

BEHAVIOR RESPONSE OF *MUSCA DOMESTICA* TO VISUAL STIMULI AND THE
DEVELOPMENT OF A NOVEL FILTH FLY CONTROL DEVICE

By

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To my family, always keeping me focused and driven

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Abstract of Dissertation Presented to the Graduate School
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House flies see reflected sunlight from objects by both their compound eyes and ocelli and use this visual input for survival. Neurological responses elicited by reflected light from plastic colored visual targets of house fly's compound eye and ocelli were measured using an electroretinogram. Light of the same intensity reflected from multiple plastic visual targets showed that house fly compound eye and ocellus were capable of color vision. The greatest neurological responses for the compound eyes and ocelli were to white, blue, and yellow.

Behavioral responses of house flies to colored plastic and fabric visual targets compared to black were determined using a two-sided light tunnel. The house fly attraction was White = Blue > Red > Green > Yellow. In a paired test blue visual target was more attractive than white. Yellow visual targets proved to be visual repellent for house flies. However, the addition of black lines, which simulated a harborage area, enhanced the attractiveness of the blue background.

Combining visually attractive color patterns with wool insecticide treated cords led to the construction of a fly control device: FAST-TC. Comparisons of active ingredients, (deltamethrin, thiamethoxam, imidacloprid, indoxacarb, fipronil, and cyfluthrin) applied to

FAST-TC in field cages effectively reduced the house fly population by >88% in 24 h compared to the 3% decrease of the untreated control. Bioassay conducted with sticky cards hung simultaneously at three heights caught 50% of the house flies released in a laboratory room at the lowest height, which demonstrated that house flies tend to fly low. At all three of the tested heights the modified version of the FAST-TC was able to attract and kill ~90% of the house flies.

In field evaluations, 2.5% imidacloprid was applied to the FAST-TC and was able to control two species of fly populations. The FAST-TC achieved >70% control for *Musca domestica* population and 79% fly control of *Megaselia scalaris*. Due to the FAST-TC efficiency in field evaluations, the University of Florida submitted a patent application for this device.

CHAPTER 1 INTRODUCTION

The U.S. Department of Defense (DOD) initiated a program in 2004 for the development and testing of tools for control of pest and disease-causing vectors called the Deployed War-Fighter Protection (DWFP) program. The house fly is a mechanical vector of 100 known pathogens (Greenburg 1971) and a major pest/disease vector during natural disasters and wartime. The purpose of the following research was to develop a new tool that effectively controls the house fly and other filth fly populations that can be useful for the DOD and as well as the civilian sector.

House fly control is best achieved using integrated management strategies combining cultural, mechanical, biological, and chemical control methods (Kolbe 2004). Baited fly traps have been available in various designs since the early 1900s and are often used in combination with other methods for fly control (Pickens 1995). There is some debate whether baited traps fit into a fly management program best as monitoring rather than a control devices. To reduce a fly population by 50-90%, traps must remove 24-58% of the house flies daily (Weidhaas and Haile 1978); however, traps alone have not been shown to reduce fly populations to acceptable levels (Smallegange 2004).

A chemical control method used in the past included the use of cords treated with insecticide. Cords treated with organophosphate and chlorinated hydrocarbon insecticides were introduced in 1947 and were found to provide good house fly control (Smith 1958). However, use of treated cords fell in popularity due to the ease of applying residual insecticide sprays directly to structures. In the 1990s there was a decrease in residual insecticide usage due to public opinion. Which spurred research to utilize modern low risk insecticides and new materials with the vintage idea of

insecticide treated cords. In field conditions low concentrations of new insecticides, such as 0.1% fipronil and 1.2% indoxacarb impregnated in natural fiber cords were shown to effectively kill flies (Hertz 2007).

Initial experimentation sought to improve a baited fly trap with the addition of a insecticide applied to a wool cord (Appendix A). Baited fly traps have two main lures to catch flies: visual lure and chemical lure. The visual lure attracts flies in from a distance, and when the fly is in close proximity to the trap, the chemical lure attracts the fly to enter the trap (Pickens 1995). The additional of the insecticide treated cord would eliminate the flies that are visually attracted to the trap even when they only contact the outside surface of the trap.

Flying insects such as the house fly rely on their compound eyes to guide them to locate food and potential mates (Nation 2002). An understanding of the physiological and behavioral responses of house flies to visual cues was used in the development of effective control device (Chapter 3). The use of electroretinograms allowed for the measurement of house fly physiological responses to reflective color with the compound eyes and ocelli. Behavioral response to reflective color was observed with the use of a two-sided light tunnel, which was designed to compare attractiveness of colors and house fly behavior in a controlled environment.

Once attractive colors were identified, a fly control device was designed and constructed. This fly control device used attractive color combinations to lure flies in from a distance and then eliminated the flies with the use of insecticide treated cords. Considerations in development of this device were to make it practical for use (Chapter 4), including construction with low cost materials and effective insecticides.

To determine the ability of this device to control fly populations, extensive field-testing was conducted in a variety of settings (Chapter 5). The goal of the device was to reduce fly populations by 50% in the first 24 h. In addition, this device was field evaluated on more than one species of filth fly.

CHAPTER 2 LITERATURE REVIEW

***Musca domestica* Linnaeus**

Musca domestica, or house fly, is one of approximately 4,200 species out of 190 genera in the Muscidae family (Moon 2009). It is a synanthropic fly, commonly found in agricultural environments and unsanitary conditions, is often viewed as a nuisance and embarrassment. Breeding sites include but are not limited to garbage, manure, rotting plants, carrion, and other decaying organic matter. In nature, house flies serve a vital function in recycling animal manure and refuse by replacing nutrients back into the soil (Hedges 1998). However, due to its close interaction with humans and propensity to harbor disease-causing pathogens, the house fly has been labeled as one of the most dangerous insects known to man (West 1951).

Identification

The house fly is classified in the class Hexapoda, order Diptera, the fourth largest order, suborder Brachycera, infraorder Muscomorpha (Cyclorrhapha), and family Muscidae (Triplehorn and Johnson 2005).

Eggs

House fly eggs are white in color measuring 1 mm in length and wider in diameter on the posterior end than on the anterior end (West 1951). The overall shape of egg being banana-like with both ends bluntly rounded. Eggs are laid in large clusters of 120-150 eggs. Female house flies are capable of producing up to six batches of eggs in their lifetime, depending on environmental factors.

Larva

House fly larva are known as maggots and have three instars (Moon 2009). Maggots have no antennae, eyes, or appendages. The body is tapered to a point at the anterior end, while the posterior end is wider and rounded. Strong mouth hooks, made of sclerotized mouthparts, are the main component of the cephalopharyngeal skeleton on the anterior end of the larvae. On the posterior end, there is a pair of black spiracular plates, used for gas exchange, shaped like two half circles (D shaped) back to back.

Pupa

The house fly, being a holometabolous insect, undergoes pupation when the third instar larvae contracts and hardens its integument to form a puparium. This process is completed within a 6-h period (West 1951). The puparium is reddish-brown in color and is 6.3 mm in length. It has a cylindrical shape with bluntly rounded ends. As in the larval instars, two button shaped spiracles are located on the posterior end that are left over from larval development but are no longer utilized for gas exchange. Between the fifth and six body segments, there is a pair of spiracles on the dorsal side used for gas exchange.

Adult

Adult house flies are dull gray in color and about 6 to 9 mm in length (Moon 2009). The top of their thorax has four black longitudinal stripes with wings that are longer than the abdomen. The wings have key identification characteristics in their venation; the first anal vein is present but does not reach the wing margin, and there is a sharp upward bend in the fourth longitudinal wing vein, m1 (Kolbe 2004).

Male and female house flies can be differentiated by the abdomen, both sexes have modifications in the last six segments (West 1951). The male house fly has a genital pouch that contains an aedeagus. The female house fly's abdomen terminates into a telescopic ovipositor.

The head has two large compound eyes with three ocelli at the dorsal midline. Male house flies compound eyes are holoptic and female compound eyes are dichoptic (Triplehorn and Johnson 2005). The antenna has a scape, pedicel, and an arista.

The house fly, a non-biting fly, has sponging mouthparts consisting of a proboscis that has a basal rostrum that articulates with a haustellum, and terminates at the labellum (Moon 2009). An important defining characteristic of the house fly, as well as other muscid flies, is the enlarged labella that are made up of two lobes with a mesal surface of pseudotracheae. Pseudotracheae assist in the digestion and scraping of food by acting as a saliva-soaked sponge. Also located within this structure are dentate prestomal teeth that assist in the scraping and liquefying of food.

Insect Vision

Visual systems in insects help relay external information to their brain to help guide behavior, such as flying and walking. In flying insects such as Diptera, the eyes serve as a way to detect movement on a vertical and horizontal plane. A visual system in most insects consists of two compound eyes and a set of ocelli. The compound eyes are structured with ommatidia which can range in number from insect species to species. In dragonflies, approximately 10,000 ommatidia can be found, whereas a *Drosophila* fly may only have 800 ommatidia (Chapman 2006). Not all insects have this type of visual system; more primitive insects and some larva may only have simple eye structures or other types of sensory organs.

Structure and Function

Ommatidia

The primary function of the ommatidium is to allow light into the compound eye which is transformed by sensory cells into electrical energy to form an image. Light enters the ommatidium by first entering a transparent cuticle also known as the cornea or lens (Nation 2002). Directly below the cornea is a cone that helps direct the light to photosensitive cells called photoreceptors. The photoreceptors are long, slender receptor neurons known as the retinular cells. These cells are considered to be the main receptors for the ommatidia because their axons are directly linked to the optic lobe of the brain with no gaps (synapses) (Chapman 2006).

Although ommatidia in many species contain similar functional cells, like the retinular cells, there is variation in structure among insect species. There are two main variations within the ommatidia: photopic and scotopic (Nation 2002). Photopic ommatidia are common with insects that are active during the day, such as house flies, and have multiple rhabdomere that extend through the retinular cell from the cone to the retinular cell axon. The light that enters a photopic compound eye is focused in the ommatidium after the cone and is not permitted to leak into other ommatidia by the presence of shielding cells or pigment cells. Pigment cells surround the retinular cells, and the proximal angles of the cone block light from spilling to other surrounding ommatidia.

Scotopic eyes are common in insects such as moths that are mostly active at night. The structural difference between the scotopic and photopic ommatidia is that, after the cone in the scotopic ommatidium, there is a crystalline structure or tract that extends to the retinular cell. Within the retinular cell, fused rhabdomere extend from the

crystalline tract to the retinular cell axon. This crystalline structure is clear and surrounded by pigment cells. However, unlike the photopic ommatidia that have little or no movement in their pigment cells, the scotoptic pigment cells contract and move their pigment distally uncovering the crystalline tract, which allows light to be shared between ommatidia and stimulate their rhabdomere as well. In dim or dark light conditions, this phenomenon gives nocturnal insects the ability to see (Nation 2002).

Ocelli

Generally, insects that have compound eyes also have a set of dorsal ocelli or simple eyes. Usually arranged as an inverted triangle in a set of three (Chapman 2006), the ocelli have some of the same functional components as compound eyes. However, unlike the compound eye the ocelli retinular cell axon is synaptically connected to the ocellar interneuron, leading to either the protocerebrum of the brain or to ganglia on the central nervous system (Mizunami 1994).

These ocellar interneurons are classified as large fibers and small fibers (Chapman 2006). The function of the ocelli is unclear but it is speculated that the configuration of the small fibers that lead to multiple areas of the brain may be able to form a crude image; whereas the large fibers that provide connections from the ocelli to larger ganglia (subesophageal, prothoracic, or meso and metathoracic) may stimulate responses when there is a change in light intensity. Due to the large fibers having a direct pathway to the ganglia, there is speculation that the ocelli may affect insect behavior because of the rapid transmission of ocellar stimulation to other areas of the insect's body (Mizunami 1994).

Optic lobes

The brain optic lobes, which receive direct information from the compound eyes and are connected to the ocelli by the small fiber's interneurons, are located in the protocerebrum. The protocerebrum has integrative centers that are thought to contain olfactory input from the antennae. The combination of the protocerebrum, deutocerebrum, and tritocerebrum make the structure that is referred to as the brain or supraesophageal ganglion (Nation 2002).

Fukushi (1975) demonstrated that house flies process visual stimulus by using monochromatic colored lights. This author used conditioning by exposing a house fly to a colored light and allowing the fly to feed on a sucrose solution. After the flies were conditioned, 70% of them responded positively by lowering their proboscis when exposed to the light, whereas the control group had only 10% positively responding. This clearly demonstrated that the visual information the brain receives is processed for learning, and that visual stimulus is important in the learning process.

Vision

Insects, especially flying insects, rely on their compound eyes to guide them by environmental elements to locate food, prey or potential mates (Tobin and Stoffolano 1973). High intensity of light can cause saturation in the photoreceptors and can limit vision. Adaptations in various insects have been made to regulate the amount of light that reaches the photoreceptors.

Goldsmith (1965) suggested that there might be different types of color receptors in the compound eyes of flies, possibly even leakage in the pigment cells with the color red. Goldsmith showed that red light passed through the accessory pigment cells and the light still reached the receptors when it should not have. This experiment suggests

that there may be information missing on how house flies form images. Wehrhahn (1984) showed that house flies with blinded compound eyes would show a positive phototaxis and edge fixation when only receiving visual stimulus through the ocelli. This may suggest that the ocelli play a larger role in visual stimulus.

Image formation

The way an insect constructs an image is by focusing light by the use of dioptric structures or light guides. As mentioned earlier, light passes through the cornea into the ommatidia. The eyes of flies, for example the horse fly, have noticeable bands that run across the compound eyes. These bands are darker across when the ommatidia have a thicker cornea. This is an adaptation to the eyes of a horse fly that enables it to see a host in an open field (Nation 2002). The cornea acts as polarizing sunglasses do for humans. Depending on the insect, the cornea filters light and only lets certain wavelengths of light into the next structure, the cone. The cone helps to further focus the light to the reticular cells. In some higher Orders of insects, such as Coleoptera and Diptera, there is a clear, hard secretion from the semper cells. Semper cells make up the cone helping in the focusing of light by the means of refraction (Chapman 2006). This secreted substance is in higher abundance in insects that have photopic ommatidia and has a gelatinous texture unlike the scotoptic ommatidia which is more crystalline in formation.

Resolution

The quality in the refraction of light by the shape of the cone and the size and shape of the rhabdom will determine the degree of resolution of the image formation (Chapman 2006). If the cone is shorter and more rounded, as it is in scotoptic ommatidia, then there is less refraction, which is due to shielding cells. However, if the

interommatidial angles are small, meaning the angles between adjacent ommatidia, then the resolution is high. If the rhabdom is not twisted and has a large diameter, then the resolution is also greater than with a smaller twisted rhabdom. Some predatory insects, like the mantids, have large rhabdom towards the front of their eyes that helps in the detection of prey movement.

Photoreceptors

Once light has been focused in the cone of the ommatidia, it enters the photoreceptors, which stimulate nerves directly linked to the optic lobe. Rhabdomere within these photoreceptors are made up of approximately 60,000 microvilli (Zuker 1995). The photons that are received by the eye cause an intercellular reaction in the microvilli called phototransduction.

Phototransduction occurs when the light that enters the eye is converted from a light signal to an electrical signal for the nervous system via a biochemical reaction (Zuker 1995). The microvilli contain a light receptor molecule, rhodopsin, made of protein called opsin, retinal, and an aldehyde of vitamin A (Chapman 2006). Rhodopsin is covalently bonded to chromophore, a light sensitive pigment. The manner in which the chromophore reacts to photons depends on the colors of light that can be attenuated. If the pigments are green-sensitive and UV-sensitive, they respond to light that falls into the green and UV-spectrum (Chapman 2006). When the photon contacts this light-sensitive pigment, there is a change in the configuration of the chromophore structure. This changes the rhodopsin into its active form metarhodopsin. Metarhodopsin is the molecule from the receptor that activates the G protein within the cell membrane. After the metarhodopsin activates the G protein, it is reverted back into its inactive state. Metarhodopsin deactivation is accomplished by absorption of the

same wavelength of photon that activated it. This process reverts metarhodopsin to rhodopsin, making the cell ready for light stimulation again. Zuker (1995) presented strong evidence that extracellular calcium is used to regulate the activation and deactivation of rhodopsin. When extracellular calcium is not present, there is a slow response in the activation of rhodopsin, but if there is an overabundance, the reaction is accelerated.

The G protein causes activation of a phospholipase by binding to a protein, most likely arrestin, which creates two intercellular messengers, inositol trisphosphate and diacylglycerol, from the catalyzed breakdown of a phospholipid membrane (Zuker 1995). These intercellular messengers signal the opening of light activated ion channels to create a depolarizing receptor potential to the axon. The messenger cascade amplifies the energy and causes an action potential to lead to a response of postsynaptic cells (Chapman 2006).

House Fly Importance

The house fly is distributed worldwide and is considered a mechanical vector capable of harboring 100 known disease-causing pathogens to man (Greenburg 1973). Mechanical vectoring of pathogens by this insect occurs when hairs on the fly's body or legs pick up microorganisms and are physically carried to food or surfaces associated with humans and animals. Ability of vectoring can also occur when house flies ingest pathogens and pass them via vomitus or fecal waste (West 1951).

The capacity of this fly to vector disease is increased because it is commonly associated with human habitation and primarily breeds in manure and other decaying organic material. Once contaminated house flies disseminate bacteria, such as *Escherichia coli* (Toshinori *et al.* 2000). The house fly is also known to vector some

viruses, such as the Poliovirus (Greenburg 1973). Under some circumstances, houseflies can help spread protozoan diseases and spirochete infections (West 1951). Fly-borne diseases have been documented throughout American history extending back to the Civil War.

Control: Baited Fly Traps

House fly populations can be minimized by simple sanitary procedures, however any break in the process that leaves any type of breeding material can lead to a serious fly infestation. It is estimated that approximately 250,000 cubic feet of flies would exist if one pair of house flies mated and every progeny lived to reproduce over one summer (West 1951).

Lawrence Pickens, who worked with filth fly traps over a thirty-year period, is internationally known as the expert on fly traps. According to Pickens (1995), traps are underutilized because attractants used in the traps cause unfavorable odors and are aesthetically displeasing. Traps require weekly upkeep, such as the unpleasant chore of re-baiting and emptying the captured, rotting flies.

There were fourteen different types of baited traps in use as non-toxic methods of controlling flies in 2006 (Welch 2006). Most commonly attractants placed in the traps consist of decomposing organic material that release gases that attract flies.

The best fly trap design tested by Pickens (1995) had the following elements: vertical bright white panels which form sharp corners and edges; a cone with a steep sixty degrees slope and a small exit hole ~1 cm; a greater than 25 cm diameter base placed 1.2 cm above the bait; 10 to 15 cm deep plastic pan that had fly entry points cut into two of its sides; and fly entry points made so flies can not look directly out of the trap once they were inside.

The differences in fly traps developed over the years usually involve a variation in size and shape of the fly entry port, which usually leads to a cone that allows the house fly to enter but not exit. Luring flies to a trap is usually achieved by a visual cue for the fly to see the trap from a distance and then odorous bait that causes the fly to enter the trap. The most important physical characteristics of a fly trap's bait are: the ability to overcome prevailing air temperature, an odor plume that is recognized by house flies as a food source or oviposition source, and the ability to stand out over other odors in the same area (Pickens 1995). Baits are placed in three categories: sugars and their fermentation products, proteins, and animal excretions.

Along with trap design Pickens (1995) suggested that fly traps work best with proper trap placement, meaning 25 to 46 cm from the ground where full sunlight will reach the trap in the morning and late afternoon so the top of the trap will be fully illuminated. The trap also should be placed as close as possible to natural fly attractants, such as breeding or feeding areas. If there is a need for more than one trap, they should be spaced no more than 18 m apart. Studies conducted on house fly activity show peak hours of activity between 9:00 a.m. to 4:00 p.m. Ninety percent of houseflies studied preferred to resting areas inside houses (Keiding 1965). On nights when the temperatures were above 20°C and the house had screened doors and windows, 80% of the house flies rested on the ground outside or in animal shelters. Jug traps in the rafters of poultry houses caught a greater number of flies during hours of 7:00 a.m., 4:00 p.m., 7:00 p.m., and 10:00 p.m. (Burg and Axtell 1984).

For sampling methods, fly traps can be made from 1 gal translucent milk jugs with four 5 cm diameter entry holes equally spread around the circumference and on the

upper third of the jug (Burg and Axtell 1984). Using a standard yeast based bait, more house flies were caught near the rafters in a narrow poultry house away from organic material, while in a residential high-rise building fly traps located at the same level of organic material caught more flies than on the rafters or walkways.

