EFFICACY OF ENTOMOPATHOGENIC NEMATODES UTILIZED FOR CONTROL OF STABLE FLIES (STOMOXYS CALCITRANS) AT ROUND BALE FEEDING SITES

By

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Abstract:

The stable fly is one of the most important livestock pests in the United States. The flies inhabit feedlots, high density livestock enclosures, dairies and hay bale feeding sites in cattle pastures. Accumulated organic materials provide excellent habitat for the stable fly to develop and complete their life cycle. The impact of the fly on the livestock, particularly cattle, causes problems for producers. A method of biological control with entomopathogenic nematodes was tested against first, second and third larval instars of the stable fly. Four distinct treatment groups were chosen on the basis of susceptibility trials. They were two single EPN genera treatments and two combined EPN genera treatments. The single genera treatments were designated as 0:1 S:H and 1:0 S:H. The combined EPN genera treatments included 1:2 S:H and 2:1 S:H. The S:H ratios refer to composition of Steinernema spp.(S) to Heterohabditis spp. (H). In all lab trials the combined EPN genera treatments increased stable fly mortality when compared to the single genera EPN treatments. Finally the information collected in the lab was administered in field trials at 8 round hay bale feeding sites in north-central Oklahoma. Based on these trials EPN treated trap site showed stable fly adult emergence to be lower in comparison to control trap sites at the same feeding sites. EPN are naturally occurring throughout the U.S. and can be administered in the field with conventional methods of spraying. Results from the trials illustrate that while control from EPN cannot compare to commercially available insecticides for stable fly mortality these naturally occurring EPN can provide background suppression that could potentially be incorporated into a sound IPM program.

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CHAPTER I

INTRODUCTION

The stable fly (*Stomoxys calcitrans*) is the most common biting fly pest of confined or pastured livestock. In the past stable flies has been a pest of livestock in confined feeding operations and dairies. In recent history stable flies have migrated into the pasture and range setting due to the practice of feeding hay bales over the winter months. Although stable flies have migrated into the pasture setting they are still prevalent in confined feeding systems. Hay bale feeding strategies, in pastures, can cause hay waste to be deposited on the ground. The wasted hay and fecal matter from the livestock combine, at these feeding sites, to produce areas that are suitable for stable fly development. Annually, in the United States, losses in the cattle industry due to stable flies have been estimated at 672 million dollars and over 1 billion dollars worldwide.

Stable flies are a blood-feeding fly species that can transmit blood-borne pathogens between animals. In addition to being disease vectors, stable flies promote blood loss and can cause secondary infection due to their feeding habits. Stable flies not only feed on livestock they also feed on any available warm-blooded mammal that is accessible to them, including human

beings. They also cause great annoyance to livestock which affect their feeding habits and temperament. The behavior observed in cattle due to the feeding process of the stable flies interrupts the efficiency of grazing in both beef and dairy breeds.

During the winter months cattle in pastures are fed round hay bales to supplement their diet when forages are limited or dormant. Due to the animals eating and defecating in the same area, over an extended period of time large quantities of efficient stable fly egg distribution and larval rearing material will be produced. These areas, around the entire hay bale feeding site, become prime habitat for adult stable flies that emerge during the spring and summer months. Also these areas provide late season generations of pupae a location for over-wintering. Insecticide treatment of these areas can be problematic since the fly larvae are found inside the substrate and underneath the surface of the soil. Viable, naturally occurring parasitoids of stable flies can be found in the pasture environment. Entomopathogenic nematodes (EPN) are found in the soil and can be effective in reaching and parasitizing the larvae that are developing in the substrate.

The overall goal of this study was to determine if the population numbers of EPN at these feeding areas can be significantly augmented and applied to the breeding material to manipulate the number of developing fly larvae. If EPN population are increased above naturally occurring rates and are successful and sustainable throughout the spring, summer and early fall months, adult emergence of stable flies may be greatly reduced at the feeding areas thus reducing the problem in pastured animals.

Objectives

1. Determine the susceptibility of stable flies developing in hay substrates to native Oklahoma EPN and compare those native EPN to commercial EPN.
2. Determine which naturally occurring EPN or combination of EPN genera could be the most successful at controlling stable flies in a hay/manure substrate when applied as single species and combined ratios at field locations.

CHAPTER II

REVIEW OF LITERATURE

General

The stable fly, *Stomoxys calcitrans* is classified in the order Diptera. This order contains families of blood feeding pests such as Tabanidae, Muscidae, Culicidae and Ceratopogonidae. The Stable fly is in the family Muscidae. This family also contains House flies, Face flies, Horn flies and Tsetse flies; all of which are considered to be significant pests in various fields of the livestock production industry. The flies in the family Muscidae are considered *synanthropic*, which indicates that they make use of environments that are generated by humans (Mullen et al. 2002). Stable flies are considered to be a significant insect pest to livestock in confined feeding/housing environments (Bruce et al.1958; Cutkomp et al. 1958; Campbell et al. 1977; Berry et al. 1983; Campbell et al. 1987; Schwinghammer et al. 1987; Wieman et al. 1992; Campbell et al. 1993). In the past half century stable flies have developed into one of the foremost insect pests in pastured livestock (Campbell et al. 2001; Broce 2005). These pests are critical in negatively affecting the health and development of various types of livestock and humans throughout the world (Campbell et al. 1977; Wieman et al. 1992). A blood meal is the protein source of nutrition for adult stable flies. Being blood-feeders, stable flies do transmit numerous pathogens and diseases, as well as affect fitness of animals in production systems (Berry et al. 1983). Stable flies are not specific to a certain host. They

will feed on any available warm-blooded animal that is within their environment. Areas which contain high populations of animals in small sections of land are prime locations for stable fly development due to the buildup of waste, fodder, urine and other biological media (Boire et al. 1988). This accumulation of materials contains materials needed for the fly to complete its life cycle. These areas also include the livestock which provide sustenance for the adult flies.

Stable flies are found worldwide, they inhabit areas that are conducive to their needs. When a location develops into ideal habitat, fly numbers can reach damaging levels. High populations can have an effect on animal health and development, which in turn affects the economics of a production system. Measures of control such as chemical control (pesticides), cultural or mechanical control (sanitation) and biological control (predators) all include a cost which must be considered by producers. While these measures of control are not completely effective they must be employed to help sustain a healthy production system, and maintain fly management.

Life Cycle/Characteristics/Behavior

Stomoxys calcitrans are known as holometabolus insects. This signifies that the insect undergoes complete metamorphosis during its life cycle. Four life stages are associated with the stable fly; they include egg, larvae, pupa and adult. Eggs are small (1mm), elongated and white and are laid in clusters that can range up to 50 eggs/mass. Between 1 and 5 days is required for larvae to emerge. During the larval stage the immature or maggot goes through three instars. This takes place over a period of 7 to 21 days. Development depends on the accessibility of provisions and climate. Third instar

larvae are 8 to 10 mm in length and have a white to cream coloration. After completion of the third instar stage the larvae pupate in the substrate that they are feeding in. Pupation takes 1 week to 1 month; temperature plays a significant role in affecting the time between pupation and adult emergence. The average life span of an adult stable fly ranges between 20 to 30 days. Adult stable flies are similar in size to house flies; they are about 7 mm in length. Other characteristics used to identify stable flies include black spots on the dorsal side of their drab colored abdomen and the manner in which their proboscis protrudes frontward (Williams 1985; Mullen et al. 2002).

Adult stable flies do not exist entirely on their host throughout their lives. Hosts are visited once in cooler temperatures and twice in warm temperatures throughout the day-light hours for feeding. Once a blood meal is taken the fly migrates to a resting area for digestion. Buildings and fences are utilized for cover in areas that contain confined animals. Tree and plant foliage provide cover in fields. Any structure available can be used to escape direct sunlight while the fly digests the blood meal. Actions observed in feeding behaviors include the flies determination to continually attempt to feed, even if disturbed, until they become replete (Bishopp 1913).

Problem Areas

Environments that contain elevated populations of animals can be attractive to the stable fly. When animals inhabit these locations manure and urine begin to accumulate. Along with fecal matter, wasted fodder is added into the mixture by animals dropping fodder from their mouths and/or stepping in or on the materials they are consuming (Suszkiw et al.2003). These actions along with the animals visiting these specific feeding

locations on a daily basis; and constantly recombining manure, soil, bedding and fodder, by walking and standing in the same general settings, convert the sections of ground around the feeding areas into an optimum site for fly development (Hogsette et al. 1987). The feed apron and edges of manure mounds produce the highest number of stable flies in Nebraska feed lots (Skoda et al. 1991). Silage that is processed, stored and fed to animals also creates a suitable environment for stable fly development as it begins to decompose. According to Williams (1980) an extensive study of stable fly breeding habitat, in Florida, showed that silage piles produced the largest number of stable flies over a three year period. Silage is organic material such as processed/chopped plant material that is normally fed to animals at confined feeding operations. It can be in bale form, housed in silos/buildings or piled and covered on the ground. It contains large amounts of water and over time, like most organic material, begins to decompose and release olfactory signals that attract stable flies.

