MANAGEMENT AND CHARACTERIZATION OF STABLE FLY LARVAL HABITATS AT ROUND HAY BALE FEEDING SITES IN PASTURES

by

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B.S., West Texas A&M University, 2000

M.S., West Texas A&M University, 2001

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Abstract

Stable flies, *Stomoxys calcitrans* (L.), are a serious pest to beef cattle in confined animal feeding operations (CAFOs) by causing economic losses in the form of reduced feed intake and feed efficiency, resulting in reduced weight gain. Integration of sanitation, parasitoids, and residual insecticides offers a much-needed reduction of this pest's impact on CAFOs. In the past two decades, stable flies have become the most important pest of pastured cattle. Further impact that stable flies have on cattle is when cattle seek protection from stable flies by standing in water, which results in water pollution with fecal matter, in addition to reduced foraging time. Sites of winter feeding of round hay bales have demonstrated to be important habitats for stable fly development during spring/summer. Cattle feeding on round bales can waste as much as 40% of the total amount of hay when fed in conventional ring feeders. Hay wastage is largely a function of the type of feeding method and the amount of agonistic behavior of the cattle. Feeding methods range from rolling hay directly onto the ground to the use of various types of feeders. Since traditional control methods utilized in CAFOs against stable flies have not been evaluated in pastures, producers rely heavily on organic insecticides in efforts to control this pest. At this time, there are no effective control methods available for stable fly management on pastured cattle. This research examined different management strategies that could minimize or eliminate stable fly larval habitats by reducing the amount of hay wasted being mixed with manure. In addition, different hay and manure mixtures were compared to characterize the larval habitat at these hay-feeding sites. Finally, the efficacy of boric acid, *Metarhizium anisopliae*, and tetrachlorvinphos in controlling the development of stable flies in hay substrates was evaluated.

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Dedication

I dedicate this work to my wife Denise because without her unwavering prayers and encouragement I would not have been able to complete this work. She is truly my best friend and life partner in all things I set out to accomplish. I also dedicate this work to my two sons, Jamison and Julius, for their smiles and laughs. I am also grateful to my parents for encouragement and financial support to help accomplish this endeavor. I would also like to send out my gratitude to Pauline and Crystal for their support and continued encouragement throughout my graduate career.

CHAPTER 1 - Introduction and Literature Review

Stable flies, *Stomoxys calcitrans* (L.) (SF), are important economic pests of livestock, especially on cattle in North America (Campbell and Hermanussen 1971, Christensen 1982). SF cause stress to cattle when blood-feeding which results in economic damages by worsening weight gain (Weiman et al. 1992) and feed conversion (Campbell et al. 1987). Harwood and James (1979) described the most important sources of annoyance to livestock as injury of various forms when the animals react to mass attacks of the flies, namely loss of blood, lowered milk production, reduced vitality, and loss of pasturing time. When cattle are under attack by SF, they may terminate eating and crowd in bunches which then causes heat stress and weight loss (Foil and Hogsette 1994). The effect of stable flies on the productivity of dairy cattle can severely decrease milk production and serious economic losses for the dairy operation. According to Bruce and Decker (1958), there was an average production loss of milk of 0.7% per SF per cow. Campbell et al. (1987) found that the economic threshold for stable flies attacking feeder heifers, when weight gain, feed efficiency, and cost of control with insecticides are considered, to be less than two stable flies per host leg. Total losses to the producer were estimated at $8.51 per animal with 5 stable flies per front leg, with worse feed efficiency accounting for 88% of the total loss. Campbell et al. (2001) reported a 7% reduction in weight gain per SF on grazing yearling cattle. If steer prices averaged $1.98/kg, the loss would be valued at $33.26 per animal or 2.33 cents per fly. These costs do not include the cost of spraying the yearling cattle indicating the need for a superior SF management plan over spraying.

The SF is a member of the dipteran family Muscidae and is a well-known cosmopolitan member of the subfamily Stomoxyinae (Zumpt 1973). Piercing-sucking mouthparts distinguish Stomoxyinae from other Muscids. SF and horn fly, *Haematobia irritans* L., are included in the

subfamily Stomoxyinae. *S. calcitrans* is known by several common names including: SF, biting house fly, dog fly, barn fly, and power mower fly (Hall and Smith 1986). Stable flies resemble the common house fly, *Musca domestica,* but have piercing-sucking mouthparts with a long, thin proboscis pointing forward from under the head. This is composed of the labium with short labella, the hypopharynx, and the labrum (Zumpt 1973). Key characteristics to the SF are the arista hairs found only on the dorsal side of the antenna (Zumpt 1973). The wing venation of the SF is quite different from other Muscidae having a slight bend upward on vein M1+2 unlike the slight curve to the house fly (Castro 1967). The SF has four longitudinal black stripes on the thorax and a checkered abdomen larger than that of the house fly (Foil and Hogsette 1994).

All species of *Stomoxys* are native to Africa. The SF is the only species found in North America (Skidmore 1985). Its introduction to the New World may have occurred during the mid 17th century (Hall and Smith 1986). The SF is a strong flier and has been observed to travel long distances (Hoffman 1968). Hogsette and Ruff (1985) documented a wind assisted flight range of 225 km in Florida. Eddy et al. (1962) recovered marked adults 8 km from the release point within 2 hours after release. Gersabeck and Merritt (1985) calculated a potential adult lifetime migration radius of 140 km. This was based on movement of 7 km per day (Bailey et al. 1973). Gersabeck and Merritt (1985) emphasized that SF movement on a spatial and temporal scale was probably a function of host activity patterns, duration of feeding, and potential of the insect to fly. When provided with an abundant host source, i.e., feedlots, 90% of marked flies were captured within 0.8 km of the release site (Gersabeck and Merritt 1985). Scholl (1986) observed that 80% of dispersing flies recovered between two feedlots 0.8 km apart were males.

Stable flies can feed on a variety of warm-blooded animals and are commonly called the biting house fly. Both sexes are vicious blood-feeders drawing blood quickly and feeding to full

capacity in 4 minutes (Harwood and James 1979) and with recent studies showing as few as 2.5 min. (Schofield and Torr 2002). Bailey and Meifert (1973) observed that the adult SF usually approaches a host only to feed. The female is anautogenous, requiring several blood meals to complete ovarian development. Parr (1962) reported that the average blood meal (25.8 mg) is three times the average body weight (8.6 mg). Ordinarily, the SF cannot obtain a full blood meal on one host because of the defensive behavior elicited by the painful bite, thus the flies alight repeatedly on the same host or fly from one animal to animal until the meal is completed (Harwood and James 1979). There are three phases in the SF's search for a host. The first one is the appetitive-searching phase, this is followed by the activation and orientation phases, when the stable flies encounter the chemical stimuli (kairomones) indicating the presence of a host. Attraction occurs when the insect (having located a host) begins to feed (Lehane 1991). Stable flies are diurnal, while most other blood-sucking insects, such as mosquitoes, are nocturnal (Lehane 1991). Holloway and Phelps (1991) have found that *S. calcitrans* has a bimodal diurnal pattern of feeding, locating hosts by responding to carbon dioxide and octenol; temperature was found to be the most important weather factor. Stable flies' highest biting activity was observed to be at approximately 30° C, whereas at 14° C the flies were no longer attracted to host animals (Zumpt 1973). Adult flies feed throughout the day, but greatest activity is observed on cattle between 10 a.m. and 4 p.m. (Hoffman 1968). The SF has been observed feeding at dawn and in the late afternoon under natural conditions, but can feed at any time during the daylight hours (Mitzmain 1913). Castro (1967) observed that stable flies predominately feed on large animals such as cattle, horses, hogs, sheep, goats, and humans. Stable flies will also bite dogs, especially on their ears (Hogsette et al. 1987). Stable flies tend to feed on the lower extremities, such as below the knees and hocks of animals but can move to the sides and back if the populations are

too high on the lower extremities (Foil and Hogsette 1994). Stable flies can also feed on nectar for immediate energy needed for flight, but cannot successfully produce eggs when a blood meal is not readily available (Jones et al. 1985).

Stable flies have a holometabolous development; their life cycle consists of the egg, three larval instars, and the pupal and the adult stages (Ross et al. 1982). After taking a blood meal, and the proper time for blood meal digestion and ovarian development, the female SF will seek out a suitable oviposition site and deposit eggs throughout the media. The ideal temperature for oviposition is between 22°C and 28°C (Zumpt, 1973). Zumpt (1973) noted that the number of eggs laid by one female was low during the cold months, but rose to about 120 eggs at 25°C, and to about 200 eggs at 30°C. The eggs are about 1 mm long and 0.2 mm wide, resembling a banana in shape, and have a median longitudinal groove (Harwood and James 1979, Hall and Smith 1986). The eggs hatch 12-24 hours after being oviposited (Foil and Hogsette 1994).

