POTENTIAL OF INSECTICIDE-TREATED CORDS AND SPRAYABLE BAITS FOR CONTROL OF HOUSE FLIES (DIPTERA: MUSCIDAE)

By

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To my wonderful family

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By

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House flies are often controlled using insecticides when the source of infestation can not be located or remedied by non-chemical methods. Historically, house flies have shown a tremendous potential to develop insecticide resistance and with few classes of insecticides currently registered for house fly control, new products and methods need to be evaluated to prevent future control failures. This research evaluated the potential use of two innovative methods to control house flies: fipronil- and indoxacarb-impregnated cords and a sprayable imidacloprid fly bait.

For the insecticide-impregnated cord studies, eight various natural and synthetic cords were evaluated to determine which cords were attractive to house flies. Natural cords were more attractive than synthetic cords; the plant-based manila cord was most attractive and the nylon parachute cord was least attractive. The most attractive cords (manila, cotton, wool, nylon, and polypropylene) were treated with 0.1% fipronil or 0.6% indoxacarb and evaluated in the laboratory to determine their effectiveness. All cords were more effective than the impregnated cotton cord except the fipronil-impregnated nylon cord (LT_{90}) and the indoxacarb-impregnated polypropylene cord. The wool cord was the most effective, LT_{50} (Fipronil = 12.9 h; Indoxacarb = 32.6 h) and LT_{90} (Fipronil = 22.4 h; Indoxacarb = 51.5 h). The wool cords were impregnated

with 0.1% fipronil and 1.2% indoxacarb and evaluated in a controlled field environment with fresh cords and cords that were aged 4 wk. No significant differences were seen between fly count reductions of either treatment. Both treatments reduced fly counts by >57% by 24 h and >87% by 48 h with both fresh and aged cords. A reduction in efficacy was seen with aged cords.

The new imidacloprid sprayable fly bait formulation was compared against two commonly used dry scatter baits in the laboratory and against a granular imidacloprid paint-on bait in a controlled field setting. Additionally, the sprayable bait was evaluated for use in impregnated cords. No differences were seen in mortality between the three scatter baits in the laboratory or between the imidacloprid baits in the field cages. Both imidacloprid baits reduced fly counts by >70% in the field within 24 h, but were not effective after treatments were aged for 2 wk. When various cords were treated with 2.5% of the new bait, the wool cord had higher mortality (74%) compared to the other natural and synthetic cords tested. Knockdown recovery was observed with all bait-treated cords in the laboratory, but was not determined to occur in the field cages. The bait-treated cords reduced fly counts by >82% with fresh cords and cords aged for 4 wk.

Impregnated cords and the new sprayable bait should prove to be valuable tools in established fly management programs in urban, agriculture, and military settings. Fipronil and indoxacarb are not currently registered for house flies, but both appear to be effective insecticides for their control.

CHAPTER 1
STATEMENT OF PURPOSE

Throughout history flies have undoubtedly been a nuisance to both man and animal alike; however, because of their propensity to frequent pathogen-rich filth they do pose a human health risk. House flies have been shown to transmit numerous pathogens and their synanthropic behavior may make house flies one of the most troublesome insect vectors (West 1951, Greenberg 1973). This is especially true in areas affected by natural disasters or conflict. Often times following these chaotic events, basic sanitation measures are out prioritized for casualty recovery and, as a result, tremendous populations of house flies emerge.

Chemical insecticides are often used in these situations or any situation where rapid house fly control is needed. Today there are more chemicals registered as insecticides than ever before, but few of these insecticides are registered for house fly control. The insecticides that are registered for house fly control only come from five chemical classes: organophosphates, carbamates, pyrethrins/-oids, triazines, and neonicotinoids. The organophosphates and the carbamate insecticides continually get further restrictions by the Environmental Protection Agency (EPA) limiting their use and their future availability in the United States may be bleak. In addition, house flies have consistently shown the ability to develop resistance to all chemicals used to kill them (Liu and Yue 2000, Scott et al. 2000, Kaufman et al. 2001) and having only 3-5 chemical classes to rotate with may prove to be detrimental to a fly management program. There is an immediate need for new insecticides registered and new techniques for house fly control to prevent future control failures.

In 2004, the Department of Defense (DOD) established the Deployed War-Fighter Protection (DWFP) program to develop and test management tools for pest and vector species, including house flies, which transmit diseases to the deployed war-fighters. The Armed Forces

Pest Management Board (AFPMB) administers the DWFP program and specifically requested research to improve or develop integrated filth fly control strategies and non-conventional pesticide methodologies. Insecticide-impregnated cords and sprayable fly bait may be beneficial tools that the deployed war fighter could use for fly management programs. The research contained herein was designed to provide new information regarding these techniques to the DOD.

CHAPTER 2
REVIEW OF LITERATURE

Classification, Origin, and Distribution

The house fly, *Musca domestica*, belongs in the class Hexapoda, order Diptera, suborder Brachycera, infraorder Muscomorpha (Cyclorrhapha), and family Muscidae (Triplehorn and Johnson 2005). It was first described by Linnaeus (1758).

It is believed the family Muscidae evolved sometime during the Permian period of the Paleozoic era (Lambrecht 1980). The exact origin may never be known, but many speculate that the house fly originated in the Middle East area of the Palearctic region (Skidmore 1985, Pont 1991) and was distributed through multiple introductions into the New World (Marquez and Krafsur 2002). Today, house flies are one of the most commonly found synanthropic pests. It is found in virtually every region of the globe that man or animal exist. The only exception is areas, such as high altitudes and the arctics, which are prone to extreme cold temperatures (West 1951).

Identification

Egg

House fly eggs are white, bluntly rounded, banana-shaped eggs approximately 1 mm in length, and often laid in clusters (Keiding 1976). The egg widens in size posteriorly to anteriorly and the dorsal surface has two longitudinal, curved ridges that narrow just prior to reaching the caudal end.

Larva (maggot)

House flies have three larval instars. Each instar has no eyes, legs, antennae, or appendages and are commonly known as maggots (Moon 2002). The maggot has a rounded posterior that tapers to a point towards its head. A pair of black spiracular plates is located

posteriorly, which progressively becomes more chitinized and "D-shaped" through molts. First and second stage larvae have two spiracular openings (slits) used for gas exchange and a third opening appears on the third instar larvae (Moon 2002). Prothoracic spiracles are fan-shaped and appear after the first molt. A cephalopharyngeal skeleton, comprised mainly of sclerotized "mouth" hooks, is located at the anterior end of the larvae.

Pupa

House fly pupae are approximately 6.3 mm in length (West 1951). At the beginning of pupation, the puparium is white in color but eventually becomes reddish-brown. The puparium is medially enlarged with bluntly rounded ends. Two pupal horns are located laterally just prior to the posterior boundary of the first abdominal segment (Siriwattanarungsee et al. 2005). Posterior spiracles are located on the posterior end and appear as two flat, circular prominences. The anterior spiracle is situated on the puparium in the same location as in the third instar larvae (Siriwattanarungsee et al. 2005).

Adult

The adult house fly is a medium-sized (6-9 mm) gray insect with large brown compound eyes (Moon 2002). On the vertex, between the eyes, lies the ocellar triangle containing the three simple eyes. The house fly's antennae are also located between the eyes, within the triangular facial depression. The antenna is six segmented, but only appears to be four. The first three segments, the scape, pedicel, and large first flagellar segment, give rise to the three-segmented arista. Segment one and two of the arista is ambiguous; segment three is bristlelike. The sponging proboscis of the house fly terminates to a heart-shaped sucker. The proboscis can be greater than the length of the head when fully extended or obscure when fully retracted. Two brownish-black maxillary palpi lie on the anterior margin of the proboscis.

The thorax of the house fly has four black longitudinal stripes that can be viewed dorsally. Attached laterally to the mesothorax are two membranous wings. The wings, when extended, are approximately twice the distance of the fly's length. At rest, the house fly pulls the wings back incompletely over the abdomen forming an overall triangular appearance from above. The fourth longitudinal wing vein sharply angles towards the wing apex. Situated below each wing is a knob-shaped organ used for equilibrium called the haltere. The legs of the house fly attach ventrally to each segment of the thorax and all legs have five-segmented tarsi. The first tarsal segment is much longer than all other segments and the fifth segment bears two claws, a hair-like empodium, and a sticky pad called a pulvillus.

The abdomen is gray, dorsally, and cream-colored ventrally. Five pairs of spiracles line the ventral surface of the female; six pairs line the ventral surface of the male. The tip of the abdomen ends in either the sclerotized genitalia of the male or the retracted ovipositor of the female.

Sex Differentiation

Adult female house flies are almost always larger than adult males. Additionally, males can be differentiated from adult females by locating the dark sclerotized genitalia plate located on the distal aspect of the abdomen. The tiny mark made by the ovipositor tip of the female is very distinctive compared to the male genitalia especially when females are gravid. Furthermore, adult house flies can be separated by the gap distance that divides the compound eyes. Females have a much wider space separating the eyes when compared to male counterparts. No differentiation can be made in the immature stages.

Life Cycle

Male and female house flies can successfully mate 24 hours after emergence from the pupae (Murvosh et al. 1964). Prior to copulation, a male will seize a resting female or *strike* a

flying female at which point they fall to a surface. If a copulating pair is disturbed while mating they may attempt to fly a short distance to an alternate surface. Copulation can last for more than 1 h, but sufficient sperm transfer can occur in less than 10 min (Murvosh et al. 1964). Once successful copulation takes place, the female is fertilized for life. Batches of up to 150 eggs are laid 4-8 days after copulation (West 1951). The female house fly carefully embeds her eggs into practically any fermenting organic material. The eggs hatch within 24 hours, and 1st instar larvae emerge and begin to feed (West 1951). The larvae undergo two molts within 3-5 days before pupation (Hogsette 1995). Pupation begins when the 3rd instar larva stops feeding and constricts within its own integument. This makes a white puparium which turns reddish-brown within 24 hours. After 3-5 days, the adult breaks through the anterior end of the puparium using a temporary, inflated sac located on its head called the ptilinum. Once free from the puparium, the newly emerged adult house fly hops around to let its wings extend and cuticle harden.

Nutrition, Longevity, and Overwintering

House flies larvae have been reared in the laboratory on practically every type of filth imaginable. Today, they are most often reared in a medium containing animal feed and water (Hogsette 1992). Fermenting odors attract gravid females to oviposit on breeding sites in the field, but understanding precisely what nourishes a maggot within the medium is not fully understood. All house fly maggots are saprophagous and feed on liquids or substrates that are readily dissolved by droplet regurgitation (Nation 2002). It was originally thought that bacteria were essential in the development of house fly maggots, however many have successfully reared them in aseptic media (Brookes 1956, Monroe 1962). Despite this, bacteria still may have provide nutritional value (e.g. vitamins) to maggots (Zurek et al. 2000).

Adult house flies are omnivorious and emerge with little stored energy and nutrients (Moon 2002). They begin to feed within 2-24 hours after emergence (Keiding 1976). In order to

survive, they must find a sugar source, or other assimilable starch, and water (West 1951). In addition, female house flies require a protein source for vitellogenesis.

When feeding, adult house flies are attracted first visually, then when they are within a detectable range, by smell using their antennae (Keiding 1976). Flies locate the source of the aroma by smelling the substrate with chemoreceptors located on the lateral aspect of their 2-5 tarsi. Once their tarsi are in contact with a suitable substance, the fly extends its proboscis and begins to feed. Liquid substances can be readily imbibed, but solids are ground down using the prestomal teeth on the proboscis and emulsified using a vomit drop originating in the crop and salivary glands. The largest particle a house fly can ingest is 40 μ (Greenberg 1973). Ingested liquid and emulsified particles enter the pseudotracheae and then pharynx. Once past the pharynx, liquids pass into the crop and emulsified food particles enter the proventriculus then the ventriculus. The crop is connected to the pharynx by a long slender tube lined with numerous sphincters that controls the flow of liquid back to the abdomen where the bilobed crop is housed. The heamolymph osmotic level dictates the rate the crop empties into the ventriculus. The more concentrated the sugar meal, the slower the crop empties (Greenberg 1973). The ventriculus empties into the longest part of the alimentary track, the proximal intestine. The proximal intestine is divided from the distal intestine by excretory organs called the Malpighian tubules. The distal intestine terminates at the anus.

Longevity of any organism can be highly variable. Food availability, environmental conditions, and activity of an individual fly greatly influences how long it will live. Of the three survival-mandated nutrients, sugar is the most critical for survival. Lysyk found that the availability of sucrose was the most important factor promoting longevity, followed by other food sources (manure, milk) and temperature (1991). Flies will live 50% longer on sucrose

alone, than they do on water alone (Greenberg 1960). However, flies without water generally die within 48 hours (West 1951).

Temperature is inversely proportional to the life span of the adult house fly; higher temperatures reduce the life expectancy, while lower temperatures increase it (West 1951). In laboratory conditions where adequate food is provided *ad libitum* and environmental conditions are controlled, male house flies can live up to 40 days and female house flies can live up to 60 days (Rockstein 1957). In the field, house flies are estimated to only live about 10 days (Hogsette 1995). Bucan and Sohal (1981) found that adult males and females isolated from the opposite sex live longer than when they are housed together.

To increase their survivability when temperatures drop below optimum levels, house flies survive by overwintering in buildings and animal confinements. All life stages are susceptible to subzero temperatures, so microclimates must exist that allow flies to propagate. Rosales et al. (1994) concluded that house flies require habitats that are above -5ºC, and must stay above 10ºC long enough for the house fly to complete its life cycle.

Flight, Movement, and Resting Behavior

Flies have two wings located on the lateral aspect of the body on the pteropleura. Directly above the metathoracic coxae are the vestigial wings, or halteres, which are used as gyroscopes for equilibrium. Like other flying insects, a house fly achieves flight by creating wing movement through indirect thorax compression and decompression caused by the flight muscles. These flight muscles comprise approximately 11% of the total body weight in the genus *Musca* (Greenberg 1973). This musculature makes house flies extremely strong fliers and very capable of flying upwind in mild and moderate winds.