Studies conducted by Romero (2006) provide information about juvenile stable flies and the requirements of an active microbial system for suitable fly development. Certain bacteria, such as *Citrobacter freundii* and *Serratia fanticola* and the olfactory cues produced by the bacteria were shown to attract gravid stable fly females. Each of these bacteria are naturally found in plant material and soil and they also exist in the gut of various ruminants. This study found that fly larvae placed in sterile natural media could not complete their life-cycle. In fact they could not survive without the presence of microbes (Romero et al. 2006). Another study conducted by Jeanbourquin (2007) tested the effects of rumen volatiles on antennal sensory receptors of the stable fly to determine if breath and body odors emitted from oxen affected the antennal receptors. Findings, in

the study, showed that stable flies are highly susceptible to these volatile cues, although rumen is not the only substance that contains and releases the cues; the odors are also associated with areas and substrate frequented by gravid flies in search of a viable media that can be used for egg placement (Jeanbourquin et.al 2007).

Livestock environments that consist of regions conducive for stable fly habitat include confined feeding operations, feed lots, horse stables, dairies, commercial chicken and swine farms and pastures(Williams et al. 1980). Specific zones in confined feeding operations such as fence lines, areas around feed and water troughs, hay feeders and silage piles and manure mounds develop into fly habitat over a period of time due to animals congregating at a specified location for feed consumption, hay or silage consumption and water intake in addition to an increased production of manure (Hogsette et.al. 1987; Skodas et al. 1991). Areas where excessive buildup of organic material, such as round bale feeding sites, result in a population increase of stable flies. This is the most important region for fly development in a pasture setting. If the bales are fed in the same locations throughout the winter months and are not unrolled or left in bale form wasted hay and cattle visiting these sites, over a period of time, begin to alter the substrate into suitable fly larvae habitat (Broce 2005).

Health and Disease

Blood-feeding dipterans are excellent vectors of pathogens. As seen with other dipteran species and the numerous pathogens that they transmit between animals and humans. Stable flies transmit a number of pathogens that are damaging to different breeds of livestock specifically because they are attracted to and mainly feed on all breeds of

livestock. In addition to transmitting viruses, bacteria, protozoa and nematodes (Thomas 1992) stable flies cause anemia in animals which can and does lead to a higher susceptibility to infection by other pathogens. Also, stable flies feeding behavior has proven to cause cutaneous lesions on cattle at feeding locations which opens the animal up to secondary infections (Moorhouse 1972). Costs associated with combating these diseases can add extra expense to a production system and these diseases can be fatal to the animals if not treated.

Stable flies are ectoparasites that feed on the underside and legs of cattle and other livestock hosts. They are considered "puddle-feeders" because they use their mouth-parts to tear open the host's skin and feed on blood that accumulates at these openings (Berry et al. 1983). Natural methods employed by cattle to evade the irritation and feeding of stable flies include bunching behavior, foot stomping and standing in bodies of water (Bishopp 1913). Other behaviors observed in cattle such as tail switching, skin and ear movements and head movements have little to no effect on stable flies because of where they feed on the animal's body (Dougherty et al. 1995). Bunching is one behavior used to avoid stable flies. By grouping together the cattle expose less of their bodies to the flies and therefore have a lesser chance of flies contacting them (Schmidtmann et al. 1982). However bunching presents a problem for the animals due to presence of stable flies in the spring, summer and early fall months. Bunching in hot weather alters heat transfer in the animals which affects feed efficiency and fodder consumption. This in turn causes the finishing time or time allotted for the animal to reach its prime marketable weight, to increase, which begins to add cost in the production system (Wieman et al. 1992; Campbell et al. 1993). Other avoidance methods that cattle utilize are moving to areas

with a lower population of flies or standing in bodies of water (Dougherty et al. 1993). By submerging lower sections of their body, cattle prevent the stable fly from gaining access to their preferred feeding sites. This behavior, like bunching behavior, also limits fodder intake. Although it does not raise body temperatures in the cattle and does not affect feed efficiency, it prevents the animals from grazing. This avoidance strategy, like bunching, can also affect weight gain and finishing time in cattle. Any obstruction of grazing negatively affects growth and production in cattle and fly avoidance behaviors exercised by cattle influence grazing habits (Dougherty et al. 1993).

Chemical Control

Due to the fact that the stable fly is a major pest in confined feeding operations and has developed into a problem in pasture settings, a variety of methods for stable fly control have been exercised throughout the years. Current and past control methods, for the stable fly include use of various insecticides to treat animals and to treat stable fly development habitat. One typical method of administering insecticides consists of spraying the animal and the substrate known to generate larvae. Some difficulties with this method are application of the insecticide to the region of the animal where stable flies feed. According to Hoggsette (2000) along with application problems residual activity of the chemicals on pastured animals is short-term. Due to precipitation and dew the residual life of insecticides is shortened in the pasture setting (Campbell et al. 1971). A separate method is the baiting/trapping of the adult flies. This method consists of attracting flies to traps with certain colors that do or do not contain insecticides. According to some research the color trapping method is not extensively used (Hogsett et al. 2008). Fly tags and tail tapes are also used to control fly problems, and have shown to

provide some control of stable flies (Hogsette 1987).These are devices that are attached to the animal and then slow-release insecticides are administered over time by the animal's natural movements to their backs, sides and necks. Similar to the problems with the spraying application methods the insecticide from the tags/tapes does not reach the areas of the animal's body where the stable flies feed. Also these devices can dislodge from the ears and tails of the animal therefore reattachment is necessary.

Another form of insecticide application is the use of "feed-through" insecticides. The chemicals used in these formulations have no effect on the cattle. Active ingredients in the insecticide are referred to as insect growth regulators (IGR's). IGR's prevent insect larvae from reaching the adult or reproductive stage. The insecticide is ingested and passed out in the animal's feces thereby distributing the insecticide onto the ground where normal walking and movement from livestock can aid in incorporating the manure into the substrate where the immature flies develop. One problem with feed through insecticides is that as manure ages the insecticide becomes degraded and ineffective (Campbell et al. 1993). According to a study by Broce and Haas (1999) stable fly females prefer to distribute eggs in aged manure. This study showed that female's visitation incidence increased as the manure aged and it was twenty days before larvae were present in the manure. Hastened aging of manure due to summer conditions increased gravid female stable fly numbers at the test locations (Broce et al.1999). As the manure ages the efficacy of the insecticide diminishes, therefore possibly having little to no effect on the larvae.

Stable fly larvae develop under the soil surface; this presents the problem of larval contact with the applied insecticide. Also chemical breakdown over time, due to sunlight

and rain hinders the effects of the pesticide. One difficulty that is associated with all pesticide use is the development of resistance by the targeted pest. Effective management practices with the use of all insecticides require the rotation between different chemistries. Also labeled rates should be administered. Employing the right practices with insecticides lessens the chance for fly resistance.

Mechanical/Cultural Control

The use of mechanical or physical control for the stable fly can be effective in reducing the number of stable flies at a given location. Sanitation in areas that contain elevated numbers of livestock is a vital step in maintaining stable fly numbers. Practices employed at confined feeding operations such as diaries and feed-lots assist producers in managing not only stable flies but other muscoid flies. These practices include removing manure, wasted hay/silage and spilled feed from pens, lots and structures that house the animals(Campbell et al. 1993) . Another step is keeping certain areas from collecting excessive moisture such as watering areas and depressions in the ground that can retain moisture and develop into suitable fly habitat. Moisture plays a critical role in the development and abundance of stable flies (Mullens et al.2005). A study conducted by Mullens (2005) showed a connection between the amounts of rainfall in early to late spring with the quantity of emerging stable flies in the summer months at a California dairy. Another study showed that proper sanitation reduced stable fly numbers by 50% from one year to the next, and in the third year numbers decreased by another 30%. The sanitary lots were in conjunction with unclean lots at the same confined feeding structure. Sanitation alone proved to play a significant role in reduction of adult stable flies and the economic injury level was only exceeded once per each year during peak stable fly

emergence (Thomas et al. 1996). The practice of sanitation must be incorporated into all aspects of the production system, although most sanitation research has been completed at confined feeding locations the same principles can be applied in the pasture setting. Placing bales at different locations in the pasture, throughout the feeding season prevents buildup of organic material in one location. It also prevents livestock from gathering in the same location in the pasture for the entire season and depositing excessive amounts of manure that can be incorporated into the soil along with the hay to increase fly developmental substrate. This practice deposits waste over a larger area and avoids heavy accumulation of the waste in a single area. If waste is spread over an increased region then it desiccates more rapidly and has less chance of becoming fly habitat. Another sanitation practice includes removing or destroying the organic accumulation at feeding locations at the end of the season. This can be accomplished by employing a cultivation implement (disk, harrow, spring tooth, etc.) in the feeding areas to aerate soil and allow moisture release. It also spreads organic material over a wider range of ground which assists in aeration of potential attractive substrate.