The hatching larva forces open the operculum and emerges rapidly in about 14 seconds (Parr, 1962). Larvae hatch from the eggs in 28 (24-48) hrs at 22°C and 50-80% RH (Hoffman 1968). According to Parr (1962), the newly-emerged larva measures an average of 1.08 mm in length, and grows to 1.7 mm; the second instar attains a length of 2.80 mm; and the third instar is 11.12 mm at maturity. First instar larvae molt to the second instar in less than 24 h (Parr 1962). Second instar larvae molt to third instar 1 d later, under optimal conditions (Parr 1962). During the third instar larvae grow from 4.4 mm to 11-12 mm in length (Skidmore 1985). Larval development ranges from 8 d (26°C and 80% RH) during summer months (Parr 1962), to several months during winter (Harwood and James 1979). Larvae are whitish-yellow, characterized by a pointed anterior with the mouth opening, and a blunt truncated posterior with the anal opening and caudal spiracles (Peterson 1960, Skidmore 1985). Larvae have 12 visible segments and a

single mouth hook (Peterson 1960, Zumpt 1973). The nutritive value of the medium and temperature play large roles in the duration of the larval stages (Parr 1962).

The pupal development takes place inside the puparium, which is the hardened cuticle of the 3rd instar larva (Castro 1967). At 21- 26°C, the pupal stage lasts from 6 to 26 d (Parr 1962, Harwood and James 1979). The reddish-brown puparia are 6 to 7 mm long and barrel shaped. Adults emerge from the puparium by inflating the ptilinum, an eversible sac on the head (Ross et al. 1982). Once the SF emerges, the body elongates. The fly has a pale gray color and crumpled wings; and the mouthparts are bent posteriorally between the forelegs (Castro, 1967). According to Castro (1967), within 30 minutes of emergence the body is darker, the wings expand, the proboscis folds forward, and the insect is ready to fly away.

To successfully produce her first batch of eggs, the female requires several blood meals from a host (Foil and Hogsette 1994). The female SF can begin mating 3-5 days after emerging and can begin laying eggs when 5-8 days old (Foil and Hogsette 1994); that is if she has had available bloodmeal. Adults assemble on sunlit objects from which the males dart out after flying females and engage in aerial interactions (Buschman and Patterson 1981). According to Buschman and Patterson (1981), males mount the females in the air or on the ground with copulation occurring on a perch. The same male can mate with more than one female, but females cannot be coupled with more than one male (Zumpt 1973). Buschman and Patterson (1981) found that flies used basking stations not only for mating, but also for thermoregulation when ambient temperatures were too high or too low. The first egg batch is mature approximately 2 d after copulation (Parr 1962). Female stable flies oviposit batches of approximately 35 eggs in moist media (Parr 1962, Hall and Smith 1986). Females can deposit

up to 600 eggs during their lifetime (Killough and McKinstry 1965). Oviposition sites include a range of decaying organic matter (Hall and Smith 1986).

Larval development sites include: grass clippings (Ware 1966), livestock manure mixed wasted feed or hay (Coffey 1966), chopped silage (Williams et al. 1980), and round hay bales (Hall et al. 1982, Broce et al. 2005). Larvae are concentrated near fencelines, drainage ditches and haylage on small Nebraska feedlots (Meyer and Petersen 1983, Skoda et al. 1992). Decomposing spilled feed is preferred in large feedlots, and stored manure at dairies (Meyer and Petersen 1983). SF larvae move into decaying organic matter to prevent freezing and continue development at a reduced rate in the winter (Berry et al. 1978). Third instar larvae and pupae have been recorded overwintering in silage, pilled manure and piled grass (Berkebile et al. 1994).

The SF is generally not considered important as a vector of animal or human diseases (Castro 1967). However, Horsfall (1962) stated that because the flies tend to probe the skin of one or more animals in their feeding, they can serve as carriers of pathogens. *S. calcitrans* can transmit numerous pathogenic organisms, including those causing cutaneous leishmaniasis, anthrax, brucellosis, equine infectious anemia, and bovine diarrhea virus (Greenburg 1971). A large number of pathogens have been recorded due to the flies interrupted feeding habits (Harwood and James 1979). They are the intermediate host of nematode worms, including *Setaria cervi,* a parasite of cattle, and of several species of *Habronema*, stomach parasites of horses (Greenburg 1973). The infective larvae of *Habronema microstoma* interfere with the ability of the fly to penetrate the skin with the proboscis and take a normal blood meal (Zumpt 1973). Another pathogen transmitted by the stable flies is *Trypanosoma evansi*, the cause of surra. This disease is always fatal to horses and mules, often affects camels and dogs seriously,

and is asymptomatic in cattle (Harwood and James 1979). Tabanids and stable flies are able to transmit a viral disease known as equine infectious anemia to equine species (Foil and Hogsette 1994).

According to Schreck et al. (1975), a priority of pest control research is to emphasize selective, environmentally acceptable methods for dealing with noxious pests. Apart from physical barriers like screens placed in human and animal houses, various control measures for SF have been developed. These include chemical compounds, traps, ecological modifications of the environmental conditions, utilization of biological control agents, and the sterile male technique (SMT) (Zumpt 1973). In addition, much can be done to reduce SF populations by managing the amount of rotting, wet manure and straw material around the premises. These types of materials can be hauled off, spread thin to dry out, composted, or burned to kill the maggots (Harwood and James 1979). In addition, roosting sites may be treated with residual sprays (Zumpt 1973). Biological control agents affecting stable flies are generally identical or similar to those attacking house flies, but they seem to exert minimal control with the exception of some hymenopterous parasites (Harwood and James 1979). Greene et al. (1989) suggests that using a parasite species can work in most situations but a survey of the habitat location is key in the developing population of the parasites. Dung beetles, belonging to the family Scarabaeidae, help to scatter the feces, which in turn dry rapidly and allow for ants to prey on the maggots (Zumpt 1973). Harwood and James (1979) pointed out that reproductive manipulation (SMT) may have some distinct possibilities because SF populations tend to be focal in nature, easily reared, and the female apparently mates only once. Buschman and Patterson (1981) contemplate that if wild males are more successful than laboratory-reared sterile males in holding favorable waiting stations, they could account for many more mating than their numbers indicated.

However, because both sexes are vicious blood feeders the sterile male technique is not ideal in controlling the stable flies.

Williams (1973) found that a box trap containing an inverted plastic cone positioned inside a plywood box was effective in catching stable flies on sandy beaches. However, the box trap was not as effective along inland sites as other traps. Although cumbersome at times because of the eight surfaces, the Williams trap, a translucent fiberglass sticky panel, has been found to be highly effective for monitoring *S. calcitrans* (Williams 1973). Broce (1988) invented the now commercial cylinder design of the Alsynite plastic traps that uses a cheaper, thinner plastic with less adhesive than the old Williams traps. He found that the new trap was equal to the Williams traps in catching house flies and stable flies; however, because of its smaller surface area, the cylinder trap had a greater trapping efficiency than the Williams trap. Hogsette and Ruff (1990) supported Broce's findings and found that the cylinder trap captured fewer total flies, but more flies per cm^2 than any of the Williams traps used in their experiments. There are several visual, thermal, and chemical cues that stable flies use in search of hosts. There are several traps for stable flies which incorporate many of these cues. The use of carbon dioxide, ultraviolet light, and plexiglass were all tested as attractants with an electrocutor grid to find the most effective combination against stable flies. The electrocutor grid trap with carbon dioxide attractant has proved highly selective against *S. calcitrans* during the winter in north central Florida (Schreck et al. 1975). Hoy (1969) found that the Malaise traps baited with CO_2 caught 3 times as many stable flies than did either of the CO and control Malaise traps. Cilek (1999) used Alsynite cylinder traps with various volatile substances, such as dry ice, acetone, and octenol, and found that CO_2 from the dry ice was a very powerful attractant for collecting stable flies. The only draw-back to these types of traps is the use of CO_2 as dry ice can be costly. When using traps of

any design, their placement is a key factor on their performance. Traps should be placed where stable flies are assembling, in a place that is easy to service, and out of the way of workers and animals to avoid being trampled or tripped on (Hogsette and Ruff 1987). Insecticides should be used as a last measure in fly control, not only because sanitation is more permanently effective, but also because insecticide resistance can develop (Harwood and James 1979). Cilek and Greene (1994) found that in Kansas feedlots resistant stable flies were found even where insecticide use was minimal. They thought the insecticide-resistant stable flies came from nearby feedlots not using proper management practices against biting flies. Permethrin-impregnated yarn wound around either Williams or cylindrical traps can also be used to reduce SF numbers (Hogsette and Ruff 1996). Tseng and Hogsette (1986) found that a distance of 2.54 cm between strands and in a continuous coil, not crisscrossed, yielded maximum catch as compared to the strands being laid right next to each other. This yarn can be left in a field for about 3 months and can be placed on several traps (Foil and Hogsette 1994). Proper sanitation, such as spreading manure out to dry, composting, and burning manure is key to reducing the number of stable flies that successfully mate and reproduce. Proper use of insecticides and traps in conjunction with reducing the breeding media can be effective in decreasing SF populations.

