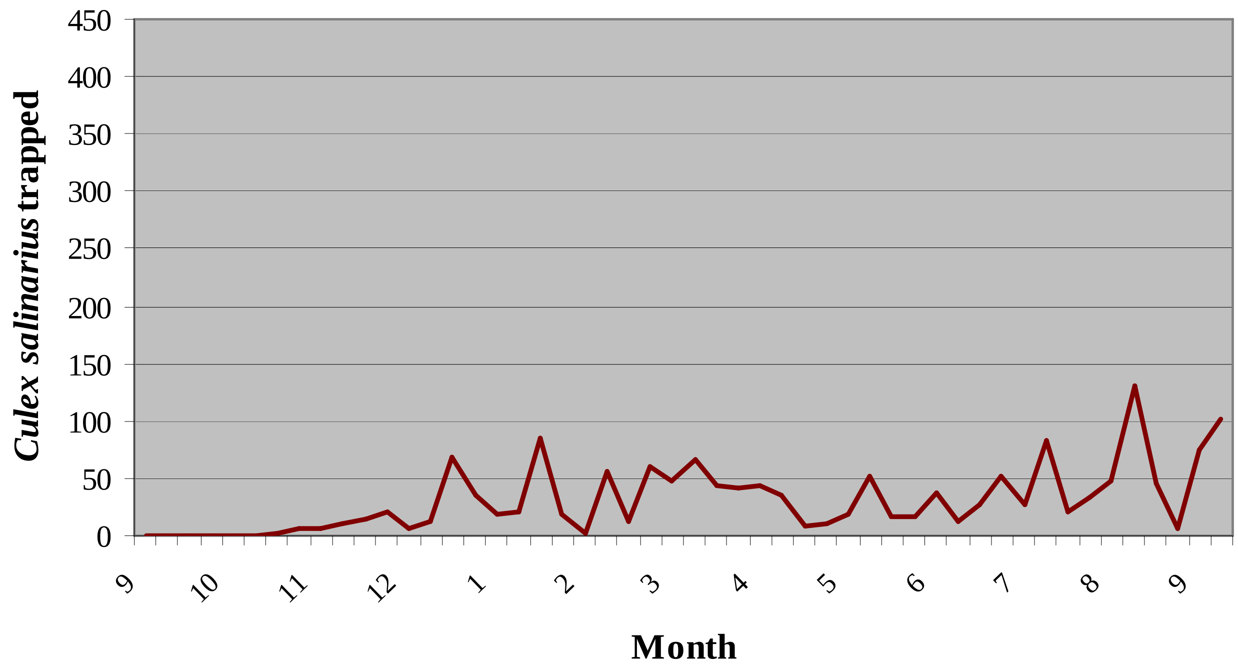

Figure 2-14. Total numbers of *Culex salinarius* trapped by the MMPro traps during the 3-Lure seasonality study conducted at the UF HTU through September 2005 through September 2006. Note: Month number equals corresponding calendar date (i.e. 1 = January).

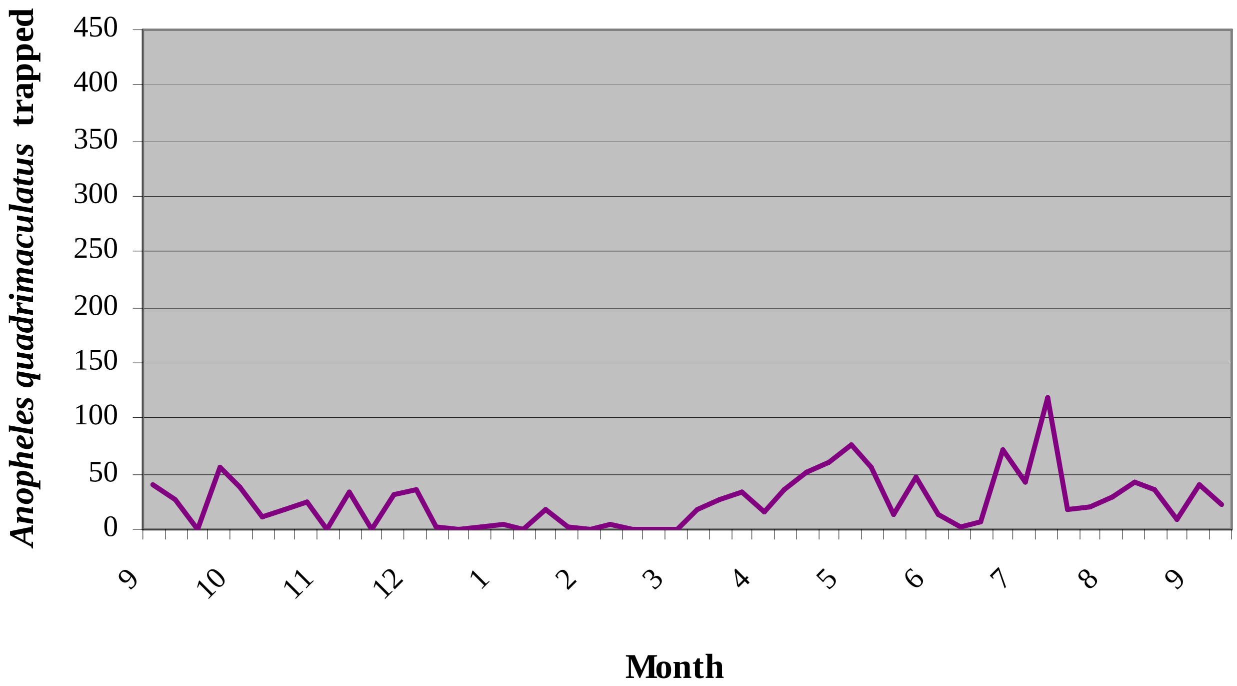

Figure 2-15. Total numbers of *Anopheles quadrimaculatus* trapped by the MMPro traps during the 3-Lure seasonality study conducted at the UF HTU through September 2005 through September 2006. Note: Month number equals corresponding calendar date (i.e. 1 = January).

Table 2-1. Four treatment rotation schedule for the MMPro Traps during the 3-Lure seasonality study at the UF HTU.

Date	Trap #1	Trap #2	Trap #3	Trap #4
09/02/05	Lurex	CO_2 alone	Octenol	Lurex^3
09/09/05	Lurex^3	Lurex	CO_2 alone	Octenol
09/16/05	Lurex	Octenol	Lurex^3	CO_2 alone
09/23/05	CO_2 alone	Lurex	Octenol	Lurex^3
09/30/05	Lurex^3	CO_2 alone	Lurex	Octenol
10/07/05	Octenol	Lurex^3	CO_2 alone	Lurex
10/14/05	Lurex	Octenol	Lurex^3	CO_2 alone
10/21/05	CO_2 alone	Lurex	Octenol	Lurex^3
10/28/05	Lurex^3	CO_2 alone	Lurex	Octenol
11/04/05	Octenol	Lurex^3	CO_2 alone	Lurex
11/11/05	Lurex	CO_2 alone	Octenol	Lurex^3
11/18/05	Lurex^3	Lurex	CO_2 alone	Octenol
11/25/05	Octenol	Lurex^3	Lurex	CO_2 alone
12/02/05	CO_2 alone	Octenol	Lurex^3	Lurex
12/16/05	Lurex	CO_2 alone	Octenol	Lurex^3
12/30/05	Lurex^3	Lurex	CO_2 alone	Octenol
01/13/06	Octenol	Lurex^3	Lurex	CO_2 alone
01/27/06	CO_2 alone	Octenol	Lurex^3	Lurex
02/10/06	CO_2 alone	Lurex	Octenol	Lurex^3
02/24/06	Lurex^3	CO_2 alone	Lurex	Octenol
03/10/06	Octenol	Lurex^3	CO_2 alone	Lurex
03/28/06	Lurex	Octenol	Lurex^3	CO_2 alone
04/11/06	CO_2 alone	Lurex	Octenol	Lurex^3
04/25/06	Lurex^3	CO_2 alone	Lurex	Octenol
05/09/06	Octenol	Lurex^3	CO_2 alone	Lurex
05/23/06	Lurex	Octenol	Lurex^3	CO_2 alone
06/06/06	CO_2 alone	Lurex	Octenol	Lurex^3
06/20/06	Lurex^3	CO_2 alone	Lurex	Octenol
07/04/06	Octenol	Lurex^3	CO_2 alone	Lurex
07/18/06	Lurex	Octenol	Lurex^3	CO_2 alone
08/01/06	CO_2 alone	Lurex	Octenol	Lurex^3
08/15/06	Lurex^3	CO_2 alone	Lurex	Octenol
08/29/06	Octenol	Lurex^3	CO_2 alone	Lurex
09/12/06	Lurex	Octenol	Lurex^3	CO_2 alone

Table 2-2. Mean numbers of total mosquitoes trapped using each attractant + CO_2 combination in the MMPro trapping study conducted from September 2005 through September 2006.

Attractant	Mean difference trapped (±Std. err)	*n*	Total trapped
Octenol	330.42 (±37.098)[a]	99	32,373
CO_2 alone (control)	210.81 (±28.674)[b]	100	21,167
Lurex[3]	160.83 (±31.161)[bc]	113	11,865
Lurex	131.86 (±25.103)[c]	90	15,435

Note: Means for attractant combination which are followed by the same number are not significantly different ($P \leq 0.05$) and n= number of observations.