Two key attractants that have been used to lure house flies to a trap are olfactory and visual stimuli. Visual stimulus is the most important in directing fly behavior (Tobin and Stoffolano 1973). After a house fly has been visually stimulated to move towards a trap, it is important to have chemical or olfactory stimuli, to draw the fly inside the trap. One of the most commonly used chemical stimuli in fly traps is the close-range sexual stimulant, (Z)-9-tricosene (Schal *et al.* 2001). Results by White *et al.* (2007) question whether (Z)-9-tricosene is truly an effective method of increasing a fly bait's attractiveness in large areas because it attracted flies at a very limited distance and was not that effective in outdoor environments.

New fly control methods are entering the market. One of the newest devices, the Fly Sentry® (Mix 2007), exposes enough fly bait to cover a large area, but also protects the bait from the sun, wind, and rain. Another trap, which seems to be a promising choice for fly surveillance, is the sheltered Quick Strike® trap. This device preserves flies intact in dry, odorless conditions (Geden 2005).

Use of fly traps continue to decline despite studies showing an increase in house fly resistance to pesticides. House flies have been documented as resistant to pyrethrins, pyrethroids, organophosphates, carbamates, fiproles, insect growth regulators, avermectins, and organochlorines (White *et al.* 2007). In 1991, Bayer CropScience developed MaxForce® Fly Spot Bait, a water-dispersible, sugar-based

bait formulation that contains 10% of imidacloprid. When ingested, this product is effective in controlling pest insects like cockroaches, fleas, crickets, termites, and flies. This chloronicotinyl class compound has shown promising results against synthetic pyrethroid-resistant house flies. Imidacloprid has a show the ability to reduce fly populations quickly (Pospischil *et al.* 2005). In experiments conducted by Hertz (2007), imidacloprid sprayable bait applied to wool cords caused a higher mortality rate in house flies than methomyl granular fly bait in the first three hours. However, over 24 hours, the methomyl granular fly bait caused an overall higher mortality rate than imidacloprid. In experiments performed by White *et al.* (2007), imidacloprid had noticeable effectiveness in 30 min after initial exposure and after two hours most of the flies were nonresponsive. However, despite significant knockdown in fly populations, some flies recovered from the initial dose.

Scientists debate whether fly traps are monitoring or control devices (Geden 2006). To reduce a fly population by 50-90%, a single trap would have to remove ~24-58% of the houseflies daily (Weidhaas and Haile 1978); however, traps alone have not been shown to reduce fly populations to acceptable levels (Smallegange 2004).

Using Visual Attraction for Control of House Flies

Understanding the physiology of insect vision can help in the development of effective control devices for house flies. There is uncertainty and disagreement when it comes to spectral reflectance of colors and their visual attractants for house flies (Geden 2006). The ideal trap has been referred to as being bright white in color (Pickens 1995), which disagrees with other studies where yellow traps caught more flies than white traps (Burg and Axtell 1984).

The physiology of the photoreceptors, suggests that surfaces with strong contrasting colors should have greater visual attractiveness. However, no studies have demonstrated this to be true. However, when house flies are given a choice of shapes to land on, they tend to land on the edges over the center of the shape (Pickens 1995). House flies also show preferences for shapes that were more complex. In addition, beaded-top traps catch more flies than traps with flat tops, and that cubical traps always outperform cylindrical traps.

House fly traps typically use a visual component as a long distance attractant, such as colors in specific wavelengths, lights, or patterns. Once the fly is in close proximity of the trap, a chemical attractant may be used to lure the house fly into the trap. A visual stimulus may be the most important attractant for most insects (Tobin and Stoffolano 1973). Since house flies are strong flyers and have large compound eyes, it is speculated that visual cues are used to search for potential feeding and breeding sites over long distances (West 1951). However, according to Keiding (1965), house fly activities are not determined by visual cues but are random and explorative. Garret (1965) observed a clear difference in house fly color preference, with greater numbers resting on primrose, green, tan, and white. This suggests that visual stimulus guides the house fly behavior.

Electroretinogram studies of the house fly's eye show sensitivity to UV light ranging from 340 nm to 370 nm, and blue-green light ranging from 480 nm to 510 nm (Bellingham 1994). The UV spectral range used in light traps for house flies and other flying insects, also is used in paints or fabrics used in baited traps. This transforms the entire trap into a visual cue that may be similar to an environmental element that would

naturally attract house flies. Black or blue UV lights are commonly used with these types of traps (Hedges 1998). Other colors may not stimulate the photoreceptors of the ommatidia. House flies exposed to green or red UV light show negative phototaxis behavior (Bellingham 1994). Aubuchon (2006) used several different types of light sources as visual attractants and showed that previous exposure to different kinds of light did not influence house fly attractiveness to light traps. In an experiment performed by Geden (2006), a known visual attractant for house flies and stable flies, alsynite, was compared to a known attractant for tsetse fly, blue fabric. The blue fabric had a maximum reflectance of 466 nm in sunlight and the alsynite was described as translucent, and reflected UV spectrum. More house flies were captured with alsynite traps than traps with blue fabric as the visual attractant. However, the blue fabric outperformed the white and black fabric traps. In addition, when the visual attractiveness of alsynite and blue fabric were combined, the trap captured as many or more than the alsynite alone (Geden 2006). Blue fabric visual targets have also shown promising results for capturing stable flies (Foil and Younger 2006).

Pickens (1995) points out that the color used with a fly trap is dependent on the weather. White and yellow colors are preferred by house flies in cooler weather, whereas red, black, and blue are preferred in hotter weather. This may show that the atmospheric temperature contributes to the manipulation of the pigment cells in the ommatidia causing them to absorb colors of different wavelengths depending on the external temperature of the insect, possibly an adaptation that associates some colors to be attractive in hot and cold conditions.

When reviewing the Pickens (1995) analysis of the best trap design, it appears that the most important factor of the trap is its visual attributes. The described ideal trap has to have edges and be a color that reflects a wavelength that is attractive to flies. The trap has to be in a location where it is fully illuminated by the sun for most of the day. Pickens also mentioned that if there is a need for more than one trap, they should be spaced at specific intervals.

CHAPTER 3
RESPONSE OF *MUSCA DOMESTICA* L. TO VISUAL STIMULI

Introduction

Visual stimulus is the most important attractant for insects (Tobin and Stoffolano 1973), especially house flies since over half of their head is comprised of two large compound eyes accompanied by a cluster of three simple eyes (ocelli). House flies rely on sunlight reflected from objects as visual input for survival. This gives flies the ability to see objects in their environment to avoid obstacles while flying, finding food, finding harborage, and being able to recognize resting areas.

Light enters the compound eyes or ocelli and stimulates photosensitive cells that trigger a process called phototransduction, which converts light to electrical signals for the nervous system, sending signals to the insect's optic lobe for interpretation (Zuker 1995). Signals received by the optic lobe result in fly behavior, like attractancy or repellency. It is unknown how insect compound eyes physiologically form images other than interpreting intensity of light, motion, and plane of polarization (Hocking 1964).

Color vision is the ability to distinguish differences in wavelengths of color and not the intensity of light (Hilbert 1992). Color vision is thought to be associated with compound eyes; whereas the ocelli are believed only to perceive differences of light intensity and are not capable of detailed image formation (Mizunami 1994). However ~200 photoreceptor cells are within a house fly ocellus produce positive phototaxis when the compound eyes are blinded (Wehrhahn 1984).

Typically, electroretinogram studies use a direct light source, such as a xenon bulb, to generate a narrow range wavelength to measure neural responses of insect eyes. Although electroretinogram studies with direct light sources showed sensitivity of

house fly compound eyes to UV light (ranging from 340 to 370 nm), and blue-green light (ranging from 480 to 510 nm) (Bellingham 1994), there is disagreement on the effect of reflected colors and their visual attractants on house fly behavior (Geden 2006). Light reflected from an object generates a wide range of wavelengths (Table 3-1) that will consist of various parts of the light source's color spectrum and filtering other parts (Hewitt 1998). Studies with specific fly trap colors have shown a direct correlation between visual sensitivity and fly trap efficiency (Agee and Patterson 1983).

Musca flies are known to have blue-green visual pigments in their compound eyes with sensitivity to ~440 to 540 nm (Salcedo et al. 1999). Blue fabric targets with the maximum reflectance of 466 nm have been shown to be more visually attractive to house flies in comparison to white and black fabric targets (Geden 2006). This same type of blue visual target has also shown promising results for capturing stable flies (Foil and Younger 2006).

Previous observations demonstrated a clear difference in color preference for house flies, which suggests that visual stimuli guide house fly behavior (Garret 1965). House fly behavior has shown an attraction to hanging cords and similar objects in field conditions as resting locations (Fehn 1958). Other studies have shown that house flies have a tendency to follow edges while foraging suggesting that the contrast of colors at the edge of lines may contribute to visual attractancy (Conlon and Bell 1991). The objectives of these studies were to: 1) measure house fly physiological response to reflective color using electroretinograms of both compound eyes and ocelli; 2) compare house fly behavioral attraction to reflective colors; 3) compare the effect of colored lines on attractiveness of the blue background.

Materials and Methods

Insects. *Musca domestica* (Linnaeus) used for these experiments were from the University of Florida Horse Teaching Unit and reared at the University of Florida, Gainesville, FL. Room conditions for all developmental stages were maintained at $26\pm 1^{\circ}\text{C}$ and 55% RH with a 12:12 (L:D) photoperiod. Adult house flies were reared by placing eggs in a basin containing freshly mixed larval medium (1.5 liters of tap water, 250 ml Calf Manna® pellets, Manna Pro Corp., St. Louis, MO, 15 ml methyl paraben, and 3 liters of wheat bran). Larvae were allowed one week to pupate. Pupae were collected by floating the basin's content in tap water. Pupae were allowed to dry and were placed in a screened cage along with granulated sugar, powdered milk, and water *ad libitum*. Adult house flies, 3 – 5 days old, were collected from screened cages by aspiration using a hand vacuum with a modified crevice tool.

Electroretinogram (ERG). Equipment similar to and techniques modified from Bellingham (1994) were used to measure depolarization of nerves in compound eyes and ocelli exposed to reflected light. Equipment consisted of two ERG micromanipulators (EAG COMBI 10X, Syntech, Hilversum, NL) with glass sheathed tungsten electrodes to record depolarization of compound eyes and ocelli. Electrodes were chemically sharpened by etching with potassium hydroxide. Recording software used to measure depolarization was ElectroAntennography Version 2.4 (Syntech, Hilversum, NL). To reduce external electro-interference, ERG equipment was enclosed in a faraday cage, which was completely draped with black theater cloth allowing no light to enter cage. Internal cage conditions were $24\pm 1^{\circ}\text{C}$ and 45% RH.

Visual target and light source. Visual targets used for ERG were constructed from twin-walled, rigid plastic sheets (20 cm x 25 cm; White C201L, Blue C505L, Black

C208L, Yellow C402T, Green C406T, and Red C404T, Coroplast™, ThyssenKrupp Material NA, Inc. AIN division Madison Heights, MI) (Beresford and Sutcliffe 2006).

Visual targets were placed 25 cm in front of the house flies head and arranged perpendicular to the floor.

Light source was four warm white LED lights (RL5-WWW7035 2940K/700 mcd/35°, Super Bright LEDs Inc. St Louis, MO) mounted on imprinted circuit board and fitted with switch. Light source was powered by a 6V battery (BSL0955 6V 10Ah rechargeable battery, Universal Power Group Inc. Carrollton, TX) and held in place by three-prong clamp 30 cm from visual target. A black piece of Coroplast™ was placed under the light source to block any light other than that reflected from the visual target from reaching the test insect. All replicates were conducted in complete darkness.

Wavelength of reflective light from visual targets was measured using USB2000® Spectrometer (Ocean Optics®, Dunedin, FL) with visual targets in place and spectrometer sensor secured to test insect (Table 3-1). Five independent readings were conducted for 1 minute each. Light intensity of reflected light from visual targets was measured with a HOBO® Light Intensity logger (Onset®, Bourne, MA) in the same manner with HOBO device in place of test insect.

ERG procedure. Seventy female house flies (40 for compound eyes and 30 for ocelli) were used for this experiment and were anesthetized with ice for 30 minutes. Each fly thorax was encased with wax and adhered to a glass slide, ensuring no visual obstruction of the compound eyes or ocelli. The slide mounted fly was clamped in place with the head facing the visual target. Electrodes were inserted into the appropriate eye using a dissecting microscope for precise placement. For compound eye bioassay, the

measuring electrode was inserted into the equatorial region of the right compound eye (Bellingham 1994) and the differential electrode was inserted into the fly's abdomen. For ocelli bioassay, the measuring electrode was inserted, just puncturing, in the cuticle of the median ocellus and the differential electrode was inserted into the fly's abdomen. After both electrodes were in place the house fly was allowed time to recover in both bioassays.

Each fly was randomly presented with every color (black, white, red, green, yellow, and blue) visual target, allowing a one-minute recovery between each 0.5 s exposure of light source on the visual targets (Figure 3-1). The ocelli ERGs were conducted separately from compound eye bioassays. Both experiments were run in a block design with each insect serving as a block, and being exposed to all colors.

Data analysis for ERG experiments. Data for the ERG consisted of measured neural responses measured in mV for the compound eyes and ocelli and averaged for each visual target. One-way analysis of variance was performed and means were separated using Tukey test ($P = 0.05$; SAS 2001).

Two-Sided Light Tunnel (TSLT). TSLT was constructed with two 42.6 L capacity heavy-duty ice chest (The Hercules ice chest No. 5345; Life-Like Products Inc., Baltimore, MD). A circular hole (10 cm diam.) was cut in one of the side walls of the ice chest centered at 16 cm from the top, 17 cm from the bottom of the ice chest, and 29 cm from the sides of the ice chest. Each ice chest then was fitted with a black pipe flange (ABS-DMV Schedule 40; NIBCO Inc, Elkhart, IN) secured in place with hot glue. Each ice chest contained a 45.7 cm fluorescent light fixture (Portfolio 18" under cabinet fluorescent light, model# GL9718-T8-BK-1, Good Earth Lighting, Inc., Wheeling, IL)

centered directly under the black flange. The fluorescent light fixture was fitted with a GE daylight fluorescent bulb (GE daylight F15 457 mm, F15T8/D/TP, General Electric Company, Cleveland, OH) as used by Shields (1989). A square hole (3 cm side) was cut in the lower corner of the side wall of each ice chest to allow the power cord from the light fixture to exit the ice chest. The area around the power cord was sealed with modeling clay (Marblex Self Hardening clay, American Art Clay Co., Inc., Indianapolis, Indiana) to prevent house flies from escaping. Two ice chests were connected by two 30.5 cm black PVC pipes (TrueFit® System 3300 3" SCH 40 COEX ABS cellular core DMV pipe Charlotte Pipe and Foundry Company, Charlotte) which connect in the center to a T-connector (NIBCO, IN). The T-connector was fitted with a cleanout adapter (NIBCO, IN) with a modified plug (NIBCO, IN) so that a capped plastic vial (50 dram Crystal capped plastic vial; Thornton Plastics, Salt Lake City, UT) could snap into the cleanout adapter and be removed when necessary. The snap-cap vial was modified with a removable tab that prevented flies from leaving the vial until the tab was pulled out. Also, the snap-cap vial was lined with wire mesh that allowed the house flies to crawl up the sides of the vial to enter the TSLT. Similar wire mesh was also inserted in the modified plug and extended from the tab in the vial to the top of the cleanout adapter. This allowed the house flies to climb from the vial to the T-connector and enter the TSLT to make a choice between the ice chests with attractive visual targets.

Visual targets. Fabric visual targets were constructed from Nautolex® skipper fabric (20cm x 25cm, OMNOVA Solutions Inc., Fairlawn, OH) and plastic visual targets were constructed from twin-walled rigid plastic sheets (20cm x 25cm, Coroplast™, ThyssenKrupp Material NA, Inc. AIN division Madison Heights, MI). Both fabric and

plastic visual targets (Table 3-1) consisted of the colors: yellow, red, green, black, blue, and white. Visual targets were mounted to the center of ice chest opposite to the pipe flange. Only the visual targets on each side could be seen from within the piping of the TSLT.

For experiments with lined visual targets, lines were added on blue visual targets by attaching 6 mm wide strips of skipper cloth spaced 1 cm apart and attached vertically on solid colored background. Vertical stripes were placed so that four lines could be seen when looking through TSLT from T-connector.

Wavelength of reflective light from visual targets was measured with a USB2000® Spectrometer (Ocean Optics®, Dunedin, FL). The spectrometer sensor was secured in place with black cardboard in a centered orientation of T-connector facing the visual target. Five readings were taken over one minute for each visual target (Table 3-1). Intensity of reflected light from visual targets was measured with a HOBO® Light Intensity logger (Onset®, Bourne, MA) in the same manner.

TSLT procedure. All TSLT experiments were conducted at the Urban Entomology lab at the University of Florida, with ambient temperature at 26.6° C. House flies (50 males and 50 females) were anesthetized with ice and placed in modified snap cap vial. The snap cap vial was attached to the TSLT and the house flies were given 1 h to recover before removing the tab that allowed the house flies to enter the TSLT. Experiments were conducted in a Latin square design and after fly release each replicate ran 2 h. Once the allotted time was reached, the pipes were disconnected from the ice chests and all pipes and chest holes were immediately capped. The flies within ice chests and pipes were knocked down with CO₂ and

counted. House flies that flew into the ice chests were considered responsive to the reflective color, whereas the flies that remained in the connecting pipe were considered nonresponsive.

Four TSLT experiments were conducted. In the first two experiments color targets were compared with black targets. Color targets included white, red, yellow, blue, and green, which were mounted in the ice chest opposite to black in the TSLT. Two experiments were conducted: the first one with all fabric visual targets and the second one with all plastic visual targets. For the third experiment, the most attractive plastic visual targets from the first experiments (blue vs. white) were compared with least attractive plastic target (yellow). The fourth experiment, color lines (red, yellow, white, black, and no lines) were added to the blue target previously selected as the preferred color and again compared with black.

Data analysis for TSLT experiments. House flies were collected and counted from capped pipes and ice chests. Total house fly response was calculated by subtracting nonresponsive flies (in piping) from total number of flies released in the TSLT. House flies collected from ice chest with visual target were divided by the total house fly response to get a percentage of house flies responding to individual visual targets. House fly response to visual targets data were arcsine-square-root transformed before analysis of variance and means were separated using Tukey's test ($P = 0.05$; SAS 2001).

Results

ERG. Measurements of the depolarization of the nerves in the compound eyes showed that house flies had the greatest stimulus from the white visual target (Table 3-2). Compound eye ERG responses to visual targets were as follows: White = Blue >

Yellow > Red > Green > Black. The compound eye had similar low neural responses in perceiving green and black visual targets.

The measurements recorded from the ocellar response showed a significantly greater in the color vision to white, blue, and yellow. Ocelli ERG responses to visual targets had the following relationship: White > Blue > Yellow = Red > Green = Black. The green visual target had no significant differences to the black visual target in responses from the ocellus.

TSLT. In the experiment comparing attraction / repellency of colors when compared to black using the fabric visual targets (Figure 3-2), more flies were attracted to the white, blue, and red visual targets, White = Blue > Red > Green > Yellow. In addition, house fly attraction to the green and red visual targets was not significantly different. The yellow visual target was repellent having more flies in the ice chest that contained the black visual target than on the side with the yellow visual target.

Replacing visual targets with plastic visual targets used in the ERG experiments, the house fly behavior was similar to that observed with fabric visual targets (Table 3-3). White and blue visual targets were the most attractive targets and had no difference in their house fly attraction, White = Blue > Red > Green > Yellow. However, different from the use of the fabric targets, the attraction of the red visual target was significantly lower than that for white and blue targets. The yellow visual target was significantly different from all other colors tested and as with the fabric visual targets was repellent.

Due to the repellency of the yellow visual target a larger percent of flies were attracted to blue and white visual targets when ran separately against yellow visual target than when compared to the black visual target (Figure 3-3). In addition, blue

visual target ran against white visual target, isolated the blue visual target as the most attractive target used in the TSLT.

The addition of a lined pattern using vertical colored lines in TSLT experiment showed that black lines significantly enhanced the blue visual target's attractiveness (Figure 3-4). Adding red or white lines to the blue targets were no more attractive than where no lines were added. However, the addition of yellow lines on the blue visual target caused repellency.

Discussion

The neurological responses measured with the ERG experiments showed that the reflected light from the color targets elicited a neurological stimulus to the house fly compound eye and ocellus. The reflected light from all visual targets used had the same light intensity to the tested insect, suggesting that the neurological responses measured with the ERG were generated by the specific colors of visual targets. This is defined as color vision.