Coinciding with the adoption of round hay bale feeding in the past two decades, stable flies have become a serious pest of pastured cattle. Decaying organic matter mixed with manure, soil, and moisture provides ideal conditions for larval development of stable flies. Considerable information is available on SF larval habitats in confined animal feeding operations, which include spilled feed, stored manure, and various forms of silage (Meyer and Petersen 1983, Skoda et al. 1991). Broce and Haas (1999) demonstrated that stable flies prefer to colonize manure at 2-weeks of age or older. While there is significant information on SF larvae

development in confined livestock operations, there is little known about their development in pastures. Hall et al. (1982) documented SF larval development in and around round hay bales stored outside. Ranchers feed these round bales in the winter for additional forage to maintain the cattle's nutritional requirements.

Harvesting and storing bales in the form of large round bales is a common production practice on Midwest farms in the United States (Buskirk et al. 2003). The first mass production of large round hay balers began in 1970 (Buchele 2005). The initial intention of using round bales was to reduce labor during harvesting and storing hay. However, round bales demonstrated to have a unique characteristic that simulated thatched roofs, thus shedding high amounts of moisture that was thought to reduce the amount of storage losses (Buchele 2005). However, in recent years, storage losses in round bales have been recorded to range from 2 to 18 % of the dry matter (Huhnke 1987, Harrigan and Rotz 1994). Another factor that influences the amount of hay that becomes watage is the type of feeding method. Buskirk et al. (2003) compared different feeding methods of large round bales and discovered that alternative designs such as cone type feeders can significantly reduce the amount of waste when compared to other feeding methods. Amount of loss from storage and feeding methods can be significant in the form of costs to the producers since feed cost is the single largest variable influencing profitability of the cow-calf enterprise in the Midwest (Miller et al. 2001).

At hay feeding sites, considerable residue remains and usually undisturbed in the following spring and summer months which in turn allows adult stable flies to oviposit at these sites. Broce et al. (2005) found high numbers of SF larvae developing in hay wastage, with adult emergence ranging from 200 to 4,866 flies/m^2/week in North Central Kansas and South Central Kansas. Hay residues on a dairy in Northwest Florida contained as high as 28,000 SF larvae/m^2

(Patterson and Morgan 1986). Hay residues can remain active larval sites for extended periods of time, especially if new bales are frequently replaced at the same site. Greene (1993) stated that cultural control is the most important method for on-site reduction of SF populations. Foil and Hogsette (1994) recommended that large round hay bales that are fed to livestock be placed on a mobile platform and moved short distances once or twice a week in order for residue accumulations to be limited to small amounts that tend to dry completely. This research examined different management strategies that could minimize or eliminate SF larval habitats by reducing the amount of hay wasted being mixed with manure. In addition, different hay and manure mixtures were compared to characterize the larval habitat at these hay-feeding sites. Finally, the efficacy of boric acid, *Metarhizium anisopliae*, and tetrachlorvinphos in controlling the development of stable flies in hay substrates was evaluated.

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List of Objectives

I. Evaluate alternative hay feeding methods to control stable flies at round hay bale feeding sites.

   a. Compare different round bale feeding methods to determine the amount of wasted hay and SF production from each feeding method.

   b. Determine if different feeding strategies that focus on movement of feeding sites affect SF production and forage quality.

II. Characterize SF larval habitats within feeding sites of round hay bales

   a. Determine environmental factors (temperature, % moisture, pH, depth to soil, concentration of fecal coliforms) associated with different hay/manure accumulations during high versus low SF production.

   b. Evaluate SF larval development and survival as a function of the hay:manure ratio of larval media.

   c. Evaluate the physical characteristics of hay as a component of larval media by simulating hay with plastic straws in laboratory bioassays.

III. Evaluate different control agents against SF larvae, including an inorganic compound, boric acid; an organophosphorous insecticide, tetrachlorvinphos; and an insect pathogenic fungus, *Metarhizium anisopliae.*

CHAPTER 2 - Alternative hay feeding methods to control stable flies (Diptera: Muscidae) developing in round hay bale feeding sites

ABSTRACT

The stable fly (SF), *Stomoxys calcitrans* (L.), has become during the last two decades a major pest of cattle on pastures in the midwestern United States. Identification of SF larval habitats in pasture environments has been limited, but recently winter feeding sites of round hay bales have been identified as suitable developmental sites due in part to the large amounts of wasted hay being mixed with manure and urine. This study evaluated different hay feeding techniques intended to reduce hay waste, and examined whether reduction of hay waste coincided with reduced SF production. Initially, two types of feeders were compared; the typical ring feeder most commonly used in the Midwest and a newer hay saving design known as a cone feeder. Cattle using the ring feeder wasted significantly more hay (23.74 %) than those using the cone feeder (10.54 %); however no differences were observed in SF production from the grounds where the two feeder types were used. Next we compared fly emergence from: 1) grounds with a typical ring feeder moved to different locations after each hay bale was consumed 2) grounds with unrolled hay bales, and 3) grounds with a ring feeder stationary during the entire feeding period. No SF emerged from any of the moved feeding sites or from the unrolled sites; but an average of 6 flies/trap/day emerged from the unmoved feeding sites in early June (24 flies/m^2/day). The promising results of moving feeders and unrolling hay bales lead us to consider whether the frequent movement of hay feeding sites has any detrimental effect on the quantity of pasture forage which is a major concern among producers. Forage analysis at the unrolled feeding sites showed no difference in forage quantity between areas inside and outside the feeding area. However, forage quantity was significantly reduced at the moved feeder sites.

Promising results from unrolling hay bales lead to further comparing this method to the other feeding techniques in terms of the amount of hay wasted that is produced by these methods. Repeated unrolling of the hay bale over the same location resulted in the highest amount of wasted hay (35.35 %), whereas unrolling each hay bale at a different location yielded similar amounts of hay waste (22.74%) as the ring feeder. These results support the fact that winter feeding sites of hay bales are viable larval habitats for stable flies. Also the results indicate the suitability of the larval habitats is not a function of the amount of hay wasted, but rather the amount of cattle feces at these sites.

Key Words: stable fly, *Stomoxys calcitrans*, hay, round bale, hay feeding

In the past two decades, stable flies, *Stomoxys calcitrans* (L.), have become a serious pest of pastured cattle. Stable flies have been implicated in causing a reduced average daily gain of 0.23 kg/d in grazing yearling cattle, when control animals were protected from stable flies by frequent insecticide treatment (Campbell et al. 2001). When cattle are attacked by stable flies they react by bunching to protect their legs, the main feeding sites of stable flies (Campbell et al. 1977). This bunching activity can have detrimental effects on forage quality in pastures by causing areas of reduced forage or even bare ground due to the concentration of animals. Another problem that arises from cattle reacting to SF bites is when cattle stand in water to avoid the bites, resulting in reduced grazing activity and increased water pollution.

Decaying organic matter mixed with manure, soil, and moisture provides ideal conditions for SF development. Considerable information is available on SF larval habitats in confined animal feeding operations (CAFOs), which include spilled feed, stored manure, and various forms of silage (Meyer & Petersen 1983; Skoda et al. 1991). Broce & Haas (1999) demonstrated that stable flies prefer to colonize manure of at least 2-weeks of age. While there is significant information on SF larval development in confined livestock operations, there is little known about their development in pastures. Hall et al. (1982) documented SF larval development in and around round hay bales stored outside. Ranchers feed these round bales in the winter as additional forage to maintain the cattle's nutritional requirements. At these sites, considerable residue remains and usually undisturbed, which in the following spring and summer months allows adult stable flies to oviposit at these sites. Recently, Broce et al. (2005) reported high numbers of SF larvae developing in hay wastage, with adult emergence ranging from 200 to

4,866 flies/m^2/week in North Central Kansas and South Central Kansas. Hay residues on a dairy in Northwest Florida contained as many as 28,000 SF larvae/m^2 (Patterson & Morgan 1986). Hay residues can remain active larval sites for extended periods of time, especially if new round hay bales are frequently replaced at the same site.

Among the different hay feeding methods, the most commonly utilized is the ring type feeder. Burskirk et al. (2003) compared the amount of wastage produced by the use of four different types of hay feeders: cradle, trailer, ring, and cone, and found that they wasted 14.6, 11.4, 6.1, and 3.5%, respectively. Whether or not feeding grounds with 3.5% hay wastage provide suitable SF larval habitats remains to be investigated. Greene (1993) stated that cultural control is the most important method for on-site reduction of SF populations. Foil and Hogsette (1994) recommended that large round hay bales that are fed be placed on a mobile platform and moved short distances once or twice a week in order for residue accumulations to be limited to small amounts that tend to dry completely. Since hay feeding sites have been identified recently as viable SF larval habitats in pasture (Broce et al. 2005), this study was undertaken to determine if different hay feeding techniques intended to reduce hay waste influence SF development.