Table 2-3. Mean numbers of total mosquitoes trapped in each trap location in the MMPro trapping study conducted from September 2005 through September 2006.

Trap location	Mean difference trapped (±Std. err)	n	Total trapped
1	259.41 (±34.817)[a]	104	27, 260
2	216.08 (±24.005)[ab]	105	22, 688
3	194.66 (±30.787)[ab]	104	20, 243
4	156.43 (±35.4599)[b]	89	10, 289

Note: Means for each trap location which are followed by the same number are not significantly different ($P \leq 0.05$) and n= number of observations.

Table 2-4. Mean numbers of total mosquitoes trapped for each month during the MMPro trapping study from September 2005 through September 2006.

Month	Mean difference trapped (± Std. err)	n	Total trapped
September 2005	795. 13 (±111.2)[a]	31	24, 649
October 2005	437.81 (68.74)[b]	27	11, 821
November 2005	430.89 (±55.88)[b]	35	15, 081
December 2005	105.37 (±18.57)[c]	35	3, 688
January 2006	121.19 (±35.75)[c]	32	4, 318
February 2006	35.59 (±9.758)[c]	32	1, 139
March 2006	71.92 (±13.85)[c]	26	1, 870
April 2006	95.53 (±14.63)[c]	32	3, 057
May 2006	118.19 (±16.98)[c]	32	3, 782
June 2006	66.64 (±10.30)[c]	33	2, 470
July 2006	164.88 (±30.70)[c]	25	4, 122
August 2006	136.10 (±21.02)[c]	31	4, 219
September 2006	139.65 (±24.10)[c]	31	4, 329

Note: Means for each month which are followed by the same number are not significantly different ($P \leq 0.05$) and n= number of observations.

Table 2-5. Mean numbers (±standard error) of mosquito species captured for each attractant + CO_2 combination for the total MMPro trapping study conducted September 2005 through September 2006.

Species Caught	Lurex Mean (±Std. err) N = 97	Lurex^3 Mean (±Std. err) N = 105	Control Mean (±Std. err) N = 104	Octenol Mean (±Std. err) N = 87
Mansonia spp. (females)	52.68 (±13.06)[b]	60.67(±10.89)[b]	91.99(±18.91)[b]	154.46(±29.22)[a]
An. crucians	18.20(±2.40)[c]	26.15(±60)[bc]	34.64(±4.86)[b]	60.73(±9.62)[a]
Cq. perturbans	8.41(±1.50)[c]	9.50(±1.58)[c]	27.94(±4.77)[b]	57.56(±8.99)[a]
Mansonia spp. (males)	4.55(±1.22)[b]	9.31(±1.88)[a]	9.94(±2.23)[a]	12.08(±2.35)[a]
Cx. erraticus	4.64(±0.96)[a]	4.29(±0.96)[a]	5.86(±1.07)[a]	9.49(±3.62)[a]
Cx. nigripalpus	4.57(±1.06)[bc]	2.90(±0.65)[c]	5.54(±1.14)[b]	7.48(±1.59)[a]
Cx. salinarius	1.81(±0.38)[b]	3.96(±0.63)[ab]	4.54(±0.84)[a]	5.03(±0.94)[a]
An. quadrimaculatus	3.29(±0.66)[ab]	2.73(±0.60)[b]	1.91(±0.45)[b]	4.65(±0.76)[a]

Note: Means for each species which are followed by the same number are not significantly different ($P \leq 0.05$) and n= number of observations. Species with total trapped numbers > 1,000 are included in table.

Table 2-6. Mean numbers (±standard error) of mosquito species captured for each trap location for the total MMPro trapping study conducted September 2005 through September 2006.

Species Caught	Trap #1 Mean (±Std. err) N = 107	Trap #2 Mean (±Std. err) N = 105	Trap#3 Mean (±Std. err) N = 104	Trap#4 Mean (±Std. err) N = 87
Mansonia spp. (females)	95.11(±22.74)[ab]	77.89(±13.49)[ab]	116.43(±24.14)[a]	66.15(±13.45)[b]
An. crucians	60.15(±9.79)[a]	42.33(4.56)[b]	20.83(±2.46)[c]	11.94(±1.67)[c]
Cq. perturbans	28.50(±5.04)[b]	41.06(±7.87)[a]	13.67(±2.76)[c]	18.46(±4.39)[bc]
Mansonia spp. (males)	5.82(±1.53)[ab]	9.20(±1.80)[b]	11.43(±2.34)[a]	9.80(±2.25)[ab]
Cx. erraticus	11.13(±3.22)[a]	4.35(±0.99)[b]	5.79(±1.53)[b]	2.20(±0.72)[b]
Cx. nigripalpus	6.75) ±1.20)[a]	5.88(±1.37)[ab]	3.66(±0.91)[b]	3.85(±1.03)[b]
Cx. salinarius	5.14(±0.88)[a]	5.39(±0.89)[a]	2.72(±0.46)[b]	1.78(±0.46)[b]
An. quadrimaculatus	3.91(±0.69)[a]	3.34(±0.63)[a]	2.30(±0.54)[a]	2.94(±0.62)[a]

Note: Means for each species which are followed by the same number are not significantly different ($P \leq 0.05$) and n= number of observations. Species with total trapped numbers over 1,000 are included in table.

Table 2-7. Total Mosquito Species Count and percent of total count of mosquito species trapped by MMPro traps in the 3-Lure seasonality study at the UF HTU conducted from September 2005 through September 2006.

Mosquito Species	Total Count	Percent of Total
Mansonia spp.	36217	50.6
Anopheles crucians	14071	19.6
Coquillettidia perturbans	10392	14.5
Mansonia spp. males	3361	4.7
Culex erraticus	2433	3.4
Culex nigripalpus	2055	2.9
Culex salininarius	1554	2.2
Anopheles quadrimaculatus	1264	1.7
Psorophora columbiae	184	0.26
Ochlerotatus infirmatus	39	0.05
Uranotaenia sapphirina	10	0.014
	71580	

CHAPTER 3
STUDIES USING HORSE ODORS TO AUGMENT MOSQUITO TRAP COLLECTIONS AT THE UNIVERSITY OF FLORIDA HORSE TEACHING UNIT, GAINESVILLE, FLORIDA

Introduction

Mosquito traps may be capable of reducing the numbers of mosquitoes near a trap, but their primary usage is for surveillance. Traps are used to catalog species composition and estimate the density of mosquitoes in an area. Various trap styles have been developed, spanning a variety of species specificity and trapping efficiencies of each species (Kline, 1999). Some of these traps are designed to mimic the natural host, usually through the release of host-seeking cues that may consist of carbon dioxide (CO_2), heat, moisture, and odors (Kline and Mann, 1998). An obstacle that helps render these traps as less effective tools for mosquito control is that they do not compete well against a natural host when the host is near the trap(s). Presumably, this is because the odor profile of the host elicits greater attraction in mosquitoes than CO_2 alone or any attractant that is comprised of simple blends.

In the past, it was found that significant differences occur between species composition and total numbers of mosquitoes collected from mosquito traps compared to those vacuumed from a horse (Campbell, 2003). This line of research has been continued in this chapter through experiments that were designed and conducted to compare simultaneously the mosquitoes captured in traps with those captured near horses. Furthermore, the addition of horse odors to the trap was examined to determine if this bait could produce the collection of the same numbers and species composition of mosquitoes which would normally be attracted to the horse.

Dilling (2004) performed several studies evaluating the use of horse odors to augment mosquito trap collections. In one study, a horse was placed in a feeding slip with a Mosquito Magnet Pro (MMPro) trap directly next to the stall. When the horse was nearby, the numbers of trapped mosquitoes declined due to a preference of the mosquitoes for the live horse. In another study, the entire body of a horse was vacuumed using a modified hand-held vacuum. Exhausted horse volatiles from the vacuum were passed through a PVC pipe and fed into the CO_2 flow of a nearby CDC 1012 trap. Trap catch numbers were not significantly increased by inclusion of the odors. It is possible that critical volatiles may have adhered to the inside of the PVC pipe, the flow through the pipe was insufficient to deliver a minimum threshold level of attractants to impart an effect, or volatiles other than CO_2 from breath are missing as are non-chemical cues such as heat, moisture, and visual ones.

Alternative sampling methods to identify horse odors may need to be developed and explored as was done to identify human odors that attract mosquitoes (Bernier et al., 2000). Some of this development involves collecting samples, analyzing and testing the biological efficacy of the samples to attract mosquitoes, followed by chemical sampling to identify the attractants that were involved.

The objectives of the studies in this chapter were to 1) Collect hair and dander samples from different locations on a horse and identify the chemical composition of compounds present in these samples using gas chromatography and mass spectrometry; 2) determine if a correlation is present between the ability of two different horses to attract insects and the chemical differences from the skin of these two different horses; 3) determine the ability of horse odor samples to attract and collect mosquitoes in a MMPro; and 4) determine if mosquito species composition and relative numbers of collected mosquitoes in the vacuum aspiration of two different horses compared to the composition and numbers of mosquitoes caught in traps augmented with collected horse odors.