Fly movement can be classified as dispersal, dispersion, or migration (Greenberg 1973). Dispersal is any active movement within a relatively small defined area. House fly problems are

often localized near a source of infestation (Howard 2001, Nazni et al. 2005). Often times, dispersal will be dependant on the sun light. Flies tend to follow the sun and more will be located where the sun is shining (Anderson 1964). This is especially true in cooler temperatures; in hot temperatures, flies may avoid the sun and search for cooler locations. Dispersion is the movement of flies between adjacent areas and often involves the movement assisted by passive transport. Passive transport can occur on garbage trucks, tractors, automobiles, or any other vehicle including strong winds. This is often seen when breeding sources are sporadic or when no breeding sources are near and flies move into the area in search of new oviposition sites. This movement is why flies are found in areas where no apparent fly breeding material is present. Migration is any directed and sustained flight that often occurs seasonally. This type of movement is not normally associated with house flies, however, many have been trapped in areas that would suggest that migration was the only possible explanation (West 1951, Jones et al. 1999). Passive transport may also play a large role in these situations.

Fly movement can be influenced by many factors such as odors, wind, weather, time of day, and population structure. Food and oviposition sites are probably the most critical factors (Bishopp and Laake 1921). However, many questions still need to be answered on what is attractive or needed by house flies since flies will often leave one oviposition site in search for an alternative site although sources are immediately available and sufficient for survival. When seeking these new food sources or oviposition sites, flies can fly upwind in mild to moderate wind speeds, but strong winds, or even shifting winds, can disperse house flies to areas where survival may not be suitable. Taylor (1974) found that house flies are day fliers and their flight activity increases with high temperatures (25-30ºC) and low humidity (50-65%). Flies always

seek temperatures above 15.5ºC, but are capable of flight at temperatures below 55ºC (Greenberg 1973).

House flies have distinct resting behavior. In warmer climates, flies prefer to rest at night outdoors on low hanging twigs of trees and bushes, but may still be seen resting indoors on wires or cords close to the ceiling (Scudder 1949, Keiding 1965). In colder climates, house flies will rest exclusively indoors. In all cases, flies tend to rest on objects with distinct edges less than 4.5 m from the ground, shielded from direct wind (Scudder 1949). Keiding and Hannine (1964) found a distinct preference for house flies to rest on objects suspended vertically from ceilings, however, Fay and Lindquist (1954) found no differences in orientation of suspended cords.

The visual orientation of house flies to objects has been widely disputed. Objects that are light in color, smooth, or metallic are highly avoided by flies; whereas, objects that are dark in color and rough are generally more frequently rested upon by flies (Arevad 1964). Hecht et al. (1968) performed a number of indoor and outdoor experiments to determine the attraction of house flies to different colored cardboards. They found that black was most preferred indoor and white was the most preferred outdoor. When combining the indoor and outdoor results, the red colored surfaces were most preferred. The least preferred colors were blue (indoor) and brown (outdoor). The attraction to the white surface outdoors was attributed to a fly's attraction to ultraviolet light because of the reflective qualities of the white cardboard. Flies see wavelengths between 350-480 mμ (McCann and Arnett 1972). Contrasts of colors (dark on light/light on dark) may be very important to the attraction of house flies. Howard and Wall (1998) counted more flies on white surfaces with black backgrounds than on any other black/white combination.

Pest Status and Health Importance

House flies are renowned for their ability to annoy anyone and anything they are near. It only takes one house fly to turn a customer away from a restaurant and only a few to disrupt

production and morale at a work site. Most see house flies as a sign of unhygienic conditions and attempt to avoid them at all costs. Flies leave fecal and vomit spots on work equipment, consumables, and personal items most of which are not be generally quantified in economic losses but their impact can easily be seen when comparing two similar establishments one with a fly problem and the other lacking a problem. Litigation cases due to house flies have increased in the United States recently due to the migration of urban dwellers deeper into rural settings where livestock and poultry farms have a relatively large abundance of flies – a situation many urban dwellers may not be familiar with.

Although primarily nuisance pests, house flies do pose a risk to the health and well-being of man and livestock. Because of a house fly's behavior and survival needs, it frequently comes in contact with pathogenic organisms. House flies are extremely capable of transmitting pathogens mechanically (West 1951). West (1951), and most recently Greenberg (1973), have compiled extensive lists of the pathogens (bacteria, viruses, fungi, protozoa, and nematodes) the house fly is capable of transmitting. The transmission of *Campylobacter spp.*, E*. coli* O157:H7, *H. pylori, C. parvum,* and *G. lamblia* are probably the most significant pathogens capable of being transmitted by the house fly recently reported (Shane et al. 1985, Grubel et al. 1997, Kobayashi et al. 1999, Graczyk et al. 2003).

Of particular importance is the exponential proliferation of house flies following situations arising from natural disasters or conflict. Following the tsunami that devastated parts of Indonesia in 2004, sewage and drainage systems were destroyed leaving sewage pools that bred numerous species of filth flies (Burrus 2005). This is often what occurs following any of these chaotic events; communities are left in shambles and multiple oviposition sites develop due infrastructural collapse or basic municipal sanitation being out prioritized. Large fly populations

then develop and epidemic levels of diarrheal cases normally follow (Thornton et al. 2005, Watson et al. 2007). This reduces military readiness and stresses health care systems (Putnam et al. 2006). The direct impact of house flies on disease transmission in these situations is either not measured or often not measurable because the same pathogens transferred by house flies can be just as easily transferred by man or other organisms.

Control

The house fly is best controlled through a fly management program based on the sound principles of Integrated Pest Management (IPM), including a combination of monitoring, cultural, biological, and chemical control measures (West 1951, Keiding 1976). Monitoring is any technique employed to determine presence/absence and peak/trough flows of house fly populations. Cultural controls are any measures that deliberately alter the life cycle of the house fly without the use of chemical or biological agents. Biological controls are agents or organisms that alter the life cycle of the house fly and chemical controls are naturally- or synthetically-derived chemicals that can alter the life cycle of the house fly.

Every aspect of the fly management program should have some form of fly monitoring to determine when and what type of approach should be employed (pre-treatment survey) and to see the effectiveness of the treatment (post-treatment survey) (Keiding 1976). In the pre-treatment survey, house fly density, distribution, and behavior should be noted to help determine which treatment option to use. Post-treatment surveys normally only need to monitor fly densities – unless failure occurred. In this case, a complete reassessment should be done to include some form of monitoring for insecticide resistance.

There are four basic methods to obtain a fly population index: counting flies, counting fly specks, netting flies, or trapping flies (Keiding 1976). If counting adult flies, several methods can be used. The classic technique is the use of a Scudder grill – a simple grid constructed of

wood that can be placed over an infestation source and then all flies landing on the grid are counted (1947). A similar technique that can be used in practically any situation is to simply mark an area (preferably near infestation or resting areas) and count flies landing on it. Counting fly specks left on index cards may be the method of choice today for indoor sampling because of its simplicity. Spot cards can be positioned in standard locations throughout the infested area and will give a good representation of the fly populations over time. Simply hang them and check back on them after a designated time period. Over time it will show peak and troughs of fly populations; in addition, the spot cards can be archived and marked with any insecticide treatment used to provide additional documentation for resistance monitoring. Some users, however, prefer to use destructive sampling. In these cases, baited traps, sticky ribbons, or even netting flies can work well.

All of the above methods work and can give consistent numbers indoors as long as the same sampling method is used for all counts. However, infestations that occur outdoors are not as easily monitored. With outdoor sampling, spot cards are an unrealistic method because placement would be difficult and precipitation would destroy them. Scudder grills and other fly count techniques are subject to wide variation depending on positional effects, time of day, and weather conditions (Geden 2005). Baited traps and net sweeping may be the best techniques to use outdoors for surveillance work, but these methods are destructive and not suitable in every situation. Beck and Turner (1985) found that using a simple visual index correlated better with absolute fly densities than spot cards, sticky ribbons, scudder grill and fly counts, but these indexes are very subjective and will vary between persons making the counts. Perhaps, the best way to monitor house flies outdoors is to use a combination of the methods described.

By far the most important aspect of a fly management program is the use of cultural controls. Cultural controls target the breeding and feeding sites of adult and larval flies. Additionally, they are used to prevent adult flies from contacting food, pathogens, and man (Keiding 1976). Garbage is the main source of infestation in an urban environment and manure is the main source in an agriculture environment, however, both sources can be found in any environment. In developed urban communities, these breeding sites are normally controlled by very established municipal sanitation measures (e.g., closed sewers, garbage removal, etc.) (Hogsette 1995). Agriculture facilities have to physically remove manure or bake it by covering dung heaps with plastic sheeting. Garbage should be removed from the area at least twice weekly or burned (Keiding 1976). Windows, screens, and doors should all be in good repair and kept closed to prevent flies from entering establishments. The installation of air curtains on doors and windows that are frequently opened and closed is an energy efficient option that can reduce the number of flies that enter.

Traditionally baited traps, light traps, electrocuting light traps, and sticky ribbons have been used as cultural control measures, but their effectiveness at reducing house fly populations are limited and their use should primarily be considered as a monitoring technique. However, these methods do trap and kill flies so using them should never be automatically ruled out. In fact, if the likelihood of large infestations does not exist, then traps are a good method for killing flies (such as in grocery stores and restaurants) but some considerations should be made prior to their use. Baited traps generally can not be used indoors or near residences because the odor associated with this method is repulsive to humans (Pickens et al. 1973). Electrocuting light traps release fly parts, bacteria, and viruses and may be just as unhygienic as the fly itself and should not be used in areas were conditions need to remain relatively aseptic (Urban and Broce

2000). Lighted traps need bulb replacement approximately every six months, and sticky ribbons need replaced frequently due to dust and fly cadaver build-up. Flies that do make their way into an establishment can be physically removed by numerous devices, but the most common physical cultural control method is the good 'ol fashioned flyswatter. A novelty gadget used for killing insects, including flies, has recently sparked numerous videos on the World Wide Web. The device is a combination electrocuting trap and fly swatter. This device may be interesting and fun, but the same risk is associated with it as the regular electrocuting light traps.

The second most important principle of a good fly management program is the use of biological controls. Every stage of a fly's life cycle is vulnerable to attack by some form of biological control (West 1951). The eggs are often predated upon by mites, earwigs, ants, and some beetles. Larvae are also attacked by mites, earwigs, and beetles, as well as some birds, wasps, and other Dipteran larvae. Pupae are often parasitized by small wasps and some beetles, others can be eaten by birds and large beetles. Many adult house flies meet their demise thanks to predatory insects (mantids, flies, dragonflies, wasps, ants) and arachnids. Many other adults are eaten by reptiles, amphibians, small mammals, and birds. House flies are also prone to infections by bacteria and fungi. Fortunately, all of these biological controls are already abundant in a fly's natural environment and the goal of a fly management program should be to maintain or supplement these existing populations (Geden 1995). Parasitic wasps can now be purchased commercially and their successful use is variable (Axtel 1999).

The use of chemical insecticides is the third component of a good fly management program. Chemical insecticides provide quick results (i.e. dead flies) and satisfactory control in one or two days, but their use should be limited to reduce the likelihood of resistance evolution and maintenance of non-target organisms. Chemical insecticides can be applied several different

ways: residual surface treatments, larviciding, space sprays (including aerial spraying), and baits. The most common method for fly control is the use of space sprays and dry insecticidal baits.

Residual surface treatments can be applied to any surface in any location the label allows but they are most effective when applied directly to fly resting areas (Keiding 1976). Neglecting to monitor and treat areas where flies are primarily resting will result in excess insecticide usage and population reductions of non-target organisms. Several insecticides are available for residual treatments; most are organophosphate based. All are generally good for long term control, but there excessive use may increase selection for insecticide resistance. One technique for residual insecticide application is the use of insecticide-impregnated cords which target the distinctive behavior of flies to rest on objects with edges (Scudder 1949, Keiding 1965). This method is thought to be less likely to select for resistance because the treated area is small and treatments can be readily removed or replaced with additional cords treated with insecticides from different chemical classes (Appendix A).

Larviciding with insecticides sounds great in theory because larvae are relatively non-mobile compared to the adult flies, which have the ability to fly to different areas if one is found unsuitable; however, larviciding is really not a practical method for extended control. Larvicides have to be applied frequently because they are applied to areas such as garbage and manure - both of which constantly accumulate. Larvicides kill non-target organisms coming in to feed on the house fly immature stages and would reduce those populations over time. In addition, if the same class of insecticide is used for larviciding that is used for adult control resistance selection would be rapid. Larvicides are effective if sites to be treated are expected to exist for a short period of time; in these instances several organophosphate insecticides and insect growth regulators can be used.

Space sprays are primarily pyrethrin- or pyrethroid-based insecticides, but some may also be organophosphate-based, that are sprayed into the air in a fly infested space or over a fly infested area. These types of insecticides target the fly nervous system and cause rapid knockdown of contacted flies (Yu 2007). Space sprays are more effective when an abundance of flies are concentrated in one area; this occurs mainly in the evening indoors and in the morning outdoors (Keiding 1976). They have little to no residual and have to be reapplied frequently (daily) in areas of large infestations. Another type of space treatment is made through the use of insecticide vaporizers. The only vaporizer currently available is formulated with the organophosphate dichlorvos, but its use is becoming more restricted and its future longevity may be short lived.

Insecticide baits are easy-to-use insecticides that have added attractants into the formulation matrix to draw flies into the treated area to contact the insecticide either by ingestion or contact. A basic bait matrix is a simple solution of sugar, water, and an insecticide. Complex bait matrices contain multiple sugars, pheromones (Z-9-tricosene), and other substances found attractive to house flies. The most widely used fly baits available are formulated in dry granules as scatter baits containing carbamates and neonicotinoids (Appendix B), however, other bait products are available and frequently used. Like space sprays and larvicides, baits have to be frequently applied because environmental conditions degrade them or they become covered by manure or garbage. Also, when baits are used in areas with large fly populations, the flies will consume the bait rapidly leaving little bait behind for the immature stages that will eventually emerge.