Materials and Methods

Feeder Comparison (01/08/04 – 01/17/04). The amount of hay wasted from the use of the conventional ring feeder (Fig. 2.1a) was compared to that of a new cone type feeder (Fig. 2.1b) (Plymouth Industries, Plymouth, NE), using four adjoining Kansas State University (KSU) pastures with ~ 25 – 30 head per pasture. Two feeders of the same design (ring or cone) were placed in each pasture. Each new bale was weighed before placing it in the feeder and all hay on the ground outside the feeder walls was collected every 24 hrs. by racking, bagging and weighing (Blasi et al. 1993). Once cattle consumed the bale of hay, a new bale was placed into the feeder. After three replications (three new bales in each feeder) of collecting hay wastage, this part of the study was terminated to allow hay to accumulate at the feeding sites for spring quantification for SF emergence. All wasted hay that was collected and weighed (kg) was converted into % hay wastage and subjected to an analysis of variance test (PROC GLM; SAS Institute, 1999). Means were separated using the Fisher's least significant difference (LSD) test to determine differences between the two feeder types. Pyramid emergence traps, with 0.25 m^2 base fitted with fly collection containers (Broce & Haas, 1999), were placed over three distinct zones of differing hay/manure accumulations and replicated 3X within each feeder site. Traps were collected daily from May to September and counts were converted to flies/trap/day and SF/m^2/day. The converted trap counts were analyzed by an analysis of variance test (PROC GLM; SAS Institute, 1999) and means were separated using the Fisher's LSD test to determine differences between the two feeder types.

Unrolled Hay Bale Waste Trial The amount of hay wasted from unrolling a single bale at different locations was compared to unrolling a bale at the same location throughout the feeding period, using ~ 25 – 30 head at two adjoining KSU pastures. Each new bale was

weighed before unrolling and all hay on the ground after a 24 hr. period was collected by racking, bagging and weighing (Blasi et al. 1993). After six sampling periods of collecting hay wastage, this part of the study was terminated to allow hay to accumulate at randomly selected feeding sites for spring quantification of SF emergence. All wasted hay that was collected and weighed (kg) was converted into percent hay wastage and analyzed by an analysis of variance test (PROC GLM; SAS Institute, 1999). Means were separated using the Fisher's LSD test to determine if there were differences among the feeding techniques.

Feeder Movement Trial (02/07/05 – 02/28/05) . Three pastures at KSU were utilized to periodically move ring feeders, unroll round bales, pile residue from an unmoved ring feeder at the end of the feeding period, and feed hay at a ring feeder continuously throughout the feeding period without being moved (control). Movement of ring feeders occurred after an entire bale was consumed (1 wk) by ~ 28 head of cattle and each site was left undisturbed until the following April when the feeder was removed for SF sampling. Round bales were unrolled at different locations daily throughout the feeding period (02/07/05 – 02/28/05) at two KSU pastures with ~ 25 h. of cattle in each pasture. These sites of unrolled bales were left undisturbed until the following sampling period of stable flies in April. Sites of selected unmoved ring feeders were piled at the end of the feeding period (02/07/05 – 02/28/05) by using a front-end loader attachment on a tractor. As previously described, pyramid emergence traps (Broce & Haas 1999) were placed at all feeding sites for SF sampling. Traps were sampled daily from April to October and SF counts were converted to flies/day/trap. The converted trap counts were analyzed by an analysis of variance test (PROC GLM; SAS Institute, 1999) and means were separated using the Fisher's LSD test to determine differences between all feeding methods.

A major concern among livestock producers is forage quality in their pastures especially if winter-feeding strategies can have detrimental effects on their pastures. To address this concern, a forage quantity trial was initiated (08/29/05 – 09/15/05) on the moved ring feeders and unrolled bale sites (schematic of forage sampling is illustrated in Figure 2.2). A 0.25 m^2 frame was used to randomly sample forage by clipping all forage within that frame to the base near the soil surface. Forage samples were separated into forbes, cool season grasses, and warm season grasses. The frames were placed at four different locations within the hay feeding area and outside the feeding area at the unrolled sites with six different sites being studied to account for pasture variability. Once again, the frames were placed at four different locations within the feeding area but at the moved ring feeder sites additional sampling was conducted at 1m from the feeder area and 10m from the feeding area at four different feeding sites. All forages were placed into brown paper bags and dried in an oven for 48 hrs at 75˚ C to obtain a dry weight measure (g) that was then converted into kg/ha. The converted forage measurement was analyzed by an analysis of variance test (PROC GLM; SAS Institute, 1999) and means were separated using the Fisher's LSD test to determine differences between forage samples inside the hay feeding area versus outside the feeding area at six individual unrolled sites. Data from ring feeder sites were also analyzed by an analysis of variance test (PROC GLM; SAS Institute, 1999) and means were separated using the Fisher's LSD test to determine differences among forage samples inside the feeding area, 1m outside the feeding area, and 10m outside the feeding area. Point sampling was carried out at 15 cm intervals at 50 consecutive points to determine the percent ground cover at both the moved ring feeding sites and unrolled sites. Three parameters arose from this sampling: percent litter, percent bare ground, and percent manure.

Results

Feeder Comparison. There were no differences in SF production between the ring and cone feeders (Table 2.1); however, percent hay wasted was significantly higher from the ring type feeder (23.74 %) than from the cone type feeder (10.54 %) ($F = 18.76$; df = 1, 22; $P = 0.0001$).

Unrolled Hay Bale Waste Trial. Comparison of hay wastage between the The unrolled hay feeding methods wasted more hay than the stationary feeding methods (specially the cone feeder (Fig. 2.6 & 2.7). Unrolling hay bales at the same location produced significantly more wastage than all other feeding methods (35.35 %); unrolling a new bale at different locations and the ring type feeder produced similar amounts of wasted hay (22.74 and 23.71 %, respectively), whereas the cone feeder produced the least wastage (10.54 %)($F = 18.76$; df = 3, 32; $P = 0.0001$)(Fig. 2.6 & 2.7).

Feeder Movement Trial. When comparing the frequency of moving the hay feeding site, such as moving a ring feeder, and unrolling a single bale at different locations versus leaving the feeding site stationary as in the case of the unmoved ring feeder sites and piling the hay residues of unmoved ring feeding sites, there were differences but the overall SF population was lower due to hot and dry conditions. There was no SF emergence from either of the moved feeding sites (moved feeder and unrolling a single bale) (Fig. 2.3) but there was SF emergence from the stationary feeding sites (unmoved ring feeder and piled residue from an unmoved ring feeder)(Fig. 2.3). The stationary feeding sites were significantly higher in SF production than those of frequently moving the feeding site ($F = 3.53$; df = 3, 424; $P = 0.0149$) (Fig. 2.3).

Forage production at Sites 1 - 5 was not significantly different when comparing forbes produced inside the feeding area versus outside the feeding area (Table 2.2). However, Site 6

yielded a significant difference in forbes production with significantly more forbes being produced inside the feeding area (2023 kg/ha; SE = 291) than outside the feeding area (801 kg/ha; SE = 203) (F = 11.85; df = 1, 6; P = 0.0138)(Table 2.2). There were no differences in cool season forage production at Sites 1, 2, 4, and 5 (Table 2.2) but there were differences observed at Site 3 with higher cool season forage being produced inside (1405 kg/ha; SE = 365) than outside the feeding area (396 kg/ha; SE = 163)(F = 6.37; df = 1, 6; P = 0.0450)(Table 2.2). Site 6 also produced more cool season forages inside (1747 kg/ha; SE = 93) than outside the feeding area (298 kg/ha; SE = 48)(F = 191.95; df = 1, 6; P = 0.0001)(Table 2.2). Warm season forage production was not significantly different between areas inside versus outside the feeding area at Sites 2, 4, 5, and 6 (Table 2.2). Warm season forage production was significantly higher inside (3078 kg/ha; SE = 254) than outside the feeding area (2125 kg/ha; SE = 161) at Site 1 (F = 10.03; df = 1, 6; P = 0.0194)(Table 2.2). However, Site 3 exhibited the only detrimental effect to forage production from unrolling the round bale with significantly lower warm season forage production inside (320 kg/ha; SE = 202) than outside the feeding area (1543 kg/ha; SE = 90)(F = 30.56; df = 1, 6; P = 0.0015)(Table 2.2). Ground cover at the unrolled bale sites was compiled from all sites with the majority of the samples being represented as plant litter (Fig. 2.4).

Forage analysis was conducted in a similar manner at the moved feeder sites except distance from the feeding site was compared at two distances (1and 10m from feeding area). Forage analysis for the moved feeder sites were pooled together due to grazing intensity and lack of fencing opportunities. Forbes production was significantly lower inside the feeding area than 1 and 10 m outside the feeding area (Table 2.2). The areas outside the feeding area were significantly different than the area within the feeding area with forbes production increasing significantly with increasing distance from the feeding area (F = 22.10; df = 2, 9; P = 0.0003).

Cool season forage production was significantly higher 10 m from the feeding area than 1 m from the feeding area and inside the feeding area (Table 2.2). However, cool season forage production was not significantly different between the areas inside the feeding area and areas 1 m outside the feeding area ($F = 16.08$; df = 2, 9; $P = 0.0011$)(Table 2.2). Warm season forage production was significantly higher at the 10 m distance than at 1 m from the feeding area and inside the feeding area but no differences were observed between areas inside the feeding area and areas 1 m from the feeding area ($F = 9.83$; df = 2, 9; $P = 0.0054$)(Table 2.2). Ground cover at the moved ring feeder sites were similar to that of unrolled bales; with the majority of the samples being represented as plant litter (Fig. 2.5). The only difference was that more samples were represented as bare ground and manure inside the ring feeding area (Fig. 2.5).