Materials and Methods

Three studies were conducted at the University of Florida Horse Teaching Unit (HTU) in Gainesville, Florida. The teaching unit houses approximately 40 quarter horses varying in age and sex on 60 acres of land. Two horses used for these experiments were an 8-year old quarter horse gelding named “Steiner” (Figure 3-1) and a 4-year old quarter horse mare named “Lodi” (Figure 3-2). Two MMPro traps and two portable vacuum aspirators were used to perform these studies.

Experimental Design

Horse Odor Collection Study

Sample odors were collected from two horses over the period of May 20 – June 30, 2006. Steiner, the eight-year old Quarter Horse gelding, was chosen to represent the animals that would be found on horse farms throughout Florida and Lodi, the four-year old black Quarter Horse mare, was chosen because of her hypersensitivity to insects such as stable flies and *Culicoides* spp. Bites from these insects resulted in loss of hair on her chest, ears, and tail (Figure 3-3).

Horses were observed at dusk to determine areas where mosquitoes landed. The sites that resulted in the greatest mosquito landings were used as the locations where samples were to be taken and analyzed. Horse odors were collected from the face, barrel/dorsal side of the abdomen, and the legs using a cotton ball of a 2” diameter. The cotton ball was rubbed in the preferred area ~ (5 in^2) for 5 min to ensure ample sample extraction (Figure 3-4). Hair was also removed from the chest of the horse with a sharp sterilized knife, using a soft, downward motion to cut hair close to the skin to scrape the dander into a clean glass vial. Mane samples were collected using scissors, with small samples taken from the underside of the mane. Samples were transported to the laboratory after collection and extracted immediately upon return to the laboratory to minimize loss of volatile compounds.

Hair samples from various locations on Steiner and Lodi were extracted with 1mL hexane and the dander samples from these horses were extracted in 250 µL. Extracts were analyzed by gas chromatography/mass spectrometry (GC/MS) on a ThermoFinnigan Trace Single

Quadrupole GC/MS system (Thermoquest Finnigan, San Jose, CA). This system consists of a GC oven, a split/splitless injection port equipped with a Programmable Temperature Vaporizer (PTV) injector.

Injections were performed manually, using 1 μL of sample with the GC injection port set at 35^{o}C prior to injection. Upon injection, the sample was loaded onto the GC column while the temperature of the injection port was ramped balistically at 14.5 ^{o}C/s to 240 ^{o}C and held there for 1.0 min. Following this loading phase, the injection port is set to clean by a second ballistic ramp at 14.5 ^{o}C/s to 240 ^{o}C, and held at that temperature for 3 min. The GC oven was held at 35^{o}C for 6 min after injection and then ramped to 250^{o}C at 10 ^{o}C/min, and held at that temperature for 25 min. The injector split was 12:1 (mL/min flow) and the carrier gas was high purity helium set to maintain a constant flow of 1.20 mL/min. Samples were injected onto a 30 mm x 0.25 mm i.d. DB-5ms column with a stationary phase film thickness of 0.25μm. The transfer line into the mass spectrometer was held at 260^{o}C throughout the analysis. The mass spectrometer was tuned and calibrated with perfluorotributylamine (PFTBA) prior to the acquisition of data. Additionally, control hexane samples were injected prior to analysis of extracted samples, and a standard hydrocarbon mixture was injected once per day to obtain retention index data to assist in compound identification.

The mass spectrometer was operated in electron ionization (EI) mode, with an average of 70 eV electrons. The ion source was set at 200 ^{o}C, the emission current was 350 μA, and the detector was set at 350 V; the detector dynode was held in the off position until 3.0 min into the analysis. A scan rate of 0.5 s per full scan was used cover the scan range of *m/z* 35-565.

Horse Odor Trapping Study

The horse odor trapping study was conducted from May 23 - September 30, 2006. Two MMPro traps were operated 4 m apart on the south side of the main body of water, just north of the large covered arena at the HTU (Figure 3-5). The MMPro is a self-powered mosquito trap that burns propane catalytically to produce CO_2, heat, and moisture. The output of combustion results in enhanced attraction of some insect species. Combustion of propane also produces electricity to power the fan. MMPros may be operated with additional lures or by simply releasing the combustion products without added lure. The principle by which the trap collects insects is patented as "counter flow geometry" where a mosquito may near the base of the outflow while one fan vacuums the insect into a 2 qt. collection net attached to the bottom of the fan inside the trap (US Patent: 7074830, Durand et al., 2006). The mosquitoes are stored in the net after the fan pulls them into the inside of the trap. A window on the front of the trap provides a view of the collection net within the trap. The trap is constructed with stainless steel with a PVC outer covering and stands about 40" tall.

Two treatments were tested: horse collected odor + CO_2 and CO_2 only. Each treatment employing horse odor was collected every 24 h to minimize volatilization of the compounds from the sample. Skin extracts of the horses were collected by rubbing a cotton ball 2" diameter in a localized area (5 in^2) for 5 minutes. Cotton balls were rotated to ensure that all surfaces were covered with the scent and the length of time confirmed the presence of the odors and oils from the skin on the cotton ball. A latex gloved hand held the cotton balls to minimize contamination from human skin (Dekker et al., 2002). The cotton ball was placed in a modified plastic cartridge, designed by American Biophysics to hold the Lurex[3] lure. The cartridge was placed at the bottom of the trap, near the exit. The control MMPro trap was operated with the normal emission of CO_2, heat, and water vapor only.

The samples were collected from the same physiological area on both horses every 24 h and tested in the traps daily for four consecutive days. The cotton balls containing collected odorants were changed after each collection was made, around 11 am EST. With each change of cotton, the nylon collection nets were also changed. The propane tanks were changed every 18 d to ensure an uninterrupted supply of propane during the experiments. Trapped mosquitoes were stored in a freezer at -25°C until counting and identification could be conducted. Data were analyzed by GLM and means separated by Duncan's Multiple Range Test using SAS 2006 after transformation by log (n + 1). Standard error of the means was calculated using SAS 2006.

Horse Vacuuming Study

Species composition and the number of mosquitoes that attempted to blood feed from two different horses were examined on the consecutive nights of September 20 and 21, 2006. Collection began at 7:30 p.m. (around dusk) both days and concluded one hour later at 8:30 p.m. Each horse was tied at the south end of the covered arena at the HTU, 61 m apart, one on the far east and the other on the far west side of the arena. Approximately 150 m from the east side of the south side of the arena was a retention pond and near the west side (20 m) was a swampy, wooded area with thick natural vegetation, down trees, and standing water.

Two individuals vacuumed the horses using portable vacuum aspirators simultaneously for 30 min (Figure 3-6). After the first 30 min, the two individuals switched horses and used portable vacuum aspirators to vacuum the other horse on the opposite side of the arena for the next 30 min. The same two individuals vacuumed each night. Mosquitoes were vacuumed from all body surfaces of the horses. On night one, individual one vacuumed horse one (Lodi) and individual two vacuumed horse two (Steiner) for the first 30 min. For the next 30 min, horse one (Lodi) was vacuumed by individual two and horse 2 (Steiner) was vacuumed by individual one. On night two, this same collection scheme was repeated; however, the locations of horses were rotated from left to right side of the arena compared to the previous night's location. Automobile batteries supplied the power, and the two vehicles were both white to eliminate bias due to vehicle color. A 1995 white Ford Ranger with a 12-V Interstate battery was parked on the east side and a 1995 white Lincoln Continental with a 12-V Auto Zone battery was parked on the west side for both consecutive nights. Mosquitoes were stored in a freezer at -25°C until counting and identification could be completed. Data were analyzed by GLM and means separated by Duncan's Multiple Range Test using SAS 2006 after transformation by log (n + 1). Standard error was calculated using SAS 2006.

Results

Horse Odor Collection Study

Figure 3-7 is a chromatogram depicting the compound peaks observed from the analysis of hair from the horse, Steiner. One of the most abundant compound peaks based on peak area is that at 13.39 min in the chromatogram. The mass spectrum corresponding to this peak could not be matched with any of the library mass spectra. Additionally, this chromatogram had significant peaks at 19.01 (geranylacetone) and at 28.60 (9-octadecenamide). Additional compounds identified in this examination of Steiner are listed in Table 3-1. There are differences in compounds on the hair between the horses examined in this study; these are reported in Table 3-2. The chromatogram in Figure 3-8 demonstrates visually the remarkable differences in compounds collected from the hair of Steiner and Lodi. Figure 3-9 shows very similar results from the dander of both horses. It also demonstrates the peak at 13.39 min, an unknown compound that has not previously been detected nor reported in horse odor, nor other host odor samples from humans, chickens, or other mammals that have been studied previously (Bernier et. al., 2000; Bernier, U.R., pers. communication).