CHAPTER 3
INSECTICIDE-IMPREGNATED CORDS FOR HOUSE FLY CONTROL

Introduction

The house fly, *Musca domestica* L., is widely considered the most common nuisance pest. Their nuisance pest status can quickly change to a public health risk if fly populations occur near inhabited areas where pathogen-rich oviposition sites are found. Areas stressed due to natural disasters, humanitarian crises, or combat are often plagued by large fly populations (Rosales and Prendergast 2000, Burrus 2005, Thornton et al. 2005). The most effective way to control house flies and reduce the risk of disease transmission is by eliminating their pathogen-rich oviposition sites. Oviposition site removal may be impractical, especially in areas affected by natural disasters and combat, where the oviposition sites are too numerous or difficult to reach.

The best control method to use when sanitation fails or when fly populations need to be rapidly controlled are chemical insecticides. Chemical insecticides provide rapid kill of house flies and markedly reduced fly densities can be achieved in as little as 1-2 days. Baits and space sprays are the primary chemical insecticide methods used for house fly control today, but both methods provide little or no residual control, and resistance to their active ingredients is well documented in house flies (Georghiou and Lagunes-Tejeda 1991, Liu and Yue 2000, Scott et al. 2000). In addition, the number of registered insecticides available for house fly control in the United States continues to decrease (Kaufman et al. 2001). New insecticides and application methods are clearly needed to avoid future insecticide resistance problems.

Insecticide-impregnated cords have been used with great success to control flies and are considered less likely to select for resistance than traditional residual sprays (Keiding 1976). Their first use was in 1947 (Baker et al.) and by the mid-1950's, insecticide-impregnated cords were commercially available and widely used (Fehn 1958, Smith 1958). The commercially

available cords contained 13.79% parathion and 3.54% diazinon (Smith 1958). Cords impregnated with high concentrations (up to 25% active ingredient) of other organophosphate and organochlorine insecticides were also widely used with great success (Kilpatrick and Schoof 1959, Keiding 1976, Rabari and Patel 1976). These products are no longer used today due to the popularity of insecticidal baits and space sprays and because the Environmental Protection Agency, acting under federal legislation, eliminated the use of their active ingredients.

The objective of this study was to investigate if cords impregnated with newer insecticides would be an effective tool for house fly control. Specifically, the objectives were to: 1) determine the attractiveness of various natural and synthetic cords to house flies, 2) determine the effectiveness of fipronil and indoxacarb on the most attractive cord materials, and 3) evaluate the effectiveness of the best cord/treatment combination in a simulated field environment.

Materials and Methods

Insects. The Horse Teaching Unit (HTU) strain of house flies, *M. domestica* L., reared at the University of Florida in Gainesville was used for all experiments. Larvae were reared on a diet medium, modified from Hogsette (1992), containing 3 liters wheat bran, 15 ml methyl paraben, 1.5 liters water, and approximately 200 g (250 ml) dairy calf feed (Calf Manna® pellets, Manna Pro Corp., St. Louis, MO). All developmental stages were held at 26 ± 1°C and 55% RH with a 12:12 (L:D) photoperiod. Adult flies emerged within screened rearing cages and were provided granulated sugar, powdered milk, and water *ad libitum*.

For all assays, adult house flies (3-5 d old) were aspirated from the screened rearing cages using a handheld vacuum with a modified crevice tool attachment. Flies used for the laboratory assays were placed into a 5°C environment for 5 min to subdue activity. Flies were then placed on a chilled aluminum tray, sexed, and counted. House flies used for field cage assays were not

anesthetized, but were aspirated from the screened rearing cages and released directly into field cages.

Laboratory Arenas. Arenas (31 x 25 x 21 cm) were constructed using PVC pipe (1.27 cm [0.5 in]) (Figure 3-1A). Rubber bands were used to establish individual treatment positions; four treatment positions were used in the cord attractiveness bioassay and five positions were used in the impregnated cord bioassay. All cords were attached to the treatment positions vertically using paper clips and were uniformly distributed along the length of the arena. The cord attractiveness bioassay held two randomly assigned cords at each treatment position and the impregnated cord bioassay held only one cord at each treatment position according to a 5 x 5 Latin square configuration. Arenas were enclosed with a transparent plastic bag (3716 cm2 [24 x 24 in], 1 mil poly, Uline, Waukegan, IL).

Cord Attractiveness Bioassay. Eight cords were evaluated: nylon (Braided, Multi-Purpose Braid 75 lb. load limit, Wellington Cordage LLC, Madison, GA), polypropylene (Braided, Multi-Purpose Rope – 56 lb. load limit, Wellington Cordage LLC, Madison, GA), cotton (Braided, Multi-Purpose Sash Cord – 28 lb. load limit, Wellington Cordage LLC, Madison, GA), cotton wick (Sterilized roll, #200209, Richmond Dental Company, Charlotte, NC), manila (Twisted, Natural Rope – 108 lb. load limit, Wellington Cordage LLC, Madison, GA), wool (Twisted, Natural Cord, Wooded Hamlet Designs, Greencastle, PA), leather (Tan laces, #6192, Rothco, Ronkonkoma, NY), and parachute cord (550 test, white, purchased locally from M & C Army Surplus Store, Gainesville, FL).

Fifty female flies were released into each arenas and 10% sugar water was provided *ad libitum*. Number of flies resting on cords was counted every 10 min for 2 hr. Arenas were lightly shaken between each count to displace flies from their resting positions. Four replications

were performed in the laboratory (28 ± 1ºC) under continuous light on separate days using different flies.

Impregnated-Cord Laboratory Bioassays. Cotton, manila, wool, polypropylene, and nylon cords were selected from the cord attractiveness experiments to be evaluated in the impregnated-cord experiments. Each impregnated-cord experiment consisted of 6 arenas. Five arenas were organized into a 5 x 5 Latin square design, blocking for treatment position, and a sixth arena was used as a control. The control arena had no treated cords and all cords within it maintained the same cord positions throughout all experiments (left to right: position 1 = cotton; 2 = wool; 3 = manila; 4 = polypropylene; 5 = nylon).

Separate experiments were done to evaluate cords (15.24 cm length [6 in], 0.6 cm [0.25] diam) impregnated with a 0.1% fipronil or a 0.6% indoxacarb solution. The 0.1% fipronil solution was prepared by combining 2.7 ml of the formulated insecticide (Termidor SC, 9.1% a.i., BASF, Research Triangle Park, NC) with 250 ml of tap water. The 0.6% indoxacarb solution was prepared by combining 5 g of formulated insecticide (DPX MP062, 30WG, DuPont, Wilmington, DE) with 250 ml of tap water. Cords were impregnated by dipping for ~2 sec in the insecticide solution and were then allowed to dry in a fume hood.

Groups of 50 female flies were placed within each arena and provided a 10% sugar water solution *ad libitum*. Mortality counts were recorded until at least 80% mortality was observed. Due to the differences in the mode of action of the insecticides, mortality for flies exposed to fipronil-impregnated cords was defined as the inability to remain standing; flies exposed to indoxacarb-impregnated cords were considered dead if they were unresponsive to touch. Each experiment was run in the laboratory (28 ± 1ºC) under continuous light and replicated twice.

Impregnated-Cord Field Cage Bioassay. Cages (1.8 x 3.7 x 1.8 m) were constructed from PVC pipe (2.54 cm [1 in] diam) and enclosed with mesh screening (Outdoor Cage, #1412A, 18 x 14 mesh, Bioquip, Rancho Dominguez, CA). Black plastic sheeting (6 mil) was used to line the floor. A sampling stage, constructed of two vertical cinder blocks and an inverted storage bin (Palletote #1721, 37 liter, Rubbermaid, Winchester, VA), was placed in the center of the cage (Figure 3-1B). On top of the sampling stage there were two 994-ml (1 qt) chick waterers, one filled with 10% sugar water and the other with tap water, and a 60-ml plastic cup filled with 8 g of previously used larval house fly medium. The chick waterers provided enough sustenance for the duration of the test and the plastic cup was used as an attractant. The plastic cup was covered with a paper towel and sealed with a rubber band to prevent flies from ovipositing on the medium.

Treatments consisted of two long (0.9 m) and eight short (0.6 m) lengths of 0.1% fipronil- and 1.2% indoxacarb-impregnated wool cords. The 0.1% fipronil solution was prepared by combining 7.7 ml of the formulated insecticide (Termidor SC, 9.1% a.i., BASF, Research Triangle Park, NC) with 700 ml of tap water. The 1.2% indoxacarb solution was prepared by combining 28 g of formulated insecticide (DPX MP062, 30WG, DuPont, Wilmington, DE) with 700 ml of tap water. Each cord was treated in the same manner as the laboratory experiments, except the cords were dipped and soaked for 1 min prior to drying.

Depending on fly availability, 27.5 – 35 ml (9.8 ± 1.8 flies/ml) of flies was released into each cage. After a 1-h acclimation period, pre-treatment fly counts were taken. Before fly counts were taken, the operator walked three laps around the interior of the cage to disturb flies from their resting positions and to recover any dead flies from the cage floor. Four consecutive fly counts were then taken from the outside of the cage 1 min after exiting. All flies that landed

on the sampling stage, chick waterers, and plastic cup attractant were counted. Treatments were then hung vertically from the mesh ceiling using paper clips in specific locations (Figure 3-1C) and post-treatment fly counts were taken at 24 and 48 h using the same method described above. After the initial 48 h evaluation, treatments were aged in the elements for four weeks, at which point residual effectiveness was re-evaluated as described above. Three replicates were performed at each treatment age (0 and 4 wk).

Statistical Analysis.

All statistical analyses were performed using JMP IN (SAS Institute 2005), except probit analysis estimates were performed using SAS (SAS Institute 2001). For the cord attractiveness experiments, the mean number of flies/cord was analyzed using a one-way analysis of variance and contrasts were performed between natural and synthetic cords and the animal- and plant-based cords. For the laboratory insecticide-impregnated cord laboratory experiments, mortality data were corrected using Abbott's formula (1925) and arcsine square root-transformed. A two-way analysis of variance was performed on the 24-h fipronil data and the 48-h indoxacarb data to determine if treatment position had an effect on mortality. LT_{50} values were estimated by probit-analysis regression (Finney 1971). Potency ratios, using the cotton cord as the standard, were performed using the method described in Robertson and Preisler (1991). Slopes, LT_{50} values, and potency ratios were considered significantly different if the 95% confidence intervals did not overlap. For the field cage experiments, percent fly count reductions were calculated from the control fly counts. Fly count reductions and mortality data (number of dead flies recovered from cage floor) were then analyzed for each treatment age (0 and 4 wk). All means were separated using the Student's T or Student-Newman-Keuls test ($\alpha = 0.05$).

Results

In the laboratory studies, all flies fully recovered from chilling after approximately 45 min at which point the flies were dispersed throughout the entire arena. Flies were more attracted to the manila cord, which had significantly more flies resting on it than any other cord (Figure 3-2). No significant differences were seen between the other natural cords or between the synthetic cords; however, all synthetic cords had significantly less flies resting on them than the natural cords (F: 112.69, df = 368, P = <0.001) and the plant-based cords were more attractive than the animal-based cords (F: 11.64, df = 368, P = <0.001). The least attractive cord was the nylon parachute cord.

The laboratory design had no position or interaction effects for either fipronil (F: 1.05; df = 4; P = 0.3982, F: 0.8347; df = 20; P = 0.6583) or indoxacarb (F: 0.71; df = 4; P = 0.5906, F: 0.32; df = 20; P = 0.9955) in the insecticide-impregnated cord experiments. At the 24 h (fipronil) and 48 h (indoxacarb) recordings, all impregnated-cords had significantly higher mortality than the controls (Figure 3-3).

House flies suffered significantly higher mortality when exposed to the fipronil-impregnated wool cord than any other fipronil-impregnated cord at 24 h (93%). The other fipronil-impregnated natural cords had percent mortalities below 15%, with manila causing only 5% mortality at 24 h. No significant differences in mortality were seen between the fipronil-impregnated nylon and polypropylene cords or the fipronil-impregnated cotton and manila cords at 24 h.

The indoxacarb-impregnated wool cord caused significantly higher mortality (85%) than any of the other cords except for the cotton cord at the 48 h recording. No significant differences in mortality were seen between the synthetic indoxacarb-impregnated cords or between the cotton and manila indoxacarb-impregnated cords. Significant differences in mortality were seen

between the wool and manila indoxacarb-impregnated cords. The indoxacarb-impregnated nylon cord caused the lowest mortality at 48 h (47%).

Fipronil- and indoxacarb-impregnated cords efficacy results can be viewed in Table 3-1. In general, the fipronil impregnated cords had lower LT_{50} and LT_{90} values than the indoxacarb-impregnated cords. Among the fipronil-impregnated cords, the wool cord had the lowest LT_{50} and LT_{90} values and the impregnated cotton cord had the highest LT_{50} and LT_{90} values. The LT_{50} values for the synthetic cords were relatively low compared to the other cords, but the LT_{90} values were no different from the cotton cord. The manila cord LT_{50} value was the second highest, but had the second lowest LT_{90} value behind the wool cord; it is important to note that it also had the highest slope compared to the other cords. All cords were more effective than the cotton cord except for the nylon cord's LT_{90} value.

All indoxacarb-impregnated cords had LT_{50} values >32 h and LT_{90} values >51 h. The indoxacarb-impregnated wool cord had lower LT_{50} and LT_{90} values than all other indoxacarb-impregnated cords except for the manila cord, which showed no significant differences in LT_{50} values. The indoxacarb-impregnated polypropylene and cotton cords each had LT_{50} values of 52 h and LT_{90} values >100 h, which were the highest values for the experiments. No differences in LT values were observed between the manila and nylon cords. All cords were more effective than the cotton cord except for the polypropylene cord's LT_{50} and LT_{90} values.

In the field cage experiments, no significant differences in fly count reductions occurred between the treatments (Table 3-2). Both treatments had >57% fly count reductions by 24 h and >87% by 48 h, independent of the treatment age. Dead flies were collected from all cages at every recording; significantly more were collected from the treatment cages than the controls. Fipronil treatments had significantly more dead flies than the indoxacarb treatments with fresh

cords at 24 and 48 h and with aged cords at 24 h. No significant differences were seen between the number of dead flies collected from the fipronil treatments and indoxacarb treatments at the 4 wk, 48-h recording.