Biological Control

Several methods of biological control are being utilized in management of livestock pests. Biological control consists of combating a pest species with a beneficial species; this includes the use of mammals (birds and bats), fish (mosquito fish), insects (parasitoids and predators), entomopathogenic nematodes (EPN) and pathogens (fungi, bacteria, viruses) to maintain and decrease populations of species that are considered pests. According to a two year study by Smith (1987), a review of natural parasitism was conducted to discover what beneficial species provided the majority of fatality in wild

populations of stable fly larvae on cattle farms in Missouri. Several different species were documented, along with the nine parasitoid wasp species identified, a beetle species (staphylinidae) and two EPN species were also collected and identified from the study (Smith et al. 1987). A separate study conducted in 1989 in Missouri examined the natural mortality of stable fly eggs and larvae and documented different enemies that naturally prey on and compete with stable flies in livestock systems. According to the results, nineteen different predacious species and sixteen competitive species were identified (Smith et al. 1989).

In livestock production systems a commonly used method of bio-control is the release of beneficial fly predators such as parasitoid wasps. Various companies produce and sell these predators. Success of these predators is based on a timely release and additional release throughout the spring and summer. By increasing parasitoid populations effective control measures can be reached. One experiment demonstrated an average of over 60% decrease in sentinel hosts over a three week period due to the preliminary and continued release of *Muscidifurax sp.* (Petersen et al. 1995). Another experiment averaged 30% population reduction of stable flies and house flies for an entire season, due to the initial and repeated release of *Spalangia sp., Muscidifurax sp., Pachycrepoideus sp.* And *Phygadeuon sp.* (Skovgard 2004).

An additional method that is utilized separately or in conjunction with the parasitoids is the use of EPN and insect pathogenic fungi. These methods are useful in attacking the stable fly larvae before pupation or sexual maturity is reached. EPN exist in the soil profile and are naturally occurring throughout the world. They use different methods of foraging for a host, an ambush method and a cruiser method. Both methods

are effective in contacting a potential host (Grewal et al. 1994). EPN can be incorporated into areas that are known reproduction sites of stable flies and other livestock pests in an attempt to lower the numbers of larvae in a specific area.

Attracting or releasing natural predators into the stable fly habitat can potentially become a more effective method of controlling the fly populations due to the activity of the predators. Mammal, bird, insect and EPN all actively seek out stable flies, pupae, larvae or eggs; unlike chemical or pathogen control methods where the fly or larvae has to come into contact with the formulations to be effected. Also degradation from climate (light, moisture, temperature) which does negatively affect chemicals and pathogens plays little to no role in affecting the animal predators during spring and summer months.

Entomopathogenic Nematodes (EPN)

As stated before fly larvae exist in regions below the surface of the soil. This makes it complicated for certain methods of control to make contact with fly larvae. EPN also exist below the surface of the soil and utilize various species of soil dwelling arthropods as hosts. According to studies by Taylor (1998) the feedlot or confined cattle feeding arrangement is an enhanced setting for stable flies due to the excessive presence of plant material from fodder and manure that accumulates and mixes with the soil profile in contrast to a poultry litter environment that lacks the necessary aspects needed for fly development (Taylor et al. 1998).

EPN in the family's Steinernematidae and Heterorhabditidae are highly infective for a wide range of insect hosts (Mullens et al. 1987). Both families of nematode are naturally occurring in the United States. Host infection is accomplished as third-stage

nematodes enter natural body openings, or intact cuticle in certain cases, and introduce symbiotic bacteria (Steinernema – *Xenorrhabdus spp.*, Heterohabditis – *Photorrhabdus spp.)* that usually kill the insect host within 24-48 h (Owuama 2001).

Poinar (1990) explains that the bacterium, which is released by EPN, multiplies rapidly killing the host. The EPN then feed on the bacteria that degrade the host tissue. As EPN feed they mature and reproduce. Up to three separate generations can emerge from a single host, as long as the host remains viable for EPN development. As each generation reaches the infective juvenile stage, the only free-living stage of the life cycle (Poinar 1990), the EPN emerge from the host cadaver and begin the search for a new host (Figure 1). Search behavior is the process in which animals locate and acquire resources necessary for development and reproduction. Both Steinernematidae spp. and Heterohabditidae spp. families of EPN are considered generalists (Kaya et al. 1993) and acquire all necessary resources from a single host; therefore, successful host location is of paramount importance (Lewis et al. 1993). Two modes of parasitism that are employed by the Steinernematidae families are the ambush method and the cruiser method. The ambush method is when the infective juvenile does not actively travel to find a new host; it utilizes a sit-and-wait behavior and infects any available host that comes into contact with it. The cruiser method is where the infective juvenile actively seeks out a new host and infects any host that it comes into contact with. Heterohabditidae EPN employ the cruiser method (Grewal et al. 1994). Cruise (widely ranging) searchers rely heavily on chemical cues to locate their remote prey, whereas the ambush (sit-and-wait) foragers rely on host mobility (Huey et al. 1981; Bell, 1991). The naturally occurring EPN used in these trials were not specieated, therefore the exact mode of parasitism cannot be

determined. In trials where low dosages of mixed inocula were utilized as treatments on wax worm moths, high porportions of cadavers produced progeny of one species only. This could be because only individuals of one sex successfully established inside the host (Koppenhoferf et al.1995). Two genera of EPN (Steinernematidae spp. and Heterohabditidae spp.) are often found in the same area, although in laboratory studies steinernematid and heterohabditid cannot coexist in the same host, whereas two steinernematid spp. may successfully parasitize the same host (Kaya et al.1993). Being that stable fly larvae are mobile and pupae are not, the two predation methods could come into play. The combination of a cruiser EPN for a sedentary pest in the soil and an ambush EPN for a soil-surface pest may be more effective that one nematode species alone (Kaya et al.1993).

Rearing the EPN in a laboratory requires minimal equipment and has proven to be a successful low-cost procedure. The development of low-cost *in vitro* mass-rearing methods for some of these EPN species (Bedding 1981) has made feasible the field treatment of some important pests (Renn et al. 1985). Poinar (1972) assessed the production cost of EPN reared on larvae of *Galleria mellonella* (Greater wax moth) at a rate of $1.00 per 1 million nematodes with one moth larvae being able to generate 100 million infective juvenile nematodes per week (Bedding 1984). One issue that arises from mass rearing EPN, is that the species specific bacterium in each subsequent generation of infective juveniles becomes less virulent than the previous generations. The nematodes can be collected in soil samples from natural habitats and coaxed out of the substrate by offering viable hosts for infective juveniles to parasitize and produce new generations.

Galleria mellonella (Greater wax moth) larvae are typically used in the rearing procedure due to their lack of defense mechanisms to soil pathogens (Poinar et al. 1967).

CHAPTER III

METHODOLOGY

EPN Collection and Rearing

Native Oklahoma EPN were collected from pastures near the hay feeding areas. Traditional survey techniques involving soil-baiting with Greater Wax Moth (Galleria mellonella) serve to indicate the presence of either Steinernematidae or Heterorhabditidae nematodes in the soil (Kaya 1997). The methods used in collection involved gathering soil samples with a standard shovel, 8 to 10 inches deep. The soil was loosened and placed into large plastic containers and wax moth (*Galleria mellonella*) larvae were placed on the soil. The larvae were checked for signs of infestation on a daily basis. Signs of EPN infestation in the moth larvae include distortion of the cuticle color due to the symbiotic bacteria transmitted by the EPN (Lacey et al. 2007). When signs of EPN infection were present the larvae were removed from the soil containers and placed into a 9 cm Petri dish containing filter paper and 1.5ml of water. The infected larvae were checked every day, under a stereo microscope, until infective juvenile EPN emerged. The bioassays were allowed to incubate for 7 days. Each bioassay was evaluated for signs of EPN infection after the incubation period and the infections were recorded and classified

tentatively by the color of the cadaver (Kaya 1997) (Table 1). Each cadaver was isolated in a separate petri dish, with moist filter paper, in the dark at 25 °C and kept for collection of emerging infective juveniles that were then maintained in solution of non-chlorinated water at 5 °C for 72 hours (Lacey et al. 2007). All trials in the experiment were tested with infective juveniles that were collected every two weeks. Wax moth larvae were constantly inoculated throughout the course of the experiments, so that viable resources were regularly on hand for field and lab experiments. When new tests were performed infective juveniles of both Steinernematidae spp. and Heterohabditidae spp. (which were collected on the same day from inoculated wax moth larvae) were always used in comparison to each other for more standardized data collection.

Susceptibility of Stable Fly Larvae to Native EPN (Objective 1)

To determine susceptibility of stable fly larvae to native EPN, the larvae were subjected to different quantities of EPN to determine parasitism rates. Twenty-five stable fly larvae, including first, second and third instars, were placed in Petri dishes containing filter paper. One ml of water was added to each dish, which ensured that moisture levels stay appropriate for the survival of the EPN. Inoculums of 300, 700, 1500, 3500, and 7500 EPN/liter of water (Renn et al. 1985; Taylor et al. 1998) were added to the filter paper in 2.0 ml of water. The previous inoculums quantities where replicated 4X and each sample contained 15 stable fly larvae / dish. Stable fly larvae were exposed to the different EPN rates for 7 days at 25°C in the dark. After 7 days live and dead larvae were observed under the microscope to verify any parasitism (Figure 2). Pupae were left for 7 days to allow time for healthy adults to emerge. After 7 days pupae were dissected to

detect any parasitism (Renn et al. 1985). The number of larvae and pupa infected were recorded from each replication.