Horse Odor Trapping Study

There were a total of 6,282 mosquitoes captured during the five month horse odor study. The species composition trapped throughout the study on Lodi and Steiner are shown in Figures

3-10 and 3-11, respectively. The three most abundant species collected on both horses was *Coquillettidia perturbans*, the *Mansonia* spp., and *Culex nigripalpus*. When odor from Lodi was collected and added to CO_2, 1,758 mosquitoes were trapped compared to the control trap with just CO_2 which caught only 1,523 mosquitoes (Figure 3-12). In this same figure, Steiner's odor + CO_2 used in the trap caught only 1,437 mosquitoes, compared to 1,564 mosquitoes for CO_2 alone. The mean numbers of mosquitoes trapped using the odors from Lodi and Steiner are in Tables 3-3 and 3-4, respectively. The breakdown of species composition trapped for both horses are listed in Tables 3-5 and 3-6, for Lodi and Steiner respectively. There was no significant difference ($P \leq 0.05$) between the treatment groups for Steiner or Lodi, including comparisons against each other, nor was there a significant difference for catches in different locations.

Horse Vacuuming Study

A total of 474 mosquitoes were vacuumed from Steiner on the east side of the arena, compared to only 411 mosquitoes when he was located on the west side. There were 437 mosquitoes aspirated from Lodi when she was located on the east side compared to 381 mosquitoes from the west side. The mean numbers for each horse and for both sides of the arena are in Table 3-7. No significant difference ($P \leq 0.05$) was found in the species composition and the number of mosquitoes aspirated when mosquitoes were compared between the two horses, nor was a significant difference in collections found for location in the arena, nor for the individual person who vacuumed the horse. The total number of mosquitoes vacuumed off of both horses for both nights was 1,703 with the most prominent species trapped being *Mansonia* spp. comprising 86% of those captured, followed by *Cq. perturbans* at just 8% and *Cx. erraticus* in third with just 4% of the total catch. Nearly the same species profile was found for both horses, with only slight differences in total percentage of species for each horse as illustrated in

Figure 3-13 and 3-14. Table 3-8 and 3-9 illustrate the total mosquito species composition trapped for each horse.

Discussion

Horse Odor Collection Study

Results from GC/MS analysis of horse hair and dander reveal that the compounds present on the horses contain some similarities and differences compared to those present on other mammals, such as humans and bovines. Aldehydes such as nonanal and decanal are common on the skin and other surfaces of just about all animals and could possibly play a role in insect attraction to host odors. Another compound class containing members that were present on the horses was the alcohols and the role of many of these in the host-seeking process remains unknown, although 1-octen-3-ol is a known mosquito attractant (Takken and Kline, 1989). An interesting aspect regarding horses as hosts for mosquito blood meals from is that they are appealing to mammal feeders, such as *Cq. perturbans* as well as avian feeders, such as those of the genus *Culex*. Therefore, examination of horse odors may reveal clues about chemicals involved in the host-seeking process.

Of notable interest is that the chromatograms of both horses' dander contained a peak at 13.39 min (Figure 3-7, 3-8, and 3-9). The identity of this compound is still under investigation. Until identification can be made, it will not be possible to determine whether or not it is crucial to mosquito location of the horses. Despite this optimism, it should not be discounted that other cues may be vital for host location, such as body temperature, respired CO_2, production of lactic acid or even other volatile compounds such as octenol, methane, or excess nitrogen excretion from bodily fluids such as urination.

The similarities in both horses' dander may explain how the equine species as a whole, combined with the aforementioned cues, has an increased ability to attract insects. Despite similarities in chemical composition of the horse dander, the hair samples taken from both horses revealed a different chemical composition, which could be attributable to the deposition of exogenous compounds on the outside surface (hair) of the horse. Lodi had several compounds on her hair that were terpene-based, similar to those found in nature on plants and trees, as well as in pressure-treated wood, like fence posts. Since Lodi and Steiner were pastured in separate fields on different ends of the HTU, this may explain the differences in the profile of compounds present in the extracts of their hair. Both horses were restricted from bathing and excessive brushing throughout the study in an attempt to minimize contamination by exogenous chemicals from shampoos and contaminants on the equipment. These precautions were also used during the horse odor trapping study. Despite these efforts, substances highly likely of exogenous origin were found in the analysis, such as compounds found in plastic gloves that were worn when samples were collected to prevent contamination of oils and other compounds present on the surface of human skin. Hair contaminants found on Steiner during the study are noted when possible in Table 3-1, Table 3-2 compares compounds that may be unique to a horse or that were found from the dander of both horses.

All of the samples were similarly for each horse with respect to location on the horse, such as the face, legs, and the neck. However, it was difficult to control for environmental conditions. Lodi was kept in a large field during the summer, with greater exposure to the environment including the sun, dirt, and exogenous chemicals, such as those from vegetation. When samples were collected from Lodi, it was apparent that they contained sweat and dirt from her habitat. Steiner was kept in a smaller paddock with greater shading, resulting collections from his hair and skin that contained less sweat than Lodi.

However, the differences in composition of the samples from each horse could not simply be attributed to the sweat of one horse versus the lack of such on the other. Bernier (1995) showed that human sweat was too aqueous, with very low volatile content and contained mostly water and salts. It could be beneficial to thoroughly analyze the sweat from horses to determine its chemical make up and then compare it to other mammals, such as the human. Another useful study that could be valuable to understanding host preference of mosquitoes may be the comparison of lactic acid content in the sweat of horses as related to others. Since lactic acid has been a component in trap studies with *Aedes* spp. mosquitoes, the combination of this compound with others identified in horse odor could be an important lure for more efficient trapping of mosquitoes.

Horse Odor Trapping Study

It has been shown that differences exist between individual people and their ability to attract mosquitoes (Schreck et al., 1990). Therefore it is likely to expect variation in attraction between individual horses. In addition to exploration of differences that exist between horses with respect to compounds present on the hair and dander, it is important to find out how a mosquito trap would fare with respect to collecting mosquitoes in close proximity of a horse.

When comparing trapping of mosquito species for either horse during this study, the results are essentially identical as seen in Tables 3-5 and 3-6 and Figures 3-10 and 3-11. The similar species profile of both horses could be due to the season of year when the particular treatment was run. *Cq. perturbans* was the most abundant species trapped; this closely follows the time of year when these mosquitoes show preference for mammals. *Mansonia* spp. was trapped effectively throughout this study, as seen in the seasonality study discussed in previous research by Dilling (2004). Finally, *Cx. nigripalpus* was the third most abundant species trapped and its appearance did not occur until mid-June, very similar to the seasonality study.

The GC/MS analysis of collected horse odors demonstrated that there is little difference in the dander between the two horses in the study and that the only difference between them was on their hair. It is extremely likely that these differences are due to exogenous chemicals from the environment.. The difference in the horses themselves, though slight, could have played a role in the small discrepancies between the total catch numbers for each horse. As previously mentioned, Dilling (2004) found a slight difference between an appaloosa mare and a paint gelding and their ability to compete against a trap. Even though there was no significant difference in that study, the appaloosa mare generally caught more mosquitoes than the paint gelding. In this study, the mare's odors when combined with CO_2 caught more mosquitoes compared to CO_2 alone and compared to the gelding's odors combined with CO_2 similar to the findings of Dilling (2004). In this study, no significant differences were found in mosquito numbers collected by odors plus CO_2 compared to CO_2 alone from either horse (Figure 3-12). Although differences were not found in this study, previous studies indicate that the differences between a mare and a gelding or a stallion and a gelding could have a direct impact on the mosquito's host-seeking behavior (Dilling, 2004). It could be very important to study hormone levels between the female and the male horse; including an intact male horse (the stallion) in future studies.

One factor that may have impacted the study is the time constraint, as the period ran from May 2006 until early October 2006. Accordingly, the time and month varied for each set of treatments, and this may have affected both the species composition as well as the total numbers of mosquitoes trapped. In addition, this area was under the conditions of a drought during this five month period, so the total number of mosquitoes present was expected and certainly lower than the previous year. Therefore, the decrease in total mosquito numbers may confound the interpretation of the results from this study. An additional factor was that only two MMPro traps were available for comparison at a time. Subsequently, the individual studies for each horse were run separately. This means that the numbers of total feeding mosquitoes on a given night during the study likely differed from one period to another. Furthermore, the traps were placed near trap #2 of the three-lure study, just south of the large body of water (Figure 3-5). This area contained varying numbers of nearby horses and these horses fluctuated throughout the entire 13 months all around the farm. The location of a horse was usually not permanent and the numbers in close proximity to the test site changed constantly. This fluctuation in natural hosts in the vicinity may have affected the trap counts.

Future studies involving horse odors combined with CO_2 used in traps could be modified by restricting the time of year by increasing the number of traps used in the comparison, as well as testing several different horses. In addition, it could be beneficial to increase the CO_2 output of the MMPro trap to more closely follow that of the horse, which is around 2000 cc/min at rest (Pelletier and Leith, 1995) compared to 200 - 500 cc/min of CO_2 that is emitted from the MMPro trap (Takken and Kline, 1989).

Horse Vacuuming Study

It has been noted that horses will more proficiently attract mosquitoes when compared to a mosquito trap using commercial bait when both are in close proximity. Therefore, it is important to investigate the ability of the factors that result in superior attraction of insects by the horse whether with olfactory cues or a combination of other factors that trigger the mosquito and affect host preference. The species composition of vacuumed mosquitoes from both horses closely followed Dilling's study (2004), again probably due to the low rainfall and warm nights.