Discussion

Insecticide-impregnated fly cords are based on a fundamental component in a fly's behavior – flies prefer to rest on objects with distinct edges, such as twigs, wires, cord, and line (Scudder 1949). Since insecticide-impregnated cords only represent a small proportion of available resting surfaces available to flies, it is assumed that factors which enhance a fly's attraction to the cords would be beneficial to the effectiveness of the treatment. Surfaces which are more attractive to flies would be expected to cause quicker mortality because of increased exposure to the insecticide. Arevad (1965) found flies to favor dark, rough surfaces over light, smooth surfaces. Specific factors influencing a fly's attraction to natural fiber cords were evaluated by Fay and Lindquist (1954). They found sisal cord to be more attractive than jute or wool cords of the same size, but less attractive than a similar sized cotton cord. When given a choice between only cotton and sisal cords, flies preferred the sisal cord. They also found that the same type of cord was more attractive to flies as the cord diameter increased between 0.13-1.1 cm.

In our attractiveness experiment, the cords we evaluated varied by fiber type (animal or plant), color, texture, and, in some cases, even diameter. All of the natural cords we evaluated were more attractive than the synthetic cords. The natural cords were "rougher" than the relatively smooth synthetic cords; in addition, the plant-fibered manila cord and the animal-fibered leather cord were darker than the other cords. These factors may have increased their overall attractiveness to the flies. If comparing the most attractive cord (manila) to the least attractive (parachute cord) the differences in texture and color are substantial (Figure 3-4).

Manila is a very rough, coarsely textured brown thatch cord made from the leaf fibers of the abaca tree, *Musa textiles,* while the parachute cord is a relatively smooth kernmantle cord made of white nylon. The parachute cord was one of two cords less than 0.64 cm, which may have decreased its attractiveness. The other cord less than 0.64 was the leather cord, but it was as attractive as the other natural cords (except manila) despite its diameter being half the size. The leather cord's dark color may have increased its attractiveness or it may have been more attractive due to animal odors that were still associated with the material.

The previously available commercial fly cords were exclusively made of cotton. Cotton was cheap, durable, absorbent and widely available. Although cotton was relatively attractive in our experiments, it had very poor efficacy for both fipronil and indoxacarb when compared to the other natural and synthetic cords tested indicating that it may not be the best type of cord to use for insecticide treatment. Fipronil- and indoxacarb-impregnated wool cords had the greatest efficacy in our experiments despite flies resting on it 50% less than the manila cord in the cord attractiveness experiments. This is contrary to the previous assumption that quicker mortality would result from increased exposure to a more attractive insecticide-impregnated cord and neglects to account for the insecticide-substrate interaction. Highly organic materials readily bind to pesticides and make them less effective (Dell et al. 1994, Gardner et al. 2000) and may have accounted for the low LT_{50} and LT_{90} values seen in the cotton cords and in the LT_{50} value of the fipronil-impregnated manila cord. The exact reason wool outperformed the other cords in our experiments was not fully investigated, but it is likely due to the insecticide-substrate interaction. The wool cord was the only animal-fibered cord evaluated and is naturally impregnated with several oils. Both fipronil and indoxacarb are very lipophilic insecticides and

probably dissolved readily within these oils which likely increased the rate of insecticide transfer from the cords through the waxy layers of the fly's cuticle.

In the laboratory, the indoxacarb cords generally provided a much slower kill than the fipronil cords, however, in the field cages differences were not as apparent. Indoxacarb is a pro-insecticide that needs to be bioactivated within the insect before it is toxic and will always cause mortality slower than an insecticide, such as fipronil, that is toxic upon contact once a lethal dose is obtained. Flies poisoned by indoxacarb in the laboratory are shielded from desiccation and predation, which may have proved to be vital to their prolonged survival in the laboratory experiments. Furthermore, the indoxacarb dose was increased in the field cage experiments and may have affected the faster results seen in the field cage experiments. Both treatments showed a decrease in efficacy in the field experiments after being aged 4 weeks, but still had adequate fly count reductions and causing significantly more flies to die than the control.

In conclusion, the use of insecticide-impregnated cords is very practical to supplement a house fly management program. Insecticide-impregnated cords ensure adequate residual coverage in areas difficult to treat with traditional residual insecticides and they can easily be removed and relocated to other fly resting areas if needed or alternated with cords impregnated with other active ingredients to reduce the possibility of resistance development. More research still needs to be done to determine adequate doses and rates of treatment, keeping in mind that these may vary depending on cord type and insecticide used. Wool cord outperformed all other cords evaluated in this study and fipronil and indoxacarb both appear to be effective insecticides for house fly control.

Table 3-1. Efficacy of various cords impregnated with 0.1% fipronil or 0.6% indoxacarb on female house flies.

				Lethal Times (h) (95% CL)[§]				Potency Ratio (95% CL)[§]	
Treatment	Cord	n[†]	Slope ± SE[§]	50	90	χ^2	P	50	90
Fipronil	Cotton	2750	9.52 ± 0.36b	39.7 (39.2-40.2)e	54.1 (52.9 - 55.5)c	9.060	0.1067	1.00 e	1.00 d
	Manila	1000	12.98 ± 0.83a	35.0 (34.5-35.6)d	44.0 (42.6 - 45.7)b	1.213	0.5445	1.13 (1.12-1.15)d	1.23 (1.21-1.25)b
	Wool	1250	5.32 ± 0.29c	12.9 (12.3-13.4)a	22.4 (21.3 - 23.8)a	0.650	0.4200	3.09 (3.01-3.16)a	2.42 (2.35-2.48)a
	Polypro	2500	4.65 ± 0.29d	26.2 (25.6-27.0)c	49.6 (46.4 - 53.8)c	3.643	0.7249	1.51 (1.45-1.57)c	1.09 (1.04-1.14)c
	Nylon	1500	3.68 ± 0.25e	23.0 (21.2-24.6)b	51.3 (48.2 - 55.4)c	1.455	0.6927	1.72 (1.66-1.79)b	1.05 (1.00-1.11)cd
Indoxacarb	Cotton	2248	4.04 ± 0.13c	52.2 (50.3-54.3)c	108.5 (102.1-115.9)c	1.3494	0.5093	1.00 c	1.00 c
	Manila	1659	5.10 ± 0.74b	36.2 (32.3-38.7)ab	64.7 (60.2 - 73.4)b	3.4030	0.3336	1.44 (1.35-1.54)ab	1.68 (1.54-1.82)b
	Wool	4250	6.44 ± 0.16a	32.6 (32.0-33.1)a	51.5 (50.1 - 53.1)a	8.6295	0.2804	1.60 (1.54-1.67)a	2.11 (2.01-2.20)a
	Polypro	2250	3.11 ± 0.20d	52.2 (49.7-54.5)c	134.8 (122.7-151.7)d	0.6717	0.7147	1.00 (0.91-1.10)c	0.80 (0.72-0.90)c
	Nylon	2000	6.57 ± 0.54a	39.2 (37.2-40.8)b	61.5 (59.4 - 64.4)b	2.9321	0.2308	1.33 (1.27-1.39)b	1.76 (1.68-1.85)b

[†] Total number of trials; 500 flies/trial except for the cotton (498) and manila (487) indoxacarb-impregnated cords (Probit [SAS Institute 2002]).

[§] Mortality was corrected using Abbott's Formula. Means within a column, in the same treatment group, followed by the same letter are not significantly different based on non-overlap of 95% confidence intervals.

Table 3-2. Cumulative number of dead flies and percent fly count reduction in relation to control fly counts of house flies exposed to 0.1% fipronil- and 1.2% indoxacarb-impregnated cords in field cages.

		% Fly Count Reduction ± SEM‡		# of Dead Flies†‡	
Treatment Age	Treatment	24 h	48 h	24 h	48 h
0 Weeks	Fipronil	80.22 ± 13.10a	98.66 ± 1.34a	83.0 ± 1.0a	95.3 ± 3.4a
	Indoxacarb	57.39 ± 6.92a	97.21 ± 1.43a	30.3 ± 4.4b	59.7 ± 2.0b
	Control			4.7 ± 4.2c	11.3 ± 8.5c
4 Weeks	Fipronil	59.25 ± 22.10a	87.43 ± 12.57a	53.0 ± 7.8a	79.3 ± 7.2a
	Indoxacarb	64.39 ± 5.89a	87.72 ± 7.34a	30.3 ± 4.7b	62.0 ± 8.1a
	Control			3.3 ± 1.7c	10.3 ± 2.0b

† Cumulative mean number of flies recovered from cage floor.

‡ Means in a column, within the same treatment age, followed by the same letter are not significantly different ($P > 0.05$; Student's T or Student-Newman-Keuls test)

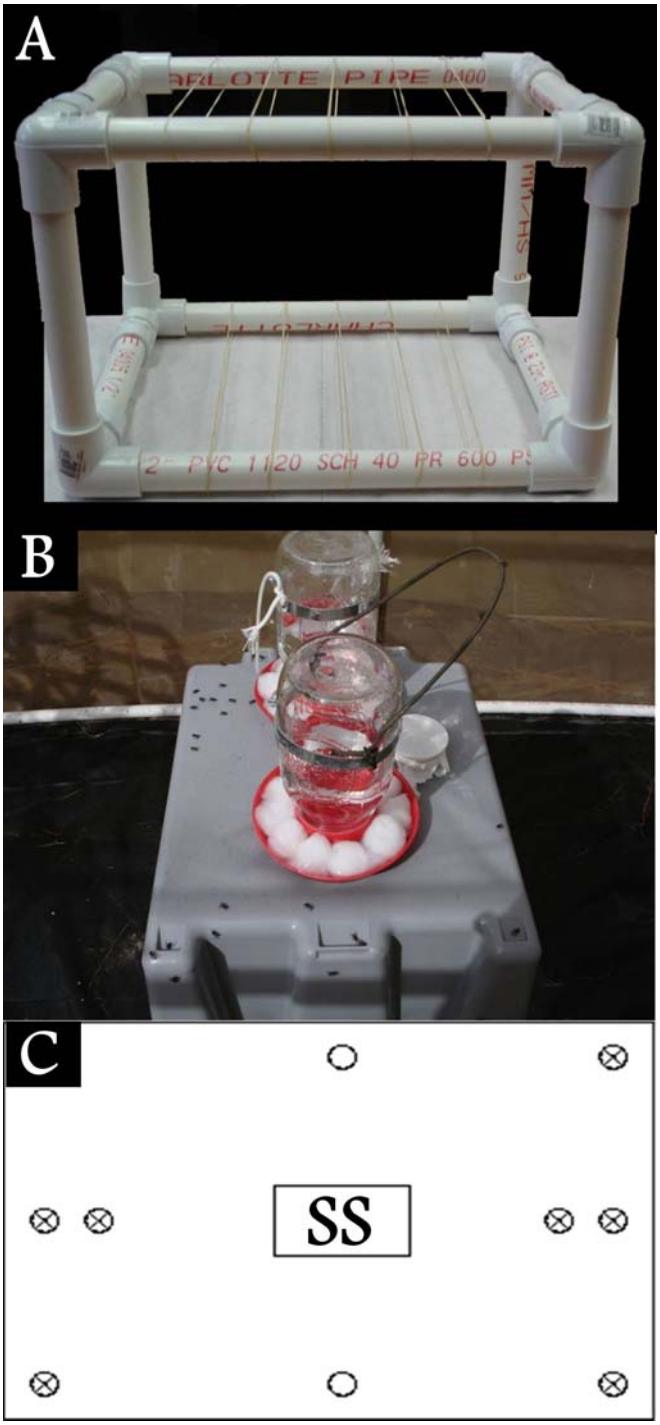

Figure 3-1. Laboratory and field experimental design elements. A). Laboratory arena constructed of PVC pipe. Cords were suspended between the rubber bands using paper clips. B) Sampling stage used in field cage experiments. Chick feeders with either 10% sugar water or tap water and a plastic cup containing previously used larval medium was used as sustenance and attraction. C). Cord placement in relation to sampling stage (SS) in the field cage bioassay. Crossed circles were short cords (0.6 m) and empty circles were long cords (0.9 m).

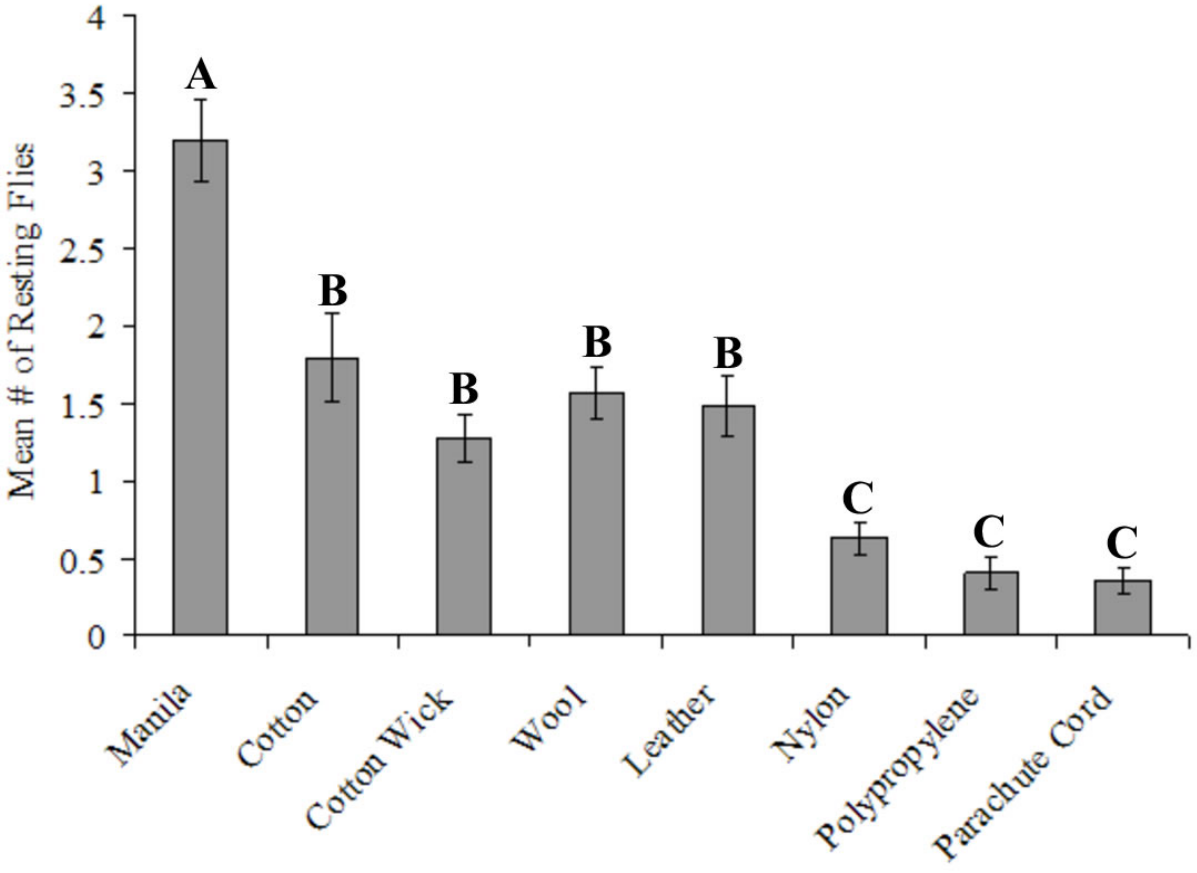

Figure 3-2. Attraction of female house flies to various natural and synthetic cords.