The ERG showed that the house fly compound eye had high neural responses to the reflected light from white, blue, and yellow targets. White (~419-738 nm) and blue (~423-574 nm) reflected wavelengths that included known visual sensitivity wavelength ranges for house flies, 450-510 nm (Agee and Patterson 1983, Bellingham 1994). The yellow visual target (~510-586 nm) gave similar physiological responses as the blue visual target, which was surprising because yellows reflectance started at ~510 nm. *Musca* flies have blue-green visual pigments in their compound eyes that have sensitivity to wavelengths 440 to 540 nm. This fact may explain why yellow targets generated a higher response with the compound eyes (Salcedo et al. 1999). However,

the lower neural response generated by the green visual target (462-571 nm) was not significantly different from black target.

In experiments where flies have had their compound eyes removed and are only using their ocelli for sight, they have a tendency to follow edges (Wehrhahn 1984). This demonstrates that the ocelli are able to form images. Visual pigments in fly ocelli have been shown to have a maximum absorption of light of 425 nm (Kirschfeld et al. 1988), which corresponds to white and blue visual targets. Typically, the ocelli are seen as having high-speed neurons that only detect changes in light intensity (Chapman 2006). However, the light intensity of reflected light used for this experiment was the same for all the targets, suggesting that the tested ocellus was detecting the colors of visual targets. Therefore, the conclusion was drawn that the ocelli are capable of color vision.

The TSLT allowed observation of the house fly's behavioral response to colors in a controlled laboratory environment. When white, blue, yellow, green, and red were compared to black, more house flies were attracted to the colors white and blue than any other color. The color that repelled house flies was yellow. In the past, laboratory experiments have shown that more house flies landed on yellow paper than other colors tested (Awati and Swaminath 1920). Field observations using painted plastic jug traps demonstrated that yellow jugs caught more house flies (Burg and Axtell 1984). Other observations using colored pieces of cardboard found that black was most attractive in the laboratory, while yellow was most attractive outdoors (Nava 1967, Hecht et al. 1968). Confirmation of yellow as a repellent color in the TSLT showed that house flies were clearly more visually attracted to the white and blue, with a ~70% behavioral attraction, compared to ~20% attraction to yellow.

A comparison of the two most attractive visual targets in the TSLT isolated blue (~60%) as more attractive than white (~25%). This is due to white having a wide range of wavelengths (Figure 3-6) that includes wavelengths peaking in the attractive blue range, but also in the repellent yellow range.

It has been argued that atmospheric conditions may contribute to the attraction of house flies to specific colors: white or yellow were preferred in cooler weather, whereas red, black, and blue were the preferred colors in warmer weather (Pickens 1995). Other studies conclude that house fly color preference is independent of temperature (Muniz 1967). The measured internal temperature before and after each TSLT replicate was $26 \pm 2^\circ$ C. If attraction to a color is temperature dependent then response to yellow and white targets should have caused the same behavioral attraction.

It has been suggested that blue is attractive to tsetse fly and other flies because it is perceived as shaded resting area (Steverding and Troscianko 2003). In the TSLT study the blue visual targets were more attractive to house flies with the addition of black lines, which could be contributed to the scototaxis tendency of house flies (Hecht 1970). The house flies perceive the blue visual target as a potential resting area and the black lines as cracks or crevices that they can use as harborage.

In other Diptera studies, similar shades of blue, which reflected in specific color bands of blue-green, and red have been shown to be effective as visual attractants (Green and Flint 1986). Sunbrella® blue fabric (peak wavelength 466 nm) was field-evaluated as a visual attractant in substitution of blue cotton fabric, which is a commonly used material in an effective tsetse fly trap due to demonstrated effectiveness of attracting flies in North America (Mihok et al. 2006, Geden 2006). These results

indicate that the material used as visual targets is not an essential factor in determining attraction to the flies, but the wavelengths of the target's reflected light is critical.

The visual information from the compound eyes and the ocelli is integrated to work together in providing information to the optic lobe that directs fly behavior in response to reflected light from objects. The studies here have shown that the ocelli are capable of color vision and contribute more to house fly vision than originally thought. Comparison of the behavioral and physiological responses (Figure 3-5) shows a direct correlation of house fly attractiveness to visual targets and the intensity of neurological response. However, the yellow visual target represents an exception, because it was repellant to house flies in the TSLT, but triggered relatively high neurological response. Yellow stimulates the same amount of neural responses as blue, but the neurological response triggers an alternative behavioral response. The triggered behavior response under the conditions of the TSLT causes house flies to be repelled by the yellow visual targets. Previous studies that have concluded that yellow was visually attractive to house flies may have been using yellow colors that were reflecting additional attractive wavelengths (Burg and Axtell 1984, Pickens 1995).

From these experiments, there is no question that the compound eyes and ocelli have the capability of color vision. Each visual target tested generated specific neurological responses, which was interpreted by the house fly brain into behavior response of either attraction or repellency. Future studies with similar experiments should investigate various adult house fly responses at different life stages to determine if colors generate different neurological and behavior responses depending on if the house fly has mated, fed, or of a certain age.

Table 3-1. Wavelengths (nm) and reflected intensity (cd/m²) of light from light sources on visual targets used in electroretinogram and two-sided light tunnel experiments.

Light Source	Material	Color	Peak(nm)	~ λ (nm)	Reflected Intensity (cd/m ²)
Used with electroretinogram					
Warm white LED lights	Plastic	Blue	460	423-574	11.8
Warm white LED lights	Plastic	White	500	419-738	11.8
Warm white LED lights	Plastic	Yellow	564	510-586	11.8
Warm white LED lights	Plastic	Green	543	462-571	11.8
Warm white LED lights	Plastic	Red	603	588-702	11.8
Warm white LED lights	Plastic	Black	**	**	11.8
Used with two sided light tunnel					
GE daylight fluorescent bulb	Fabric	Blue	436	397-586	35.5
GE daylight fluorescent bulb	Fabric	White	518	395-725	248.6
GE daylight fluorescent bulb	Fabric	Yellow	571	504-585	153.9
GE daylight fluorescent bulb	Fabric	Green	544	450-571	28.0
GE daylight fluorescent bulb	Fabric	Red	612	583-752	28.0
GE daylight fluorescent bulb	Fabric	Black	**	**	19.4
GE daylight fluorescent bulb	Plastic	Blue	482	394-583	59.2
GE daylight fluorescent bulb	Plastic	White	522	380-812	232.5
GE daylight fluorescent bulb	Plastic	Yellow	575	502-593	106.6
GE daylight fluorescent bulb	Plastic	Green	544	457-570	28.0
GE daylight fluorescent bulb	Plastic	Red	610	578-738	19.4
GE daylight fluorescent bulb	Plastic	Black	**	**	12.1

** No measurable peak or wavelength.

Table 3-2. Measured depolarization of female house fly equatorial region of right compound eye and median ocellus from white LED light source reflected off of plastic visual target.

Visual Target Color	Compound Eye Response (mV)	Ocelli Response (mV)
White	-8.3 ± 0.97a	-4.9 ± 0.22a
Blue	-7.4 ± 0.97ab	-3.6 ± 0.17b
Yellow	-6.8 ± 0.94b	-3.0 ± 0.19c
Red	-5.2 ± 0.64c	-2.7 ± 0.21c
Green	-4.3 ± 0.49cd	-1.8 ± 0.12d
Black	-3.3 ± 0.39d	-1.7 ± 0.05d

Means within columns followed by different letters were significantly different at P=0.05.

Table 3-3. Comparison of house fly response to fabric visual targets and plastic visual targets used in two-sided light tunnel with GE daylight fluorescent bulb.

Visual target color	Fabric target fly response	Plastic target fly response
White	67.0 ± 3.86a	67.3 ± 4.12a
Blue	64.4 ± 3.17a	66.4 ± 3.44a
Red	57.3 ± 2.13ab	55.3 ± 2.20b
Green	48.2 ± 3.12b	45.2 ± 3.61b
Yellow	30.8 ± 3.81c	32.9 ± 2.86c

Means within columns followed by different letters were significantly different at P=0.05.

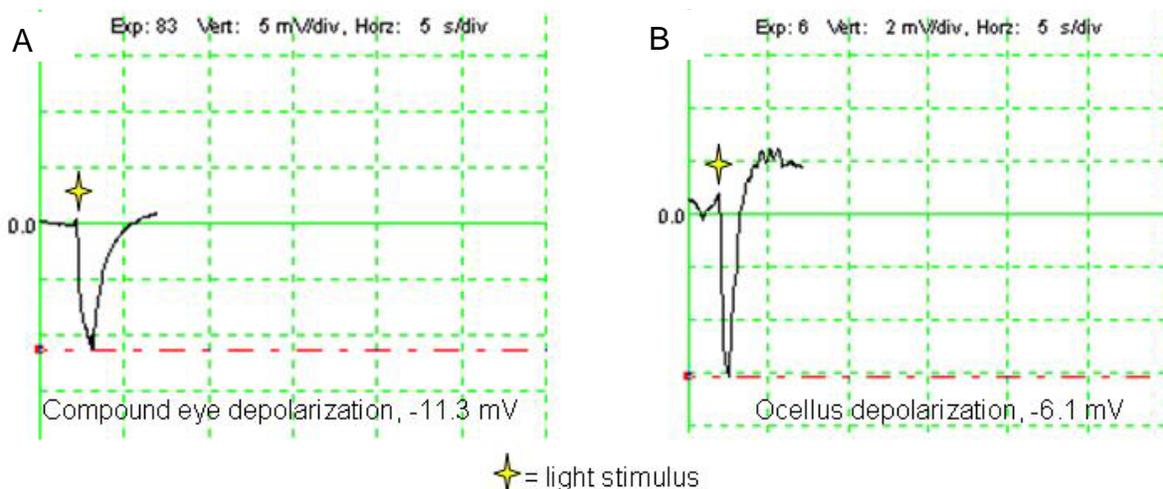


Figure 3-1. Typical electroretinogram elicited from female house fly equatorial region of compound eye A) and median ocellus B) after 0.5 s stimulus of white reflected light.

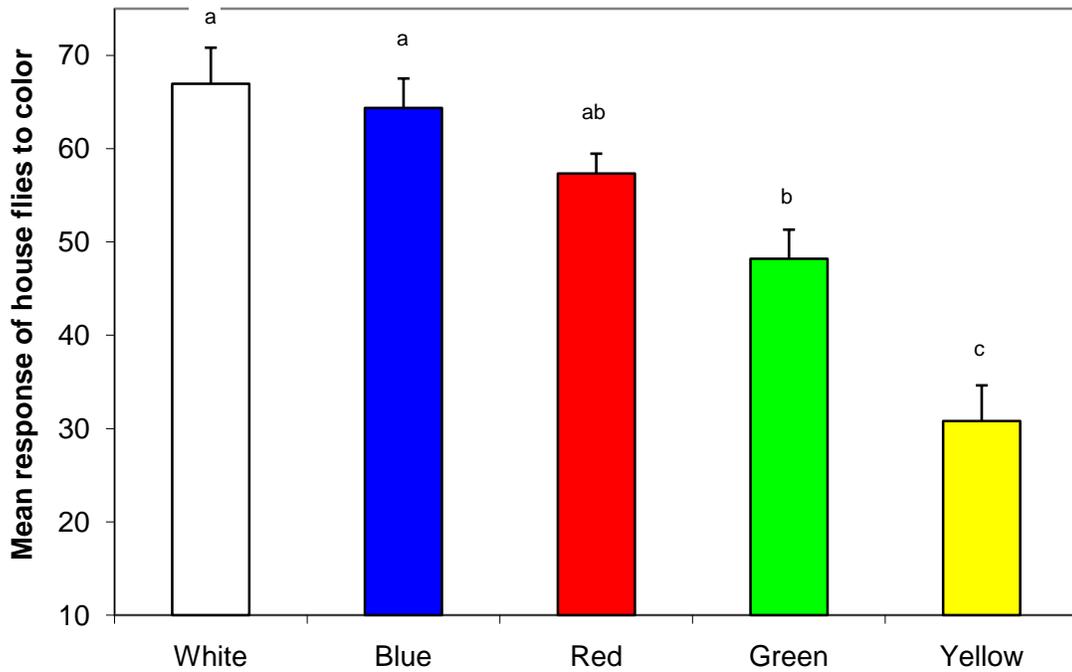


Figure 3-2. Mean behavioral response of house flies to fabric visual targets compared to black after 2 h in two-sided light tunnels.

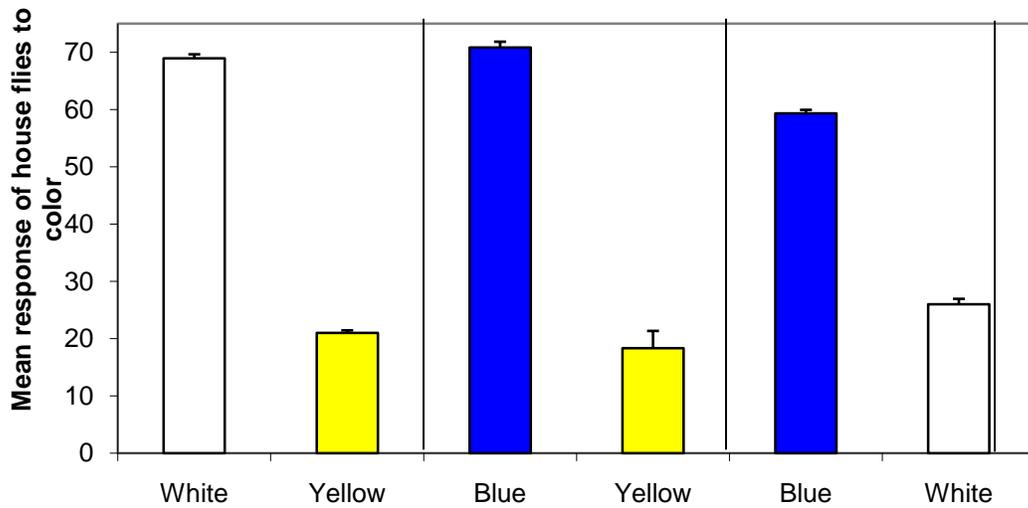


Figure 3-3. Mean behavioral response of house flies to fabric visual targets in two sided light tunnel; white vs. yellow, blue vs. yellow and blue vs. white.

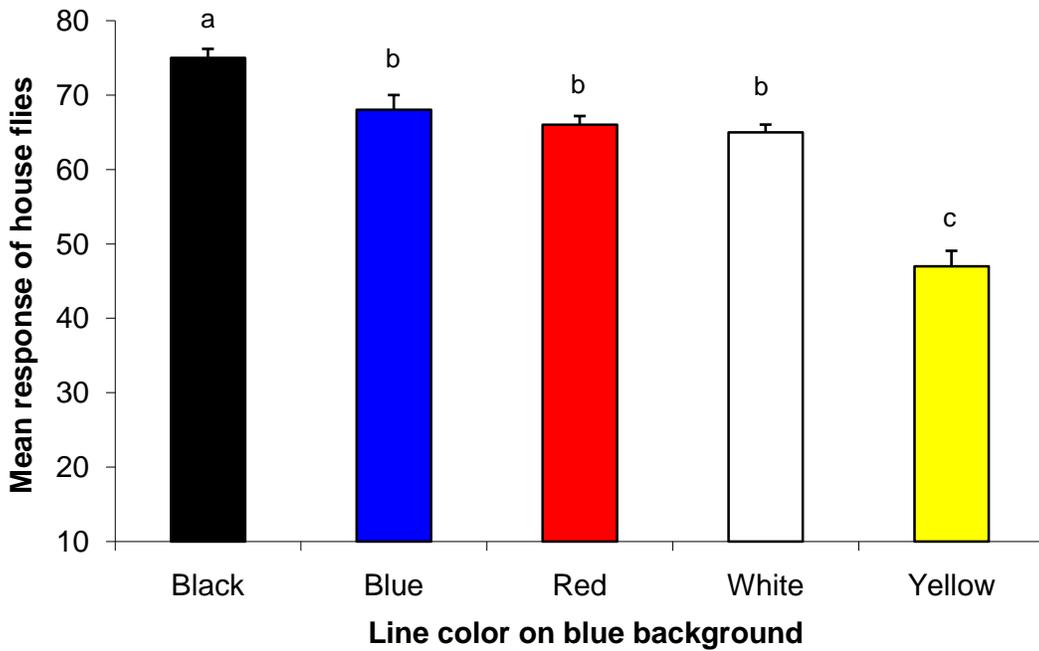


Figure 3-4. Mean behavioral response of house flies to different colored lines on blue backgrounds in two-sided light tunnels compared to solid black.

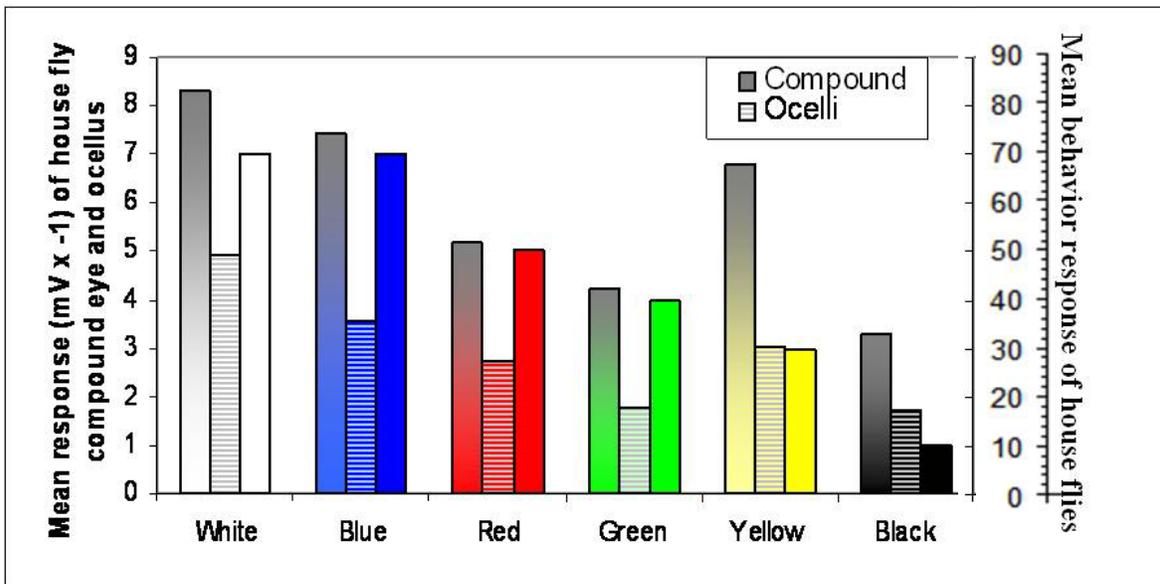


Figure 3-5. Mean physiological response of house flies from electroretinogram experiments with house fly behavioral response in two-sided light tunnels. Third column of color group represents mean behavior response of house flies to plastic visual targets compared to black in TSLT.

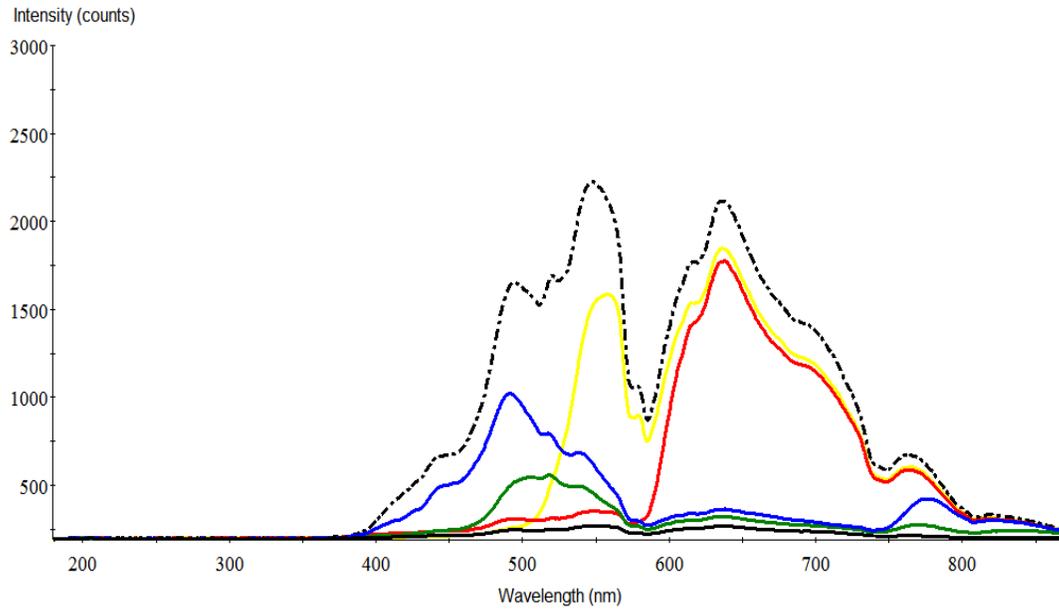


Figure 3-6. Spectral reflectance of sunlight from colored plastic visual targets. Each visual target color is represented by its color of line; white is represented as a dotted line.