There were slight numerical differences of mosquitoes aspirated from the two horses yet there was no statistical difference noted, as seen in Table 3-7. Contrary to the horse odor trapping experiment using the two horses, more mosquitoes were aspirated from Steiner on the east side of the arena when compared to Lodi. This also held true when Steiner stood on the west side of the arena.

The mosquitoes showed similar increases in activity both nights with respect to the time frames of collection. The early time from 7:00 - 7:30 pm had less mosquitoes feeding with a drastic increase as dusk set in. With this increase in landings and biting, the horses became agitated and used their tails to swat off mosquitoes. Both horses also stomped and flinched, which made it more difficult to aspirate mosquitoes and collect them in the container. The feeding locations were similar for both horses; the smaller *Cx.* spp. tended to prefer the legs and near the hooves and came out to feed during the first 30 min period. The feeding location of the *Cx.* spp. was similar to what Dilling (2004) found, near the coronet band of one appaloosa mare. Very little mosquitoes fed on the coronet band in this study; however, it was obvious that the smaller mosquitoes preferred the legs rather than the other portions of the body. This could be because *Cx.* spp. are avian feeders and adapted to feeding in areas of the host with less skin and muscle tissue, like the legs of the horses which are more similar to the physiological makeup of birds than the larger areas on the body. The larger species, such as the *Cq. perturbans* and *Ma.* spp., were later feeders and preferred the higher up sections of the body, including the face, forehead and the top of the hip.

A small difference, though not significant, was noted between the two far sides of the arena; regardless of which horse was there, a greater number of mosquitoes were vacuumed from the east side when compared to the west. There was a large retention pond near the east side which still had some standing water. This may have attributed to the greater number of mosquitoes nearby, leaving the breeding ground, in search of a blood meal. When the mosquitoes left their resting site, they may have stopped at the first host they came upon, which in both cases was the horse on the east side of the arena at the south end.

It was very difficult to draw any conclusions from two nights, so it would be beneficial to repeat the aspirating experiment an additional number of repetitions throughout the season to determine if differences really do exist between horses and their ability to attract more mosquitoes than another.

Conclusions

Mosquitoes affect livestock species and humans all over the world through disease transmission and resultant morbidity and possibly mortality. When mosquito traps are used on various livestock facilities, they become less effective when natural hosts are in close proximity. It is important to find ways to improve trapping so that they are more effective in decreasing the numbers of mosquitoes that are able to feed on animals. When various samples were collected and analyzed using GC/MS, it was found that horses were very similar to other mammals including humans and that there could be a compound found on the dander of horses that is unique to them. This compound, in combination with other host-seeking cues, could be an important key in the amazing ability of the horse to attract the mosquito. Further analysis will need to be completed to determine the nature of the compound and the role that it may or may not play in mosquito attraction. Once this compound has been discovered it could be used with other cues in mosquito traps in order to increase their ability to collect mosquitoes and other haematophagous insects.

When odor samples were collected from the horses and used in the mosquito trap, it was found that the trap count did not exhibit a significant difference between the uses of odors from either horse. Additional field research is needed to confirm that horse odor can increase a trap count and that the mosquito is not just feeding opportunistically. Furthermore, a horse exhales

CO_2 at a rate of 2000 cc/min at rest, over 4 times the amount that the MMPro trap releases (Pelletier and Leith, 1995). It could be beneficial to adjust the CO_2 output from the MMPro to be more quantitatively similar to that of the horse.

To further investigate the differences between two horses, mosquitoes were aspirated off of each horse and the numbers were compared, with no statistically significant differences observed in the numbers and species aspirated. There appeared to be a location factor so further research needs to be completed in order to confirm the differences between horses. A better understanding of horse odors and the role that they play in host-seeking could lead to more efficient traps designed to compete against natural hosts.

Figure 3-1. Steiner, sorrel quarter horse gelding used for odor collections and mosquito trapping studies.

Figure 3-2. Lodi, black quarter horse mare used in the odor collections and mosquito trapping studies.

Figure 3-3. Illustration of hypersensitivity found on Lodi, black quarter horse mare used for odor collections and mosquito trapping studies.

Figure 3-4. Method of collecting horse odors from different locations on the body using cotton balls to collect horse odor for mosquito trapping studies.

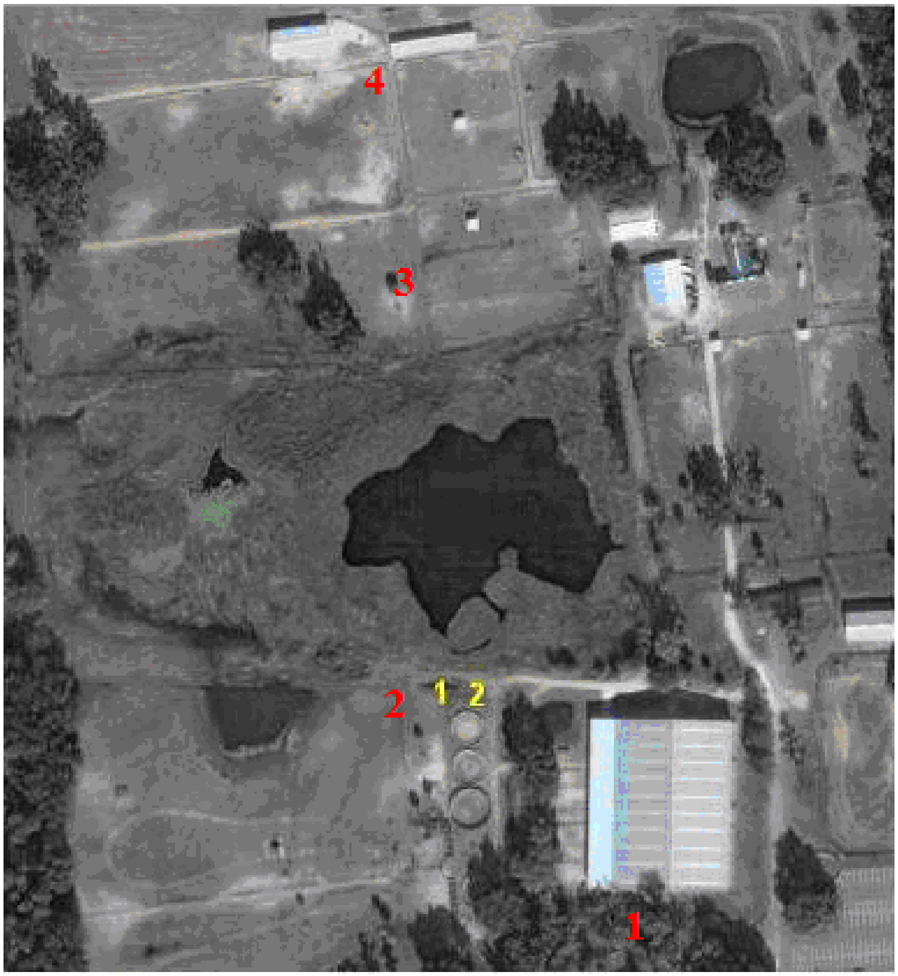

Figure 3-5. Aerial view of the University of Florida HTU showing the location of the two MMPro traps used during the horse odor study (yellow) and those used during the seasonality study (red).

Figure 3-6. Portable vacuum aspirator (DC Insect Vac. BioQuip, Rancho, Dominguez, CA) and technique of aspirating mosquitoes off of the horses used for horse vacuuming studies.

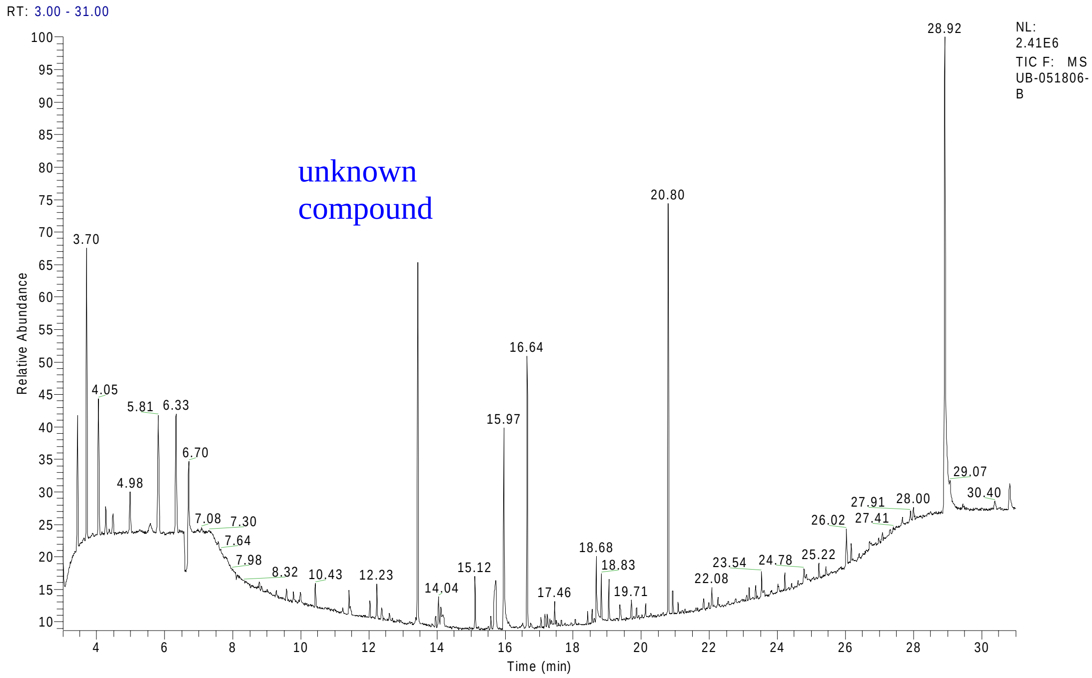

Figure 3-7. Chromatogram from the analysis of extracts from collected hair from “Steiner,” Equus caballus at the University of Florida HTU.