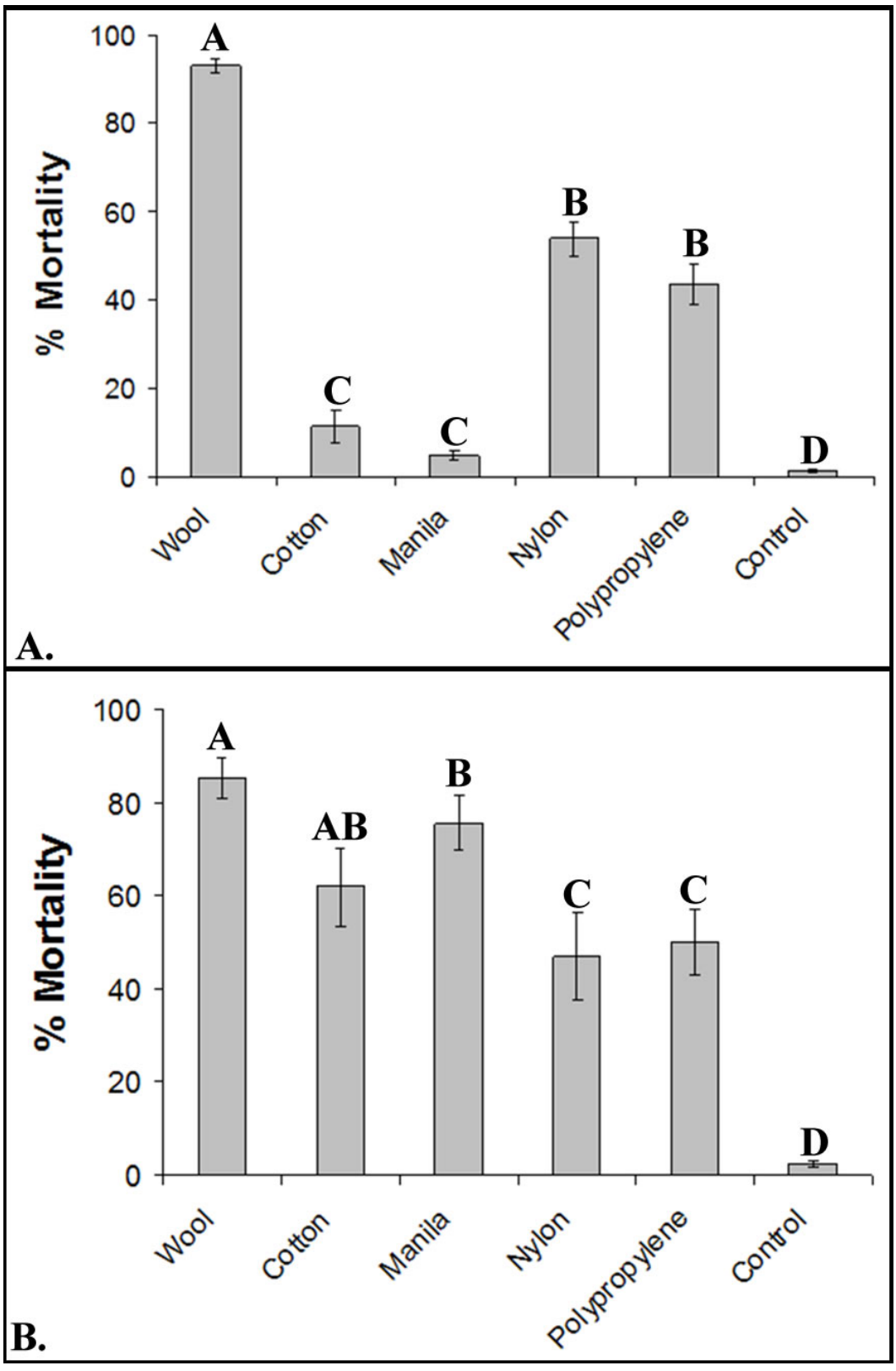

Figure 3-3. Female house fly mortality exposed to various natural and synthetic cords treated with 0.1% fipronil for 24 h (A) and 0.6% indoxacarb for 48 h (B).

Figure 3-4. Comparison of the most attractive cord (manila) and the least attractive cord (nylon parachute) in the cord attractiveness experiments.

CHAPTER 4
EVALUATION OF A NEW IMIDACLOPRID BAIT FOR HOUSE FLY CONTROL

Introduction

The house fly, *Musca domestica* L., is the most commonly encountered pest of the generalized group of Diptera called filth flies. Large numbers of house flies are frequently found in areas where manure, garbage, and other decaying organic matter are abundant. Although primarily a nuisance to people and animals, house flies can pose a health risk by mechanically transferring pathogens picked up from their breeding sites, particularly when they enter homes or eating establishments. When the source of infestation is inaccessible or sanitation measures are not effective, house fly control is often achieved using dry insecticidal scatter baits.

Two widely used scatter baits are Maxforce® Granular fly bait and Golden Malrin® fly bait. Maxforce® Granular is an imidacloprid-based bait containing the fly attractant (Z)-9-tricozene, the bittering agent Bitrex®, and other attractants and inert ingredients. It is currently the only imidacloprid-based scatter bait available. Golden Malrin® contains 1.1 % methomyl, 0.049 % (Z)-9-tricosene, as well as other attractants and inert ingredients, and is one of several methomyl-based scatter bait formulations available.

In general, dry scatter baits have many advantages over other types of insecticidal fly control products: they are easier to work with in field environments, they can be more attractive to flies than liquid baits, and they usually have a longer storage shelf life (Gahan et al. 1954, Darbro and Mullens 2004). However, dry scatter baits need to be replaced frequently in some areas when granules become covered by manure or other debris (Barson 1987). The U.S. Environmental Protection Agency (EPA), acting under legislative mandates, also requires the scatter bait granules be dyed to distinguish them from other non-toxic materials. For example, the Maxforce® Granular fly bait is formulated as red granules and the Golden Malrin® is

formulated as blue granules. When these granules become wet, the dye often bleeds onto the surrounding surface and may be unsightly for the user.

Label restrictions are also very different and can limit the uses of certain active ingredients or insecticide products. Golden Malrin® can only be applied as scatter bait or within bait stations, whereas, the Maxforce® Granular can be applied as scatter bait, within bait stations, or it can be mixed with water and painted onto surfaces allowing it to be applied directly to distinct fly resting areas, such as on ceilings or rafters. However the use of Maxforce® Granular is more restricted than that of Golden Malrin® because its label restricts its use in food establishments. Golden Malrin®, despite being the only carbamate-based insecticide not classified as "restricted-use", can be used within food establishments when used in bait stations placed at least 1.2 m from the ground in areas where food processing or preparation does not occur.

An imidacloprid sprayable bait, Maxforce® Fly Spot, has recently become commercially available. It contains 10% imidacloprid, 0.1% Z-9-tricosene, Bitrex®, and inert ingredients. This formulation still maintains the advantages of traditional scatter baits, while eliminating some of the disadvantages of the currently available products. Once applied, Maxforce® Fly Spot bait dries clear and the label allows for application within food establishments when the facility is not in operation.

Our objectives were to compare the effectiveness of the new sprayable bait in relation to the two most commonly used dry scatter baits. In addition, we compared the performance of the imidacloprid sprayable and granular baits in a controlled field environment and tested the imidacloprid sprayable bait impregnated in cords.

Materials and Methods

Insects. The Horse Teaching Unit (HTU) strain of house flies, *M. domestica* L., reared at the University of Florida in Gainesville was used for all experiments. Larvae were reared on a diet medium, modified from Hogsette (1992), containing 3 liters wheat bran, 15 ml methyl paraben, 1.5 liters water, and approximately 200 g (250 ml) dairy calf feed (Calf Manna® pellets, Manna Pro Corp., St. Louis, MO). All developmental stages were held at 26 ± 1°C and 55% RH with a 12:12 (L:D) photoperiod. Adult flies emerged within in screened rearing cages and were provided granulated sugar, powdered milk, and water *ad libitum*.

For all assays, adult house flies (3-5 d old) were aspirated from the screened rearing cages using a handheld vacuum with a modified crevice tool attachment. Flies used for the laboratory assays were placed into a 5°C environment for 5 min to subdue activity. Flies were then placed on a chilled aluminum tray, sexed, and counted. House flies used for field cage assays were not subdued, but were aspirated from the screened rearing cages and released directly into field cages.

Laboratory Arena Design. Arenas (31 x 25 x 21 cm) were constructed using PVC pipe (1.27 cm [1/2 in] diam) (Figure 3-1A). Rubber bands were used to establish five uniformly distributed cord positions along the length of the arena. Each position held one cord which was vertically attached to the rubber bands using paper clips. Five cords were used with all laboratory experiments: nylon (Braided, Multi-Purpose Braid 75 lb. load limit, Wellington Cordage LLC, Madison, GA), polypropylene (Braided, Multi-Purpose Rope – 56 lb. load limit, Wellington Cordage LLC, Madison, GA), cotton (Braided, Multi-Purpose Sash Cord – 28 lb. load limit, Wellington Cordage LLC, Madison, GA), manila (Twisted, Natural Rope – 108 lb. load limit, Wellington Cordage LLC, Madison, GA), and wool (Twisted, Natural Cord, Wooded

Hamlet Designs, Greencastle, PA). Arenas were enclosed with a transparent plastic bag (3716 cm2 [24 x 24 in], 1 mil poly, Uline, Waukegan, IL).

Field Cage Design. Cages (1.8 x 3.7 x 1.8 m) were constructed from PVC pipe (2.54 cm [1 in] diam) and enclosed with mesh screening (Outdoor Cage, #1412A, 18 x 14 mesh, Bioquip, Rancho Dominguez, CA). Black plastic sheeting (6 mil) was used to line the floor. A sampling stage, constructed of two vertical cinder blocks and an inverted storage bin (Palletote #1721, 37 liter, Rubbermaid, Winchester, VA), was placed in the center of the cage (Figure 3-1B). On top of the sampling stage there were two 994-ml (1 qt) chick waterers, one filled with 10% sugar water and the other with tap water, and a 60-ml plastic cup filled with 8 g of previously used larval house fly medium. The chick waterers provided enough sustenance for the duration of the test and the plastic cup was used as an attractant. The plastic cup was covered with a paper towel and sealed with a rubber band to prevent flies from ovipositing on the medium.

Fly Bait Comparisons. Three fly baits, 2 dry scatter baits and 1 sprayable bait, were applied to polystyrene Petri dishes (100 by 15 mm; Fisher Scientific, Pittsburgh, PA). The methomyl granular bait (Golden Malrin®, Methomyl 1.1%, (Z)-9-Tricosene 0.049%, Wellmark International, Schaumburg, Illinois; dose: 0.23 g/0.9 m^2) and the imidacloprid granular bait (Maxforce® Granular fly bait, Bayer CropScience, Kansas City, MO; dose: 30.17 g/0.9 m^2) were sprinkled on the Petri dish. The Imidacloprid sprayable bait (Maxforce® Fly Spot bait, Imidacloprid WG 10, Lab Code: 342/207-7, Bayer CropScience, Monheim am Rhein, Germany; dose: 0.45 g/0.9 m^2; rate: 0.12 g Pr/ml/0.9 m^2) was suspended in tap water, sprayed on the Petri dish bottom using an airbrush (Paasche, Type H, Chicago, IL), and allowed to dry in a fume hood prior to being placed in the arena. Bait dishes were placed on the bottom rubber bands in the center of the arena. A separate arena with an untreated Petri dish was used as the control.

Cords in these experiments served only as resting positions for the flies, they were untreated and hung in the same configuration for all repetitions: (left to right: position 1 = cotton; 2 = wool; 3 = manila; 4 = polypropylene; 5 = nylon).

Groups of 50 female flies were placed within each arena and a 10% sugar water solution was provided *ad libitum*. Mortality was recorded at 1, 3, 5, and 24 h. Flies were considered dead if they were unable to stand or fly. Each experiment was run in the laboratory (30 ± 1ºC) under continuous light and replicated three times.

The two imidacloprid baits were evaluated in the field cages. Treatments consisted of two plastic lattice squares (0.19 m^2) treated with imidacloprid granular bait, imidacloprid sprayable bait, or tap water (control). Treatments were applied on only one side of the lattice at the same rates as the laboratory fly bait comparison assays. The imidacloprid granular bait was mixed with tap water (1.44 g: 1 ml) and painted on. The tap water (3.78 ml/0.9 m^2) and imidacloprid sprayable bait (0.12 g Pr/ml/0.9 m^2) were sprayed on using an airbrush. All treatments were allowed to thoroughly dry outdoors in the open air before being hung on the ceiling PVC pipes using cable ties. Each lattice square was placed medially along the length of the cage, approximately 0.5 m away from each side of the sampling stage and positioned so that the treated surfaces of the lattice squares faced opposite directions.