Discussion

The cone type feeder did reduce the amount of wasted hay, a fact in agreement with Buskirk et al. (2003) findings that the cone feeder wasted less hay. However, no differences were observed in SF production (Table 2.1). While the cost of the cone feeder is significantly higher (~$600-800) than that of the ring feeder (~$150-200), its use could be justified by the long-term savings incurred by utilized hay. However, it may not be justifiable for SF management. Broce et al. (2005) demonstrated that hay feeding sites are viable developmental habitats for stable flies but results from comparing the ring and cone feeders suggest that the amount of wasted hay is not as important in the production of SF as is the concentration of cattle feces at these sites.

Since no differences were observed in SF production between the ring and cone feeders, it was determined that utilization of the less costly ring type feeder would be rational in testing the hypothesis that moving the feeding site could diminish suitable oviposition sites of stable flies. It could be hypothesized that, whether from moved ring feeders or unrolling bales at different locations, this study suggests that when cattle are concentrated at a particular site, more urine and feces are deposited at unmoved feeding sites, thus increasing the likelihood of successful SF development. Hogsette et al. (1987) indicated that the mixing of hay, manure, urine, and precipitation events provide ideal conditions for SF larvae. This study suggests that when manure and urine are limited or spread over larger areas, the feeding sites become less suitable for SF development. This study also agrees with Foil & Hogsette (1994) recommendation of periodically moving large round hay bales short distances once or twice a week in order for residue accumulations to be limited to small amounts that tend to dry completely.

Since frequently moving the feeding site is beneficial in reducing SF developmental sites, a major concern of producers is the impact of feeding sites on pasture forage quality. Results of this study suggest that if cattle while feeding on the hay are spread out over a larger area, as in the case of unrolling bales out, and not concentrated, forage quality is unaffected and in some cases improved (Table 2.2). However, although ring feeder sites that are moved do not provide viable habitats for SF development, they do detrimentally affect forage quality (Table 2.2) as could be expected whenever cattle are more concentrated in a particular area. While this study identified benefits of unrolling hay bales in terms of SF management and limited effects to forage quality, a disadvantage to this feeding method is its higher labor costs due to the feeding of bales on a daily basis. While most producers in the Midwest (U.S.A.) have adapted truck beds for hay feeding that limit labor and time unrolling the hay bales, this could be an additional cost if a producer has to invest in the initial cost of these truck beds.

Successful SF management has been demonstrated through this study but additional results of wasted hay suggest that unrolling bales at the same location can increase the amount of wasted hay (Fig. 2.7). Also, unrolling a bale at a different location wasted similar amounts of hay as the ring feeder (Fig. 2.6 & 2.7) and were similar to Blasi et al. (1993) findings that unrolling bales wasted 23%. This suggests that unrolling hay bales could be disadvantageous when considering labor inputs with no hay wastage benefit. Ball et al. (1998) suggested that feeding losses of 3-6% were acceptable when feeding round bales, but Broce et al. (2005) suggested that these low feeding losses were obtained through increased labor inputs which is also implied in this study of unrolling hay bales as labor intensive. Further studies are needed to determine the cost-benefit analysis of unrolling hay bales as a viable management technique to reduce SF populations in pastures.

Greene (1993) suggested that the cultural control practice of sanitation is the most important technique for on-site reduction of stable flies. While this is beneficial in reducing SF populations, most producers in the Midwest would be reluctant to adopt this technique since this would have to be carried out during spring. This activity coincides with calving season, which is usually before stable flies become a problem, but are becoming established at hay feeding sites in late April to early May (Broce et al. 2005). This study suggests that altering feeding techniques rather than sanitation efforts can be a viable alternative in reducing SF developmental sites within pastures.

It is evident that further studies are needed to assess the cost-benefit analysis of utilizing these feeding methods in a SF management plan. Also, since stable flies can travel intermediate distances (Hogsette & Ruff 1985), area wide implementation of altering feeding methods needs to be assessed to obtain the true impact these findings have on stable flies. Moreover, characterization of the larval habitat at hay feeding sites needs to be studied to identify factors that influence SF development at these feeding sites. Identification of potential chemical alternatives needs to be assessed at these feeding sites for an effective SF management plan.

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Table 2.1 Comparison between ring and cone type hay feeders in relation to stable fly production and wasted hay residues

	Ring ± SE	Cone ± SE
Total Daily SF emergence	317.40 ± 99.70	313.10 ± 68.96
SF/m^2/day	14.89 ±4.12	15.02 ±3.11
Surface Area of Hay Residues (m^2)	11.55 ±1.55	10.58 ±5.54
SF/season/feeding site[£]	6,672.52 ± 894.09	6,527.98 ± 1,125.77
% Hay Wastage*	23.71 ±2.17 [a]	10.54 ±1.49 [b]

*Read within row. Means followed by the same letter are not significantly different (P = 0.05, LSMeans test [SAS Institute 1999]).
[£] SF/m^2/day X # of days X surface area

Table 2.2 Comparative forage production at six unrolled hay feeding sites and moved ring feeders

	Forbes kg/ha (SE)			**Cool Season kg/ha (SE)**			**Warm Season kg/ha (SE)**		
	Inside Feeding Area	Outside Feeding Area	*P-value*	Inside Feeding Area	Outside Feeding Area	*P-value*	Inside Feeding Area	Outside Feeding Area	*P-value*
Site 1	478 (266)	421 (149)	0.8580	396 (109)	287 (78)	0.4478	3078 (254)	2125 (161)	0.0194*
Site 2	1046 (303)	770 (247)	0.5061	2401 (257)	2116 (208)	0.4217	396 (226)	967 (290)	0.1710
Site 3	776 (276)	415 (320)	0.4250	1405 (365)	396 (163)	0.0450*	320 (202)	1543 (90)	0.0015*
Site 4	1580 (316)	1170 (197)	0.3133	2508 (133)	2436 (221)	0.7898	774 (316)	1316 (229)	0.2141
Site 5	1409 (240)	989 (242)	0.2638	1466 (454)	1755 (172)	0.5734	1982 (554)	967 (223)	0.1400
Site 6	2023 (291)	801 (203)	0.0138*	1747 (93)	298 (48)	0.0001*	2856 (91)	2335 (193)	0.0505
	Inside Feeding Area	1 m Outside Feeding Area	10 m Outside Feeding Area	Inside Feeding Area	1 m Outside Feeding Area	10 m Outside Feeding Area	Inside Feeding Area	1 m Outside Feeding Area	10 m Outside Feeding Area
Moved Ring Feeders£	229 c (12)	432 b (17)	595 a (64)	79 b (16)	166 b (30)	443 a (75)	159 b (11)	231 b (29)	536 a (106)

* Statistically significant (P<0.05 PROC GLM SAS Institute, 1999)

£ Read within row and within forage category. Means followed by the same letter are not significantly different (P = 0.05, LSMeans test [SAS Institute 1999]).

Fig. 2.1 The different feeding methods of round hay bales. (a) Ring type feeder; (b) Cone type feeder; (c) site of unrolled round bale.