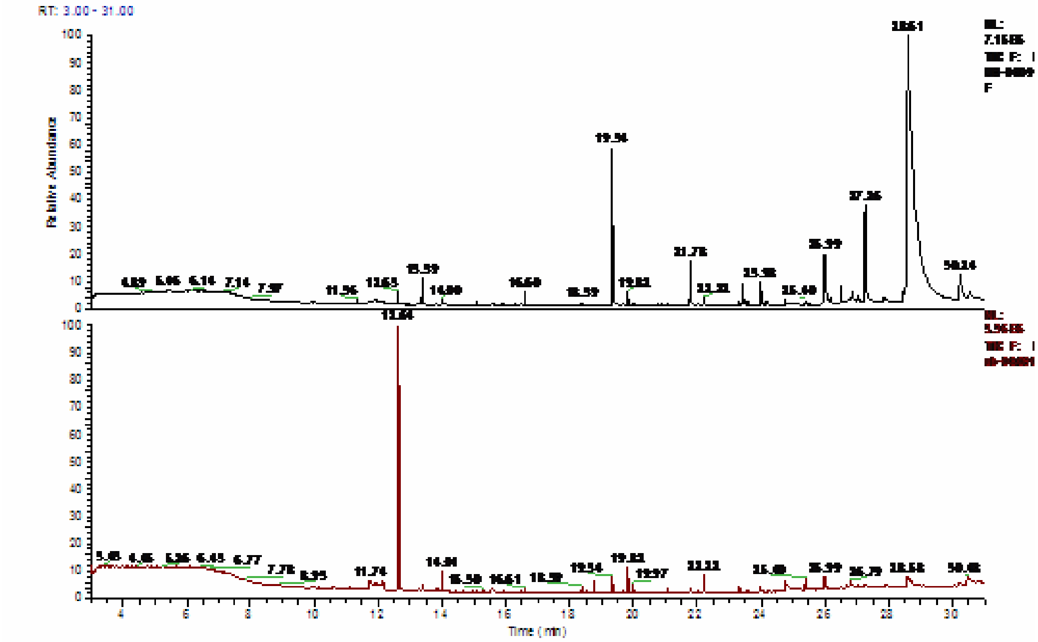

Figure 3-8. Chromatograms illustrating differences in peaks and abundances of compounds from the chest hair from Steiner (top), to that of Lodi (bottom).

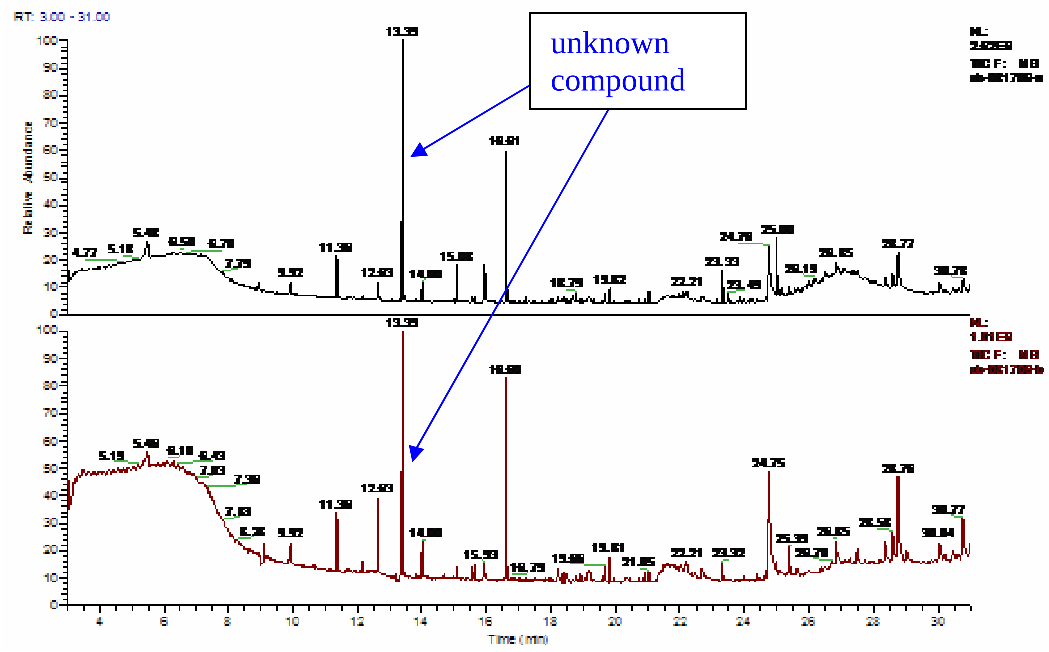

Figure 3-9. Chromatograms comparing the dander from Steiner (top), to that of Lodi (bottom)

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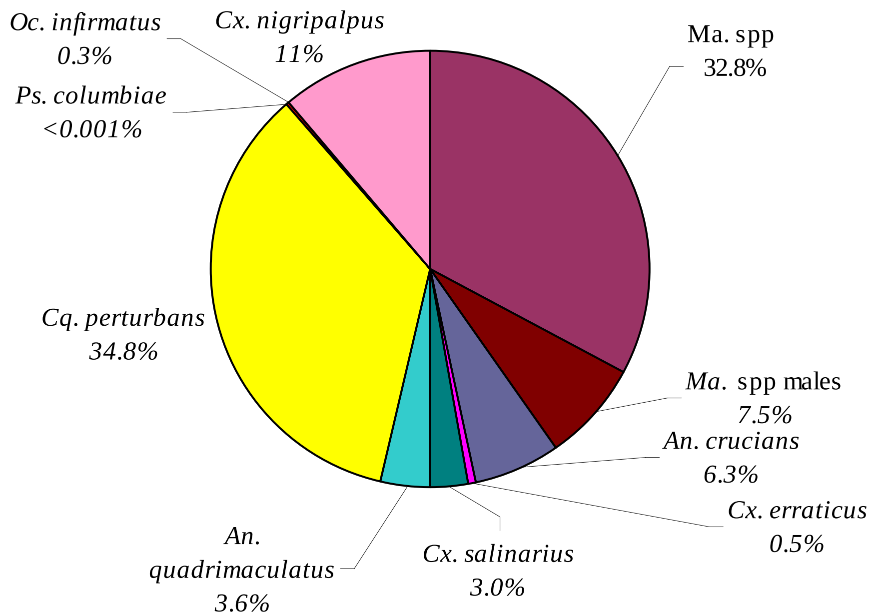

Figure 3-10. Total mosquito species composition for horse odor trapping study using samples from Lodi in the MMPro traps from May 2006 until October 2006 at the UF HTU.

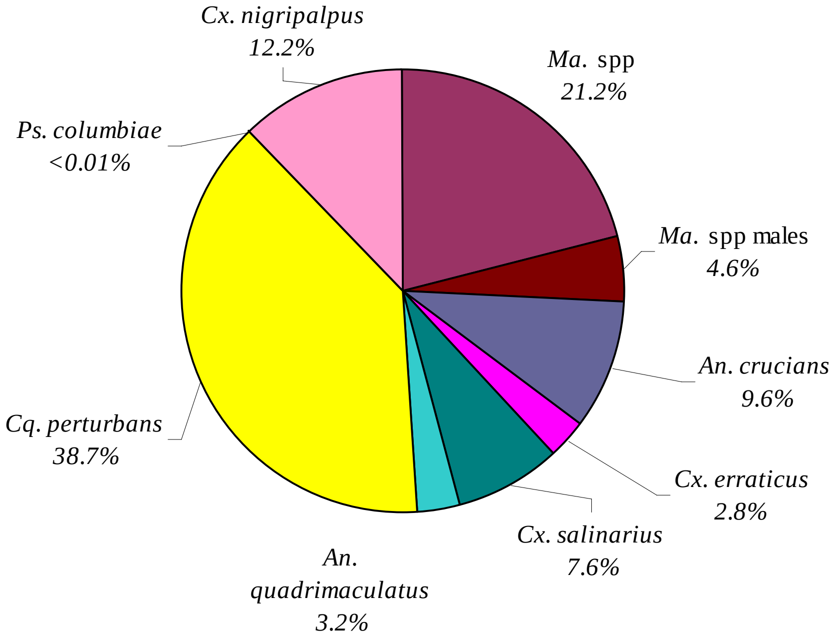

Figure 3-11. Total mosquito species composition for horse odor study using samples from Steiner in the MMPro traps from May 2006 until October 2006 in trapping study at the UF HTU.