Depending on fly availability, 27.5 – 35 ml (9.8 ± 1.8 flies/ml) of flies were released into each cage. After a 1-h acclimation period, pre-treatment fly counts were taken. Before fly counts were taken, the operator walked three laps around the interior of the cage to disturb flies from their resting positions and to recover any dead flies from the cage floor. Four consecutive fly counts were then taken from the outside of the cage 1 min after exiting. All flies that landed on the sampling stage, chick waterers, and plastic cup attractant were counted. Treatments were

then hung within the cages and post-treatment fly counts were taken at 1 and 24 h using the same method described above. After the initial 24 h evaluation, treatments were aged in the elements for two weeks, at which point residual effectiveness was re-evaluated as described above. Three replicates were performed at each treatment age (0 and 2 wk).

Bait-Treated Cords. Five laboratory arenas were organized into a 5 x 5 Latin square design, blocking for treatment position, and a sixth arena was used as the control. Each treatment consisted of a cord (15.2 cm length, 0.6 cm diam) impregnated with a 2.5% solution of imidacloprid sprayable bait. The imidacloprid solution was prepared by combining 25 g of the formulated insecticide with 100 ml of tap water. Cords were impregnated by dipping for ~2 sec in the insecticide solution and were then allowed to dry on aluminum foil covered trays in a fume hood prior to being placed into the arenas. The control arena had no treated cords and had the same cord configuration as the fly bait comparison bioassay described above.

Laboratory tests were conducted with groups of 60 female flies/arena. Flies were provided a 10% sugar water solution *ad libitum*. Morbidity (knockdown) was recorded at 2-5 h post-treatment and mortality was recorded at 24, 48, and 72 h. Flies were considered knocked down if they did not move when touched at the 2-5 h recordings. Flies that were unresponsive to touch at the 24, 48, and 72 h recordings were considered dead. Each experiment was run under continuous light in the same laboratory conditions as described above and replicated twice.

In the field cages, treatments consisted of two long (0.9 m) and eight short (0.6 m) lengths of imidacloprid-impregnated wool cords. Each cord was treated in the same manner as the laboratory experiments, except the cords were dipped and soaked for 1 min prior to drying. Flies were released and fly counts were taken in the same manner as the imidacloprid bait field cage experiments. Cords were hung vertically from the mesh ceiling using paper clips in specific

locations, which remained constant throughout the experiment (Figure 3-1C). Post-treatment sampling counts were done at 24 and 48 hrs. After the initial 48 h evaluation, treatments were aged in the elements for four weeks, at which point residual effectiveness was re-evaluated as described above. Three replicates were performed for each treatment age (0 and 4 wk).

Data Analysis. All analyses were done using a one-way analysis of variance with JMP IN (SAS Institute 2005). For the fly bait comparison and the bait-treated cord experiments, percent morbidity (bait-treated cords) and mortality data were arcsine square root-transformed and analyzed for each time interval. For the field cage experiments, percent fly count reductions were calculated from the control fly counts. Fly count reductions and mortality data (number of dead flies recovered from cage floor) were then analyzed for each treatment age (0 and 2 wk for the imidacloprid comparisons; 0 and 4 wk for the bait-treated cord experiments) (Conover and Iman 1981). Means for all analyses were separated using the Student's T test or the Student Newman Kuels (SNK) method ($\alpha = 0.05$).

Results

In all laboratory experiments, flies did not fully recover from chilling until roughly 1 h after entry into the arenas. Flies first contacted the baited Petri dishes in the bait comparison experiments approximately 35 min post recovery in the following order: imidacloprid granular bait, imidacloprid sprayable bait, methomyl granular bait. Initial fly contact on the treated cords in the bait-treated cord experiments was not observed.

Fly Bait Comparisons. The imidacloprid granular and the imidacloprid sprayable baits had higher fly mortality than the methomyl granular fly bait at 3 h, but by 24 h the methomyl granular bait had the highest overall mortality (Figure 4-1). At 24 h, fly mortality with the imidacloprid sprayable bait was not significantly different from mortality with either the imidacloprid granular or the methomyl granular fly baits, but significant difference in fly

mortality did exist between the methomyl and the imidacloprid granular fly baits. All treatments were significantly different than the control fly mortality at all observations ≥ 3 h.

In the imidacloprid field cage experiments, no differences were seen between either treatments with fresh or aged cords (Table 4-1). Both treatments had >35% fly count reductions at 24 h and >70% fly count reductions by 48 h with fresh cords, but fly count reductions did not exceed 8% with aged cords for either treatment. A fly count increase was observed with the imidacloprid granular treatment at 48 h with aged cords. The number of dead flies collected in the treatment cages was significantly different from the control with fresh cords, but no differences were observed between the aged treatments and controls 2 wk post-treatment.

Bait-Treated Cords. Morbidity increased on a time-dependent basis until approximately 3-4 hours post-treatment, at which time flies recovered from being knocked down by all of the imidacloprid bait-treated cords except for the cotton cord (Figure 4-2). Flies exposed to all imidacloprid bait-treated cords had knockdown recovery by 24 h. All cords caused significantly more mortality than the control cords at every 24 h recording (Figure 4-3). Beyond 24 h, mortality with the imidacloprid bait-treated nylon, cotton, and wool cords increased more sharply than the mortality caused by the bait-treated manila and polypropylene cords. The imidacloprid bait-treated wool cord caused the highest overall fly mortality (74%). All other cords resulted in house fly mortalities <60% with the imidacloprid bait-treated polypropylene cord showing the lowest overall fly mortality (25%).

In the field cages, the imidacloprid bait-treated cords caused >87% fly count reductions by 24 h with fresh and aged cords (Table 4-2). The aged bait-treated cords fly count reductions decreased by ~6% by 48 h, whereas fly count reductions increased by ~6% with the fresh cords.

The number of dead flies collected was significantly different than the control at 24 and 48 h for both fresh and aged cords, except for the 48 h recording with the aged cords.

Discussion

When insecticides are used for house fly control, most users expect to see satisfactory results (i.e. dead flies) within hours and markedly reduced populations within 1-2 days. Thus, an effective fly bait will attract flies quickly and cause high mortality within a relatively short period of time. In our bait comparison experiment, flies contacted the imidacloprid baits sooner than they contacted the methomyl bait, which may have been a contributing factor to the higher fly mortality at 3 h with the imidacloprid baits than with the methomyl bait. However, the higher fly mortality with the methomyl bait after 24 h suggests that methomyl may be a more potent, although slower acting, active ingredient.

Other studies comparing imidacloprid and methomyl baits have also shown the same mortality trends we observed in flies exposed to technical and bait formulations of imidacloprid and methomyl (White et al. 2007). In those experiments, White et al. observed up to 50% of the flies that were knocked down by imidacloprid formulations recovered. They hypothesized innate characteristics, independent of resistance mechanisms, may make some flies tolerant to neonicotinoids. We observed knockdown recovery in the bait-treated cord experiments with all cords, but no recovery was seen in the house flies exposed to any of the baits we tested in the bait comparison experiments. Recovery may have occurred in these experiments, but was not observed because recordings were not taken between the 5 h and 24 h recordings. Flies that were knocked down were not isolated from the arena in our experiments and could have received a second dosing before having the opportunity to fully recover.

Differences in cord material or treatment application technique may have also attributed to fly recovery in the bait-treated cord experiments. Cord saturation is dependent on the cord

composition and may have lead to sublethal dosing. We observed that cord composition varied between the types of cords we used, and even among individual cords. Distribution of oils and other materials on the surface of each cord make it difficult to have the bait uniformly distributed over the surface of the cord. When bait is sprayed onto a solid surface, such as the Petri dish, a precise amount of bait remains on the surface after the water evaporates. However, when a cord is dipped into a bait solution, the bait may be absorbed deep into the fibers, disperse throughout, or pool in areas on the cord. Thus, some bait may not be available for flies to contact. This is evident when hand-dipping cords in dyed insecticides materials, such as in an indoxacarb wettable granule (WG) solution, which is grayish-brown in color. Despite being fully submerged in the insecticide solutions, some of the cord often remains its natural color, apparently void of any bait. Once the same cords are allowed to completely dry and are removed from the drying trays, brown staining surrounds were they once lay indicating that some of the insecticide may be lost during the drying process as well.

It is undetermined if knockdown recovery occurred in our field cage experiments. If flies are knocked down in the field, natural enemies may prey upon them before they are able to fully recover. We observed knocked-down flies being preyed upon by ants, spiders, and lizards. Others, undoubtedly, became victim to desiccation after being knocked down. Barson (1987) found that flies knocked down by methomyl in the field often lost their ability to fly, but still had the ability to reproduce. White et al. (2007) commented that 10% of the flies knocked-down by imidacloprid in their laboratory studies fully recovered and resumed normal behavior when protected from a second exposure to imidacloprid. The inability to fly would make flies more vulnerable to predation by natural enemies. However, if reproduction is still occurring, it will be detrimental to any fly control program because of a fly's prolific reproductive capabilities. Field

studies to determine the effect of knock down and recovery on a fly management program would be beneficial.

Insecticide-impregnated cords have been used extensively in the past to control flies and have recently been examined using new insecticides not yet registered for use against house flies (*Unpublished,* Chapter 3). In those experiments, indoxacarb and fipronil were more effective on wool cords than any other natural and synthetic cords tested. Wool cords also showed higher efficacy than the other natural and synthetic cords tested when treated with the imidacloprid sprayable bait. Exact LT_{50}'s were not determined in these experiments because of the knockdown and subsequent recovery observed, but based on Figure 4-3, we estimate that 50% of the flies died after approximately 60 hours (2.5 d) with the wool cord. With such a long period to reach 50% fly mortality, imidacloprid-treated cords were slower acting than any of the fipronil- or indoxacarb-impregnated cords previously tested. However, in the field cages, the imidacloprid bait-treated cords reduced fly counts by 80% in 24 h. The high lipid content of wool cord may facilitate the transfer of insecticide through the cuticle of the house fly, but this does not explain the differences in results between the laboratory and field assessments. Differences in the rate of cords per cage area may explain the differences in the laboratory and field results. The cords in the field cages were hung at a rate of 9.1 m of cord/9.3 m^2 area based on the recommended rate of the insecticide cords used in the 1950's (Fehn 1958, Smith 1958, Weinburgh et al. 1961). The rate in the laboratory arena was comparatively much lower, 0.02 m of cord/m^2 vs. 1.0 m of cord/m^2. This rate appears to be quite high and may vary between different cord/insecticide combinations. Additionally, the aforementioned predation and desiccation of the knocked down flies probably was significant factor contributing to the rapid fly count reductions in the field cages.

When comparing the imidacloprid bait-treated cords and the imidacloprid bait-treated lattice squares, the bait-treated cords were more effective. The lattice squares did not reduce the fly counts to any significant degree after being aged 2 weeks, but the imidacloprid bait-treated wool cords had good fly count reductions even after being aged 4 weeks. The plastic lattice squares were selected as a treatment surface to represent the material found on many portable toilets, latrines, or dumpsters. Bait treatments on this type of surface are very vulnerable to environmental conditions because the material does not allow the bait to penetrate as in the cord treatments. Damp conditions in the mornings and unexpected precipitation (6.5 cm) that occurred between evaluation intervals washed away most of the bait product from the lattice squares. With the imidacloprid granular bait application, the lattice squares were almost completely void of the red dye following these moisture events and red staining was seen on the cage floor. We assume that the imidacloprid sprayable bait was also washed off the lattice squares given the results of the fly count reductions and the dead flies recovered, but was not observed because the bait has no color. The bait-treated cords were exposed to 3.5 cm less precipitation than the bait-treated lattice squares, which may have also affected the bait available on the cord. When dipping cords in a bait solution, the bait is absorbed in between individual cord fibers and even deeper into the core of the cord, making the bait more protected from environmental conditions. When the cords are then subjected to these moisture events, the bait may concentrate in specific areas of the cord (such as the cord end) instead of completely leaving the cord as seen with the lattice. Additionally, flies prefer to rest on cords and probably receive a larger dose of insecticide in this manner as compared to when they land on the flat surfaces of the lattice. When a fly lands on a flat surface they are exposed only to the precise amount of

toxicant that absorbs through their tarsi or is imbibed; however, when resting on cords, their thorax and abdomen are also brushed by the treated cord fibers.

In conclusion, the imidacloprid sprayable bait was found to be as effective as the traditional commercial scatterbaits compared in this study. Its unique formulation and less restrictive product label allow this bait to be used in areas where other fly baits are prohibited. Unless a more rain-fast formulation becomes available, the imidacloprid sprayable bait will need to be reapplied frequently in areas with high moisture or precipitation especially when applied to non-absorbent surfaces such as portable latrines or dumpster lids. The bait's potential effectiveness in insecticide-impregnated cords needs further investigation due to differing laboratory and field results. Regardless, this new imidacloprid sprayable bait should prove to be a very useful tool in any fly management program.

Table 4-1. Number of dead and percent fly count reduction in relation to control fly counts of house flies exposed to imidacloprid bait-treated lattice squares in field cages.

		% Fly Count Reduction ± SEM‡		# of Dead Flies†‡	
Treatment Age	Treatment	1 h	24 h	1 h	24 h
0 Weeks	Imidacloprid granular bait	47.1 ± 6.3a	70.9 ± 4.4a	36.0 ± 10.0a	117.0 ± 9.5a
	Imidacloprid sprayable bait	36.6 ± 20.5a	80.2 ± 4.7a	36.3 ± 2.0a	113.0 ± 10.1a
	Control			0.3 ± 0.3b	1.7 ± 0.7b
2 Weeks	Imidacloprid granular bait	0.8 ± 8.2a	-3.8 ± 20.1a	1.0 ± 0.6a	19.7 ± 11.2a
	Imidacloprid sprayable bait	7.6 ± 10.8a	6.3 ± 19.5a	0.7 ± 0.7a	14.0 ± 11.6a

† Mean number of individuals recovered from cage floor.

‡ Means in a column, within the same treatment age, followed by the same letter are not significantly different ($P > 0.05$; Student's T test or Student-Newman Kuels Method)

Table 4-2. Number of dead and percent fly count reduction in relation to control fly counts of house flies exposed to imidacloprid bait-treated cords in field cages.