Hay/Manure Substrate Trials (Objective 1)

After susceptibility trials were concluded, the most promising EPN were utilized in laboratory trials in a hay/manure substrate. Four distinct treatment groups were chosen on the basis of the susceptibility trials for this experiment. They were two single EPN genera treatments and two combined EPN genera treatments. The single genera treatments were designated as 0:1 S:H and 1:0 S:H. The combined EPN genera treatments included 1:2 S:H and 2:1 S:H. The S:H ratios refer to composition of Steinernema spp.(S) to Heterohabditis spp. (H). These were based on color descriptions of wax moth larvae from Lacey and Kaya (2007). The hay/manure mixture was constituted in a standardized method according to Talley et al. (2009). Manure was collected from animals that were free from any pesticide or anthelmintic applications and placed into a freezer for ~ 2 months to ensure all original biota were no longer viable. The hay was also collected from areas that were not introduced to any insecticides. The hay/manure substrate contained a ratio of 2 parts hay to 1 part manure (2.6 L by volume of hay and 1.3 L manure each totaling to 3.9 L) as well as enough water (600 ml) to prevent the substrate from desiccation. The hay/manure substrate was placed into plastic cups (300ml) with a 1 inch dry hay layer atop the hay/manure substrate (Figure 3). This hay/manure substrate was used to simulate the conditions observed in pasture environments where hay feeding occurs (Figure 4). Forty stable fly larvae were placed on top of the dry hay layer and allowed 24 hours to descend into the hay/manure substrate. After the larvae moved into the mixture the EPN treatments were added to the top of the

substrate at rates of 7,500 EPN/L, in ratios (S:H) of 0:1, 1:0, 1:2 and 2:1. Four replications of each treatment and a single control were assessed. The stable fly larvae were exposed for 3 weeks, in normal dark/light cycles (12 hours light/12 hours dark) at a temperature of 25°C. After the allotted time period the stable fly larvae, puparia and adult flies were separated from the substrate and counted. As mentioned before all stable fly larvae were examined under a stereo microscope to observe any infectivity and pupa were set aside 7 to 10 days to allow for adult emergence. If adults did not emerge the pupae were dissected to determine infectivity. Adults were counted as uninfected if they completed their life cycle. This data was recorded and subjected to data analysis as described below.

EPN Comparison (Objective 1)

EPN Trials were conducted to compare and contrast the native EPN to commercially available EPN. The commercial species were ordered and obtained from Arbico Organics LLC (Oro Valley, AZ) and included *Heterohabditis bacteriophora (Hb), Steinernema carpocapsae (Sc)* and *Steinernema feltiae (Sf)*; the native EPN were *Steinernema spp.* (*S*) and *Heterohabditis spp. (H).* A trial was conducted in artificial hay/manure substrate, as described earlier, that consisted of 15 stable fly larvae /cup to determine % infectivity. The stable fly larvae were then added to the cups 24 hours before EPN treatment applications. After 24 hours a dry hay layer was added to the top of the media and the EPN were administered. A standard application rate of 7,500 EPN/L was administered to the top of the dry hay layer. The cups were then covered with paper towels and secured with rubber bands. The paper towels were dampened every other day to ensure that the media did not dry out over the course of the trial. The stable fly larvae

were exposed to the EPN for 5 days at a constant temperature (25 °C) and humidity (70%) as well as normal light/dark cycles. Seven different treatments of EPN (Native: 1:0 S:H, 0:1 S:H, 1:2 S:H, 2:1 S:H; Commercial: Hb, Sc, Sf) as well as an untreated control were replicated four times. After five days the cups were emptied and sorted through to record % infectivity.

Field Trials (Objective 2)

Sites used in the field experiments were located in the north central region of Oklahoma, specifically Payne and Noble counties. These locations included both Oklahoma State University North and South Animal Science Ranges and the Oklahoma State University Dairy. Two privately owned pastures that were located in the same area were utilized as well.

At the hay feeding sites 5 EPN treatments plus an untreated control were applied to each site resulting in 8 unique replications. The hay feeding sites were areas that were actively utilized by cattle during the hay feeding period (Dec. – Mar.) prior to the spring months of 2008 and 2009 (Figure 5). Screen mesh cone emergence traps were randomly placed in a circular pattern: at optimum regions of the feeding site as described by Talley et al. (2009). The traps were built from a screen mesh that consists of a mesh size small enough to not allow adult stable flies to pass through but still allow natural climate conditions to be a factor in the field experiments (Figure 5). The base of the trap was plastic garden edging material that was buried in the ground 4 to 6 inches in depth to prevent stable fly larvae as well as adults from escaping. The apex of the trap consisted of a collection jar placed upside-down so that emerging adults could be gathered easily. The

lids of the collection jars had the centers removed and were then attached, with the use of silicone, to the peak of the mesh traps. This allowed the jars to be screwed onto the traps so that they could not be dislodged from the traps.

Each experiment location was inspected for the presence of fly pupae. Any pupae that were found were collected and reared in the lab to ensure that these sites were conducive to stable fly development. A pea size clump of stable fly eggs were placed inside each trap at each location (Figure 6). This established a baseline population of stable fly larvae for testing. Further applications (mid-June, mid-July, & late August) of lab reared stable fly eggs were added to the trap areas when the populations of adult flies emerging from the traps decreased. Additional EPN applications were added to the traps each time period that corresponded with a stable fly egg application to all trap areas 24 hrs prior to being reseeded with additional fly eggs throughout the course of the trial. The 4 EPN treatments (1:0 S, 0:1 H, 2:1 S:H, and 1:2 S:H) plus an untreated control was replicated 8X. The traps were checked twice a week from May 1st to September16th and the number of adult flies that emerged was recorded from each replication at each feeding site.

Data Analysis

Susceptibility of stable fly larvae to native EPN: Probit analysis (PROC PROBIT, SAS Inst. 2009) was used to calculate LC_{50} values for the selection of the EPN genera or ratio using a natural log transformation of the EPN concentrations. Overlap of the 95% fiducial limits was used to determine significance (Taylor et al. 1998).

Hay/Manure substrate trials and EPN comparison: An analysis of variance (ANOVA, PROC GLM, SAS Inst. 2009) was used to analyze stable fly larval infectivity and mortality. Infectivity and mortality data are presented as a percentage. A *t*-test was used to separate the means and an LSD test was used to compare EPN treatments with controls.

Field trials: An analysis of variance (ANOVA, PROC GLM, SAS Inst. 2009) was used to analyze daily stable fly emergence per day per trap. Since there were 8 different field sites (8 replications) with each EPN treatment and an untreated control at each site these were analyzed at each sampling event. Average daily emergence trap data for each EPN treatment as well as an untreated control were separated with a *t*-test and a LSD test.

CHAPTER IV

RESULTS

Susceptibility of Stable Fly Larvae to Native EPN (Objective 1)

The single EPN genera treatment consisting of *Steinernema spp.* (S) (1:0) resulted in a LC_{50} value of 81,504/L and a X_2 value of 67.78 and was higher than the single genera EPN treatment of *Heterohabditis* spp. (H) (0:1) 19,346/L with aX_2 value of 107.42. While these two single genera EPN treatments (1:0 S:H & 0:1 S:H) demonstrated a low virulence towards stable flies in filter paper bioassays these results are inconclusive due to the EPN treatment 0:1 S:H (*Heterohabditis* spp.) did not calculate within a probit analysis because stable fly mortality was highly variable among the different doses and the data did not fit the logarithmic curve for the assumptions of the probit analysis to work properly. Stable fly larvae mortality increased in both combined EPN genera ratios (1:2 S: H and 2:1 S:H) compared to the two single EPN treatments (1:0 S:H and 0:1 S:H). The LC_{50} values of the two EPN genera ratios measured 3879/L (2:1 S:H) with a X_2 value of 62.20 and 4127/L (1:2 S:H) with a X_2 value of 32.42. Although the lower LC_{50} value in the higher ratio of *Steinernema spp.* (2:1 S:H) indicates that this EPN genera treatment was more virulent against stable fly larvae, they were not significantly different

from the higher ratio of *Heterohabditis* spp. treatment (1:2 S:H) due to overlap in the 95% fiducial limits (Table 2). It should be noted that the combined EPN genera treatments were more virulent overall than the single EPN genera treatments and this agrees with Taylor et al. (1998) findings that *Steinernema spp.* are more virulent against the closely related house fly.