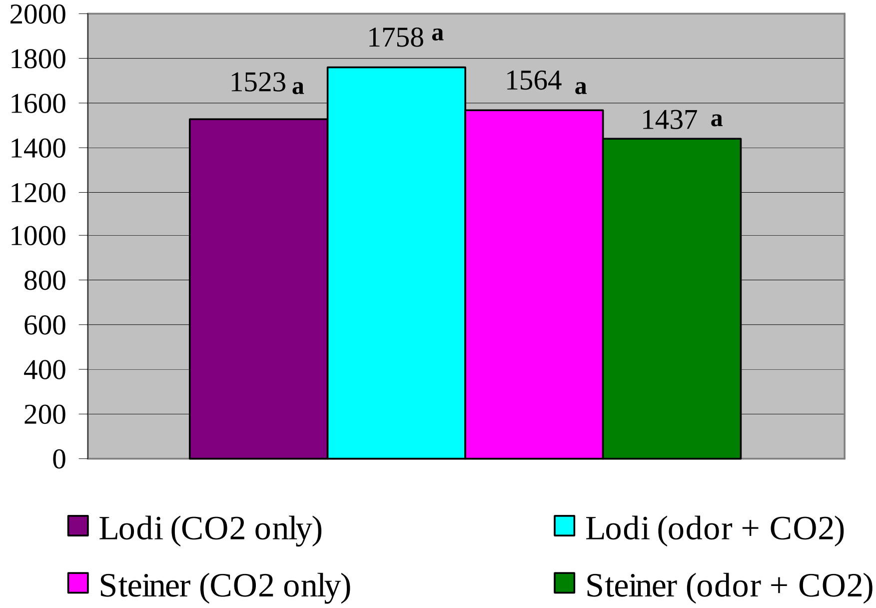

Figure 3-12. Total mosquitoes trapped using the horse odors in the MMPro traps; samples from Lodi and Steiner. Note: Totals were not found to be statistically different ($p<0.05$), and these are indicated by the same letter.

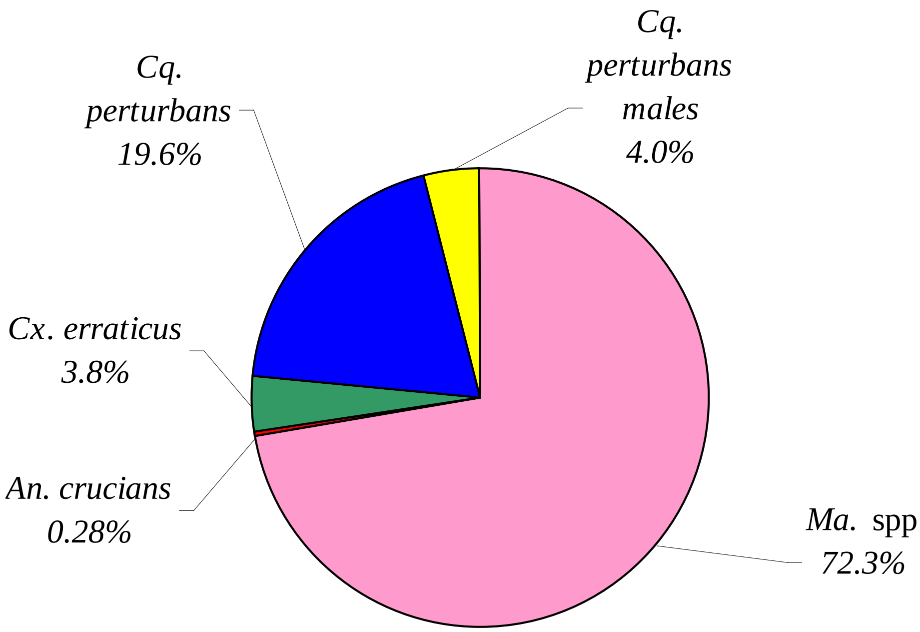

Figure 3-13. Mosquito species comparison (represented as a percent of the total mosquitoes collected) aspirated from Lodi during the horse vacuuming study conducted at the UF HTU.

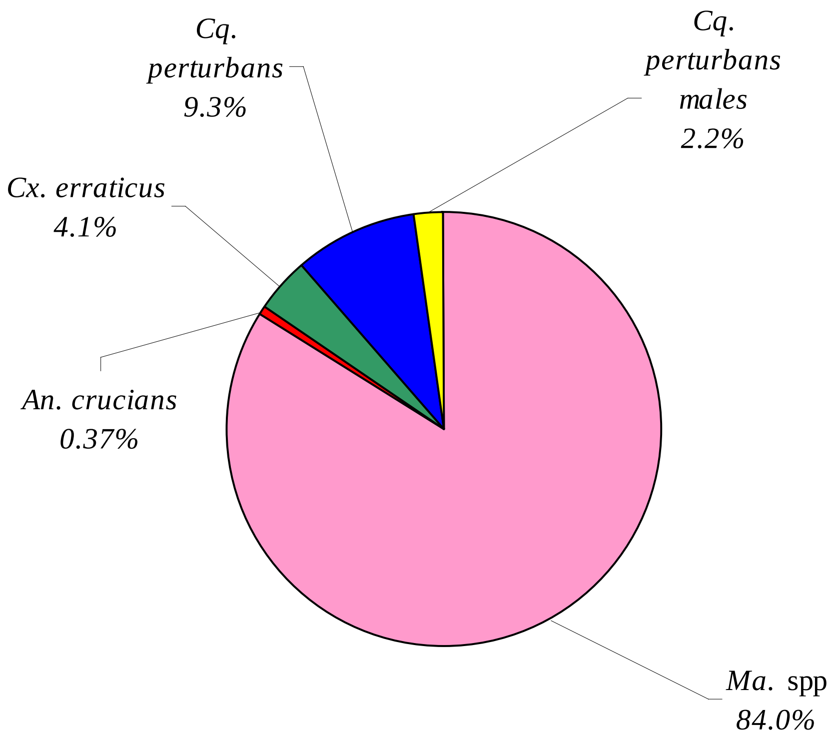

Figure 3-14. Mosquito species comparison (represented as a percent of the total mosquitoes collected) aspirated from Steiner during horse vacuuming study conducted October 2006.

Table 3-1. Compounds found on Steiner, *Equus caballus*, from samples collected for analysis by gas chromatography and mass spectrometry.

Compound	GC (t_R) (min)
Unknown	13.44
Nonanal	14.04
Decanal	15.12
Decanal	15.73
Nonanoic acid	15.97
Undecanal	16.64
Undecenal	17.24
Undecanoic acid	18.68
Dodecenal	18.82
Geranylacetone	19.05
Diethyl phthalate, contaminant	20.80
Unsaturated alcohol, or an aldehyde	23.35
Farnesol related compound	23.54
1-heptdecanol	26.01
Tetradecanal	27.91
Dioctyladipate	28.92
Long Chain Hydrocarbon	30.40
Diisooctyl phthalate (contaminant)	30.83
Suspected long chain amide	36.06
Cholesterol	49.30
Cholestanol	49.78

Table 3-2. Comparison of compounds found on the dander of two horses.

Compound	GC (t_R) (min)	Horse[1]
Terpene, alpha-pinene	10.61	L
2-methylnonane	11.36	S
Cyclosiloxane	11.73	B
Octanal	12.15	B
2-ethyl-1-hexanol	12.64	B
Unknown Compound	13.39	B
Terpene, 4-Carene	13.69	L
2-hydroxyacetophenone	13.84	B
Nonanal	14.00	B
Terpene, p-menth-1-en-4-ol	14.59	L
Benzyl ester of acetic acid	14.96	L
Unsaturated alcohol	15.08	B
Decanal	15.64	B
Terpene or terpene-like, e.g. geraniol or myrcene	15.91	L
Terpene or terpene-like, e.g. geraniol or myrcene	16.28	B
Unsaturated aldehyde	16.48	B
Undecanal	16.60	B
Caryophellene	18.78	L
1-dodecanol	19.34	B
Tridecanal	19.81	B
4-methoxy-6-(2-propenyl)-1,3-benzodioxole	19.96	B
Terpene-related	20.06	L
Aldehyde or unsaturated alcohol	21.05	B
Tridecanol	21.78	B
Pentadecanal	22.22	B
Aldehyde	23.32	B
Alcohol	23.98	B
Hexadecanoic acid	24.75	B
Aldehyde, long chain	25.40	B
Alcohol, long chain	25.99	S
Diisoctyl maleate or related	26.49	S
Dioctyl maleate or related	27.26	B
9-octadecenamide	28.60	B

[1]Key: Lodi (L), Steiner (S) and Both (B).

Table 3-3. Mean numbers (±standard deviation) of mosquitoes captured per trapping interval using the odors from Lodi in the MMPro traps.

Interval	Mean difference trapped (± SD)	n
Treatment	109.88 (± 18.33)[a]	16
Control	95.20 (± 15.62)[a]	16

Note: Means followed by the same number are not significantly different ($P \leq 0.05$) and n= number of observations.

Table 3-4. Mean numbers (±standard deviation) of mosquitoes captured per trapping interval using the odors from Steiner in the MMPro traps.