		% Fly Count Reduction ± SEM		# of Dead Flies†	
Treatment Age	Treatment	24 h	48 h	24 h	48 h
0 Weeks	Bait-Treated Cords	90.4 ± 4.1	96.8 ± 3.2	97.7 ± 17.2a	114.3 ± 19.8a
	Control			12.7 ± 4.6b	19.7 ± 7.7b
4 Weeks	Bait-Treated Cords	87.9 ± 0.9	82.4 ± 14.7	50.7 ± 14.0a	69.3 ± 23.7a
	Control			8.7 ± 3.8b	13.0 ± 4.7a

† Mean number of individuals recovered from cage floor. Means in a column, within the same treatment age, followed by the same letter are not significantly different (P > 0.05; Student's T test)

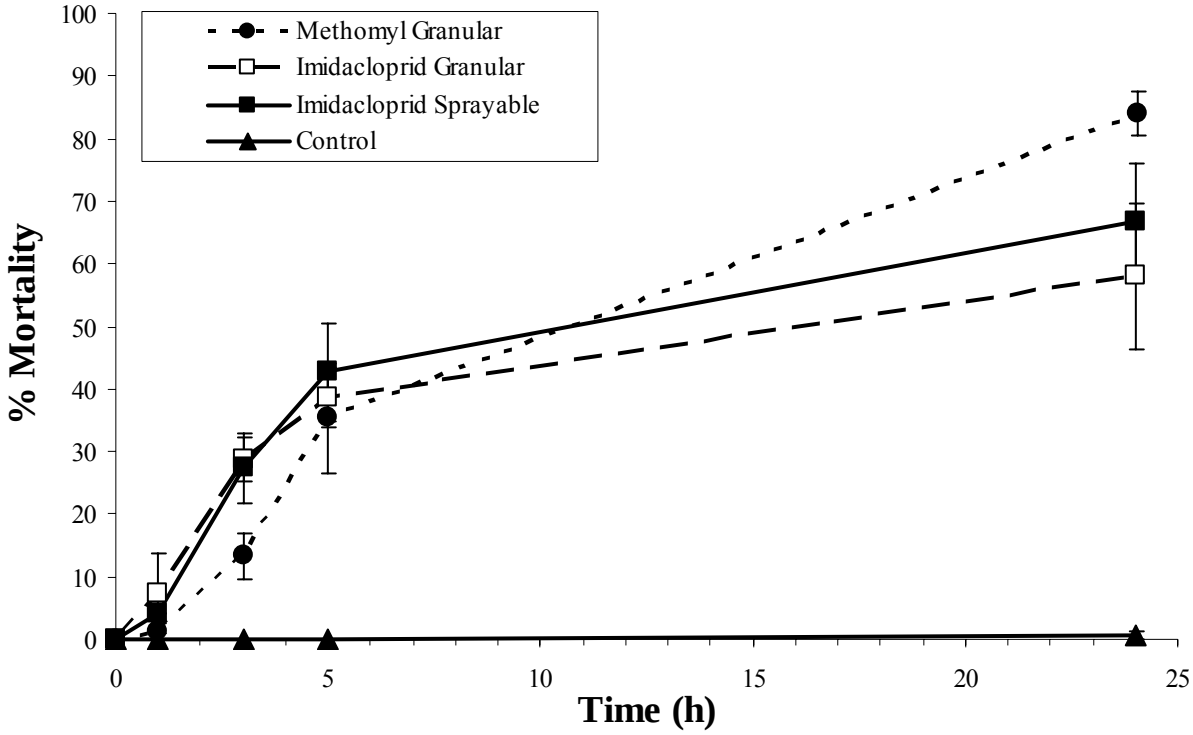

Figure 4-1. Mortality of female house flies exposed to imidacloprid and methomyl granular scatter baits and a sprayable imidacloprid bait.

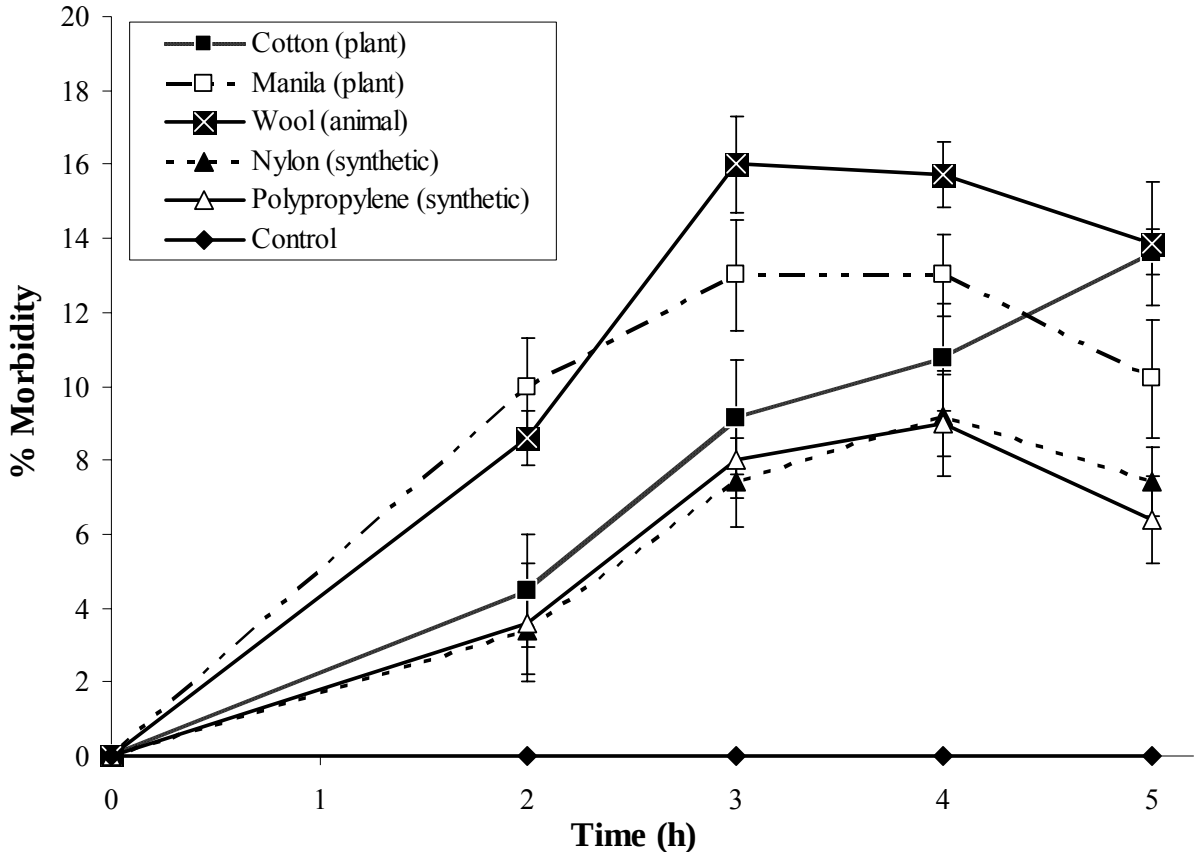

Figure 4-2. Morbidity (knockdown) of female house flies exposed to natural and synthetic cords dipped in a 2.5% solution of imidacloprid sprayable bait.

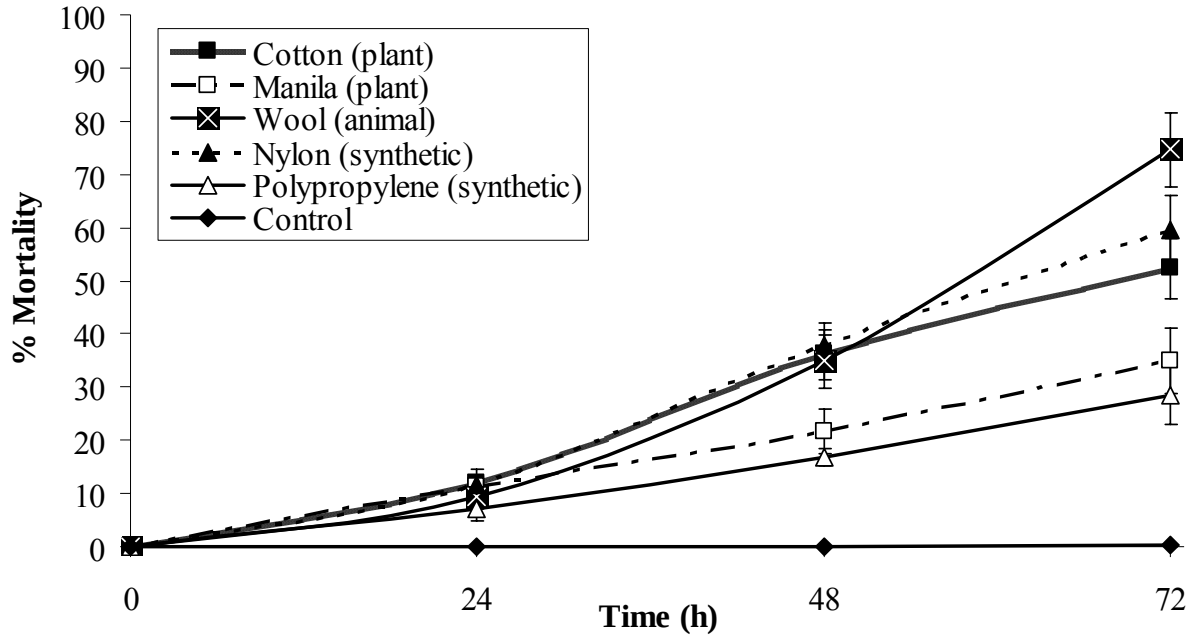

Figure 4-3. Mortality of female house flies exposed to natural and synthetic cords dipped in a 2.5% solution of imidacloprid sprayable bait.

CHAPTER 5
SUMMARY AND CONCLUSIONS

House flies are often found in tremendous numbers in situations where U.S. troops are most frequently deployed, such as areas stressed by conflict or natural disasters. House flies are nuisance pests that decreases troop morale and they pose a health risk to deployed troops that may disrupt mission objectives if diseases that are associated with their presence stress the health care systems. The main objective for this research was to evaluate insecticide-impregnated cords and sprayable fly bait as new methods to control the house fly and provide usable information to the DOD that will help protect the deployed war fighter.

First and foremost, house flies can be controlled using insecticide-impregnated cords and sprayable fly bait and their use would benefit agricultural, urban, and military fly management programs. Insecticide-impregnated cords and sprayable fly baits are both very easy to use products. Impregnated cords can be hung using a staple gun or other similar method and sprayable bait is mixed with water and sprayed onto any surface. Impregnated cords can be removed and relocated quickly, which may be beneficial in relatively mobile troop deployments or in situations were resistance is suspected. Sprayable baits can be removed with simple water wash down and easily reapplied when needed.

One of the most interesting findings in this research is the further understanding in insecticide-impregnated cord toxicity. Previous insecticide-impregnated cords were made exclusively of cotton because it was cheap, durable, and relatively attractive to house flies, however, the cotton cords in our experiments were the least effective cord. Wool cords consistently showed they were more effective than any of the other cords we evaluated despite the fact that they were not the most attractive cord to the house flies. House flies were most attracted to the highly organic manila cord and preferred to rest on it more than any other natural

or synthetic cord evaluated. The wool cord was the only animal-based cord impregnated with insecticides and possibly gave it a distinct advantage over the other cords because it is naturally coated with oil, which probably helped facilitate insecticide transfer through the fly cuticle.

Two fly scatter baits, Maxforce® Granular and Golden Malrin® fly bait, are both listed on the DOD pesticide contingency list for house fly control but both have limitations. Only one can be applied in food service areas and both can stain materials and equipment which can compromise camouflage and substrate appearance. Eliminating these disadvantages, while maintaining bait efficacy, would appear to provide advantages to a fly management program and benefit the deployed war fighter. The new sprayable imidacloprid bait, Maxforce® Fly Spot, is as effective as the two previously mentioned scatter baits in the laboratory and as effective as its counterpart, Maxforce® Granular, in the field. Its unique formulation allows it to be used within food serving areas and it will go unnoticed because it dries clear. Unfortunately, like the other bait products, environmental factors, such as rain, decrease the efficacy over time. Residual efficacy did improve when the bait was applied to the cords rather than the non-absorbent plastic surfaces often found on latrines and dumpsters.

We anticipated that the research completed here would provide some information that could be further used to develop future products that could be benefit the DOD. The insecticides evaluated in the impregnated cord studies are both non-registered for house fly control. House flies have shown little to no resistance towards fipronil and indoxacarb and both insecticides appear to be very effective against this pest. At this time, no information has been obtained on whether or not the manufacturers of fipronil or indoxacarb are seeking registrations for these products to control flies. However, a Colorado company has informed me of their interest in indoxacarb-impregnated cords and has begun conversations with DuPont® regarding further

research into this type of product. The sprayable imidacloprid fly bait is not currently listed on the DOD pesticide contingency list but it received its EPA registration in 2006 and became commercially available in mid 2007. A formal request will be submitted to the Armed Forces Pest Management Board this summer to request that it be assigned a National Stock Number (NSN) and be placed on the DOD pesticide contingency list. If successful, Maxforce® Fly Spot will be readily available to the deployed war fighter for use in their fly management programs.

APPENDIX A
REVIEW OF INSECTICIDE-IMPREGNATED CORDS

The use of insecticide-impregnated cords to control house flies was first tried with DDT in 1947 (Baker et al.). By the early 1950's, impregnated materials for fly control became increasingly common (Pimentel et al. 1951). Insecticide-impregnated cords were being used on dairies, at rural residences, military mess halls, state fairs, and state prisons with great success (Kilpatrick 1955, Maier and Mathis 1955, Soroker 1955, Kilpatrick and Schoof 1956). Commercial Fly-Cords distributed by Fly-Cord Inc. (Savannah, Georgia) were widely available and used by 1957 (Fehn 1958, Smith 1958). These cords were considered the treatment of choice for use in buildings housing animals because of the economy and efficiency (Fay and Kilpatrick 1958).