Hay Manure Substrate Trials

Data collected from the susceptibility trials as well as findings from Taylor et al. (1998) suggests that the rate (7,500 EPN/liter) was to be utilized in an artificial substrate experiment. Single genera EPN treatments of Steinernema spp. produced 29.63% infectivity compared to 30.31% infectivity generated by Heterohabditis spp. (Figure 7). The combined ratios of the EPN genera produced an infectivity of 48.69% for 1:2 S:H ratio and 46.44% for the 2:1 S:H ratio (Figure 7). The increase in infectivity in the combined genera treatments were significantly different from the single genera EPN treatments but not from each other (Figure 7; $F= 13.84$; $df = 3, 159$; $P < 0.0001$). . Infectivity rates recorded from the substrate trials showed the combined ratios of EPN outperformed the doses that contained only single genera of EPN. Although the Heterohabditis spp. dominant ratio (1:2 S:H) infectivity percentages was higher in all replications, mortality of the stable fly larvae was lower in the susceptibility trials (Table 2). Based on these results the Heterohabditis spp. might have better foraging behavior than the Steinernema spp. within a hay/manure substrate to locate developing stable fly larvae.

EPN Comparison

Three additional commercially available strains of EPN were tested in the hay/manure substrate bioassay and compared with the native EPN previously tested. The three commercial EPN were *Steinernema feltiae (Sf), Steinernema carpocapsae (Sc)* and *Heterohabditis bacteriophore (Hb).* Stable fly mortality was significantly different among the different native EPN and commercial EPN (Table 3; $F = 15.98$; $df = 7, 95$; $P<0.0001$). All five different strains showed different stable fly mortalities. The stable fly larvae showed susceptibility to all strains of EPN in the hay/manure substrate cups with no mortality recorded in the control (Table 3). When viewing the 7 different EPN treatments based on stable fly adult mortality, the commercial strain of S. *feltiae* (Sf) produced significantly higher mortality than the other EPN treatments (55.59 %). This data coincides with the findings of Mullens et.al. that demonstrated that *S.feltiae* showed the highest infectivity rate against four different species of manure breeding flies (Mullens et al. 1987). The second highest mortality rate belonged to the native EPN treatment that consisted of the single genera of Steinernema 1:0 S:H (38.25 %) although lower than S. *feltiae* this EPN treatment was significantly different from the other two commercial species of *Steinernema carpocapsae* (Sc) (26.00%) and *Heterohabditis bacteriophore* (Hb) (11.29%)(Table 3; $F = 15.98$; $df = 7, 95$; $P<0.0001$). The two combined ratio of native EPN treatments also outperformed the aforementioned commercial species and were significantly higher (Table 3; $F = 15.98$; $df = 7, 95$; $P<0.0001$). The native EPN treatments that consisted of a combined ratio 1:2 S:H (36.48 %) and ratio 2:1 (32.74%) produced similar mortality in the hay/manure substrate but were also significantly higher than the single genera EPN treatment that consisted mainly

of *Heterohabditis* (0:1 S:H; 20.35%)(Table 3; F = 15.98; df = 7, 95; P<0.0001). Comparatively Steinernematidae genera outperformed Heterohabditidae genera on average in hay/manure substrates (Table 3). Although the highest rate of mortality only achieved 55% these methods could be used in conjunction with other biological control agents to achieve suppression of stable flies.

Field Trials

Data collected over two consecutive stable fly seasons (2008-2009) illustrates that the average adult stable fly emergence, for the season, was always higher in the control traps (Figure 8 & 9). Results collected from field experiments coincide with results found in the lab trials. However, the 2008 field trial did not yield any statistical differences between the control group and EPN treatments (F = 1.67; df = 4, 19; P = 0.2080). Consistently over a period of 3.5 months in 2009, stable fly emergence was always lower where EPN treatments were applied when compared to the control (Table 4 and Figure 9). In July stable fly emergence was significantly different between the EPN treated areas and the untreated control ([6 Jul 2009: F = 6.03; df = 4, 39; P = 0.0008] [8 Jul 2009: F = 3.10; df = 4, 39; P = 0.0276] [13 Jul 2009: F = 8.84; df = 4, 39; P <0.0001] [15 Jul 2009: F = 5.34; df = 4, 39; P = 0.0018]. Increases of adult stable flies were present in the controls at three separate time periods over the 3.5 month period after initial seeding took place: late June; early – mid July; and late August to early September (Figure 9). Results reviewed over the 3.5 month period show that increases in adult stable fly populations occurred in all traps at all locations (Figure 9). Although the increase in the controls was always higher than in any of the EPN treated trap sites (Table 4 and Figure 9).

CHAPTER V

SUMMARY

Results obtained were compared with results and conclusions found in other published studies. This biological control study shows that future research is needed if we are to realize the susceptibility of stable flies to EPN in a natural hay bale feeding site, as well as in confined feeding operations. Further analysis of the project might consist of recording climate factors such as temperature, precipitation and soil moisture throughout the sampling period to determine what time of the season are the EPN most effective. Also administering EPN with another form of biological control method such as parasitic wasp, Staphylinidae beetles or a pathogenic fungus could provide interesting data. A study of how chemical control methods affect naturally occurring EPN might be beneficial as well. Further studies of soil types and compaction at the feeding locations would also advance our knowledge about the efficacy of the EPN on stable fly larvae.

Ultimately this research offers information that may perhaps be valuable in further analysis of stable fly and EPN behavior, biology and control methodology in the future. The conclusions derived from this study illustrate that

EPN can have a negative effect on stable fly populations at round bale feeding sites. The EPN, being soil dwelling species, can function as successful suppressors in the developmental substrate of stable flies at hay bale feeding sites. By increasing the population of EPN in the substrate at these feeding sites the population of developing stable flies can be reduced. In this study naturally occurring trapped and reared EPN (S and H) were not as effective as one of the commercially available species *Steinernema feltiae* in the laboratory setting.

While results of these EPN trials cannot compare to commercially available insecticides, for stable fly mortality, these naturally occurring EPN can provide background suppression that could potentially be incorporated into a sound IPM program. Rearing methods, dosage rates, aspects of stable fly and EPN development and behavior coincide with findings that were observed in current and past literature.

REFERENCES

Bedding, R. A. (1984). Large scale production, storage and transport of the insect-parasitic nematodes Neoaplectana spp. and Heterorhabditis spp. Annals of applied biology 104(1): 117-120.

Bell, W. J. 1991. Searching Behaviour: The Behavioral Ecology of Finding Resources. Chapmanand Hall Animal Behavior Series, London.

Berry, I. L., D. A. Stage, et al. (1983). Populations and economic impacts of stable flies on cattle. Transactions of the ASAE - American Society of Agricultural Engineers 26(3): 873-877.

Bishopp, F. C. (1913). The stable fly (Stomoxys calcitrans L.), an important live stock pest. Journal of economic entomology 6: 112-U115.

Boire, S., D. E. Bay, et al. (1988). An evaluation of various types of manure and vegetative material as larval breeding media for the stable fly. Southwestern Entomologist 13(4): 247-250.

Broce, A., Hogsette J., Paisley,S. (2005). Winter feeding sites of hay in round bales as major developmental sites of Stomoxys calcitrans (Diptera : Muscidae) in pastures in spring and summer. Journal of economic entomology 98(6): 2307-2312.

Broce, A. B. and M. S. Haas (1999). Relation of cattle manure age to colonization by stable fly and house fly (Diptera: Muscidae). Journal of the Kansas Entomological Society 72(1): 60-72.

Bruce, W. N. and G. C. Decker (1958). The relationship of stable fly abundance to milk production in dairy cattle. Journal of economic entomology 51(3): 269-274.

Campbell, J. B., I. L. Berry, et al. (1987). Effects of stable flies (Diptera: Muscidae) on weight gain and feed efficiency of feedlot cattle. Journal of economic entomology 80(1): 117-119.

Campbell, J. B., M. A. Catangui, et al. (1993). Effects of stable flies (Diptera: Muscidae) and heat stress on weight gain and feed conversion of feeder cattle. Journal of agricultural entomology 10(3): 155-161.

Campbell, J. B. and J. F. Hermanussen (1971). Efficacy of insecticides and methods of insecticidal application for control of stable flies in Nebraska. Journal of economic entomology: 1188-1190.

Campbell, J. B., S. R. Skoda, et al. (2001). Effects of stable flies (Diptera: Muscidae) on weight gains of grazing yearling cattle. Journal of economic entomology 94(3): 780-783.

Campbell, J. B. and G. D. Thomas (1993). Control of flies at confined production units. Agri-Practice 14(8): 38.

Campbell, J. B., R. G. White, et al. (1977). Effects of stable flies on weight gains and feed efficiency of calves on growing or finishing rations. Journal of economic entomology: 592-594.

Cutkomp, L. K. and A. L. Harvey (1958). The weight responses of beef cattle in relation to control of horn and stable flies. Journal of economic entomology 51(1): 72-75.

Dillman, A.R., Chaston, J.M., Adams, B.J., Ciche, T.A,, Goodrich-Blair, H, Stock, S.P., and Sternberg, P.W. (2012). An Entomopathogenic Nematode by Any Other Name. PLoS Pathog 8(3): e1002527. doi:10.1371/journal.ppat.1002527

Dougherty, C. T., F. W. Knapp, et al. (1993). Stable flies (Stomoxys calcitrans L.) and the behavior of grazing beef cattle. Applied Animal Behaviour Science 35(3): 215-233.