Interval	Mean difference trapped (± SD)	n
Treatment	119.75 (± 22.75)[a]	12
Control	130.33 (± 26.07)[a]	12

Note: Means followed by the same number are not significantly different ($P \leq 0.05$) and n= number of observations

Table 3-5. Total mosquito species and percent of total mosquitoes trapped using the odors from Lodi in the MMPro traps during the horse odor trapping studies.

Mosquito Species	Total trapped	Percent of total (%)
Coquillettidia perturbans	1143	34.8
Mansonia spp.	1076	32.8
Culex nigripalpus	362	11.03
Mansonia males	246	7.5
Anopheles crucians	207	6.3
Anopheles quadrimaculatus	117	3.6
Culex salinarius	100	3.04
Culex erraticus	16	0.49
Ochlerotatus infirmatus	10	0.31
Psorophora columbiae	4	0.12
Total	3281	100.0%

Table 3-6. Total mosquito species count and percent of total mosquitoes trapped using the odors from Steiner in the MMPro traps during the horse odor trapping studies.

Mosquito Species	Total trapped	Percent of total (%)
Coquillettidia perturbans	1162	38.7
Mansonia spp.	636	21.2
Culex nigripalpus	365	12.2
Anopheles crucians	288	9.6
Culex salinarius	229	7.6
Mansonia males	137	4.6
Anopheles quadrimaculatus	98	3.3
Culex erraticus	83	2.8
Ochlerotatus infirmatus	10	0.33
Psorophora columbiae	3	0.1
Total	3001	100.0%

Table 3-7. Mean numbers (±standard deviation) of mosquitoes captured per trapping interval for the vacuum aspirator study conducted in October 2006.

Interval	Mean difference trapped (± SD)	n
Lodi	204.50 (± 181.41)[a]	4
Steiner	221.25 (± 139.92)[a]	4
West	198.00 (± 152.07)[a]	4
East	227.75 (± 170.14)[a]	4

Note: Means followed by the same number are not significantly different ($P \leq 0.05$) and n= number of observations

Table 3-8. Total mosquito species count and percent of total mosquitoes trapped using the vacuum aspirator on Lodi in October 2006 at the UF HTU.

Mosquito Species	Total Count	Percent of Total (%)
Mansonia spp.	687	84.0
Coquillettidia perturbans	76	9.30
Culex erraticus	34	4.15
Culex nigripalpus	18	2.20
Anopheles crucians	3	0.37
Total	818	100.0%

Table 3-9. Total mosquito species count and percent of total mosquitoes trapped using the vacuum aspirator on Steiner in October 2006 at the UF HTU.

Mosquito species	Total count	Percent of total (%)
Mansonia spp.	774	87.6
Coquillettidia perturbans	58	6.60
Culex erraticus	41	4.63
Culex nigripalpus	7	0.79
Anopheles quadrimaculatus	2	0.23
Total	885	100.0%

CHAPTER 4
CONCLUSIONS AND IMPLICATIONS

Mosquito trapping is an effective tool used to monitor species composition in the area and allows professionals to predict possible disease outbreaks in a population. Many different commercial traps and lures are available to attract and trap the mosquitoes. It is important to study the efficacy of these different traps and lures in different situations, some that are effective on North Florida Horse farms. Disease prevention and a decrease in nuisance biting are crucial for minimal economic loss to equine owners. Several lures were tested in comparison to a natural host at the University of Florida HTU. Octenol, Lurex, and Lurex^3 were combined with CO_2 and tested against a control trap the Mosquito Magnet Pro trap operated with CO_2 alone. It was found that throughout the study, octenol proved to be the most effective lure when used in close vicinity of a natural host. There was a significant difference between the three lures tested; octenol was more effective, followed by the control trap (CO_2 alone), with no difference between Lurex and Lurex^3.

An additional set of studies examined the odors of a horse as an attractant. Odor samples from two different horses were collected and used in the MMPro traps. The traps were operated for 24 h. No significant difference in mosquitoes trapped was found between the odors of the two horses. An additional study was conducted where the same two horses were vacuumed to determine if either horse had an increased ability to attract mosquitoes. No significant differences were found between the two horses.

The series of research studies conducted at the UF HTU have indicated possible directions for future studies. The horse odor studies answered several questions, yet raised several more. It was found that the hair and dander of the two horses was very similar chemically. When the analyzed samples were used in the traps, the results were less clear. It would be beneficial to continue studying different horses, both for chemical analyses and efficacy in mosquito traps.

In addition to increasing the number of horses in these studies, it would be beneficial to compare attraction to various locations of the horse, similar to studies in humans. Alternatively, horse sweat samples could be analyzed and then compared to humans and other mammals. Then the samples could be used in the mosquito traps and compared to the other horse hair and dander samples. Other sample collection methods could be explored as well. Actual pieces of hair and shavings of horse hair could be used in the traps.

More could be done with the horses themselves. In these series of studies, a castrated male and a female were used, both with different hormonal profiles. A stallion could be used as well as a gelding and a mare, to compare the different effects of hormones and the ability to attract mosquitoes. More repetitions of the studies conducted here would support the results and make the horse a more valuable tool for mosquito surveillance and control.

Mosquito trapping is an effective tool at monitoring local species composition and predicting potential disease outbreaks. However, when the natural host is in the area, the trap's effectiveness decreases and other methods must be used. If the horse is in fact effective at increasing trap numbers, more experiments are needed to refine the use of the horse and horse odors as trap lures.

APPENDIX
ADDITIONAL INFORMATION ABOUT FLORIDA MOSQUITOES

Table A-1. Classification of the family Culicidae

	Tribe	Genera
Anopheline		*Anopheles, Bironella, Chagasia*
Culicinae	Aedeomyiini	*Aedeomyia*
	Aedini	*Aedes, Ochlerotatus, Verrallina, Ayurakitia, Armigeres, Eretmapodites, Haemagogus, Heizmannia, Opifex, Psorophora, Udaya, Zeugnomyia*
	Culcini	*Culex, Deinocerites, Galindomyia*
	Culisetini	*Culiseta*
	Ficalbiini	*Ficalbia, Mimomyia*
	Hodgesiini	*Hodgesia*
	Mansoniini	*Coquillettidia, Mansonia*
	Orthopodomyiini	*Orthopodomyia*
	Sabethini	*Sabethes, Wyeomyia, Phoniomyia, Limatus, Trichoprosopon, Shannoniana, Runchomyia, Johnbelkinia, Isostomyia, Tripteroides, Malaya, Topomyia, Maorigoeldia*
	Uranotaeniini	*Uranotaenia*
Toxorhynchitinae		*Toxorhynchites*

The classification of all mosquitoes into 3 subfamilies, 10 tribes of Culicinae, and 38 genera is based on Knight and Stone (1977).

Table A-2 List of mosquitoes in Florida

Genus	species
Anopheles	*albimanus, atropos, barberi, bradleyi, crucians, diluvialis, georgianus, grabhamii, inundatus, maverlius, nyssorhynchus, perplexens, punctipennis, quadrimaculatus, smaragdinus, walkeri*
Aedes	*aegypti, albopictus, cinereus, vexans*
Ochlerotatus	*atlanticus, bahamensis, canadensis, dupreei, fulvus pallens, hendersoni, infirmatus, mathesoni, mitchellae, scapularis, sollicitians, stiticus, taeniorhynchus, thelcter, thibaulti, tormentor, tortilis, triseriatus,*
Psorophora	*ciliata, columbiae, cyanescens, discolor, ferox, horrida, howardii, johnstonii, mathesoni, pygmaea*
Culex	*atratus, bahamensis, biscaynensis, cedecei, erraticus, iolambdis, mulrennani, nigripalpus, peccator, pilosus, quinquefasciatus, restuans, salinarius, tarsalis, territans*
Deinocerites	*cancer*
Culiseta	*inornata, melanura*
Coquillettidia	*perturbans*
Mansonia	*dyari, titillans*
Orthopodomyia	*alba, signifera*
Wyeomyia	*mitchellii, smithii, vanduzeei*
Uranotaenia	*lowii, sapphirina*
Toxorhynchites	*rutilus septentrionalis, rutilis rutilis,*

The classification of Florida mosquitoes by Genus species was taken from Richard Darsie, 2006.

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BIOGRAPHICAL SKETCH

Aimee Camille Holton was born in Gainesville, Florida, in 1981 to Quinn and Suzanne Holton. She has lived in Gainesville, Florida her whole life, attended and graduated from Santa Fe High School in Alachua, Florida in 2000. She went to Santa Fe Community College and earned an Associate of Arts degree in 2002, and then she transferred to the University of Florida. She earned a Bachelor of Science degree in animal science with a major in equine industry. Following graduation in December of 2004, she was accepted into a graduate program under Saundra TenBroeck with a concentration in equine science management. Throughout the course of her graduate program, she served as a teaching assistant for equine reproductive management, sales preparation of thoroughbred yearlings, and psychology and training I and II under the supervision of Mr. Joel McQuagge. She graduated with a Master of Science degree in animal science with a minor in veterinary entomology in August of 2007. Aimee plans to pursue a career as a teacher of biological sciences for secondary students.