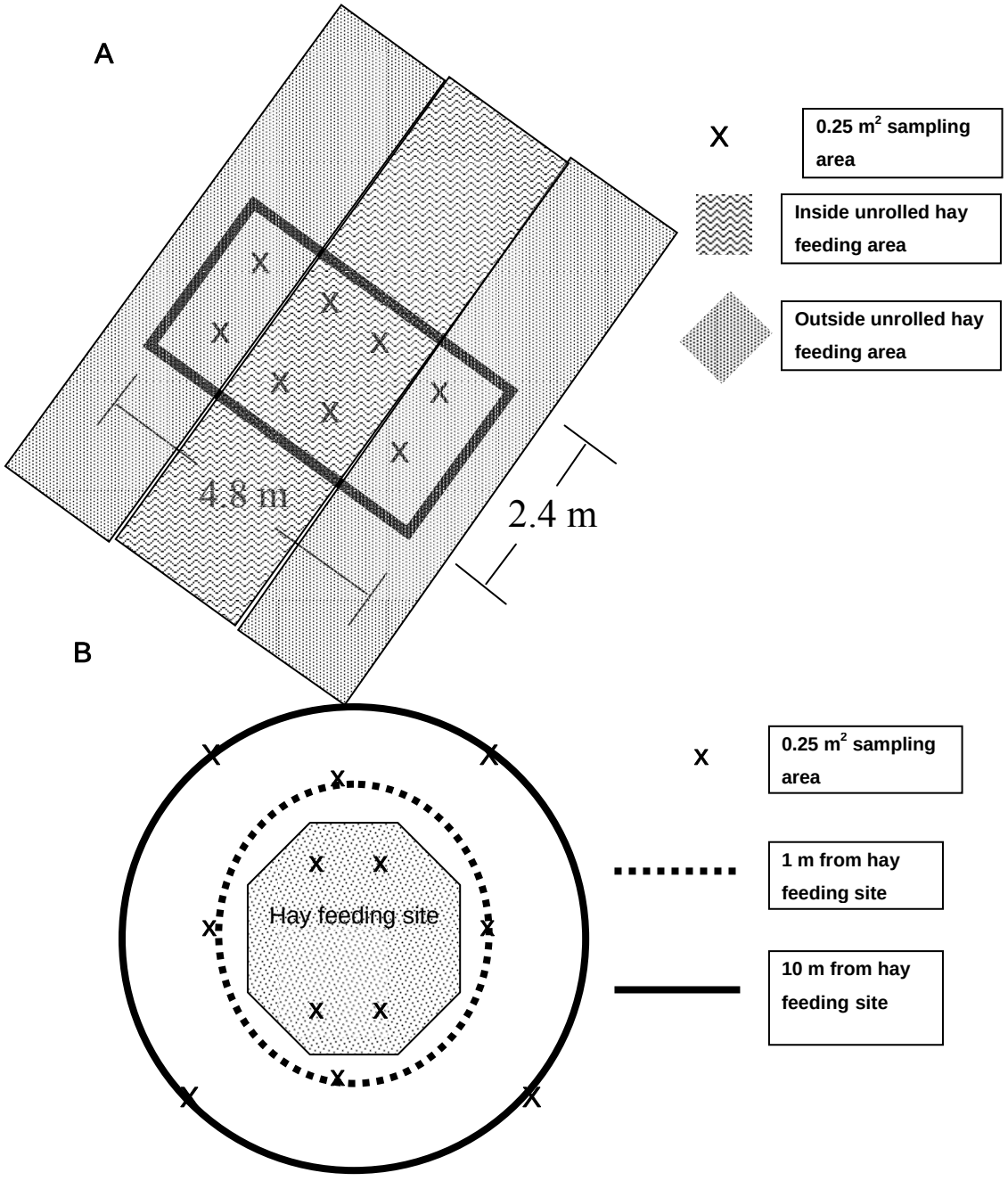
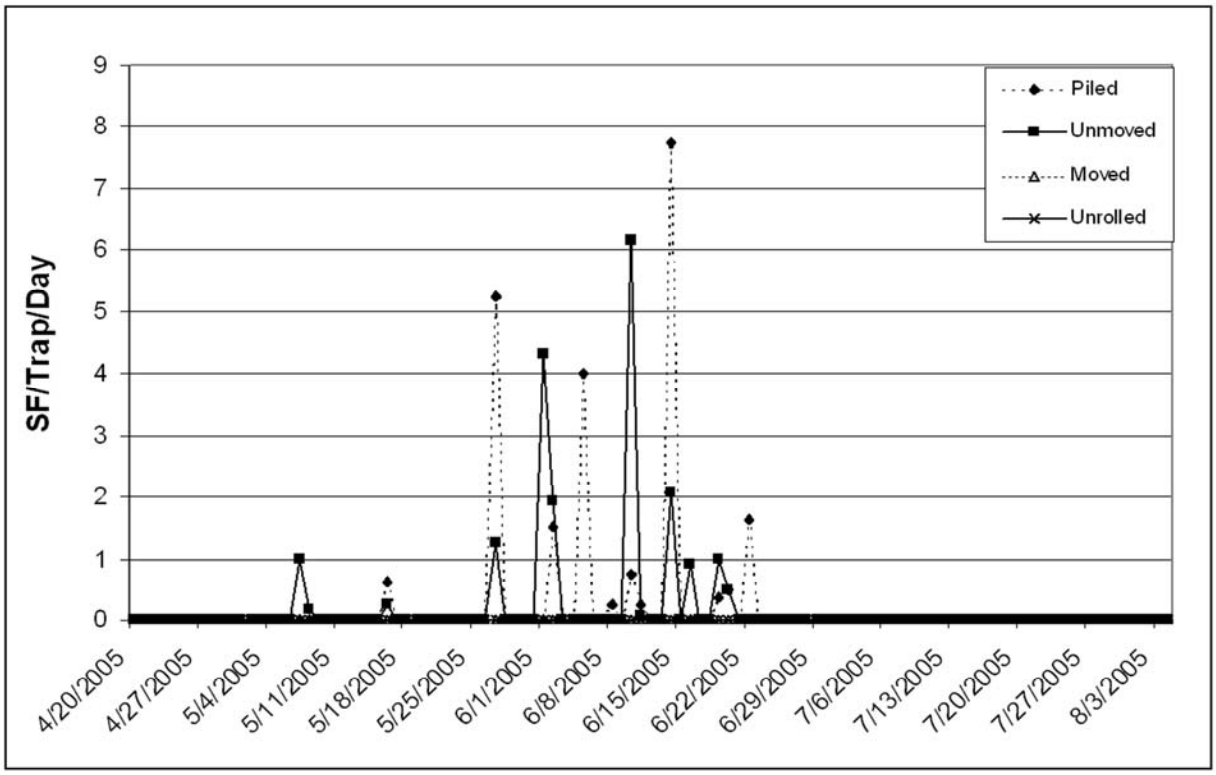


Fig. 2.2 Schematic of forage sampling methods (a) for unrolled feeding sites and (b) moved ring feeder sites



Total Stable Fly Production	
Piled	**183**
Unmoved	**236**
Moved	**0**
Unrolled	**0**

Fig. 2.3 Average number of stable flies caught daily in emergence traps at different hay feeding sites representing different feeding techniques (piled, unmoved, moved, and unrolled). Total stable fly production from each feeding technique is represented in the bottom half of the figure.

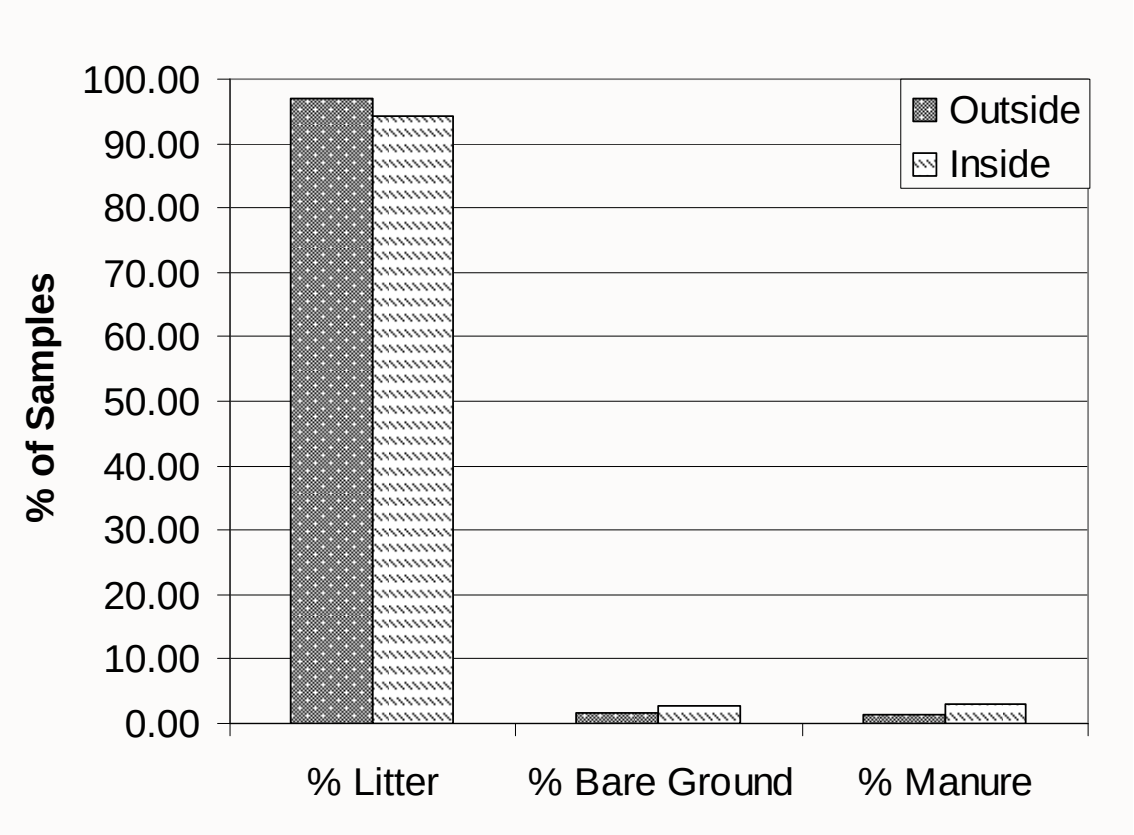


Fig. 2.4 Point sampling at 15 cm intervals at 50 consecutive points both outside and inside the feeding area of unrolled hay bale feeding sites to determine the ground cover.