CHAPTER 4 FLY KILL CAUSED BY TOXICANTS IN WOOL YARN APPLIED TO A BLUE COLORED FLY CONTROL DEVICE

Introduction

Movement of house flies is primarily influenced by attraction to specific conditions, such as resting sites, food sources, and breeding media (Keiding 1965). House flies have been observed to prefer resting areas near the ceiling on electrical cords or the darkness of rafters in barns (Mathis et al 1967, Burg and Axtell 1984). These behaviors are often taken into account in order to optimally place traps. For instance, foraging flies are usually found close to the ground (Driggers 1971, Geden et al 2009), whereas resting flies are caught on sticky traps hung high in structures. Consequently, there has been mixed information on the best height placement of fly control devices.

In 1947, the observation was made that house flies preferred to rest on edges of various structures and objects (Baker et al 1947). Taking advantage of this type of fly behavior, the use of insecticide treated cords was introduced. Cords were initially treated with DDT, but once commercially prepared, the insecticide treated cords were cotton and dipped in solutions such as parathion and/or diazinon (Fehn 1958). Insecticides that were commonly used on cords for fly control, such as organophosphate and organochlorine insecticides, are no longer registered by the Environmental Protection Agency (Rabari and Patel 1976). Research reviving the vintage insecticide treated cord demonstrated that modern insecticides were effective in killing flies when applied to wool cord (Hertz 2007).

Baits and insecticide sprays are typically used as chemical methods for house fly control, but neither method provides long lasting residual effects. Insecticide treated cords has been viewed as an alternative to traditional insecticide sprays and have been

successful in controlling flies for long periods (Keiding 1976). In heavy infestations of flies the installation of 91 m/ 93 m² of insecticide treated cords, was able to reduce the house fly population by 70% in one week and able to maintain control for at least a month (Fehn 1958).

Insecticide resistance to a variety of active ingredients has been well documented in house flies (Georghiou and Lagunes-Tejeda 1991, Liu and Yue 2000, Scott et al. 2000). In order to avoid resistance developing in house fly population, chemical groups of insecticides should be alternated in set time intervals routinely. However, the number of registered insecticides for house fly control has drastically decreased making insecticide rotations a challenge (Kaufman et al. 2001).

Combining known house fly visual attractive colors, a blue background with black lines (Chapter 3), with an effective insecticide delivery method of wool cords (Hertz 2007) a fly attractant system with toxicant treated cords (FAST-TC) can be constructed (Figure 4-1). To make this device available for use by military and civilian personnel it needs to be cost effective and versatile. This study's objectives were to: 1) compare efficiency of active ingredients (AI) on FAST-TC, 2) evaluate a less expensive alternative to wool cord, and 3) determine suitable height for use of FAST-TC.

Materials and Methods

Insects. *Musca domestica* (Linnaeus) used for these experiments were from the University of Florida Horse Teaching Unit and reared at the University of Florida, Gainesville, FL. Room conditions for all developmental stages were maintained at 26°C and 55% RH with a 12:12 (L:D) photoperiod. Adult house flies were reared by placing eggs in a basin containing freshly mixed larval medium (1.5 liters of tap water, 250 ml Calf Manna ® pellets, Manna Pro Corp., St. Louis, MO, 15 ml methyl paraben, and 3

liters of wheat bran). Larvae were allowed one week to pupate. Pupae were collected by floating the basin's content in tap water. Pupae were allowed to dry and placed in a screened cage along with provisioned granulated sugar, powdered milk, and water *ad libitum*. Adult house flies, 3 – 5 days old, were collected from screened cages by aspiration using a hand vacuum with a modified crevice tool. After collection, house flies were anesthetized by being placed on ice for 30 min.

AI comparison: No-choice laboratory bioassay. This experiment was conducted in laboratory cages (31 x 25 x 21 cm) constructed with PVC pipe (1.27 cm). A rubber band was used to establish individual treatment positions in center top of cage, one position for each cage. All pieces of yarn used were 10 cm in length and were attached to the treatment positions vertically using paper clips hanging in the top center of the laboratory cage.

Eight treatments were evaluated: 1) cyfluthrin (CY-Kick CS, Whitmire Micro-Gen Research Laboratories, Inc., St. Louis, MO), 2) deltamethrin (Suspend SC, Bayer Environmental Science, Research Triangle Park, NC), 3) fipronil (Termidor SC, BASF, Research Triangle Park, NC), 4) imidacloprid (Premise 75, Bayer Environmental Science, Research Triangle Park, NC), 5) indoxacarb (Indoxacarb 150 SC, Du Pont, Wilmington, DE), 6) thiamethoxam (Optigard Flex, Syngenta, Greensboro, NC), 7) chlorantraniliprole (E2Y45 200 SC, Du Pont, Wilmington, DE), 8) cyfluthrin and imidacloprid combination (C&I). All products diluted to 1% active ingredient when mixed with bait solution, except cyfluthrin and imidacloprid combination that contained 1% of each AI. Bait solution contained 10% sucrose and 10% powdered milk in tap water. Controls consisted of wool yarn pieces dipped in tap water and dried overnight.

Experimental design was configured in a Latin square, conducted under continuous light at room temperature of 23°C.

Fifty female house flies were released in each laboratory cage and mortality of house flies was recorded 1, 2, 3, and 6 h. House fly mortality data was analyzed by one-way analysis of variance. Means were separated using Fisher's protected LS difference test (JMP 2007).

AI comparison: Choice laboratory bioassay. This experiment was conducted in laboratory cages used in AI no-choice bioassay. Three rubber bands were used to establish twelve positions for wool yarn pieces that were uniformly distributed along the top of the laboratory cage, four pieces of yarn per row. All pieces of yarn used were 10 cm in length and were attached vertically to the treatment positions using paper clips. One of the twelve pieces of yarn was treated with AI and randomly placed in center row. The AI-treated piece of yarn rotated position with each replicate.

Seven treatments were evaluated: 1) cyfluthrin, 2) deltamethrin, 3) fipronil, 4) imidacloprid, 5) indoxacarb, 6) thiamethoxam, 7) cyfluthrin and imidacloprid combination (C&I). All AI treatments and control pieces of yarn were prepared in same manner as in no-choice experiment. Experimental design was configured in a Latin square, but blocking for treatment position for AI impregnated yarn treatment. The experiment was conducted under continuous light at room temperature of 23°C.

Fifty female house flies were released in each laboratory cage. Two 59 ml containers with cotton wicks containing 10% sugar water were provided *ad libitum* at each end of the cage. Mortality of house flies was recorded 1, 2, 3, 6, and 24 h. House fly mortality data were analyzed by one-way analysis of variance; data was arcsine

square root transformed. Means were separated using Fisher's protected LS difference test (JMP 2007).

AI comparison: Field bioassay. This experiment was conducted in field cages (1.8 x 3.7 x 1.8 m; Outdoor Cage, #1412A, 18 x 14 mesh, Bioquip, Rancho Dominguez, CA) with translucent plastic sheeting (6 mil) used to cover the floor built on a grassy area shaded by pine trees. Food (1 L of 10% sucrose), water (1 L), and 60 ml container of spent oviposition media (covered with paper towel to prevent house fly access) were placed in the cages to provide nutrition and to compete with the treatments for fly activity.

Treatments consisted of FAST-TC (Figure 4-1) treated with different active ingredients. Each device was constructed with two blue corrugated plastic boxes (54 x 8 cm, Coroplast®, blue, number: C505L-8000.9000; Dallas, TX) each folded and hot glued into an open box with a 13 x 13 cm piece of blue plastic glued in place to close one side. The two boxes were connected by one 30 cm piece of wool yarn (Wool-Ease®, color# black 153; Lion Brand® Yarn Company, New York, NY.) centered on each side and hot glued into place. The top box was mounted inverted with the open end facing down and bottom box was mounted with the opening facing up. Across the open end of the bottom box two 13 cm pieces of wool yarn were crisscrossed and hot glued in placed over 170 g of food source (Calf Manna® pellets, Manna Pro Corp., St. Louis, MO).

Six active ingredients treatments were evaluated: 1) cyfluthrin, 2) deltamethrin, 3) fipronil, 4) imidacloprid, 5) indoxacarb, 6) thiamethoxam. All AI and control wool yarn

was prepared in same manner as in no-choice and choice bioassays. In the morning the experiment was started, AI-treated yarn was hot glued to its corresponding device.

All treatments were placed in the field cages 1 h after 350 house flies of mixed sex were released in each cage. Treatments were hung 1 m in front of the cage entrance and with the top of the filth fly control device 1.2 m from the floor of field cage. At 6 and 24 h fly counts were conducted, dead flies were collected from the floor of each cage and placed in a Mason jar (118 ml) to allow knocked down flies to recover in the cage. At each sampling time, flies on the floor of the cage, those remaining in the jar, and those collected in the bottom of the FAST-TC were counted. Percent mortality data for each time after trap placement was analyzed by one-way analysis of variance after arcsine square root transformation. Means were separated using Fisher's protected LS difference test (JMP 2007).

Wool cord vs. wool yarn. This experiment was conducted in laboratory cages (31 x 25 x 21 cm) constructed with PVC pipe (1.27 cm). A rubber band was used to establish individual treatment at the center of cages. Treatments consisted of 10 cm wool cord pieces (Twisted, Natural Cord, Wooded Hamlet Designs, Greencastle, PA; 0.6 cm diameter) and 10 cm wool yarn pieces (Patons® Classic Merino Wool Yarn, Ontario Canada, winter white, #N4W3H3). Nine pieces of wool yarn were braided to match the diameter of one wool cord. Wool cords and wool yarn were prepared using 2.5% imidacloprid bait (Maxforce® Fly Spot bait, Bayer CropScience, Kansas City, MO 64120). Cords and yarn pieces were dipped for 1 min in the insecticide solution and then dried overnight in fume hood. Treatments were secured in position vertically using paper clips, one treatment per cage. Controls consisted of 10 cm wool cord pieces that

were dipped in tap water and dried in fume hood overnight. Laboratory cages were enclosed with a transparent plastic bag (3716 cm², 1 mil poly, Uline, Waukegan, IL).

Thirty female house flies were released in each laboratory cage and allowed 1 hour for acclimation before first mortality count. Mortality of house flies was recorded at 1, 3, 5, and 24 h after fly release in cages. Four replications were conducted under continuous light at room temperature of 23°C. The experiment was conducted in a randomized complete block design. Mortality data was analyzed by analysis of variance, and means were separated using Fisher's protected LS difference test (JMP 2007).

Trap height test: indoor bioassays. Comparison of three trap heights simultaneously was conducted in laboratory room (4.8 x 20.1 x 2.7 m) with ambient temperature of 23°C. Treatments consisted of sampling cards (10 x 10 cm white corrugated plastic, Coroplast®, number: C505L-8000.9000; Dallas, TX) assembled with a piece of fly ribbon (Aeraxon® fly catchers fly strips, #0-18441-00005-3, The Tanglefoot Company, Grand Rapids, MI) pinned horizontally across each side. Sampling cards were arranged in three locations equally spaced from one another around the center of the room. Sampling cards were hung vertically from ceiling with fishing line at three heights of 0.91, 1.8, and 2.44 m simultaneously per location. The laboratory window was blocked out with aluminum foil and closed blinds to prevent external light from entering room. Entry door was sealed from inside with masking tape gasket to prevent house flies from escaping.

Three hundred house flies of mixed sex were released in the center of the room on top of a fixed, centrally-located counter (0.79 x 2.9 x 0.91 m). After the house flies were

released, the room was sealed and left undisturbed with continuous illumination by ceiling mounted fluorescent light. After 24 h, all sampling cards were collected and the captured house flies were counted for each height/location combination. Six replications were conducted.

Percentage of flies caught at each height was calculated by dividing the total number of flies caught at each height by the total number of flies released. Statistical analysis of percentage of house flies caught at the three heights was done with one-way analysis of variance after being arcsine square root transformed and means were separated using Fisher's protected LS difference test (JMP 2007).

Comparison of trap heights was also conducted at each height individually. This experiment was conducted in rearing rooms (2.6 x 3.5 x 2.7 m) with temperatures maintained at 23-26°C. Each room received a modified FAST-TC, consisting of only the bottom box, which was hung at the appropriate height. The trap was constructed with blue corrugated plastic (54 x 8 cm, Coroplast®, blue, number: C505L-8000.9000; Dallas, TX) folded and hot glued into an open box with a 13 x 13 cm piece of blue plastic glued in place as the box's floor. Across the open end of the blue box were two 30 cm pieces of black 2.5% imidacloprid-treated yarn that crisscrossed and was hot glued in place. Treated black yarn was prepared the night before by soaking in 2.5% imidacloprid (Maxforce® Fly Spot bait, Bayer CropScience, Kansas City, MO 64120) for 1 min. Untreated wool yarn was dipped in tap water. All wool yarn pieces were dried overnight in a fume hood before attachment to blue box. Treated black yarn extended the opening of the box forming a plus sign as well as extending over the center of box sides down to bottom edge of box. Blue boxes were hung open side up by fishing line

from ceiling and held 50 g of food attractant (Calf Manna ® pellets, Manna Pro Corp., St. Louis, MO). Rooms had no windows and the entry doors were sealed from inside with masking tape gasket to prevent house flies from escaping.

Three treatments were evaluated: 0.91, 1.8, and 2.44 m. Each treatment trap was hung from center of the ceiling in each room. The experiment was conducted using a 4x4 Latin square configuration. Three hundred mixed sex house flies were released in the center of room at floor level floor level and left undisturbed. Fly counts were taken after 24 h by counting dead flies recovered on the floor and from within the modified bottom box of the filth fly control device. House fly mortality percentage data was arcsine square root transformed and one-way analysis of variance was performed. Means were separated using Fisher's protected LS difference test (JMP 2007).

Trap suitable height tests: Semi outdoor experiments. Comparison of individual heights in semi-outdoor conditions were conducted in a four-room screened shade house (3.1 x 3.7 x 2.7 m per room) constructed with a metal frame and screen mesh walls and ceiling. Atmospheric conditions were sunny and clear with average temperature during the day of 29°C during the four days this experiment was conducted. Treatments consisted of a modified bottom box of the FAST-TC as described previously.

Three treatment heights were evaluated: 0.91, 1.8, and 2.44 m, and each modified trap was hung from center of ceiling in each screened room. The experiment was conducted using a 4x4 Latin square configuration. Three hundred mixed sex house flies were released in center of room at the floor level and left undisturbed. Fly counts were taken after 24 h counting dead flies recovered on floor and from within the

modified bottom box of the filth fly control device. House fly mortality percentage data was arcsine square root transformed and one-way analysis of variance was performed. Means were separated using Fisher's protected LS difference test (JMP 2007).

Results

AI comparison. House fly mean mortalities were not significantly different between indoxacarb (100%), fipronil (96%), cyfluthrin and imidacloprid combination (92%), and imidacloprid (92%) when house flies were given no choice of yarn pieces (Figure 4-2A). In all four treatments, the population was quickly reduced within 6 h. The mortality of cyfluthrin (88%) without the addition of the imidacloprid killed just about the same number of flies when combined with imidacloprid. Deltamethrin (56%) and thiamethoxam (70%) did not kill as many house flies in six hours but did eliminate over 50% of the population. However, chlorantraniliprole was significantly less effective than the other treatments with a mean mortality only matching the control.

When additional untreated pieces of yarn were added to the lab cages, the mean mortality was similar at 6 h between cyfluthrin and imidacloprid combination (84%), fipronil (86%), and cyfluthrin (78%) but these treatments caused significantly higher mortalities than the other treatments (Table 4-1). Thiamethoxam (48%), indoxacarb (42%), imidacloprid (38%), and deltamethrin (22%) killed about the same number for flies. Deltamethrin eliminated the lowest proportion of the house fly population.

The highest mean mortalities in the choice test at 24 h were caused by indoxacarb (94%), fipronil (100%), cyfluthrin and imidacloprid combination (98%), imidacloprid (90%) and deltamethrin (88%) (Figure 4-2B) with no significant differences. The lowest mean mortality was caused by cyfluthrin (82%) which was not significantly different from deltamethrin, thiamethoxam (84%), imidacloprid, or indoxacarb. The cyfluthrin and

imidacloprid combination were not significantly more effective than imidacloprid alone in 1 h, only knocking down 8% of the fly population. Cyfluthrin alone knocked down ~20% of the house fly population in 1 h but by 24 h had lower mortality than both imidacloprid and the combination of imidacloprid and cyfluthrin.

In the field cages with AI applied to FAST-TC, treatments reduced the house fly population by greater than 88% at 24 h compared to the 3% decrease of the control (Figure 4-2C). No significant difference was found between number of dead flies by deltamethrin (94%), thiamethoxam (97%), imidacloprid (94%), and indoxacarb (93%). There was no significant difference between fipronil (91%) and deltamethrin, imidacloprid, and indoxacarb. Cyfluthrin (89%) was not significantly different from fipronil and indoxacarb.

Wool cord vs. wool yarn. No difference was found between the uses of the wool cord as an insecticide delivery method verses the wool yarn (Figure 4-3). There was no significant difference at each mortality count. Both treatments eliminated ~70% of the fly population within 24-h compared to close to 0% mortality in the control.

Suitable height. In the laboratory test with the three heights simultaneously in the room significantly more house flies (~50%) were caught at 0.91 m (Figure 4-4A) than the other heights. There was no significant difference in the number of flies caught at 1.8 (~20%) and 2.44 m (~30%). There were no differences among the room locations of treatments.

Height test conducted with individual heights per room (Figure 4-4B) showed significantly more flies killed (95%) when traps were placed at 2.44 m, compared to 1.8 m (~70%), but neither height was significantly different from traps at 0.91 m that killed

80% of the flies. Individual heights evaluated in the semi-outdoor conditions of the screened shade house (Figure 4-4C) demonstrated there was no significant difference between the three heights having an average mortality rate of ~90% at 24 h.

Discussion

Varieties of house fly strains have shown resistance to tetrachlorvinphos, permethrin, cyfluthrin, and methomyl (Kaufman et al. 2001). Permethrin elimination for 30 generations of house flies under laboratory conditions stopped the natural selection for permethrin resistant house flies, and a reversal in insecticide resistance levels was observed (Meyer et al. 1987). A control technique to avoid flies developing insecticide resistance is to change the class of AI after set periods of time, such as one month rotations. In order for the FAST-TC to be a useful tool in fly control, a variety of active ingredients should be used to produce adequate fly mortality. This would allow AI rotation to avoid or counteract potential insecticide resistance in fly populations.

In the no-choice comparisons, seven out of eight AIs decreased the fly population in laboratory cages by >50% in 6 h. Chlorantraniliprole, which is anthranilimide insecticide, was the only AI that did not cause significant fly mortality. This insecticide was dropped from subsequent tests. Fipronil, a phenylpyrazole insecticide that causes insect death by ingestion and absorption through the cuticle is not registered for house flies. It caused no mortality within the first hour of the choice assay, but then close to 50% mortality at 3 h. Indoxacarb, an oxadiazine insecticide also not registered for house fly control, causes mortality of insects by ingestion and cuticle absorption. It caused mortality similar to that of fipronil. However, house fly reduction with indoxacarb did not reach 45% until 6 h.

Imidacloprid fly spot bait which is registered for house fly control kills flies by ingestion and is fast acting and has not shown any signs of house fly insecticide resistance (Gao et al 2007). In comparisons with spinosad and methomyl, house flies were affected as quickly as 30 min by the imidacloprid. However, house flies exposed to imidacloprid had a higher recovery rate than flies exposed to other insecticides (White et al. 2007). Additionally house flies force fed imidacloprid to house flies in laboratory test have shown insecticide resistance (Gerry and Zhang 2009). An attempt to enhance imidacloprid by combining it with cyfluthrin in laboratory conditions showed no synergistic or additive effect.

The FAST-TC eliminated >88% of the field cage house fly population within 24 h with all six AIs evaluated. Field cage findings suggest that the FAST-TC can be used with a wide range of AIs available on the market. This may prove useful for control of insecticide-resistant fly populations, or in prevention of insecticide resistance.

In an evaluation of natural and synthetic materials, wool proved effective in reducing house fly populations by 50% in 18 h when used as an insecticide treated cord (Hertz 2007). However, the handmade wool cord previously used was costly at ~\$5.10/m, whereas store bought 100% wool knitting yarn cost ~\$0.03/m. The comparison of the handmade wool cord and store bought wool knitting yarn of the same diameter showed that both materials eliminated ~90% of the house fly population.

Along with improvements to its design and material selection, the height the FAST-TC during its operation was evaluated. It has been demonstrated in field cage studies with light traps that twice as many house flies were caught at the height of 0.6 m than at higher heights (Driggers 1971). House fly monitoring devices in field conditions have

shown to be more effective at heights of 1 m or less (Geden 2005). Also, commonly used sticky fly traps have been shown to work best for other Muscidae at 0.3 m (Broce 1988). Typically, house flies feed and emerge from organic matter located on the ground (West 1951), which makes it logical that more house flies would be caught near the ground if all of the breeding and foraging sources are located there. When house flies were indoors and simultaneously given a choice of sugar-based attractants at three heights. The sticky cards hung at 0.91 m were nearest the floor where the house flies were released and the sticky cards hung at 2.4 m were near the visually attractive fluorescent lights mounted in the ceiling. This experiment suggests that house flies can be attracted to a colored target hung at any height.