Dougherty, C. T., F. W. Knapp, et al. (1995). Behavior of grazing cattle exposed to small populations of stable flies (Stomoxys calcitrans L.). Applied Animal Behaviour Science 42(4): 231-248.

Grewal, P. S., E. E. Lewis, et al. (1994). Host finding behaviour as a predictor of foraging strategy in entomopathogenic nematodes. Parasitology 108(2): 207-215.

Hogsette, J. A., A. Nalli, et al. (2008). Evaluation of different insecticides and fabric types for development of treated targets for stable fly (Diptera : Muscidae) control. Journal of economic entomology 101(3).

Hogsette, J. A. and J. P. Ruff (1987). Control of stable flies and horn flies (Diptera: Muscidae) with permethrin tapes applied to tails of beef and dairy cattle. Journal of economic entomology 80: 417-420.

Huey, R. B., and Pianka, E. R. 1981. Ecological consequences of foraging mode. Ecology 52:991-999.

Jeanbourquin, P. and P. M. Guerin (2007). Sensory and behavioural responses of the stable fly Stomoxys calcitrans to rumen volatiles [electronic resource]. Medical and veterinary entomology 21: 217-224.

Kaya, H. K. and R. Gaugler (1993). Entomopathogenic nematodes. Annual Review of Entomology 38: 181-206.

Kaya, H., Stock, P., 1997. Techniques in insect nematology. In: Lacey, L.A. (Ed.), Manual of Techniques in Insect Pathology. Academic Press, London, pp. 281–324.

Lewis, E. E., R. Gaugler, et al. (1993). Response of cruiser and ambusher entomopathogenic nematodes (Steinernematidae) to host volatile cues. Canadian journal of zoology 71(4): 765-769.

Moorhouse, D. E. (1972). Cutaneous lesions on cattle caused by stable fly. Australian veterinary journal: 643-644.

Mullen, G. R. and L. A. Durden (2002). Medical and veterinary entomology. Amsterdam, Boston.

Mullens, B. A., J. A. Meyer, et al. (1987). Infectivity of insect-parasitic nematodes (Rhabditida: Steinernematidae, Heterorhabditidae) for larvae of some manure-breeding flies (Diptera: Muscidae). Environmental Entomology 16: 769-773.

Mullens, B. A. and N. G. Peterson (2005). Relationship between rainfall and stable fly (Diptera : Muscidae) abundance on California dairies. Journal of Medical Entomology 42(4): 705-708.

Nielsen, C., T. Steenberg, et al. (2005). Effect of Metarhizium anisopliae (Deuteromycotina: Hyphomycetes) on survival and reproduction of the filth fly parasitoid, Spalangia cameroni (Hymenoptera: Pteromalidae).Environmental entomology 34(1): 133-139.

Owuama, C. I. (2001). Entomopathogenic symbiotic bacteria, Xenorhabdus and Photorhabdus of nematodes. World Journal of Microbiology and Biotechnology 17(5): 505-515.

Petersen, J. J. and J. K. Cawthra (1995). Release of a gregarious Muscidifurax species (Hymenoptera: Pteromalidae) for the control of filth flies associated with confined beef cattle. Biological control : theory and applications in pest management 5(2): 279-284.

Poinar, G. O. and P. T. Himsworth (1967). Neoaplectana parasitism of larvae of the greater wax moth, Galleria mellonella. Journal of Invertebrate Pathology 9(2): 241-246.

Renn, N., G. Barson, et al. (1985). Preliminary laboratory tests with two species entomophilic nematodes for control of Musca domestica in intensive in intensive animal units. Annals of applied biology 106(2): 229-233.

Romero, A., A. Broce, et al. (2006). Role of bacteria in the oviposition behaviour and larval development of stable flies. Medical and veterinary entomology 20: 115-121.

Schmidtmann, E. T. and M. E. Valla (1982). Face-fly pest intensity, fly-avoidance behavior (bunching) and grazing time in Holstein heifers. Applied animal ethology 8(5): 429-438.

Schwinghammer, K. A., F. W. Knapp, et al. (1987). Physiological and nutritional response of beef steers to combined infestations of horn fly and stable fly (Diptera: Muscidae). Journal of economic entomology 80(1): 120-125.

Skoda, S. R., G. D. Thomas, et al. (1991). Developmental sites and relative abundance of immature stages of the stable fly (Diptera: Muscidae) in beef cattle feedlot pens in eastern Nebraska. Journal of economic entomology 84: 191-197.

Skovgard, H. (2004). Sustained releases of the pupal parasitoid Spalangia cameroni (Hymenoptera: Pteromalidae) for control of house flies, Musca domestica and stable flies Stomoxys calcitrans (Diptera: Muscidae) on dairy farms in Denmark. Biological control : theory and application in pest management 30: 288-297.

Smith, J. P., R. D. Hall, et al. (1987). Field parasitism of the stable fly diptera muscidae. Annals of the Entomological Society of America 80(3): 391-397.

Smith, J. P., R. D. Hall, et al. (1989).A review of natural mortality and enemies of the stable fly (Diptera: Muscidae) in Missouri. Florida entomologist 72(2): 351-360.

Suszkiw, J. and J. Core (2003). Project aims to clean house on filth flies.Agricultural research (Washington, D.C.) Agricultural research 51(11): 20-22.

Taylor, D. B., A. L. Szalanski, et al. (1998). Susceptibility of house fly (Diptera: Muscidae) larvae to entomopathogenic nematodes (Rhabditida: Heterorhabitidae, Steinernematidae). Environmental Entomology 27(6): 1514-1519.

Talley, J., L. Zurek, et al. (2009). Characterization of Stable Fly (Diptera: Muscidae) Larval Developmental Habitat at Round Hay Bale Feeding Sites [electronic resource]. Journal of medical entomology 46(6): 1310-1319.

Thomas, G. D., S. R. Skoda, et al. (1996). Scheduled sanitation to reduce stable fly (Diptera: Muscidae) populations in beef cattle feedlots. Journal of economic entomology 89(2): 411-414.

Thomas, G. D. S. S. R. (1992). The stable fly : a pest of humans and domestic animals, proceedings of a symposium presented at the annual meeting if the Entomological Society of America December 1992, Baltimore, Maryland. Lincoln, Neb., Agricultural Research Division, Institute of Agricultural and Natural Resources, University of Nebraska--Lincoln.

Watson, D. W., D. A. Rutz, et al. (1995). Efficacy of Beauveria bassiana for controlling the house fly and stable fly (Diptera: Muscidae). Biological control : theory and applications in pest management 5(3): 405-411.

Wieman, G. A., J. B. Campbell, et al. (1992). Effects of stable flies (Diptera: Muscidae) and heat stress on weight gain and feed efficiency of feeder cattle. Journal of economic entomology 85(5): 1835-1842.

Williams, D. F., A. J. Rogers, et al. (1980). Preferred breeding media of the stable fly Stomoxys-Calcitrans in northwestern Florida USA. Mosquito News 40(2): 276-279.

Williams, R. E. (1985). Livestock entomology, Wiley.

APPENDICES

Table 1: Characteristics of common EPN and infected host cadavers, taken from Lacey and Kaya (2007).

Nematode species	ij length (µm)	host cadaver color
S. carpocapsae	**558 (468-650)**	**Beige**
S. riobrave	**622 (561-701)**	**Beige**
S. texanum	**756 (732-796)**	**Tan/walnut brown**
S. feltiae	**849 (736-950)**	**Tan/walnut brown**
S. glaseri	**1130 (864-1448)**	**Grayish- dark brown**
S. kraussei	**951 (797-1102)**	**Tan/walnut brown**
H. bacteriophora	**588 (512-670)**	**Brick red to dark purple**
H. indica	**528 (479-573)**	**Dark red**
H. megidis	**768 (736-800)**	**Orange brown**
H. zealandica	**685 (570-740)**	**Pale mint green**

Table 2: Probit analysis with LC_{50} values of stable fly larvae exposed to native EPN genera.

EPN genera*	LC_{50}	95% Cl†	X^2
0:1 Natural (S:H)	19,346	---*---	107.42
1:0 Natural (S:H)	81,504	24,364-1,618,810	67.78
2:1 Natural (S:H)	3879	2962-5294	62.20
1:2 Natural (S:H)	4127	3166-5615	32.42

* S:H ratios refer to composition of Steinernema spp.(S) to Heterohabditis spp. (H) and were based on color descriptions of wax moth larvae from Lacey and Kaya (2007).

†Overlap of 95% confidence intervals signifies no statistical difference between the different EPN genera treatments applied to stable flies.