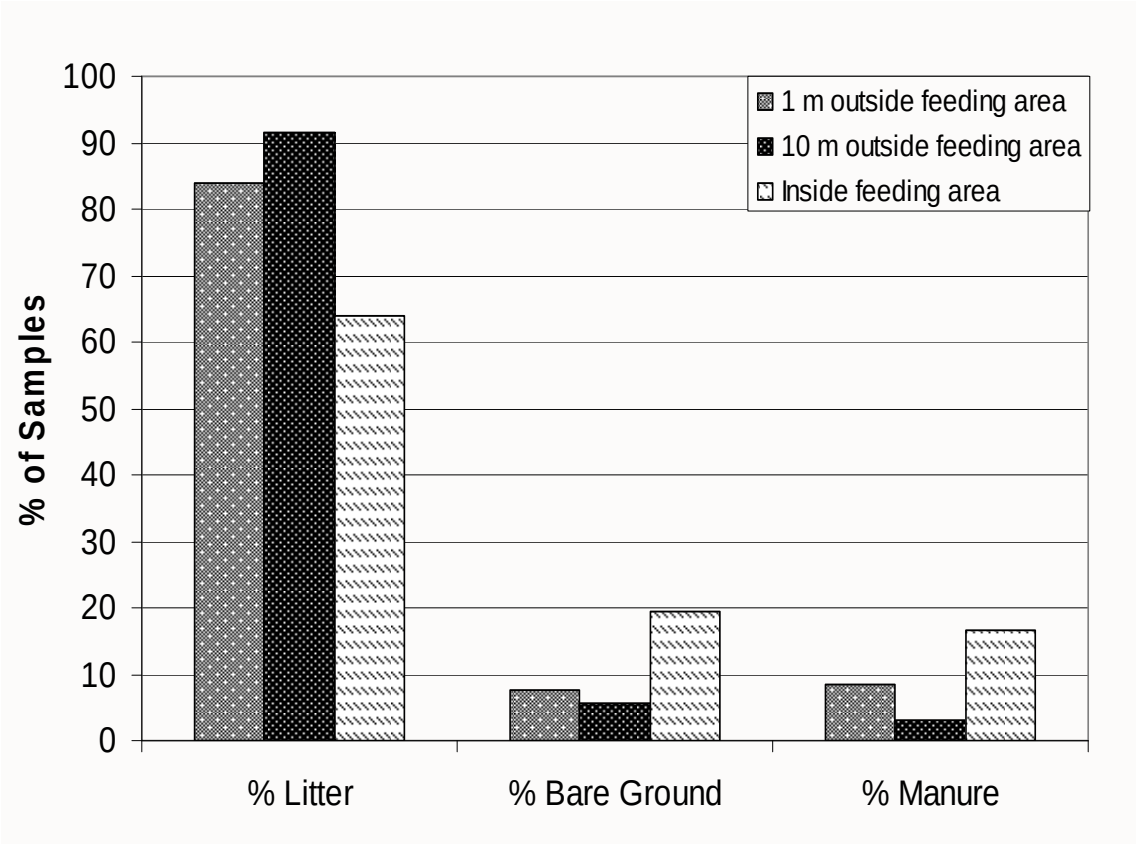


Fig. 2.5 Point sampling at 15 cm intervals at 50 consecutive points at 10 m from the feeding area, 1 m from the feeding area, and inside the feeding area of moved ring feeder sites to determine ground cover.

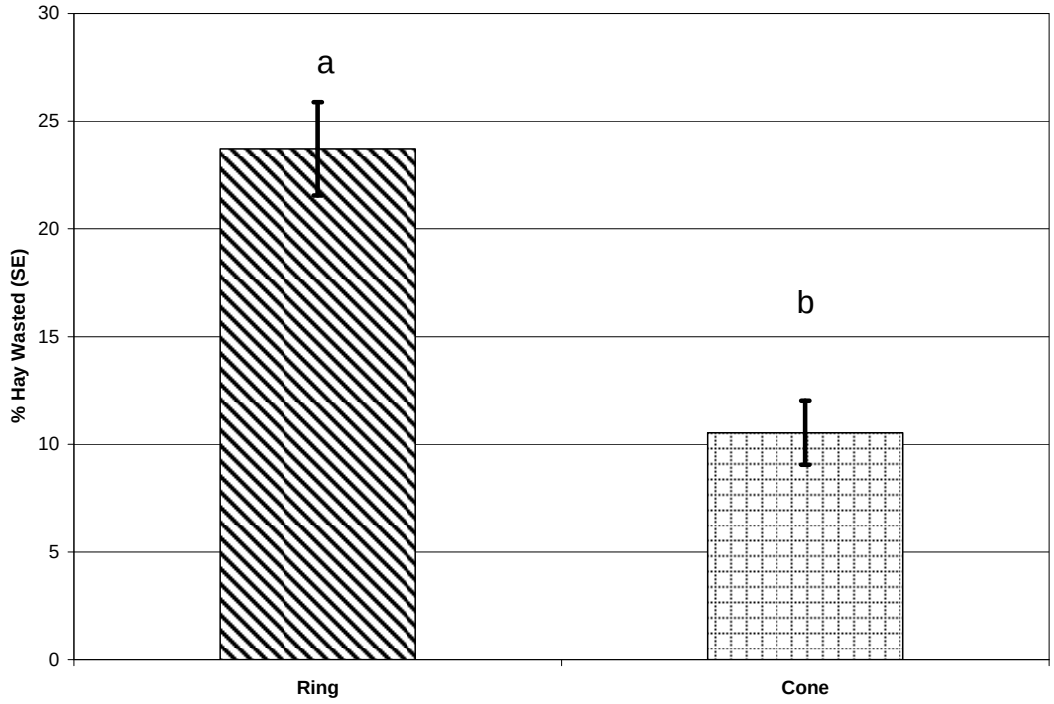


Fig. 2.6 Percent hay waste from the ring and cone feeders. Bars followed by the same letter are not significantly different.

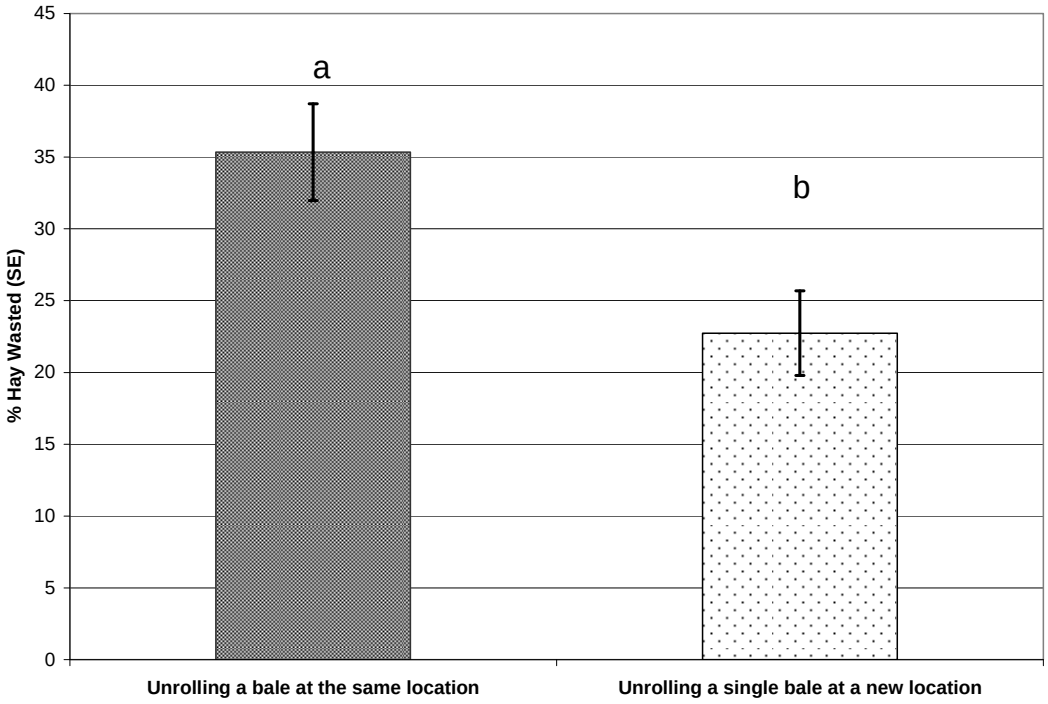


Fig. 2.7 Percent hay waste from unrolled feeding methods. Bars followed by the same letter are not significantly different.