Individual height tests conducted in rearing rooms demonstrated that there was no difference between the fly mortality at 0.91 m and the other two heights. This leads to the conclusion that the height is not important when hanging a fly control device inside a building. In semi-outdoor conditions in a screened shade house, there was no difference in house fly mortality at any of the three heights. In comparison of the three experiments, it was concluded that the FAST-TC at all three heights was visually attractive enough to lure foraging and resting house flies to it.

Using the combination of attractive colors, the FAST-TC can be constructed for house fly control. The FAST-TC can be effective with a variety of insecticides and can be constructed at low cost. In addition, when in use the FAST-TC is visually attractive to be used at a wide range of heights indoor or outdoor conditions.

Table 4-1. House fly mortality in choice laboratory cage study using twelve 10 cm pieces of black wool yarn distributed uniformly across ceiling of cage. One of the center four pieces impregnated with 1% AI and 10% sucrose and powdered milk.

AI	1 h	3 h	6 h
Deltamethrin	1.5 ± 0.3 bc	4.5 ± 3.0 de	11.3 ± 4.4 bc
Thiamethoxam	3.8 ± 1.1 b	15.0 ± 0.7 bc	24.3 ± 4.9 b
Cyfluthrin	10.0 ± 6.2 a	39.8 ± 7.6 a	38.8 ± 6.5 a
Imidacloprid	4.3 ± 1.3 b	13.3 ± 2.7 cd	18.5 ± 2.3 b
C&I	3.5 ± 0.3 bc	13.3 ± 0.6 cd	42.8 ± 2.0 a
Fipronil	0.0 ± 0 c	23.8 ± 4.0 b	42.8 ± 2.1 a
Indoxacarb	0.0 ± 0 c	4.5 ± 3.2 de	20.8 ± 8.9 b
	F = 7.19	F = 12.91	F = 10.87
	P = .0001	P < .0001	P < .0001

Treatments within a column followed by the same letter are not significantly different.

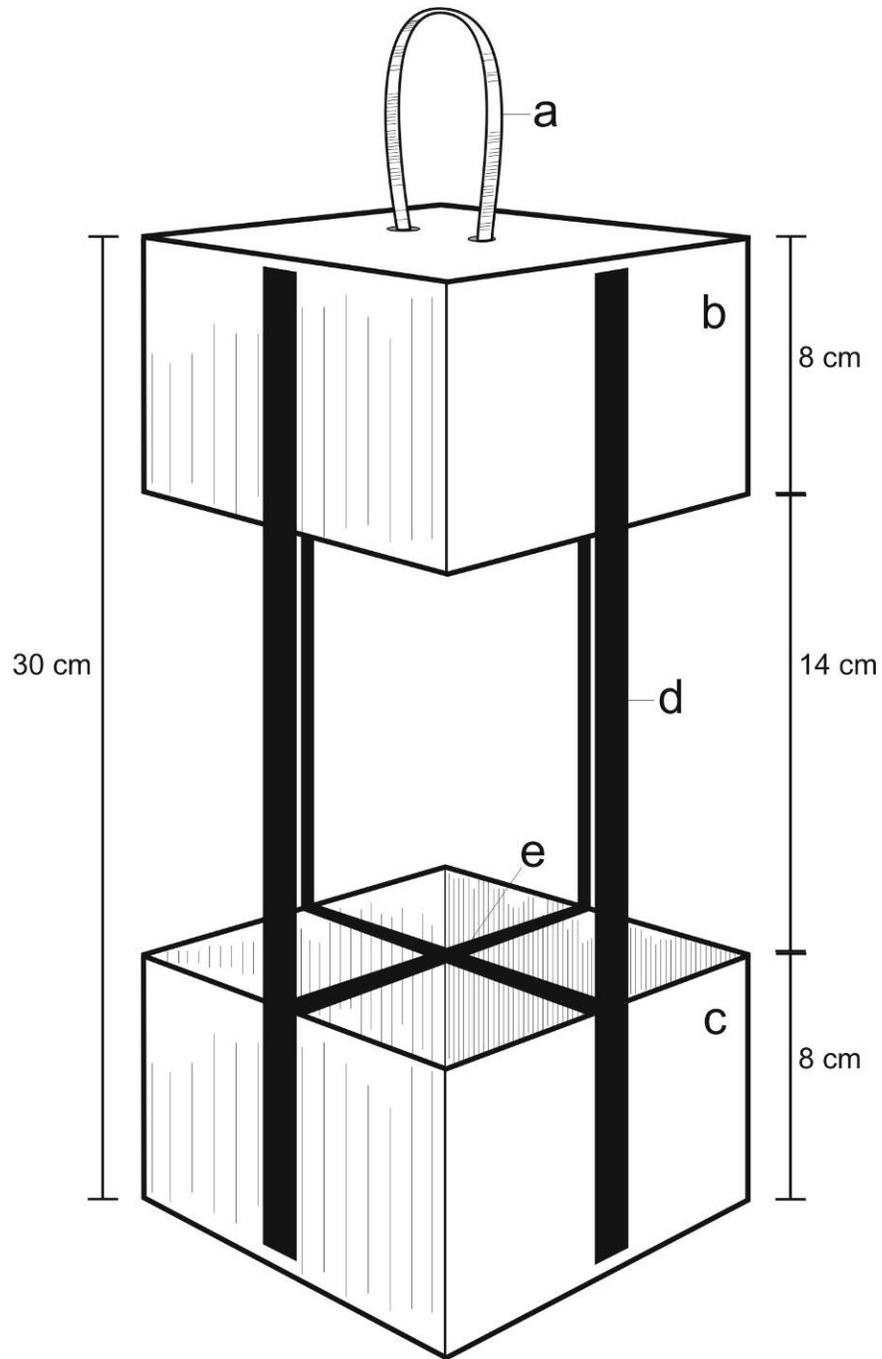
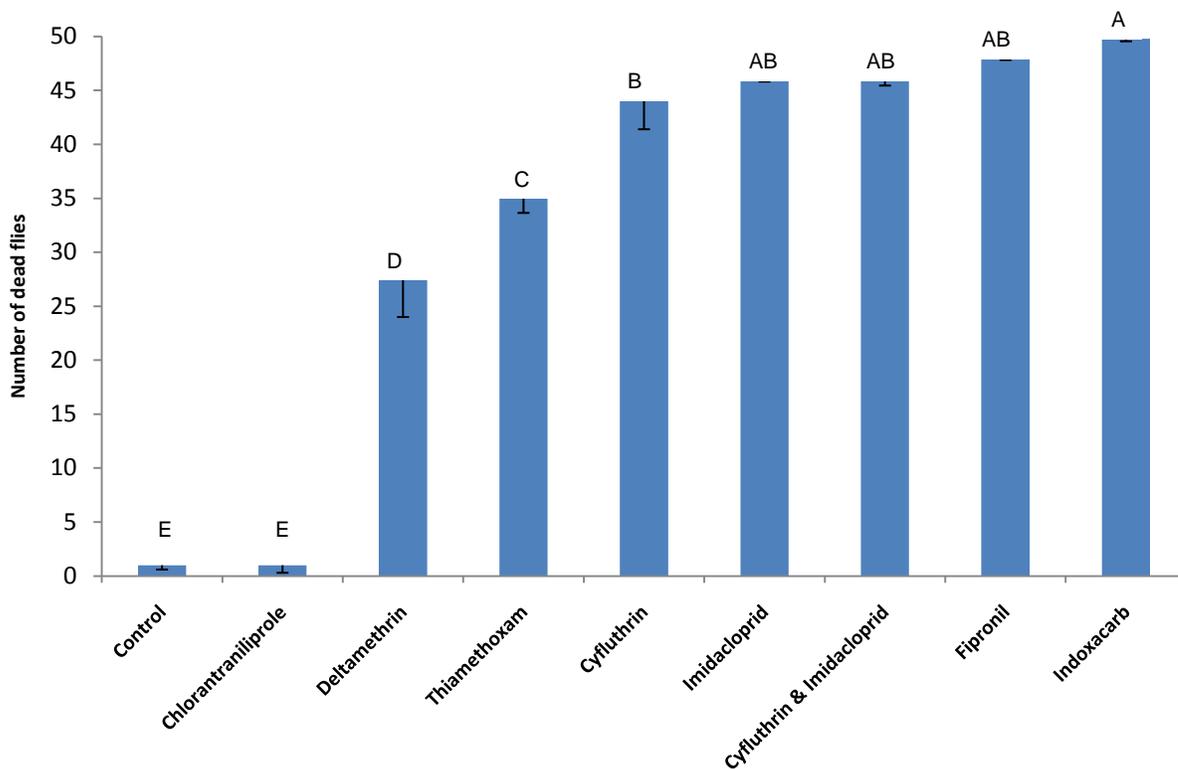
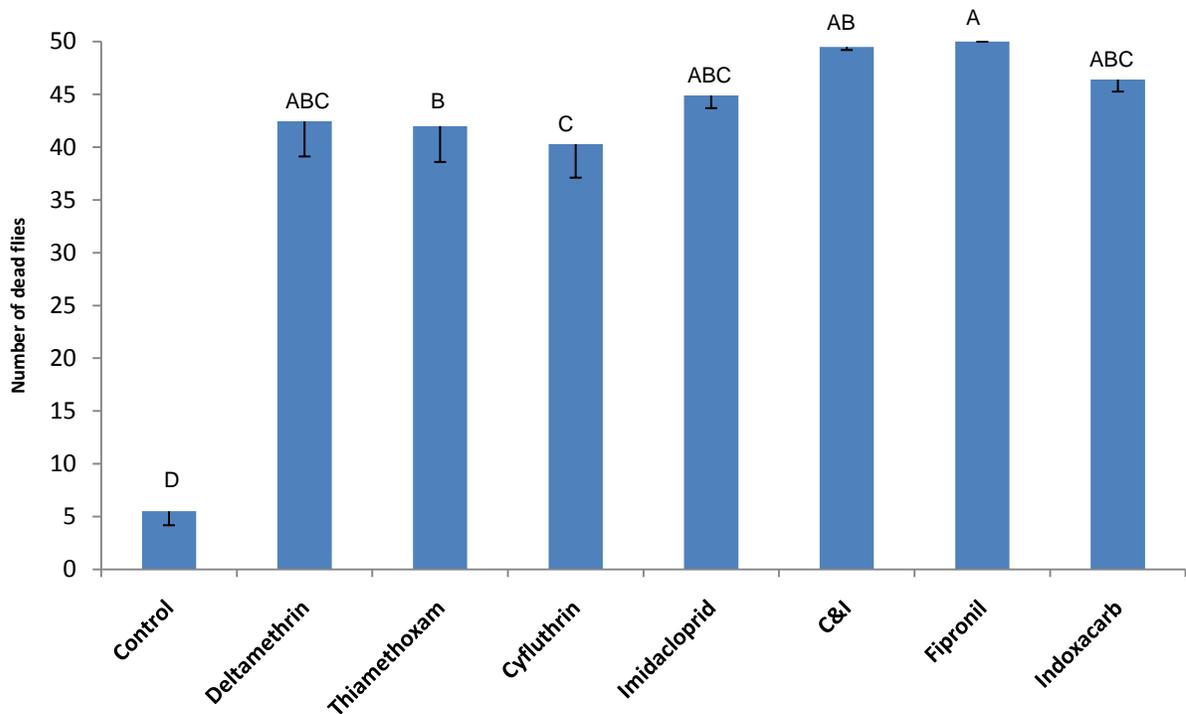


Figure 4-1. Schematic drawing of collapsible FAST-TC. a. Plastic cable for hanging. b&c. Blue corrugated plastic. d. Insecticide-treated wool yarn. e. Insecticide-treated wool yarn crisscross opening of box over food source.

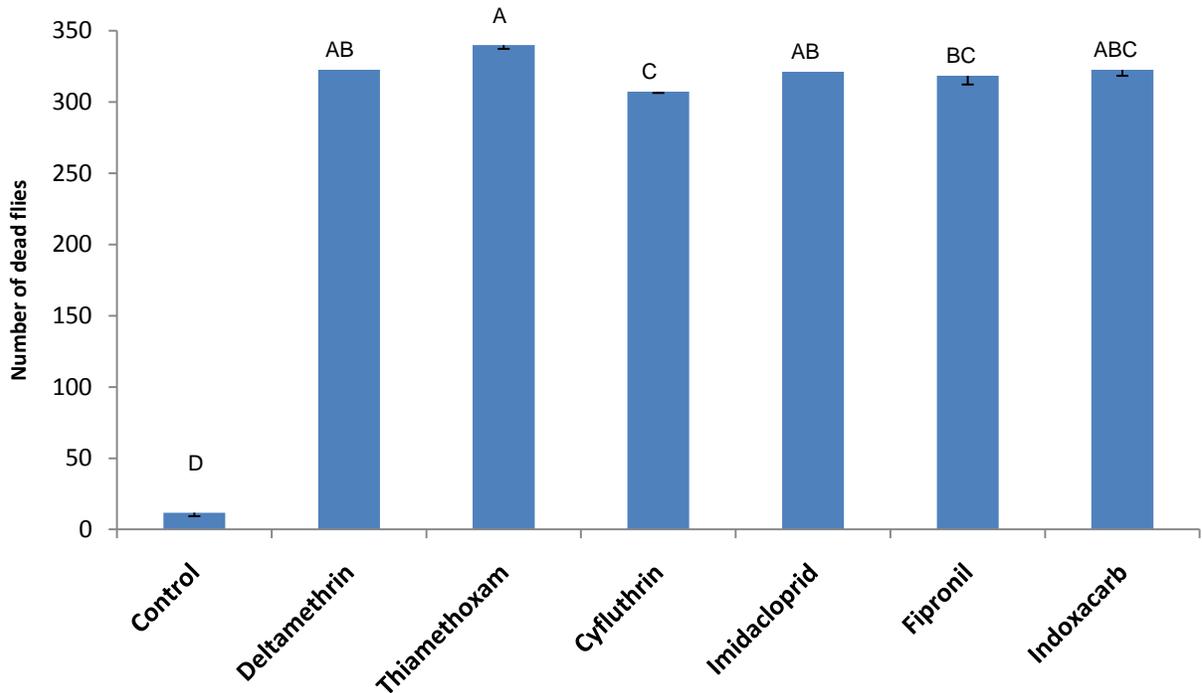


A

Figure 4-2. Mortality of flies exposed to cords treated with different active ingredient. A. No-choice laboratory cage study with solitary 10 cm black wool yarn impregnated with 1% AI, 10% sucrose, and 10% powdered milk. B. Choice laboratory cage study with eleven untreated 10 cm pieces of black wool yarn distributed uniformly across the cage, one center piece treated with 1% AI and 10% sucrose and 10% powdered milk. C. Field cage study with FAST-TC.



B



C

Figure 4-2. Continued

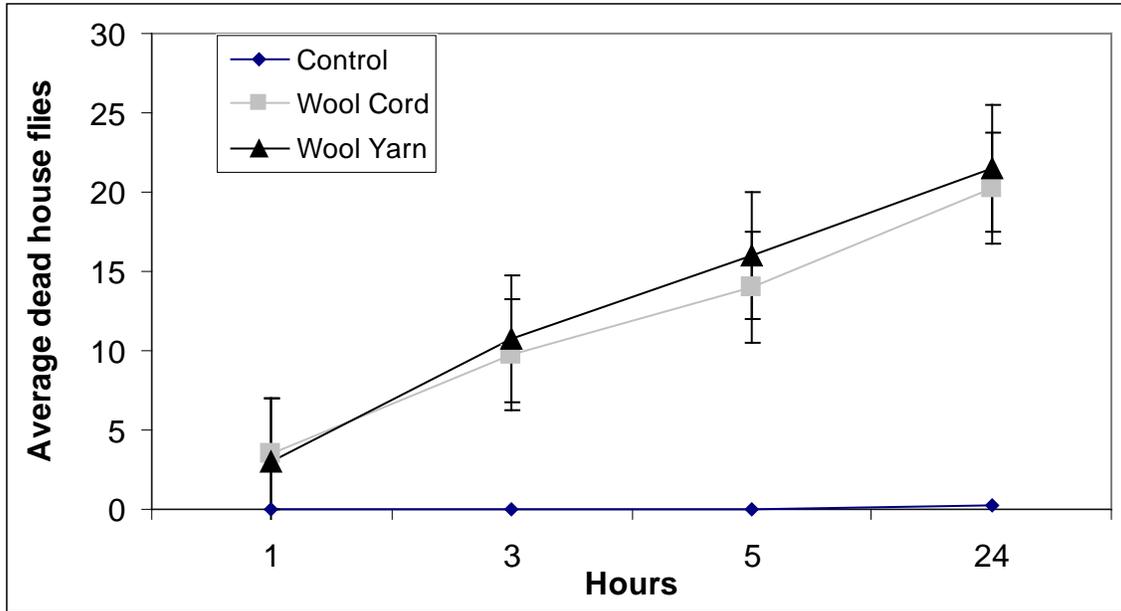


Figure 4-3. Mean number of dead house flies after 1, 3, 5, and 24 h exposure to wool cord or wool yarn treated with 2.5% imidacloprid fly bait.