Fay and Lindquist (1954) recognized that impregnated cords offer only a small percentage of the surfaces available for flies to rest. Exploiting the factors which enhance a fly's attraction to a particular cord would subsequently lead to higher mortality on impregnated cords. They found that cord type, thickness, and color significantly influenced a fly's attraction to a particular cord. Sisal and cotton cords were more attractive than jute or wool cords. Cord attractiveness increased with cord diameters between 3/64" and 7/16". Flies preferred red and black cords over blue, yellow, green, or white cords. No preference was evident between vertically or horizontally hung cords.

Although several other organophosphate insecticides have been evaluated for their effectiveness, all provided satisfactory results. However, only one cord was available commercially (Kilpatrick and Schoof 1959, Gratz et al. 1964, Rabari and Patel 1976). The commercial Fly-Cord was a 3/32" diameter, red cotton cord impregnated with 13.79% parathion and 3.54% diazinon (Fehn 1958, Smith 1958). The cord was supplied on a reel containing 300

feet; each linear foot of cord contained 75-100 mg of parathion (Youngblood 1960). The manufacturer recommended a rate of 30 linear feet of Fly-Cord per 100 square feet of floor area in locations were adult flies congregate (Fehn 1958, Smith 1958). Cords were normally hung about three feet apart using staple guns or simply tied on to the structure (Fehn 1958, Youngblood 1960). If multiple competing resting surfaces were available to the flies, most applicators increased the amount of impregnated cords in the area. In places where flies were not seen resting or where conditions were unsuitable (i.e. areas with drafts), no cords were placed.

The use of impregnated-cords was an attempt to find new methods to reverse the resistance associated with residual spraying of chlorinated hyrdrocarbons such as DDT (Keiding and Jespersen 1986). Fly-Cords offered an easy-to-use control method that restricted and concentrated a residual insecticide. This method lowered the selection pressure for resistance because flies which avoided contact with the cords diluted the remaining population of resistant individuals (Keiding and Jespersen 1986). Other methods, such as paint-on baits, non-residual space sprays, and combining baits and larvicides, were evaluated and determined effective (Keiding and Jespersen 1986). Paint-on baits and non-residual space sprays are widely available and used today in the United States.

No commercially available insecticide-impregnated cord products are currently available in the United States. This is partially because the main active ingredients, parathion and diazinon, are not registered for filth fly control. In addition, the use of selective insecticidal baits has become increasingly popular. In Denmark, the use of impregnated cords was abandoned because of newer construction techniques that allowed for more ventilation in the animal shelters and more effective residual sprays became available (Keiding and Jespersen 1986). The World

Health Organization and the United States Military continue to recommend the use of insecticide treated or impregnated cords (Rozendaal 1997, AFPMB 2006)

APPENDIX B
REVIEW OF FLY BAITS

Insecticidal baits have long been used for fly control. One of the original bait formulations contained either 1-2% formaldehyde or sodium arsenite mixed with milk or sugar water (Keiding 1976). Residual insecticides have been mixed with sugar to make them more attractive to flies and serve as a type of bait, but this type of mixture is often not as effective as baits specifically formulated to attract flies. Today's fly baits are loaded with many different attractants including pheromones, sugars, and other substances that specifically attract house flies. Most of these fly baits are formulated as either dry scatter baits, but many also come in easy-to-use bait station devices. Some of the dry scatter baits can be mixed with water and painted on a surface.

Insecticidal baits have many advantages over other chemical control methods. Baits are relatively inexpensive, usually have a longer storage shelf life, are more attractive to flies than other chemical control methods, and are easier to work with in field environments (Gahan et al. 1954, Darbro and Mullens 2004). The dry scatter baits simply get scattered on the ground in the infested areas or placed within a bait station while bait station devices normally only need to be opened and hung in infested locations. Keiding (1976) considered baits less likely to select for resistance than residual sprays. He was most likely referring to the physiological resistance seen in many of the organophosphate and carbamate insecticides at that time. Today, many insects, including flies, have been shown to develop behavioral resistance to baits (Darbro and Mullens 2004).

Fly baits do have disadvantages. Dry scatter baits do not target fly resting areas unless they can be painted on and this type of application often leads to stained surfaces because the U.S. Environmental Protection Agency (EPA) requires the scatter bait granules to be dyed to distinguish them from other non-toxic materials. Staining can also occur when these granules

become wet by rain and bleed onto the surrounding surface, which may be considered unsightly for the user. Bait station devices are also degrade rapidly in environments with intense sun or precipitation. Dry scatter baits also need to be replaced frequently in some areas when granules become covered by manure or other debris such as garbage (Barson 1987).

As with any chemical insecticide, label restrictions can be disadvantageous (although necessary in most cases) and limit the needs of the applicator. For example, the two most widely used dry scatter baits in the United States are Maxforce® Granular fly bait and Golden Malrin® fly bait. Maxforce® Granular is an imidacloprid-based bait (neonicotinoid class) containing the fly attractant (Z)-9-tricozene, the bittering agent Bitrex®, and other attractants and inert ingredients. Golden Malrin® contains 1.1 % methomyl (carbamate class), 0.049 % (Z)-9-tricosene, as well as other attractants and inert ingredients, and is one of several methomyl-based scatter baits available. Maxforce® Granular has a more restricted label than that of Golden Malrin® because its label restricts its use in food establishments. Golden Malrin®, despite being the only carbamate-based insecticide not classified as a "restricted-use" insecticide, can be used within food establishments when used in bait stations placed at least 1.2 m from the ground in areas where food processing or preparation does not occur.

Recently, a new fly bait has become commercially available that may offer advantages over some of the other current fly baits available. Maxforce® Fly Spot bait contains 10% imidacloprid, 0.1% Z-9-tricosene, Bitrex®, and inert ingredients. Once applied, Maxforce® Fly Spot bait dries clear and the label allows for application within agricultural livestock production facilities and serving areas of food establishments when the facility is not in operation.

APPENDIX C
REVIEW OF INSECTICIDES EVALUATED

Fipronil

Fipronil is a phenylpyrazole insecticide that was first registered in the United States in 1996 (Connelly 2001). It is used to control termites, ants, roaches, fleas, ticks, and various other agriculture and turf pests. No fipronil products are currently registered for house fly control.

Fipronil causes mortality by contact and ingestion (Vargas et al. 2005). Insects exposed to fipronil show extreme neural excitation that eventually leads to insect paralysis and death. Death is caused by the disruption of the normal passage of chloride ions through the γ-aminobutyric acid type A (GABA) receptor system of insects (Scharf et al. 2000). Hainzl et al. showed fipronil to have a tighter binding affinity toward insect GABA-regulated chloride channels over mammalian receptors (1998). Fipronil-sulfone, an important active metabolite of fipronil, was also found to block the glutamate receptors in cockroaches (Zhao et al. 2004). Glutamate-gated chloride channels are only found in invertebrate systems at skeletal neuromuscular junctions of both the peripheral and central nervous system (Raymond and Sattelle 2002, Scharf 2003). The unique quality of fipronil to affect two target sites makes it a highly selective insecticide and potentially important factor limiting the development of detectable resistance (Zhao et al. 2004).

To date, resistance to fipronil appears to remain at low levels or even be non-existent in house flies (Scott and Wen 1997, Scott et al. 2000, Kristensen et al. 2004). Low levels of cross-resistance have been reported in multi-resistant house flies and attributed to monoxygenase-mediated detoxification, decreased insecticide penetration, and target site mutations (Wen and Scott 1999, Liu and Yue 2000, Kristensen et al. 2004). Resistance surveys in New York found house flies susceptible to fipronil even at LC_{99} levels (Scott et al. 2000).

Indoxacarb

Indoxacarb (DPX-MP062) is an oxdiazine insecticide that was first registered in the United States in 2000 (EPA 2000). It is a 75:25 mixture of the active S-isomer (DPX-KN128) and the inactive R-isomer (DPX-KN127). A less effective formulation (DPX-JW062) contains a 50:50 mixture of the two stereoisomers. Indoxacarb was originally formulated to control lepidopteran pests of fruits and vegetables, but newer registrations include cockroach, mole cricket, and fire ant baits. It is not currently registered for house flies.

Indoxacarb is considered an organophosphate replacement and designated as a "reduced-risk" insecticide by the EPA. It is a pro-insecticide that must be biochemically converted to a toxic decarbo-methoxyllated metabolite (Dias 2006). Toxicological effects are dependent on the conversion of the inactive metabolite to its toxic form within the insect body. In mammals, indoxacarb metabolites are rapidly excreted; whereas in insects, indoxacarb is rapidly converted by an esterase and amidase into DCJW, which is the more insecticidally active metabolite. Insects exposed to lethal doses of indoxacarb experience impaired nerve function, feeding cessation, paralysis, and eventually death. Indoxacarb poisoning occurs through contact or ingestion and it works by blocking the sodium channel of the insect nervous system. This mode of action is distinct from other insecticides that target the sodium channels of insects (DDT, pyrethroids) because DCJW disrupts the sodium channels without modifying the activation or deactivation kinetics (Lapied et al. 2001). It works by blocking the channel pore, and prevents normal sodium ion flow.

Because indoxacarb is a new chemistry, not much work has been done on insecticide resistance. Shono et al. (2004) selected house flies that had >118-fold resistance in as little as three generations and concluded that the resistance mechanism was associated with a major

factor on autosome 4 and a minor factor located on autosome 3, both of which are not linked to any resistance mechanisms previously described.

Imidacloprid

Imidacloprid is a chloronicotinyl nitroguanidine (neonicotinoid) that was first registered in the United States in 1994 (NPTN 1998). It is used to control a wide variety of agricultural, urban, public health, and veterinary pests and is estimated to account for 11-15% of the total global insecticide market (Tomizawa and Casida 2005). Several formulations are available for different treatment applications, only two are available for house fly control: Maxforce® Granular fly bait and Maxforce® Fly Spot bait. Both products are baits, but differ from one another by their formulation. Maxforce® Granular fly bait is a red granule that can be applied as a traditional scatter bait, within a bait station, or mixed with water and painted onto a surface. The Maxforce® Fly Spot bait, alternatively, is white wettable powder that is mixed with water and sprayed onto a surface. When the Maxforce® Granular fly bait is painted on a surface, or if the granules become wet, it stains the surface red, whereas the Maxforce® Fly Spot bait is clear and does not stain.

Imidacloprid kills insects through contact and ingestion by agonizing the nicotinic acetylcholine receptor (nAChR) (Fossen 2006). In house flies, imidacloprid is metabolized by oxidation to the "olefin" metabolite, which has the same toxicological activity as imidacloprid (Nishiwaki et al. 2004). Insects exposed to lethal doses of imidacloprid experience nervous system excitability, modified feeding behavior, and death. Imidacloprid is considered a selective insecticide because: (1) imidacloprid has a higher affinity for the insect nAChR's than mammalian nAChR's, and (2) there are more nAChR's located in the insect nervous system than what are found in mammalian systems (Yamamoto et al. 1995).

Resistance has yet to be reported in house flies (Gao et al. 2007), but it is well-documented in Drosophila (Daborn et al. 2001). Cross resistance has been observed in multi-resistant house flies (Wen and Scott 1997). Multiple resistance mechanisms are suspected in house flies. Monoxygenase-mediated detoxication seems to be a primary mechanism in some strains of house flies, but not in others (Wen and Scott 1997, Liu and Yue 2000).

Methomyl

Methomyl is a carbamate insecticide that was first registered in the United States in 1968 (EPA 1998). It is used to control a wide variety of agricultural, urban, public health, and veterinary pests. Several methomyl formulations are available, but the 1% fly bait formulation is the only one which is not classified as a restricted-use pesticide.

Methomyl causes mortality by contact and ingestion by inhibiting the acetylcholinesterase (AChE) enzyme, which occurs in the central nervous system. Methomyl binds to AChE and prevents it from binding to acetylcholine. This results in acetylcholine saturation at its neural receptor, which results in a dramatic increase in generation of nerve impulses. Insects exposed to methomyl show signs of hyperexcitability, convulsions, paralysis, and death. Decarbamoylation of AChE is rapid and, therefore, carbamates are considered reversible AChE inhibitors and recovery from sub-lethal poisonings can occur quickly (Yu 2007).

Little resistance to methomyl has been seen in house flies despite its frequent use and the high levels of resistance seen in house flies to other carbamates (Barson 1989, Webb et al. 1989, Scott et al. 2000, Darbro and Mullens 2004). Flies feeding on methomyl granules have been found to receive a super-lethal dose that may play a large roll in why resistance has not been as widespread (Price and Chapman 1987). Behavioral resistance, or bait aversion, has started to become more apparent. In 1989, Barson (1989) reported that 8% of the resistant flies were repelled by methomyl. In a study comparing the mortality of 35 field strains of house flies fed

methomyl in choice and no-choice tests, mortality decreased by nearly 30% when the flies were given the choice test (Darbro and Mullens 2004).

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BIOGRAPHICAL SKETCH

Jeffrey Conrad Hertz was born in 1976 in Peoria, Illinois. His parents, Marion Conrad "Butch" Hertz and Margaret Eloise "Weezie" Hertz (Hilton), raised him in Lewistown, Illinois. They moved to Bernadotte, Illinois where he continued to attend school in neighboring Lewistown until he graduated from Lewistown Community High School in 1994. He entered the United States Navy and reported to basic training at Recruit Training Command, Great Lakes, Illinois in November later that same year. Over the last 12 years, he served with the United States Marine Corps, at Naval hospitals, and most recently, he was assigned to the medical staff at the United States Capitol. He received his Associate of Science degree in medical laboratory technology from George Washington University, Washington D.C. in 2002 and his Bachelor of Science in interdisciplinary studies, majoring in biology from Mountain State University, Beckley, West Virginia in 2003. In 2004, he was selected as the very first enlisted Sailor selected to study entomology under the Medical Service Corps In-service Procurement Program (MSC-IPP). Upon graduation HM1 (FMF) Jeffrey Hertz will be commissioned to the rank of Lieutenant Junior Grade as a medical entomologist in the Medical Service Corps. He enjoys running and is an active member of Centennial Lodge #174 of Ancient Free and Accepted Masons located in Upper Marlboro, Maryland. He, his wife, Karina, and two children, Conrad and Kyra, are excited about their upcoming move to Jacksonville, Florida where he will be working at the Navy Entomological Center of Excellence.