Table 3: Percent mortality of native EPN genera (S:H ratios refer to composition of Steinernema spp.(S) to Heterohabditis spp. (H)) **vs. commercial spp. EPN toward stable fly larvae developing in hay substrate**

Treatment	% Mortality +/- SD*
Control	0 +/- 0 f
0:1 Natural (S:H)	20.35 +/- 12.07 ede
1:0 Natural (S:H)	38.25 +/- 7.78 b
2:1 Natural (S:H)	32.74 +/- 17.43 bc
1:2 Natural (S:H)	36.48 +/- 20.93 bc
Commercial (Hb)	11.29 +/- 14.49 ef
Commercial (Sc)	26.00 +/- 11.66 cd
Commercial (Sf)	55.59 +/- 22.25 a

***Mortalities followed by the same letter are not significantly different P<0.05, LSD test.**

Table 4: Average daily emergence of stable fly/trap/day from hay feeding sites where EPN treatments were applied. (S:H ratios refer to composition of Steinernema spp.(S) to Heterohabditis spp. (H))

	Control	1:0 S	0:1 H	2:1 S:H	1:2 S:H
6/24/2009	0.75	1.75	0.63	1.50	1.38
6/26/2009	7.25	2.00	5.00	3.25	0.50
6/29/2009	1.75	1.38	2.25	0.88	1.25
7/1/2009	1.50	0.50	0.75	1.00	0.38
7/6/2009*	**7.38 a**	**1.88 b**	**0.50 b**	**1.00 b**	**1.25 b**
7/8/2009*	**3.75 a**	**1.25 b**	**1.75 ab**	**0.63 b**	**0.50 b**
7/13/2009*	**4.75 a**	**1.50 b**	**0.63 b**	**1.38 b**	**0.50 b**
7/15/2009*	**4.75 a**	**1.50 b**	**0.63 b**	**1.38 b**	**0.50 b**
7/20/2009	0.13	0.13	0.25	0.00	0.25
7/23/2009	0.13	0.13	0.38	0.00	0.00
7/28/2009	0.13	0.25	0.00	0.38	0.13
8/4/2009	1.38	1.38	0.00	0.00	0.25
8/10/2009*	**3.50 a**	**1.00 b**	**0.88 b**	**0.75 b**	**0.38 b**
8/12/2009	1.75	2.00	0.75	0.88	0.13
8/17/2009	2.50	1.38	1.38	0.88	0.50
8/20/2009*	**1.63 a**	**0.63 b**	**0.63 b**	**0.25 b**	**0.63 b**
8/24/2009*	**1.13 a**	**0.75 ab**	**0.13 c**	**0.50 bc**	**0.00 c**
8/26/2009	0.50	0.63	0.63	0.25	0.00
8/31/2009	0.38	0.38	0.38	0.25	0.38
9/3/2009	0.38	0.38	0.25	0.13	0.00
9/8/2009*	**2.25 a**	**1.50 a**	**1.00 ab**	**1.13 ab**	**0.13 b**
9/10/2009	1.38	0.75	1.00	0.50	0.50
9/14/2009	1.13	1.25	0.75	0.88	0.50
9/16/2009	0.38	0.38	0.25	0.25	0.25

***Daily emergence values with the same letter within rows are not significantly different P<0.05, LSD test.**

6 Jul 2009: F = 6.03; df = 4, 39; P = 0.0008

8 Jul 2009: F = 3.10; df = 4, 39; P = 0.0276

13 Jul 2009: F = 8.84; df = 4, 39; P < 0.0001

15 Jul 2009: F = 5.34; df = 4, 39; P = 0.0018

10 Aug 2009: F = 12.81 df = 4, 39; P < 0.0001

20 Aug 2009: F = 3.23 df = 4, 39; P = 0.0233

24 Aug 2009: F = 5.25 df = 4, 39; P = 0.002

8 Sept 2009: F = 2.74 df = 4, 39; P = 0.0442

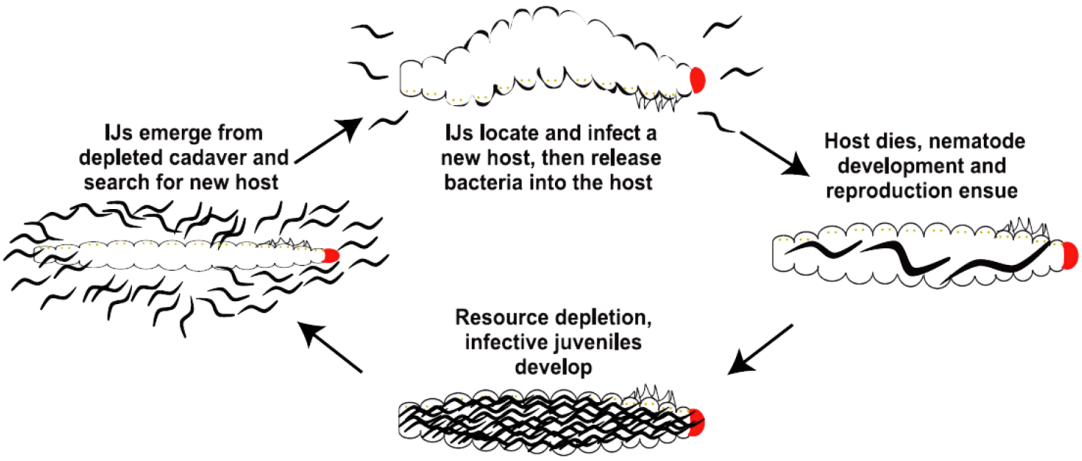

Figure 1: The life cycle of EPN within an insect host. (Dillman et al. 2012).

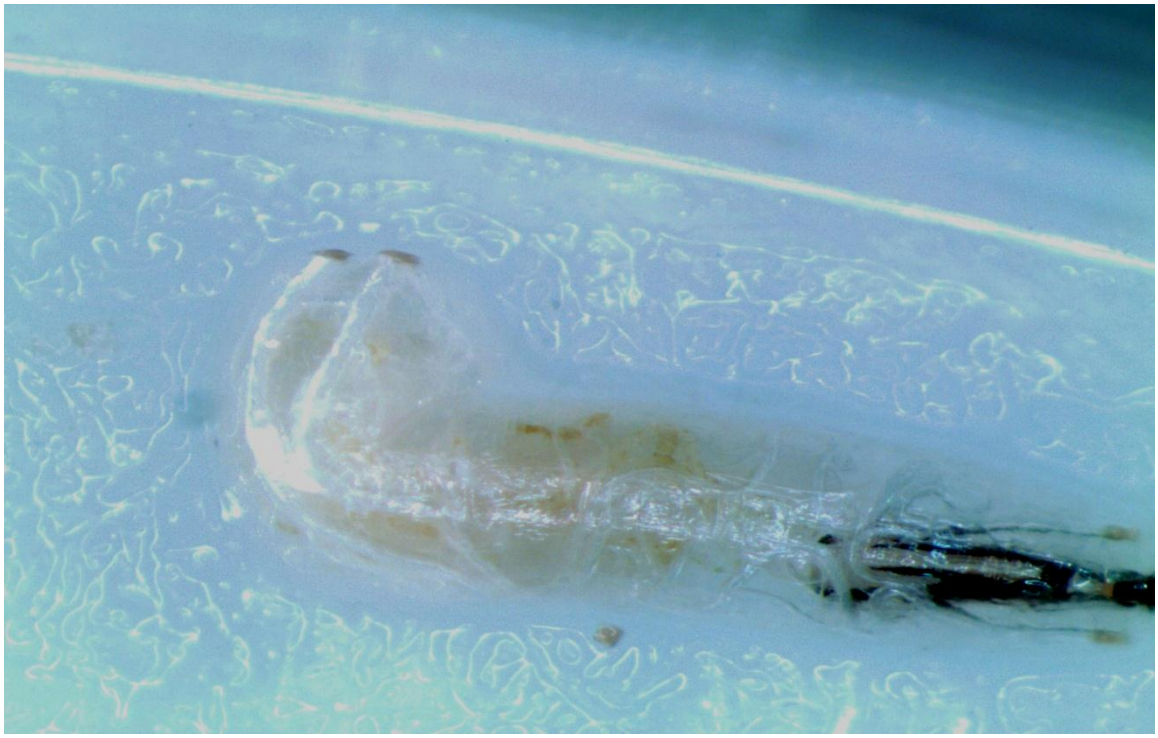

Figure 2: Lab reared stable fly larvae infected with third generation lab reared entomopathogenic nematode (Steinernematidae)

Figure 3: Cups with the hay/manure substrate.

Figure 4: Round bale feeding site located in a pasture setting. The area surrounding the feeder generates viable stable fly substrate.

Figure 5: Screen mesh cone emergence traps placed at a round hay bale feeding site in the spring and summer months during active stable fly development.

Figure 6: Seeding areas under cone emergence traps with lab reared stable fly eggs.