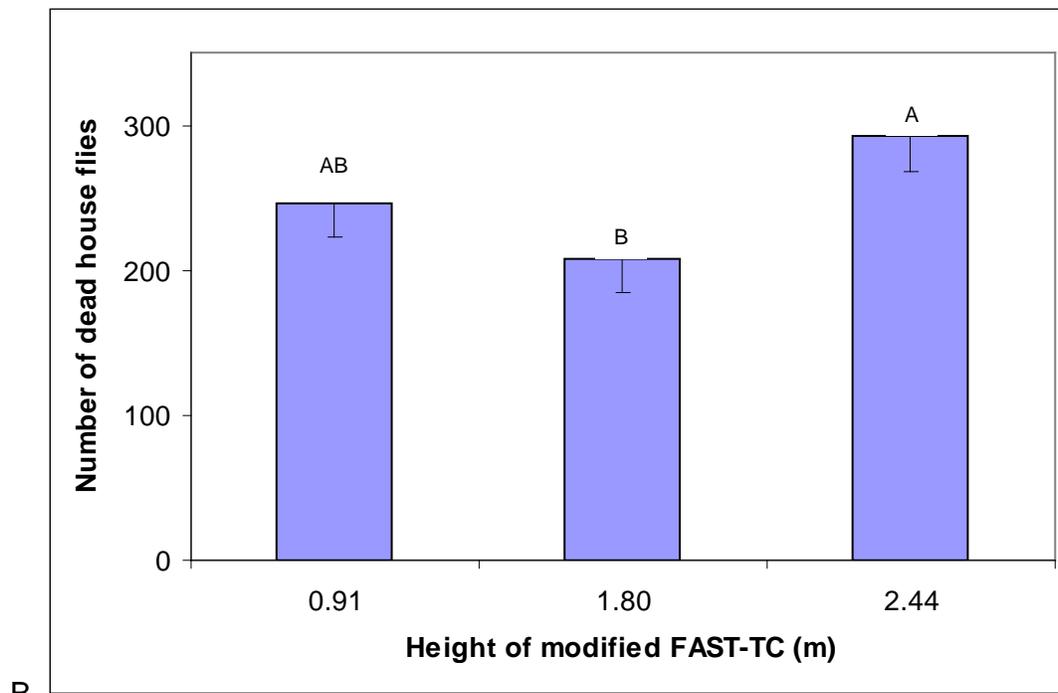
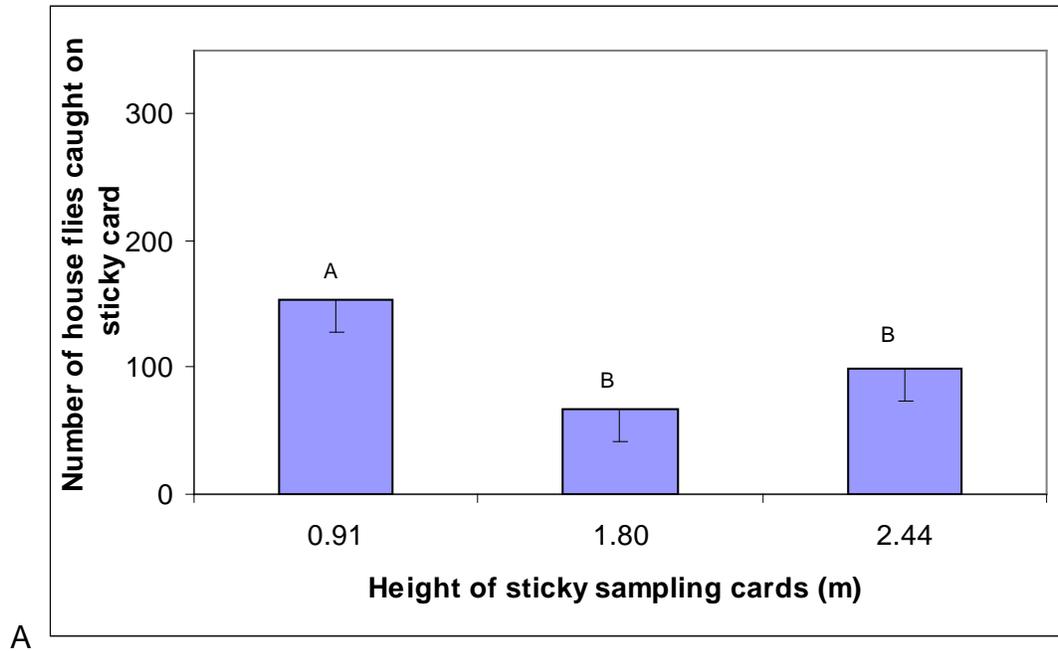
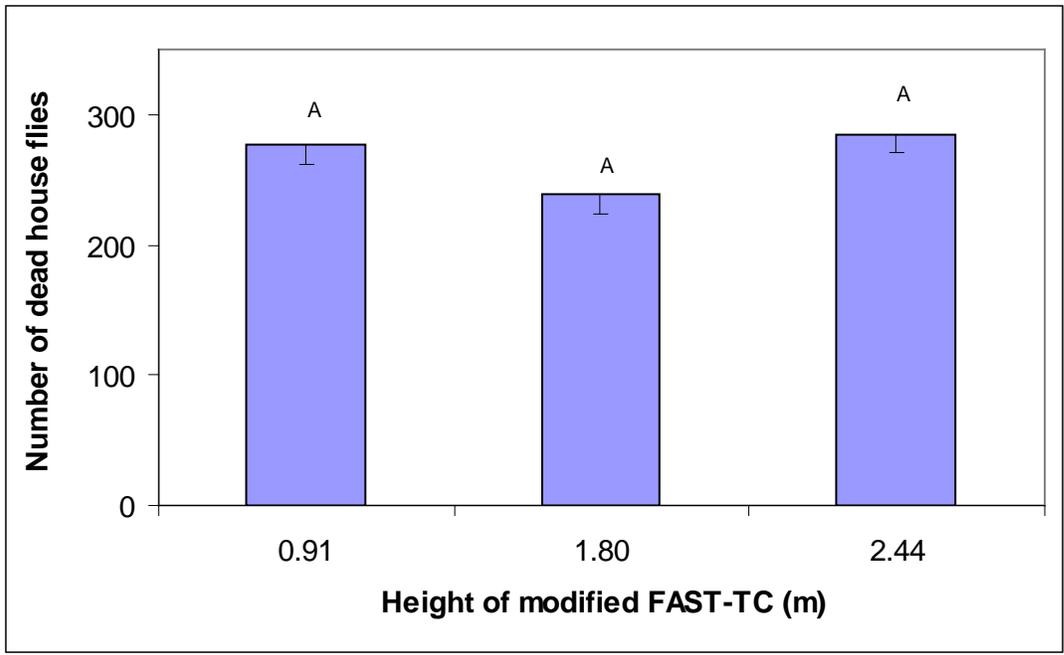


Figure 4-4. Indoor and semi-outdoor trap height comparisons over 24 h. A. Number of flies caught on sampling cards hung at three heights simultaneously indoors. B. Number of dead house flies from modified FAST-TC hung indoors. C. Number of dead house flies from modified FAST-TC hung semi-outdoors.



C

Figure 4-4. Continued

CHAPTER 5 FIELD EVALUATION OF A FLY ATTRACTANT SYSTEM WITH TOXICANT TREATED CORDS

Introduction

The house fly is an important pest in markets, stores, and animal facilities due mostly to its annoyance (Hogsette 2003). Controlling house fly populations is important because they can serve as mechanical vectors for one hundred known pathogens (Greenberg 1971). The scuttle flies are known to breed in minimal amounts of various decaying organic matter and are often a pest of dirty floor drains and mausoleums (Christenson 1988; Disney 2008). Whereas the blow fly is often associated with decaying animals and other high protein organic matter (Hall and Gerhardt 2009). The scuttle and blow flies are widely considered an annoyance pest, but can also cause facultative myiasis; an invasion of vertebrate tissue by the larval stages (Day et al 2004; Hall and Gerhardt 2009). After natural disasters and during wars, fly populations can become quite large, and control options may be limited (West 1951).

Baited fly traps are considered monitoring tools and cannot be used as a control method alone (Geden 2005). However, these fly traps are still commonly used to monitor fly populations during concurrent use of various control measures to determine the effectiveness of a management strategy. Baited traps work with two basic components: visual attraction and chemical attraction (Pickens 1995). The visual component attracts flies from a distance and the chemical attractant lures the flies into the trap.

The fly attractant system with toxicant treated cords (FAST-TC) was developed from research conducted at the University of Florida (Chapter 4). This device was constructed using a visually attractive blue corrugated plastic background with black

lines made of the insecticide treated wool cord (Hertz 2007). This objective of this study was to evaluate the field effectiveness of the FAST-TC in areas with known fly populations of the: 1) house fly, *Musca domestica*, 2) scuttle fly, *Megaselia scalaris*, and 3) blow fly, *Lucilia cuprina*.

Materials and Methods

Fly attractant system with toxicant-treated cords (FAST-TC)

Treated yarn. Treated yarn was prepared by soaking yarn (6 mm diameter, Wool-Ease[®], color# 153 black; Lion Brand[®] Yarn Company, New York, NY.) in 2.5% imidacloprid solution (Maxforce[®] Fly Spot bait, Bayer CropScience, Kansas City, MO) for 1 min and allowing the cords to drain and dry in a fume hood overnight.

Fly attractant system assembly. The fly attractant system was constructed with two cylinders connected by black insecticide-treated wool yarn. The cylinders were made with a blue 38 x 11 cm strip of plastic sign board (Coroplast[®], blue, number: C505L-8000.9000; Dallas, TX) and hot glued to form a 12.5 cm diameter cylinder. A 237 ml clear plastic deli cup (8 oz clear plastic container, #APCTR08; American Plastic Industries Ltd. Chattanooga, TN) was hot glued inside each cylinder with the opening pointed towards the center of the system. A single 13 cm piece of black treated yarn was extended across the center of the open end of the top cylinder and hot glued in place. Across the open end of the bottom portion, two 13 cm pieces of black treated yarn were crisscrossed and hot glued in place over 170 g of additional food source (Calf Manna[®] pellets, Manna Pro Corp., St. Louis, MO). The two cylinders were vertically connected by four evenly spaced parallel 30 cm long treated pieces of wool yarn extending from the top to bottom cylinders of the device, separated by a 11 cm space.

Field evaluation procedure. A FAST-TC was suspended 0.3 m from the ground over a plastic collection tray (46 x 76 x 10.5 cm) for five consecutive days in various field locations. The collection tray was placed under the device to catch dead flies falling from the FAST-TC to assess mortality estimates. Choice of location was determined by live population sampling prior to placement of FAST-TC, following the device placement suggestion of Lysyk and Axtell (1986). Live population sampling consisted of observing and counting live flies resting and landing in predetermined sampling areas for 5 min. For smaller fly species the use of sticky cards (Sticky Aphid trap, Seabright Laboratories Emeryville, CA) was employed. Fly counts and dead fly collections were conducted every approximately 24 h at the same time every day, beginning one day prior to FAST-TC placement, extending through five days of its deployment, and two days after its removal.

Flies were considered dead if found ataxic in the collection pan, in the bottom cylinder containing calf manna, or on the FAST-TC device itself. Dead flies were collected and placed into labeled zipper locked bags to be counted in the laboratory. Dead flies were collected each day after live fly counts were recorded. Treatment replicates consisted of a nine day period during which fly populations (live and dead) were monitored. Each day received a number representing the presence (+) or absence (-) of the FAST-TC, -1, 0, +1, +2, +3, +4, +5, -2, -3. The zero represented the day of FAST-TC placement. Days -1 and 0 were considered pre-treatment counts and days +1, +2, +3, +4, +5, -2 and -3 were considered post-treatment counts.

Field sites by species.

The house fly. There were three sites used to test FAST-TC for control of house flies. A herpetology facility in Alachua County, Florida that produces and distributes ~10,000 snakes per month had a large house fly population breeding around the facility's garbage dumpster. This dumpster was only emptied once every two weeks and contained organic debris including animal waste and snake corpses, providing optimal feeding and breeding sites for house flies. The garbage dumpster lid (lid area 2.6 x 1.5 m) served as the live fly sampling area at this facility. A 1 m Sheppard's hook was used to suspend the FAST-TC at this site over a collection pan 2 m on the north side of the dumpster.

The herpetology facility also operated a rat rearing building, also with waste receptacles located on its east side of its rat rearing building. These receptacles contained rat feces and used cage bedding, and were also emptied once every two weeks. However, they were occasionally overlooked by sanitation workers resulting in larger periods of accumulated waste. The live fly sampling areas near the rat rearing facility were marked on the top of two receptacles (0.8 x 0.8 m each), one on each side of the treatment location. A 1 m Sheppard's hook was used to suspend the FAST-TC over a collection pan 2 m to the east of the two receptacles used for live fly sampling.

In Chesapeake, VA a compost pile located in the backyard of a residential house maintained a continuous population of house flies. Two live fly sampling areas (0.5 x 0.5 m each) were marked on the ground with wooden stakes and string on each side of the compost pile. The FAST-TC was suspended from a 1 m Sheppard's hook over a collection pan 2 m on west center side of compost pile.

During military field exercises conducted at U.S. Army Fort McCoy, WI, military preventive medicine personnel evaluated the FAST-TC around their military berthing and dining tents. For evaluation at this site, the wool yarn on the FAST-TC was treated at the location by the Army medical entomologist. This was done using a spray bottle to apply a 2.5% imidacloprid spot bait suspension instead of dipping the cords prior to FAST-TC assembly. The FAST-TC were hung from the tent lines on the side of the dining tent and between the two berthing tents and were monitored following the field protocol.

The scuttle fly. The aforementioned Alachua County, FL, herpetology facility experimental site also had a severe infestation of scuttle flies which persisted for many years prior. The flies were present in the snake egg incubators and ovipositioned on dead snake eggs and other organic materials. The incubators provided optimal conditions that allowed *M. scalaris* to breed in high numbers (Trumble and Pienkowski 1979). Thousands of adult flies were released in the room whenever the incubators were opened, dispersing flies which bred on the snake fecal waste and in other incubation chambers.

The FAST-TC devices were evaluated in the two main snake rearing rooms (213 m² each) referred to as the Annex and Colubrid rooms. A wall separated the Annex and Colubrid rooms with a single door connecting them which was kept closed when not in use. Destructive sampling was used to monitor in-house live adult scuttle fly populations. Nine sticky cards were evenly distributed throughout each room with three cards located on each of two edge and one center aisle. Sticky cards were spaced evenly from one another, one card being in the center and one on each side of the aisle.

Sticky cards were placed in a vertical orientation on snake cage shelves, 1.5 m from floor. Sticky cards were removed and replaced every 24 h during sampling periods. Cards were returned to the laboratory where flies were counted under a dissecting microscope.

Due to staff traffic and normal operations of the facility, the FAST-TC in each room was hung from a plant hook 0.4 m from the wall over a collection pan. In the Annex, the FAST-TC was placed next to the west wall 0.2 m from the staff restroom and a door leading to a smaller rearing room. This location was near the southwest corner of the Annex and was 1 m from the closest set of shelves containing snake cages. In the Colubrid room, the FAST-TC was placed on the center of the south wall between two windows, directly 1 m from the south edge aisle of the shelves containing snake cages.

The blow fly. A carport located in Gainesville, FL, at a residential lot where the residents set out paper plates daily containing moist cat food experienced waves of blow fly invasions when cat food was present. Two of the plates used for the cat food were designated as live fly sampling areas. The FAST-TC was suspended from the railing of a boat parked in the carport, over a collection pan 0.5 m from the plates of cat food.

Data analysis. Live fly live population data was used to calculate the percent control for each treatment. Percent control was calculated as the average number of live flies sampled two days prior to FAST-TC placement (days -1 and 0) divided by the average live population over the seven days post-FAST-TC deployment (days 1, 2, 3, 4, 5, -2, and -3). A Paired t-test was performed on live fly numbers sampled during pre- and post-treatment. Average number of dead flies was calculated collectively by

species per sampling day. The total number of dead flies collected on a particular sampling day was divided by the total number of dead flies collected. Numbers of dead flies were not analyzed statistically.

Results and Discussion

The house fly. The FAST-TC was very effective in controlling house flies with an overall estimated control of >70% for all sites (Table 5-1). Approximately 50% of all flies were killed during the first 24 h of the FAST-TC deployment at each location (Figure 5-1). The herpetology facility had the largest number of house flies observed during this study. The house fly populations at the dumpster and behind the rat rearing facility declined rapidly after FAST-TC deployment. At both sites, the average number of dead flies collected everyday decreased while FAST-TC was deployed, suggesting a decline in the house fly population, as demonstrated by the estimated percent control. At the VA residential compost pile, FAST-TC also reduced the house fly population within the first 24 h. However, the average number of dead flies collected on the fourth day of FAST-TC deployment increased suggesting a new house fly emergence that was also eliminated within 24 h.

The FAST-TCs used at Fort McCoy during a U.S. Army field exercise were hung throughout the camp. Unlike the other field evaluations with the FAST-TC the insecticide was mixed in a spray bottle and sprayed on the wool yarn until it was saturated. The application of insecticide resulted in equal killing efficiency as when cords were dipped into pesticide solution. This demonstrates that the FAST-TC can be recharged if needed with insecticide spray applications. Two days after treatment was removed, a small percentage of house fly mortality was observed, likely due to the residual imidacloprid spot bait that was left behind on the tent cord.

The scuttle fly. The scuttle fly is the most medically important phorid fly as they are associated with sporadic cases of facultative human myiasis (Hall and Gerhardt 2009). Before FAST-TC deployment the average number of scuttle flies captured in a 24 h period on an individual sticky cards was >500. After five days of the FAST-TC deployment, the average number of scuttle flies captured on an individual sticky card was <20. The average overall estimated control for both snake rearing rooms was 79% (Table 5-1).

The FAST-TC efficiently reduced the scuttle fly population, killing >40% of the total number of scuttle flies in the first 24 h (Figure 5-2). Scuttle flies are opportunistic and can breed in small amounts of organic matter (Disney 2008). Prior to the FAST-TC testing, the herpetology use of insecticide or other control measures by facility staff was limited. Unsuccessful attempts to control the unmanageable fly population were made with aphid sticky cards. The sticky cards offered no noticeable control but did prove to be an excellent population-monitoring device while the FAST-TCs were used.

The blow fly. Blow flies are normally associated with dead animals and were highly attracted to during moist cat food as it likely mimicked carrion (Scholl et al 2009). The average estimated percent control for blow flies did not show reduction, although the on-site observations indicated that there were fewer flies in the area when the FAST-TC was deployed. The number of dead flies collected near the moist cat food while the FAST-TC was deployed also demonstrated that the blow fly population fluctuated (Figure 5-3). This may be due to blow fly emigration from off-site areas. *Lucilia spp.* usually appears within minutes on remains of animals (Anderson 2010). In

addition, the presence of the cat food was dependent on time of placement, and on the speed of consumption by the cats.

In summary, to reduce a filth fly population by 50-90%, traps must remove 24-58% over 24 h (Weidhaas and Haile 1978). Using the visually attractive blue background in combination with the insecticide-treated black yarn proved effective in controlling two species of filth flies. The FAST-TC demonstrated that it could be used in conditions where insecticide use is limited as well as having the ability to be recharged by spray applications when necessary. This novel approach can be refined as a useful tool for filth fly control.

Table 5-1. Estimated percent fly control calculated per FAST-TC location separated by fly species.

Replicate	Pre-treatment live sampling*	Post-treatment live sampling*	Percent fly control
House fly			
Garbage dumpster			
1	17.0	23.0	-35%
2	69.0	35.9	48%
3	23.5	15.1	36%
4	30.0	19.1	36%
5	26.0	18.4	29%
6	117.0	19.3	84%
7	169.0	18.4	89%
8	123.5	20.1	84%
9	130.0	20.3	84%
10	233.0	22.9	90%
11	206.5	20.4	90%
Rat rearing facility			
1	107.5	12.1	89%
2	92.5	10.9	88%
3	137.0	19.7	86%
4	61.5	34.7	44%
5	59.5	13.1	78%
Residential compost pile			
1	34.0	4.0	88%
2	16.5	2.4	85%
3	5.5	4.0	27%
Ft McKoy military camp			
1	10.0	3.7	63%
2	21.3	7.7	64%
3	1.7	1.5	10%
4	8.0	1.2	85%
5	11.0	0.8	92%
6	23.7	0.8	96%
7	11.0	1.5	86%
8	14.0	2.3	84%

Total mean pre-treatment count = 65.1

Total mean post-treatment count = 19.0

Total estimated house fly control = 71%

*Pre-treatment live sampling consist of sampling days -1 and 0.

**Post-treatment live sampling consist of sampling days 1,2,3,4,5,-2, and -3.

Bold numbers represent increase in fly population.

Table 5-1 continued.

Replicate	Average Pre-treatment count*	Average Post-treatment count**	Percent fly control
Scuttle Flies			
Annex snake rearing room			
1	1284.5	297.6	77%
2	756.5	127.6	83%
3	334.0	108.6	67%
CLB snake rearing room			
1	267.0	99.9	63%
2	193.5	309.9	-60%
3	968.0	122.6	87%
4	1772.5	360.3	80%
5	1660.0	579.1	65%
6	1163.0	686.4	41%
7	1484.0	730.1	51%
8	908.0	763.9	16%
9	1751.0	796.3	55%
10	745.0	790.0	-6%
Total mean pre-treatment count = 1022.0			
Total mean post-treatment count = 213.5			
Total estimated scuttle fly control = 79%			
Blow flies			
Near moist cat food			
1	16.0	28.0	-75%
2	48.5	10.1	79%
3	8.5	23.4	-176%
4	81.5	34.1	58%
5	7.0	45.3	-547%
6	11.0	46.4	-322%
7	12.5	43.6	-249%
8	8.0	38.6	-382%
9	15.5	5.6	64%
10	15.5	5.7	63%
11	5.5	4.1	25%
12	7.0	5.6	20%
13	6.0	5.7	5%
14	2.0	5.7	-186%
15	7.5	5.7	24%
Total mean pre-treatment count = 16.8			
Total mean post-treatment count = 11.1			
Total estimated blow fly control = 34%			

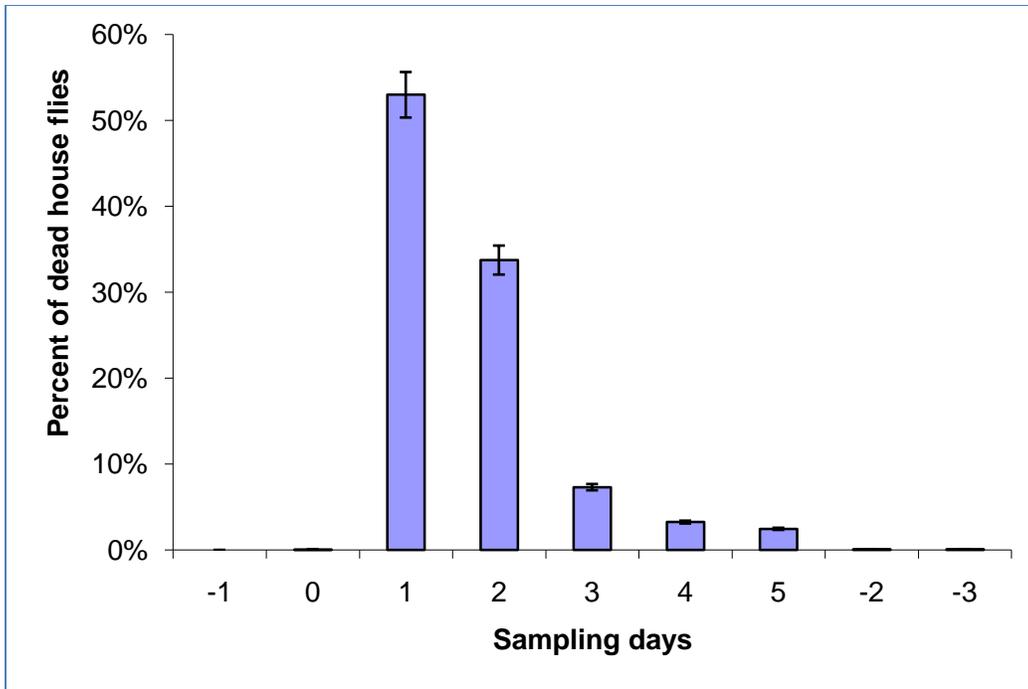


Figure 5-1. Daily percent of dead house flies collected under FAST-TC in relation to total dead flies collection (based on 44,598 total flies).

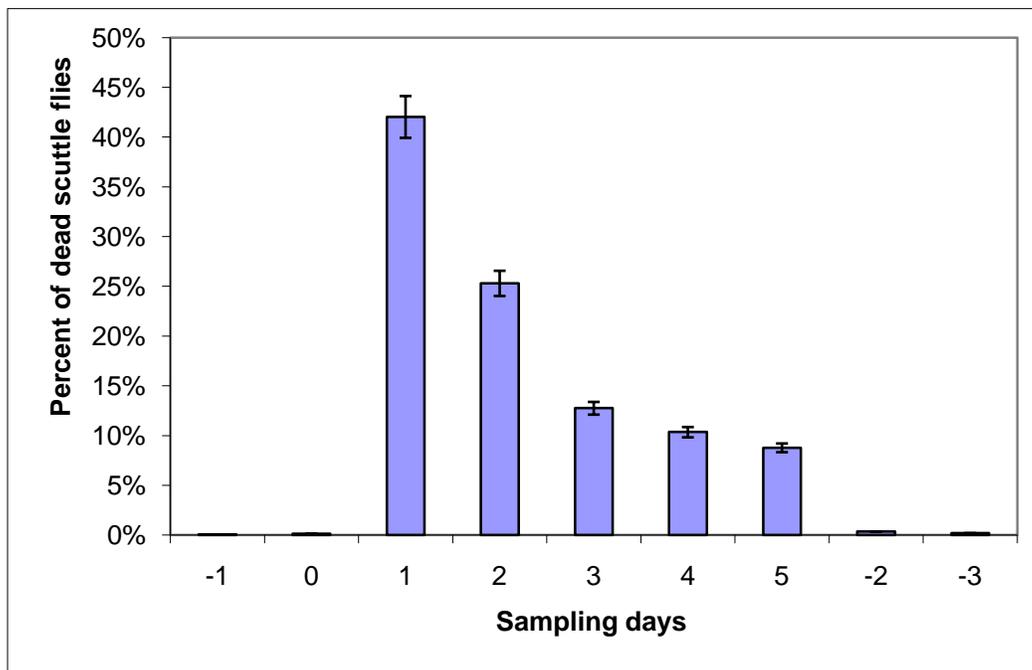


Figure 5-2. Daily percent of dead scuttle flies collected under FAST-TC in relation to total dead flies collection (based on 61,705 total flies).

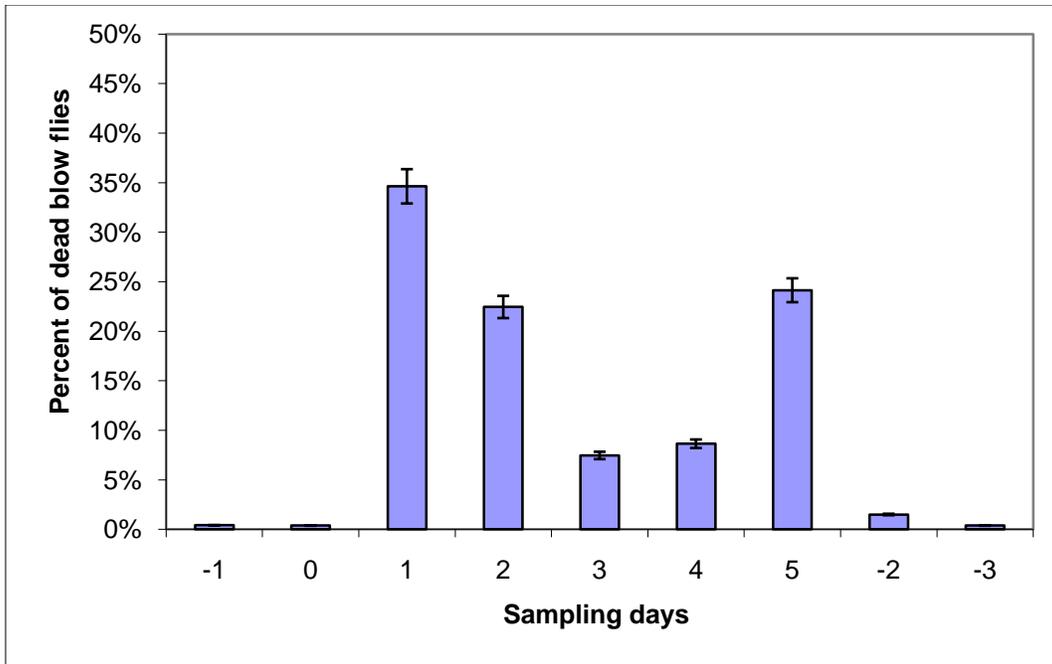


Figure 5-3. Daily percent of dead blow flies collected under FAST-TC in relation to total dead flies collection (based on 3,136 total flies).

CHAPTER 6 CONCLUSION

House fly control is best achieved using integrated management strategies combining cultural, mechanical, biological, and chemical control methods. Typically, in an effort to break the life cycle chain of house flies the removal of fly breeding and resting areas is achieved with sanitation. When sanitation fails insecticides are utilized to spray or bait areas, where flies feed or rest. The development of the fly attractant system with toxicant treated cords (FAST-TC) gives the ability to target flies that are seeking foraging or harborage sites while utilizing minimal amounts of insecticides. In addition, upon removal of the FAST-TC no residual chemicals will be left to be harmful to the environment.

House flies perceive reflected sunlight from objects as visual input for survival. This gives flies the ability to navigate through their environment to avoid obstacles while flying, find food, find harborage, and be able to recognize resting areas. Neurological responses of house fly's compound eye and ocelli were measured from reflected light from colored visual targets using an electroretinogram. Light of the same intensity reflected from multiple plastic visual targets showed that house fly compound eye and ocellus were capable of color vision. The greatest neurological responses for the compound eyes and ocelli were to white, blue, and yellow (Chapter 3).

Behavioral responses to colored plastic and fabric visual targets compared to black were determined using a two-sided light tunnel. The house fly attraction to colored visual targets was White = Blue > Red > Green > Yellow. In a paired test the blue visual target was more attractive than white. Yellow visual targets proved to be repellent to house flies. An observation of more house flies on the black visual target

side of the two-sided light tunnel when compared to yellow further demonstrated the yellow visual target repellency. The addition of yellow lines on to an attractive surface also repelled flies. However, the addition of black lines, which simulated a harborage area, enhanced the attractiveness of the blue background. The visual attraction to the black lined blue background suggests that house flies are able to form images to guide their behavior (Chapter 3).

Combining visually attractive color patterns with previously studied wool insecticide treated cords led to the construction of the FAST-TC (Chapter 4). In a laboratory cage study with house flies, knitting wool yarn performed as well as previously used wool cord as a insecticide delivery method. The comparison of active ingredients, (deltamethrin, thiamethoxam, imidacloprid, indoxacarb, fipronil, and cyfluthrin) applied to FAST-TC in field cages showed a reduction of house fly population >88% in 24 h compared to the 3% decrease of the untreated control. Bioassay conducted with sticky cards hung simultaneously at three heights caught 50% of the house flies released in a laboratory room at the lowest level, demonstrating that house flies tend to fly low. Modified version of the FAST-TC was able to attract house flies that were foraging at the low heights and flies that were seeking resting areas at the higher heights.

The FAST-TC was field tested and with very low amounts of insecticide and was able to control two species of flies in a variety of conditions (Chapter 5). Due to the efficiency that the FAST-TC demonstrated in the field in controlling house, scuttle, and blow flies the University of Florida submitted a patent application for this device.

The research conducted here to develop the FAST-TC will provide a novel approach in the control of filth flies and will provide a useful tool in the arsenal of fly control. Hopefully this research will stimulate further investigation of fly vision to help in the improvements and development of other control measures.

APPENDIX A
EFFECTIVENESS OF FLY TRAPS AND TREATED CORDS IN CATCHING AND
KILLING HOUSE FLIES (DIPTERA: MUSCIDAE)

Introduction

There is some debate whether baited traps fit into a fly management program as monitoring or control devices. To reduce a fly population by 50-90%, traps must remove 24-58% of the house flies daily (Weidhaas and Haile 1978); however, traps alone have not been shown to reduce fly populations to acceptable levels (Smallegange 2004).

Insecticide-treated cord can also be used to control house flies. Cords impregnated with organophosphate and chlorinated hydrocarbon insecticides were introduced in 1947 and were found to provide good house fly control (Smith 1958). However, use of treated cords fell in popularity due to the ease of applying residual insecticide sprays to structures. Low concentrations of new insecticides, such as 0.1% fipronil and 1.2% indoxacarb impregnated in cords (Hertz 2007), have been shown to effectively kill flies in field cages.

The objective of this study was to evaluate the enhancement of a fly trap using insecticide-treated cord to control house flies in field cages.

Materials and Methods

Insects. For the laboratory experiments, house flies were collected from the University of Florida Horse Teaching Unit about 1 month prior to experiment, reared in the laboratory, and used at the F3 through F5 generation. Flies were reared following a method modified from Hogsette et al. (2002) with no powdered egg yolk in the adult diet, and Calf Manna (MannaPro Products, Chesterfield, MO) substituting for alfalfa meal and corn meal in the larval diet. Larvae and adults were held in separate

containers at $26.2 \pm 0.5^\circ\text{C}$ and $51.2 \pm 3.5\%$ RH with a 12:12 (L: D) photoperiod. When collecting flies from the colony, 3 to 5 d old house flies were aspirated and placed in a freezer (-30°C) until inactive (~1-5 min).

Fly trap enhancement with treated cord. Enhancement of fly traps with treated cords was evaluated on populations of flies established in field cages. Field cages (1.8 x 3.7 x 1.8 m; Outdoor Cage, #1412A, 18 x 14 mesh, Bioquip, Rancho Dominguez, CA) with translucent plastic sheeting (6 mil) used to cover the floor were built on a grassy area shaded by pine trees. Food (1 L of 10% sucrose), water (1 L), and 60 ml container of spent oviposition media (covered with paper towel to prevent fly access) were placed in the cages to provide nutrition and to compete with the treatments for fly activity.

Musca-Doom Disposable Fly Traps (Farnam Companies, Inc. Product code 057117; Phoenix, AZ 85067) (identical to Trap n' Toss fly traps) were used in this experiment. The label sticker was removed from each trap to allow maximum visual fly response to the trap. Six treatments were evaluated: 1) fly trap, no attractant, untreated wool cord, 2) fly trap, attractant, no cord, 3) fly trap, attractant, untreated cord, 4) fly trap, attractant, treated cord, 5) fly trap, no attractant, treated cord, and 6) no fly trap, no attractant, treated cord hung in the shape of a halo. Treatments with attractant were prepared the morning of the experiment by filling fly traps with 5 ml of MuscaDoom attractant solution mixed, as directed by the manufacturer, in tap water.

Treated cords were prepared using imidacloprid bait (Maxforce® Fly Spot bait, Bayer CropScience, Kansas City, MO 64120) with 25 g of product dissolved in 100 ml of tap water. Wool cord pieces (Twisted, Natural Cord, Wooded Hamlet Designs, Greencastle, PA; 0.6 cm diameter) were cut to the length (46 cm) to match the

circumference of the outside center of the fly trap and treated by dipping for 1 min in the insecticide solution. Untreated cords were dipped in tap water. All cords were dried overnight in a fume hood. Cords were then wrapped around the fly traps or formed into a halo for the treatment without traps.

All treatments were placed in the field cages 1 h after ~ 300 flies (35 mL volumetrically) were released in each cage, corresponding to ~ 45 flies per m² of cage area. Fly traps were hung 1 m in front of the cage entrance and 14 cm from the cage ceiling; the cord halo was hung 28 cm from the cage ceiling. At 1, 24, and 48 h after trap placement, flies were collected from the floor of each cage and placed in a Mason jar (118 ml) to allow knocked down flies to recover in the cage. At each sampling time, flies on the floor of the cage, those remaining in the jar, and those collected in traps were counted. After mortality data were collected, a fly annoyance index was estimated by a person walking around the inside of the field cage for one complete revolution while tapping the cage mesh. During the following 1 m, annoyance was rated using the following scale: 1 = no flies (0 per m²), 2 = few flies seen in cage (~10 per m²), 3 = several flies around face and arms (~20 per m²), 4 = many flies around face and arms (~30 per m²), 5 = numerous flies around the face, arms (~40 per m²), and many seen in cage. Mortality data for each time after trap placement were analyzed by analysis of variance, and means were separated using Student Newman-Keuls test (P = 0.05; SAS 2001). Linear regression was used to correlate the annoyance index with the number of flies observed in cage.

Results

Flies released into field cages initially oriented to the top sides and corners of the cage. Eventually, flies were attracted to the traps or cords, food, water, and oviposition

media. Within 1 hour, significant differences in fly mortality ($df = 5$, $F = 5.12$, $P = 0.0016$) were seen among treatments (Figure A-1); treatments with treated-cords had higher mortality (21%) than those with traps alone (1%). After 24 h, all treatments containing treated cords had significantly higher mortality (68%) than fly traps alone (12%) ($df = 5$, $F = 16.82$, $P < 0.0001$). There were no significant increases in trap kill when attractant was added to either the trap alone (14% with attractant vs. 6% without attractant) or traps with treated cords (64% with attractant vs. 70% without attractant). After 48 h, there was a significant difference between the treatments with treated cords (97% fly mortality) and the ones without cords (17% fly mortality) ($df = 5$, $F = 87.41$, $P < 0.0001$). Traps with treated cords killed 97% of flies; whereas, traps without treated cords killed only 17%. The annoyance index (Figure A-2) decreased as flies per m^2 decreased; traps with the treated cords decreased the annoyance index to 1 after 48 h.

Discussion

Whether baited house fly traps can be efficient as a control device or just a monitoring device depends on the house fly population reduction it can provide (Weidhaas and Haile 1978). This experiment showed that, total catches with commercial traps were within a range insufficient to provide fly population control. Traps used according to the manufacturer's instructions only retained a very small percentage of the fly population after 48 h. Therefore, trap performances must be enhanced if they are to be used for control, rather than as monitoring devices only.

The addition of an insecticide element, such as imidacloprid treated cords, increased fly trap efficacy. Imidacloprid has residual effects (Pospischil et al. 2005) that in combination with the wool cord reduced the fly population quickly. In this experiment, the treatments with the imidacloprid-treated wool cord also reduced the fly population

quickly as observed previously with imidacloprid use (White et al. 2007). The fly annoyance index was negatively correlated with fly mortality in cages; as flies per m² decreased the annoyance decreased. Furthermore, the treated wool cord alone was shown to be just as effective as the traps with the treated cord addition, demonstrating that the trap itself was not a critical factor in reducing the fly population.

This research has demonstrated that house fly trap design is important for attraction, trapping, and retaining flies, and that these factors can greatly influence whether a trap can be an efficient method for fly population reduction. Once a house fly has been attracted to and trapped in a fly trap, the traps' capability to remove attracted insects from the infesting population can be improved by increasing the traps' killing power. The addition of insecticide-treated cord is a simple method to improve traps efficacy in the field, and may provide sufficient improvement to make house fly traps into control devices rather than simple population monitor. However, insecticide-treated cords alone or in combination with other visual and odor attractants, but without a trapping mechanism, may cause sufficient fly mortality to provide efficient fly control.

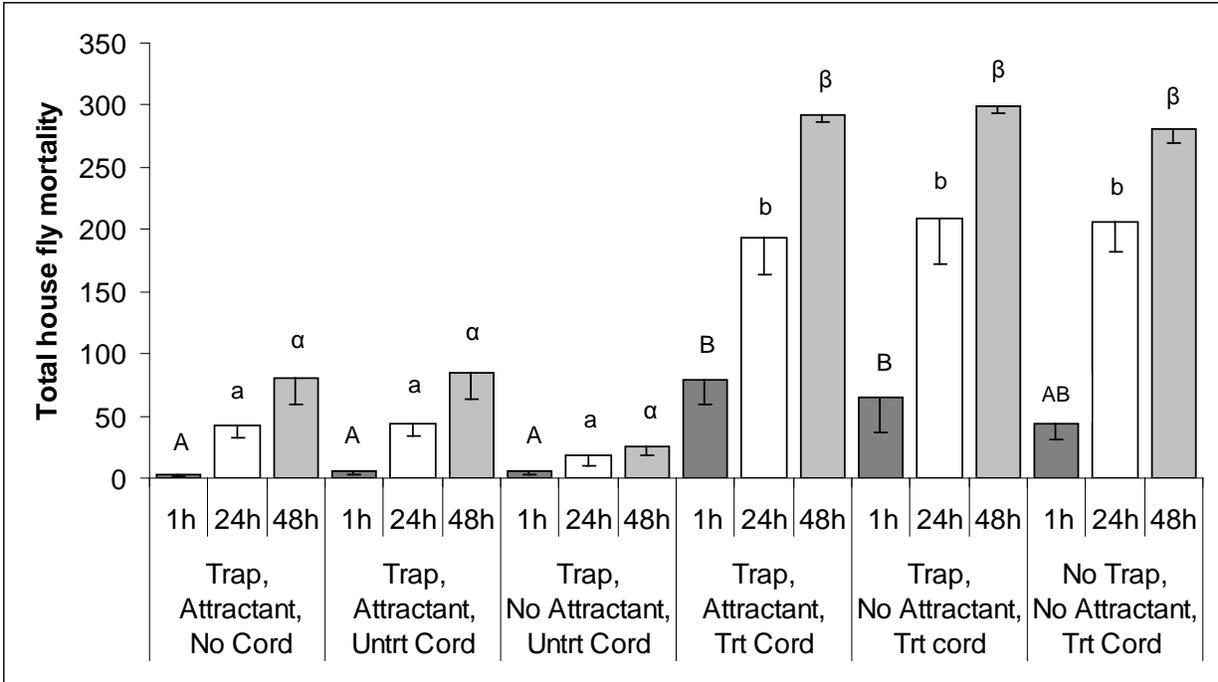


Figure A-1. Mortality of house flies at 1, 24, and 48 h after initial exposure to different fly trap treatments in field cages.

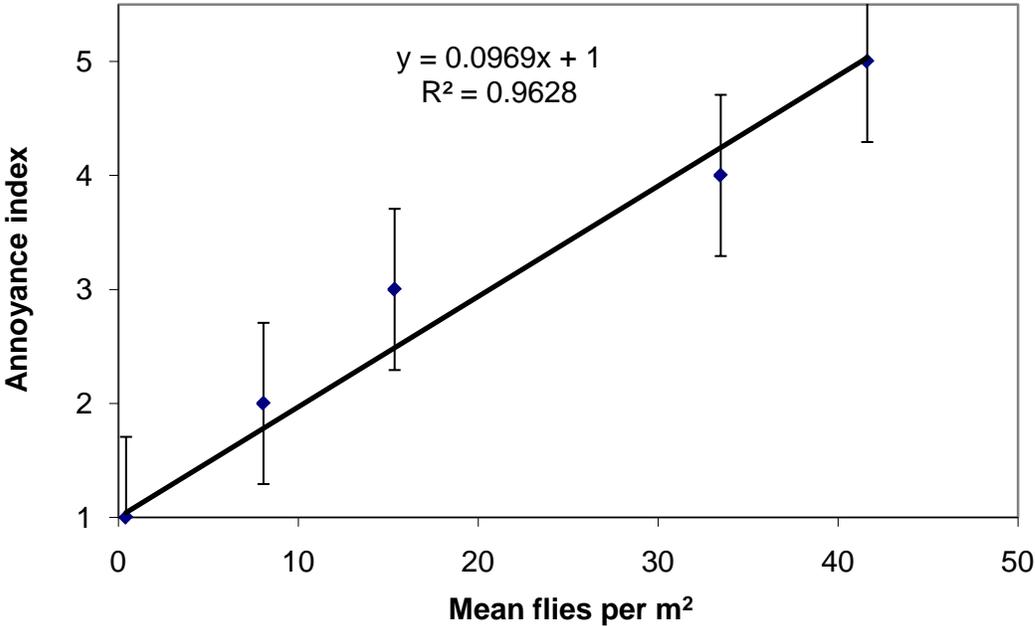


Figure A-2. Correlation between annoyance index and number of surviving flies observed in field cages.