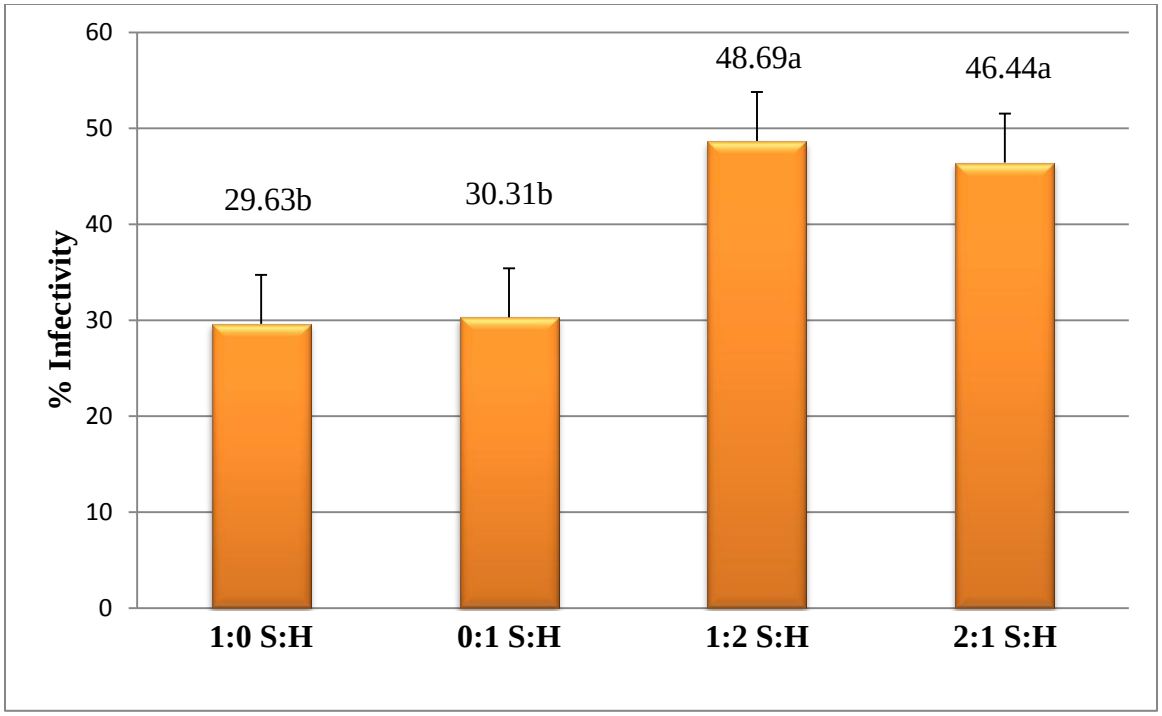

Figure 7: Average % infectivity of stable flies exposed to different EPN treatments in hay/manure substrate.

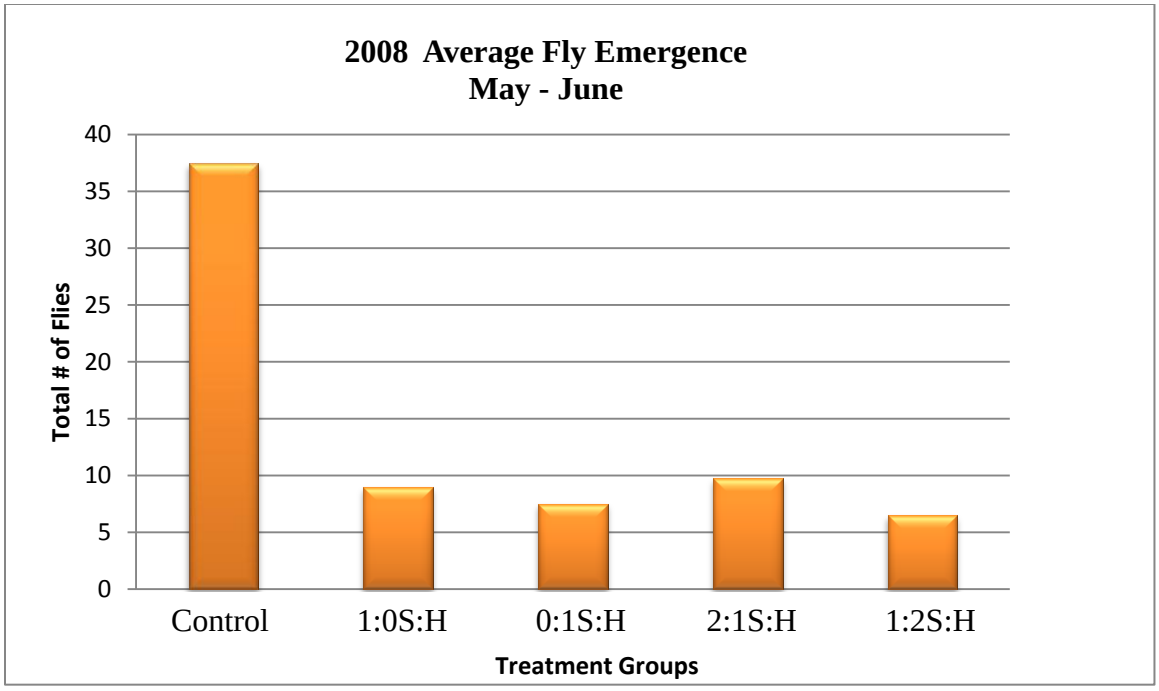

Figure 8: Average weekly emergence of adult stable flies for the months of May through June 2008 captured in traps at field locations.

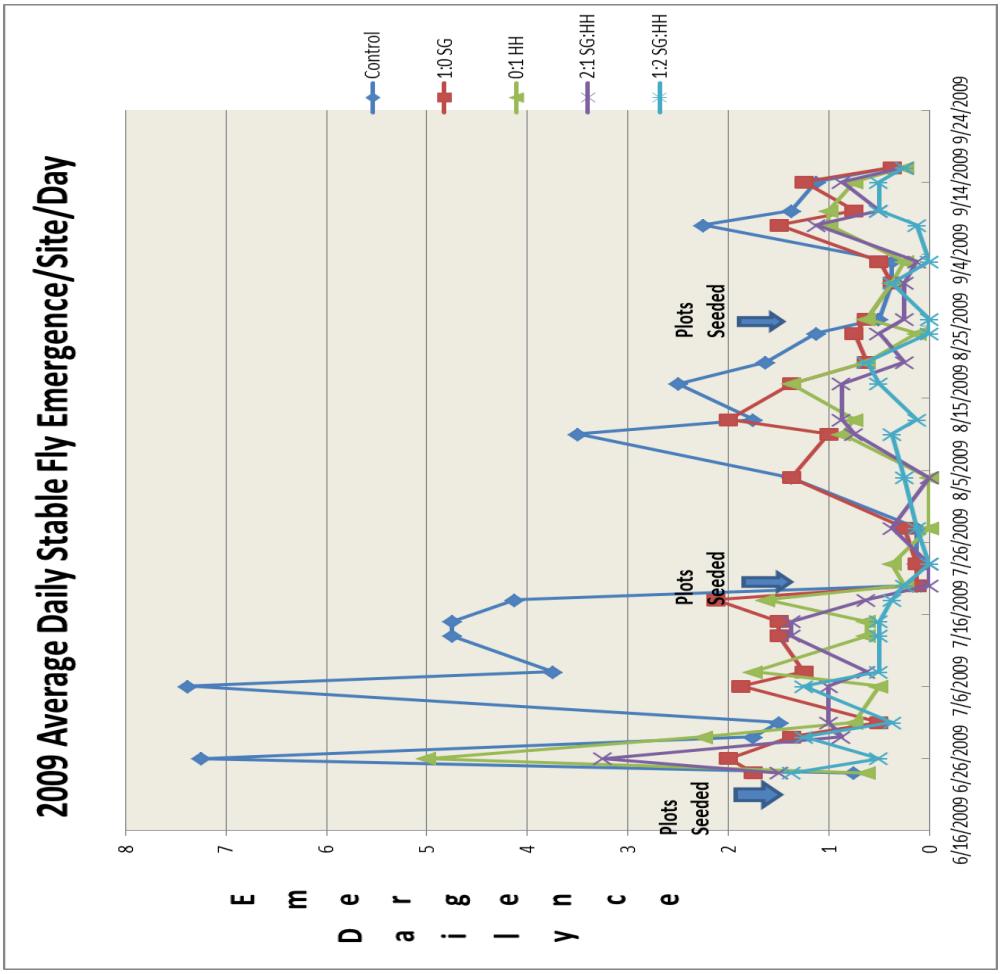

Figure 9: Average daily emergence of adult stable flies at round hay bale feeding sites. Stable fly eggs were introduced under each trap as indicated by the Plot Seeded arrows.

VITA

Lucas Ryan Pierce

Candidate for the Degree of

Master of Science

Thesis: EFFICACY OF ENTOMOPATHOGENIC NEMATODES UTILIZED FOR CONTROL OF STABLE FLIES (*STOMOXYS CALCITRANS*) AT ROUND HAY BALE FEEDING SITES

Major Field: Entomology/Plant Pathology

Education

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- Major: Entomology (Livestock Entomology)

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Experience

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Professional Memberships:

Professional Memberships

- Division II colligate student/athlete *Aug. 1997– 1998*
- Member of Sanborn Entomology Club at OSU *Aug. 2001*
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