APPENDIX B
CONCISE DESCRIPTION OF THE INVENTION

Fly Attractant System with Toxicant-Treated Cords

This fly attractant system incorporates a visual and chemical attraction to lure nuisance flies to the device. From a distance, flies are attracted visually to the color blue and the edges of the device. As the fly approaches the device the black yarn against the blue background, give the appearance of a crack or crevice, which a fly will perceive as shelter and fly even closer. Once the fly is in close proximity to the device the chemical attractant lures the fly in closer attracting the fly to land on the device with the assumption it is a food source. The fly will investigate the black yarn and will feed. Once it feeds on the yarn that is treated with imidacloprid insecticide, the fly will die within seconds to minutes.

Treated yarn. Treated yarn was made by soaking yarn (Wool-Ease®, color# black 153; Lion Brand® Yarn Company, New York, NY.) in 2.5% imidacloprid bait (Maxforce® Fly Spot bait, Bayer CropScience, Kansas City, MO 64120) for 1 min.

Fly attractant system assembly. Cylindrical fly attractant system with toxicant-treated cords is constructed with two cylinders connected by black insecticide-treated wool yarn. The top portion of the attractant system was made with blue plastic sign board (Coroplast®, blue, number: C505L-8000.9000; Dallas, TX) cut in 38 x 11 cm strip and rolled to form a cylinder with the diameter of 12.5 cm, with ends hot glued together. A 237 ml clear plastic deli cup (8 oz clear plastic container, #APCTR08; American Plastic Industries Ltd. Chattanooga, TN) was hot glued inverted to the bottom of the top portion of the device. A 13 cm piece of black treated yarn was extended across the center of the open end of the deli cup and glued in place with hot glue. The bottom

portion of the attractant system was prepared as described above for the top portion with a 38 x 7 cm blue plastic sign board. A deli cup was hot glued inside the bottom portion of the device. Across the open end of the bottom portion two 13 cm pieces of black treated yarn were crisscrossed and hot glued in place. The two cylinders were connected by four evenly spaced parallel 30 cm long treated pieces of wool yarn 5 mm in diameter extending from the top to bottom portions of the device, which were separated by a 11 cm space.



Figure B-1. Cylindrical fly attractant system with toxicant-treated cords.



Figure B-2. Fly attractant system with toxicant-treated cords placed near fly infested garbage cans, which eliminated 3006 flies in 24 hours.

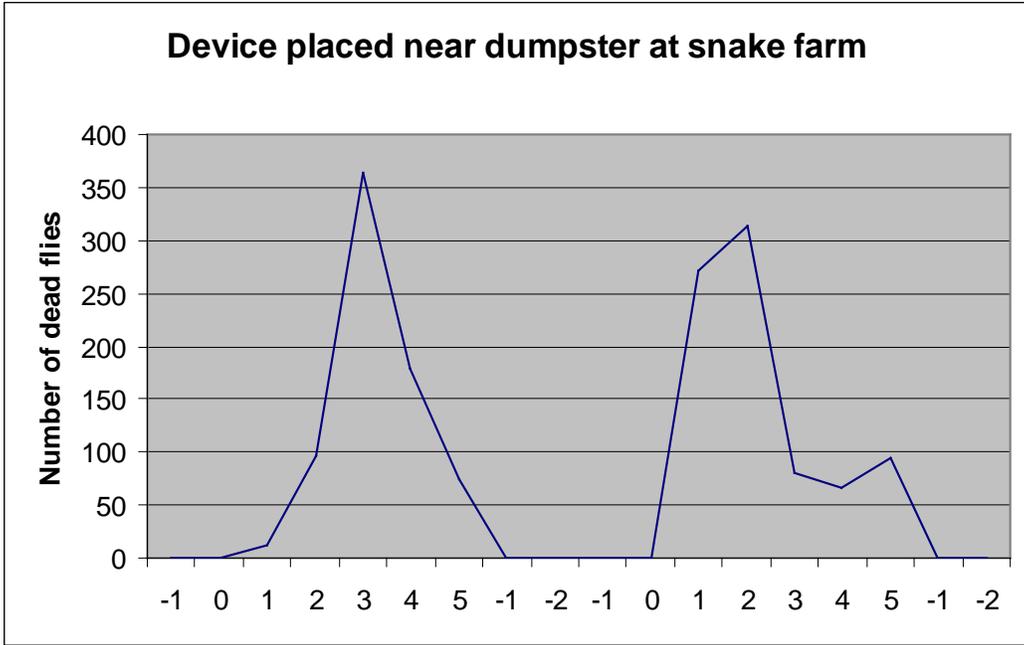


Figure B-3. Dead flies killed by the fly attractant system with toxicant-treated cords near a house fly infested dumpster.

LIST OF REFERENCES

- Agee, H. R. and R. S. Patterson. 1983.** Spectral sensitivity of stable, face, and horn flies and behavioral responses of stable flies to visual traps (Diptera: Muscidae). *Environ. Entomol.* 12: 1823-1828.
- Anderson, G. S. 2010.** Factors that influence insect succession on carrion, 201-250. In J. H. Byrd and J. L. Castner (eds). *Forensic entomology: the utility of arthropods in legal investigations*. CRC Press. Boca Raton, FL.
- Aubuchon, M. D. 2006.** Biological and physical factors affecting catch of house flies in ultraviolet light traps. Ph.D. dissertation. University of Florida, Gainesville, FL.
- Awati, P. R., and C. S. Swaminath. 1920.** Bionomics of houseflies. III. A preliminary note on attraction of houseflies to certain fermenting and putrefying substances. *Indian J. Med. Res.* 7:560-567.
- Baker, W. C., H. I. Scudder, E. L. Guy. 1947.** The control of house flies by DDT sprays. *Public Health Rep.* 62: 597-611.
- Bellingham, J. 1994.** A comparative study of the spectral sensitivity, antennal sensilla, and landing preferences of house-fly, *Musca domestica* (L.) (Diptera: Muscidae), and the lesser house-fly, *Fannia canicularis* (L.) (Diptera: Fanniidae). Ph. D. dissertation. University of Birmingham. Edgbaston, UK.
- Beresford, D. V. and J. F. Sutcliffe. 2006.** Studies on the effectiveness of coroplast sticky traps for sampling stable flies (Diptera: Muscidae), including a comparison to alsynite. *J. Econ. Entomol.* 99: 1025-1035.
- Brach, E. J. and R. M. Trimble. 1985.** Effect of adhesive on the spectral reflectance of insect traps. *Can. Entomol.* 117: 1565-1568.
- Broce, A. B. 1988.** An improved alsynite trap for stable flies, *Stomoxys calcitrans* (Diptera: Muscidae). *J. Med. Entomol.* 25: 406-409.
- Burg, J. G. and R. C. Axtell. 1984.** Monitoring house fly, *Musca domestica* (Diptera: Muscidae), population in cage-layer poultry house using a baited hug-trap. *Environ. Entomol.* 13: 1083-1090.
- Chapman, R. F. 2006.** *The insects: structure and function*, 4th ed. Cambridge University Press. Cambridge, UK.
- Christenson, C. 1988.** Phorid flies: tiny pests that pose big problems. *Pest Con. Tech.* 3: 52-57.

- Conlon, D. and W. J. Bell. 1991.** The use of visual information by house flies, *Musca domestica* (Diptera: Muscidae), foraging in resource patches. *J Comp Physiol A*. 168: 365-371.
- Day, J. F., J. D. Edman, S. E. Kunz, and S. K. Wikel. 2004.** Direct injury: phobias, psychoses, annoyance, allergies, toxins, venoms, and myiasis, pp 99-149. In B. F. Eldridge and J. D. Edman (eds). *Medical Entomology: A textbook on public health and veterinary problems caused by arthropods*. Kluwer Academic Publishers. Dordrecht, NE.
- Disney, R. H. 2008.** Natural history of the scuttle fly, *Megaselia scalaris*. *Annu. Rev. Entomol.* 53: 39-60.
- Driggers, D. P. 1971.** Field evaluation of blacklight electrocutor grid traps for the control of flies associated with poultry. Ph.D. dissertation, University of Florida. Gainesville, FL.
- Fehn, C. F. 1958.** House fly control at scout camps with insecticide impregnated cord. *Public Health Rep.* 5: 62-64
- Foil, L. D. and C. D. Younger. 2006.** Development of treated targets for controlling stable flies (Diptera: Muscidae). *Veterin. Parasitol.* 137: 311-315.
- Fukushi, Tsukasa. 1975.** Classical conditioning to visual stimuli in the housefly, *Musca Domestica*. *J. Insect Physiol.* 22: 361-364.
- Gao, J. R., J. M. Deacutis, and J. G. Scott. 2007.** The nicotinic acetylcholine receptor subunit *Mdα6* from *Musca domestica* is diversified via post-transcriptional modification. *Insect Mol. Biol.* 16: 325-334.
- Garrett, D. A. 1965.** The sensory responses of the house fly, *Musca Domestica* Linn., to attractants. Ph.D. dissertation Oklahoma State University. Stillwater, OK.
- Geden, C. J. 2005.** Methods of monitoring outdoor populations of house flies, *Musca domestica* L. (Diptera: Muscidae). *J. Vect. Ecol.* 30: 244-250.
- Geden, C. J. 2006.** Visual targets for capture and management of house flies, *Musca domestica* L. *J. Vector Ecol.* 31: 152-157.
- Geden, C. J., D. E. Szumilas, and T. W. Walker. 2009.** Evaluation of commercial and field-expedient baited traps for house flies, *Musca domestica* L. (Diptera: Muscidae). *J. Vect. Ecol.* 34: 99-103.
- Georghiou, G. P., and A. Lagunes-Tejeda. 1991.** The occurrence of resistance to pesticides in arthropods: An index of cases reported through 1989. Food and Agriculture Organization of the United Nations.

- Gerry, A. C. and D. Zhang. 2009.** Behavioral resistance of house flies, *Musca domestica* (Diptera: Muscidae) to imidacloprid. *Army Med. Dept. J.* 54-59.
- Goldsmith, T. H. 1965.** Do flies have a red receptor. *J. Gen. Physiology.* 49: 265-287.
- Green, C. H. and S. Flint. 1986.** An analysis of colour effects in the performance of the F2 trap against *Glossina pallidipes* Austen and *G. morsitans morsitans* Westwood (Diptera: Glossinidae). *B. Entomol. Res.* 76: 409-418.
- Greenberg, B. 1971.** Flies and disease: Ecology, classification and biotic association, vol 1. Princeton University Press. Princeton, NJ.
- Greenberg, B. 1973.** Flies and disease: Biology and disease transmission, vol 2. Princeton University Press. Princeton, NJ.
- Hall, D.C. and R. R. Gerhardt. 2009.** Flies (Diptera), pp 137-152. In G. Mullen, and L. Durden (eds). *Medical and veterinary entomology.* Academic Press. San Diego, CA.
- Hecht, O. 1970.** Light and color reactions of *Musca domestica* under different conditions. *B. Entomol. Soc. Am.* 16: 94-98.
- Hecht, O., R. Muniz, and A. Nava. 1968.** Contrary responses of *Musca domestica* L. concerning their selection of different shades and hues. *Entomol. Exper. App.* 11:1-14.
- Hedges, S.A. 1998.** Field Guide for the management of structure-infesting flies. G.I.E. Cleveland, OH.
- Hertz, J.C. 2007.** Potential of insecticide-treated cords and sprayable baits for control of house flies (Diptera: Muscidae). M.S. thesis University of Florida, FL.
- Hewitt, P. G. 1998.** Conceptual physics, 8th ed. Addison-Wesley Longman Inc. Reading, MA.
- Hilbert, D. R. 1992.** What is color vision. *Philos. Stud.* 68: 351-370.
- Hocking, B. 1964.** Aspects of insect vision. *Canad. Ent.* 96: 320-334.
- Hogsette, J. A., D. A. Carlson, and A. S. Nejame. 2002.** Development of granular boric acid sugar baits for house flies (Diptera: Muscidae). *J. Econ. Entomol.* 95: 1110-1112.
- Hogsette, J. A. 2003.** United States Department of Agriculture—Agricultural Research Service research on veterinary pests. *Pest Man. Sci.* 59:835-841.

- JMP. 2007.** JMP user's guide version 7.02. SAS Institute. Cary, NC.
- Kaufman, P.E., S. G. Scott, and D. A. Rutz. 2001.** Monitoring insecticide resistance in house flies (Diptera: Muscidae) from New York dairies. *Pest Manag. Sci.* 57: 514-521.
- Keiding, J. 1965.** Observation on the behavior of the housefly in relation to its control. *Riv. Parasit.* 26: 45-60.
- Keiding, J. 1976.** The house-fly: biology and control., pp. 82, Technical Report Series. World Health Organization.
- Kirschfeld, K., R. Feiler, and K. Vogt. 1988.** Evidence for a sensitizing pigment in the ocellar photoreceptors of the fly (*Musca, Calliphora*). *J. Comp. Physiol. A.* 163: 421-423.
- Kolbe, W. A. 2004.** Flies, gnats, & midges, pp. 825-881. In S. Hedges (ed), *Handbook of Pest Control* 9th ed. GIE Media. Richfield, OH.
- Komori, K., K. Hara, K.G. Smith, T. Oda, and D. Karamine. 1978.** A case of lung myiasis caused by larvae of *Megaselia spiracularis* Schmitz (Diptera: Phoridae). *Roy. Soc. Trop. Med. Hyg.* 72: 467-470.
- Liu, N., and X. Yue. 2000.** Insecticide resistance and cross-resistance in the house fly (Diptera: Muscidae). *J. Econ. Entomol.* 93: 1269-1275.
- Lysyk, T. J. and R. C. Axtell. 1986.** Field evaluation of three methods for monitoring populations of house flies (*Musca domestica*) (Diptera: Muscidae) and other filth flies in three types of poultry housing systems. *J. Econ. Entomol.* 79: 144-151.
- Mathis, W., A. D. Flynn, and H. F. Schoof. 1967.** Parathion resistance in house fly populations in the Savannah, Georgia, area. *J. Econ. Entomol.* 60: 1407-1409.
- Meyer, J. A., G. P. Georghiou, and M. K. Hawley. 1987.** House fly (Diptera: Muscidae) resistance to permethrin on southern California dairies. *J. Econ. Entomol.* 80: 636:640.
- Mihok, S., D. A. Carlson, E. S. Krafur, and L. D. Foil. 2006.** Performance of the nzi and other traps for biting flies in North America. *B. Entomol. Res.* 96: 387-397.
- Mix, J. 2007.** Busy PCO launches new invention: Fly sentry. PCO. 18-20.
- Mizunami, M. 1994.** Functional diversity of neural organization in insect ocellar systems. *Vision Res.* 35: 443-452.

- Moon, R. D. 2009.** Muscid flies (Muscidae), pp. 275-295. In G. Mullen and L. Durden (eds.), Medical and veterinary entomology. Academic Press, London, UK.
- Muniz, R. 1967.** Observaciones sobre la busqueda de sitios de reposo y estudio experimental sobre la seleccion de colores por *Musca domestica* en el ambiente natural. M.S. thesis, Institución Politecnico Nacional, Mexico, DF.
- Nation, J. L. 2002.** Insect physiology and biochemistry. CRC Press. Boca Raton, Florida.
- Nava, A. 1967.** Preferencias de *Musca domestica* en la seleccion de superficies de color. M.S. thesis, Institucion Politecnico Nacional, Mexico, DF.
- Pickens, L.G. 1995.** Baited fly traps – 1900 to 1995. IPM Practitioner. 17: 1-6.
- Pospischil, R., J. Junkersdorf, and K. Horn. 2005.** Control of house flies, *Musca domestica* (Diptera: Muscidae), with imidacloprid WG 10 in pig farms (Germany). Proc. 5th Conf. Urban Pests. P&Y Design Network, Malaysia. 309-317.
- Rabari, P. M., and R. C. Patel. 1976.** Control of housefly (*Musca domestica* L.) in dairy barn of Jersey cattle. Gau Res. J. 2: 21-28.
- Salcedo, E., A. Huber, S. Henrich, L. V. Chadwell, W. Chou, R. Paulsen, and S. G. Britt. 1999.** Blue and green absorbing visual pigments of *Drosophila*: ectopic expression and physiological characterization of the R8 photoreceptor cell-specific Rh5 and Rh6 rhodopsin. J Neurosci. 19: 10716-10726.
- SAS. 2001.** SAS stat user's guide version 8.01. SAS Institute. Cary, NC.
- Schal, C., V. Sevala, M.L. Capurro, T.E. Snyder, G.J. Blomquist, and A. Bagneres. 2001.** Tissue Distribution and lipophorin transport of hydrocarbons and sex phermones in the house fly, *Musca domestica*. J. Insec. Sci. 1:12.
- Scholl, P. J., E. P. Catts, and G. R. Mullen. 2009.** Myiasis (Muscoidea, Oestroidea), pp 309-338. In G. Mullen, and L. Durden (eds). Medical and veterinary entomology. Academic Press. San Diego, CA.
- Scott, J., T. Alefantis, P. Kaufman, and D. Rutz. 2000.** Insecticide resistance in house flies from caged-layer poultry facilities. Pest Manag. Sci. 56: 147-153.
- Shields, E. J. 1989.** Artificial light: Experimental problems with insects. B. Entomol. Soc. Am. 35: 40-45.
- Smallegange, R. 2004.** Fatal attraction. Control of the housefly (Diptera: Muscidae). Entomol. Berichten 64: 87-92.

- Smith, A. C. 1958.** Fly-cord studies in California. Calif. Vect. 5: 57-62.
- Steverding, D. and T. Troscianko. 2003.** On the role of blue shadows in the visual behavior of the tsetse flies. Proc. R. Lond. B. 271: S16-S17.
- Tobin, E.D. and J.G. Stoffolano. 1973.** The courtship of *Musca* species found in North America: The house fly, *Musca domestica*. Ann. Entomol. Soc. Am. 66: 1249-1257.
- Toshinori, S., M. Kobayashi, and N. Agui. 2000.** Epidemiological potential of excretion and regurgitation by *Musca domestica* (Diptera: Muscidae) in the dissemination of *Escherichia coli* O157: H7 to food. J. Med. Entomol. 37: 945-949.
- Triplehorn, C. A., and N. F. Johnson. 2005.** Borror and Delong's Introduction to the Study of Insects. Thomson Brooks/Cole, Belmont.
- Trumble, J.T. and R. L. Pienkowski. 1979.** Development and survival of *Megaselia scalaris* (Diptera: Phoridae) at selected temperatures and photoperiods. Proc. Entomol. Soc. Wash. 81: 207-210.
- Wehrhahn, C. 1984.** Ocellar vision and orientation in flies. Proc. R. Soc. Lond. 222: 409-411.
- Weidhaas, D. and D. Haile. 1978.** Theoretical model to determine the degree of trapping required for insect population control. Bull. Ent. Soc. Amer. 24: 18-20.
- Welch, R.M. 2006.** Relative Effectiveness of Commercial Fly Traps and Ammonium Carbonate/Yeast in Catching House Flies (Diptera: Muscidae). M.S. thesis, University of Florida, FL.
- West, L. S. 1951.** The housefly: its natural history, importance, and control. Comstock Publishing Company. Ithaca, NY.
- White, W. H., C. M. McCoy, J. A. Meyer, J. R. Winkle, P. R. Plummer, C. J. Kemper, R. Starkey, and D. E. Snyder. 2007.** Knockdown and mortality comparison among spinosad-, imidacloprid-, and methomyl-containing baits against susceptible *Musca domestica* (Diptera: Muscidae) under laboratory conditions. J. Econ. Entomol. 100: 155-163.
- Zuker, C. S. 1995.** The biology of vision in *Drosophila*. Proc. Natl. Acad. Sci. 93: 571-576.

BIOGRAPHICAL SKETCH

Joseph W. Diclaro II was born in the coal-mining city of Beckley, West Virginia. He was the second youngest of four children. After leaving home at a young age, he moved to Cincinnati, Ohio and graduated from Mt. Healthy High School in 1989. In 1990, he enlisted in the United States Navy and was proud to become a Hospital Corpsman. Over the last 20 years, he has served overseas at Naval hospitals, with Naval Mobile Construction Battalion, United States Marine Corps, as well as various shore duties. Most recently, he was assigned to the medical staff at the Attending Physician Office at the United States Capitol. He received his Associate of Science degree in general studies from Northern Virginia Community College in 2000. He received his Bachelor of Science in interdisciplinary studies in biology from Mountain State University, Beckley, West Virginia in 2004. He attended George Mason University and received his master's in biodefense in 2007. In 2006, he was the second enlisted Sailor selected to study entomology under the Medical Service Corps In-service Procurement Program (MSC-IPP) and became the first to obtain a PhD in this program. Upon graduation HM1 (SCW) Joseph W. Diclaro II will be commissioned to the rank of Lieutenant as a medical entomologist in the Medical Service Corps. He enjoys the time he can spend with his kids and looks forward to seeing them succeed. He is also a proud active member of Centennial Lodge #174 of Ancient Free and Accepted Masons located in Upper Marlboro, Maryland.