CHAPTER 3 - Characterization of stable fly (Diptera: Muscidae) larval developmental sites in round hay bale feeding areas

ABSTRACT

Stable flies, *Stomoxys calcitrans* (L.), have traditionally been pests of livestock in confined operations but, in the last two decades they have become serious pests of pastured and range cattle in the Midwestern United States. The increase in these flies' population size is due in part to the adoption of intensive hay feeding practices on rangelands: producers feed cattle hay in the form of round bales that result in significant wasted hay being mixed with high concentrations of manure at these feeding sites. Subsequently, in the spring and early summer, this hay/manure mixture becomes ideal larval habitat for stable flies. This study examined larval habitats within these hay feeding sites and determined that three zones with distinct hay/manure composition exist. While these sites provide ideal larval habitats for stable flies, a reduction of larval production latter on in the fly season was recorded. Thus, various environmental parameters (temperature, % moisture, pH, depth of hay/manure layer, concentration of fecal coliforms) were monitored during periods of high (5/17 – 6/3/2004) and low (7/26 – 8/12/2004) stable fly production. Stable fly productions during these high and low periods were compared, as was also fly production from the different hay/manure zones. Temperature and fecal coliform counts were the two most distinguishable characteristics that differed between the high and low stable fly production periods with temperatures ranging from 21 - 25° C during the low stable fly production versus 25 - 30° C in the high stable fly production period. Fecal coliform counts ranged from 4.15×10^3 to 4.07×10^4CFU/g during high stable fly production from <10 to 0.09×10^2 CFU/g during low stable fly production. In addition, stable fly larval development and survival were evaluated as a function of the hay:manure ratio (0:1, 1:1, 2:1, and 5:1) of larval

media. Temperature was significantly higher in all media containing hay, but no differences were observed in stable fly emergence from media with different hay:manure ratios; although developmental time from egg to adult was less in all ratios containing hay as compared to the manure only medium (0:1). These results support the hypothesis that both, temperature and microbial communities are important factors in stable fly larval habitats and further demonstrate that certain media with specific hay/manure composition support a higher level of larval development.

Key Words: stable flies, environmental factors, hay feeding sites, larval habitats

Increasingly, stable flies, *Stomoxys calcitrans* (L.), have become a serious pest of cattle in pastures and rangeland. Hall et al. (1982) reported stable flies in large numbers attacking pastured cattle in Missouri, numbers that were much larger than observed previously. Recently, Broce et al. (2005) demonstrated hay feeding sites as major larval development sites for stable flies. Skoda and Thomas (1993), Meyer and Petersen (1983), and Skoda et al. (1991) among others, have listed a variety of media in which stable flies develop with all having the common factor of being decomposing plant material. Decaying organic matter mixed with manure, and soil provides ideal conditions for stable fly development. Although considerable information is available on stable fly larval habitats in confined animal feeding operations (CAFOs), little is known about the larval development sites within hay feeding areas in pastures.

Rasmussen and Campbell (1981) studied the effects of moisture, temperature, organic matter, pH, and interspecific competition on stable fly larval development in Nebraska feedlots. Lysyk (1998) determined that temperature plays an important role in determining the rate of population change and Berry et al. (1976) modeled immature stable fly development using relationships between minimum developmental time and temperature. Broce and Haas (1999) reported that stable fly oviposition is restricted to cattle manure that has undergone an advanced decomposition process. Hay:manure media at hay feeding sites may undergo a similar decomposition process since hay feeding usually occurs in the winter months but stable fly development at these hay feeding sites is not observed until spring (Broce et al. 2005). Hay residues on a dairy in Northwest Florida contained 28,000 stable fly larvae/m^2 (Patterson and Morgan 1986). Hay residues can remain active larval sites for extended periods of time, especially if new round hay bales are frequently offered at the same site.

While temperature is an important factor that influences stable fly development, it has been reported recently that bacterial associations are vital in stable fly development and selection of oviposition sites. Laboratory studies have confirmed the nutritional dependency of muscoid fly larvae, including stable fly, on live bacteria (Lysyk et al. 1999). Romero et al. (2006) recently reported that stable flies oviposited more eggs in a natural substrate (a mixture of hay, soil and manure) with a live microbial community than on the same but sterilized natural substrate. While these studies suggest the importance of microbial associations with stable fly oviposition and/or development, limited work has been done to determine if these microbial communities influence stable fly development in the field.

Several studies involving grazed pastures have noted increases in coliform concentrations in surrounding bodies of water soon after the introduction of cattle to pastures (Stephenson and Street, 1978; Doran and Linn, 1979). In fact, Lenehan et al. (2005) reported higher fecal coliform counts in soil samples as the distance from round hay bale feeding sites decreased. Since the feeding of hay concentrates cattle in small areas, an increase in manure accumulation should also be expected at these hay feeding sites.

The purpose of this study was to characterize stable fly larval habitats within round hay bale feeding sites to: 1) determine environmental factors (temperature, % moisture, pH, depth of hay/manure layer, concentration of fecal coliforms) associated with different hay/manure media compositions during periods of high and low stable fly productivity; 2) evaluate stable fly larval development and survival as a function of the hay:manure ratio of the larval media; and 3) evaluate the significance of hay as a physical component of larval media by using a plastic hay surrogate.

Materials and Methods

Field Trial. Comparison of two different feeder types (ring and cone feeders) was conducted at the Kansas State University (KSU) cow/calf unit as described in previous studies (Chapter 2). The feeding sites were monitored from May through September, 2004. During this initial field trial, the presence of three well-delineated concentric zones of distinct hay/manure composition at each feeding site became apparent. The three concentric zones were characterized as follows: *Inner*- area covered by a medium of greater amounts of hay than manure composition. *Intermediate* – area where the composition of the medium was an even ratio of hay and manure. *Outer* – area covered by a medium of an even composition of hay, manure, and soil (Fig. 3.1). Pyramid emergence traps, with 0.25 m^2 base fitted with fly collection containers (Broce & Haas 1999), were randomly placed at three different locations within these zones (Fig. 3.1). Environmental parameters monitored during the field trial included temperature, moisture, pH, depth of the hay layer, and fecal coliform concentrations. Hourly temperature was monitored with a data logger with external probes (HOBO® H8 Outdoor/Industrial 4-Channel External Logger, Onset Computer, Bourne, MA) inserted into the three different zones at each feeding site.

Sampling events were conducted at two different time periods with the first occurring during high stable fly activity with sampling of stable fly emergence and temperature taking place from 17 May 2004 to 3 June 2004 and sampling for moisture, pH, depth of hay/manure substrate, and fecal coliforms occurring on 3 June 2004. The other sampling period was during low stable fly activity with sampling of stable fly emergence and temperature taking place from 26 July 2004 to 12 August 2004 and sampling for moisture, pH, depth of hay/manure substrate, and fecal coliforms occurring on 12 August 2004. Sampling was carried out in this manner due

to previous results (Chapter 2) that demonstrated that although these feeding sites support high stable fly populations in early summer months the populations decline over time even though the substrates were left undisturbed. Core samples of the media over the 3 zones were taken using a spade shovel (30 cm deep by 20 cm diameter). Composite samples were taken from each medium (~ 100 g) and spooned into brown paper sacks. Samples were weighed, oven-dried for 48 h at 75° C, reweighed and the percent moisture was calculated. pH was monitored with a portable data logger (Acorn® pH 6 series, Omni Controls Inc., Tampa, FL) at the excavation site of the core sample. Samples for quantification of fecal coliform bacteria were obtained from the core sample by taking a composite sample from the media. For this, 10 g of the medium were aseptically spooned into 500 ml sterilized glass flasks and suspended in 40 ml of phosphate-buffered saline (PBS) (pH 7.2; MP Biomedicals, Eschwege, Germany). The concentration of fecal coliforms were determined by a drop plate technique using mFC (membrane fecal coliform) agar (Oxoid Limited) incubated at 44.5° C for 18 to 24 h. Fecal coliform colonies (blue) were counted from dilutions and expressed as Colony Forming Units/g (CFU/g) and counts were log-transformed for analysis. All environmental factor data were compared among the three zones within each feeder type and between the two sampling periods by ANOVA and means separated by Fisher's Least Significant Difference (LSD) (SAS Institute, 1999).

Laboratory Bioassays with Varying Ratios of Hay and Manure. Bioassays were conducted with four different artificially-created ratios of hay:manure to determine if media composition influences stable fly development. The four different ratios evaluated were based on varying amounts of hay mixed with a set amount of manure (volumetrically). The four hay:manure ratios used were: 0:1, 1:1, 2:1, and 5:1. Manure was obtained from individual manure pats from the KSU Pure Bred Beef Teaching Research Center Unit from cows which had

not received any pesticide treatment recently. Manure was frozen (−20° C) to kill any existing arthropods. Hay was obtained from the KSU cow/calf unit and consisted of native smooth brome grass that was chopped and sieved through a No. 4 sieve (1.5 holes/ linear cm; W.S. Tyler Company, Mentor, OH) for uniformity. Approximately 1- 1.5L of distilled water was added to all media samples for appropriate moisture for stable fly development. All substrates were inoculated with 200 stable fly eggs then kept at 25 – 26° C, 70% RH, and a 14:10 L:D photoperiod. Temperature and pH were recorded daily with a portable data logger (Acorn® pH 6 series, Omni Controls Inc., Tampa, FL) until pupation. Each medium representing a different hay:manure ratio was replicated by independently mixing each medium 4X and after pupation was documented in all media types the substrates were then transferred to plastic containers (20 x 20 x 25 cm) each with two screened emergence cups. Development times were recorded in all media samples by recording the number of days to pupation and adult emergence. Cumulative temperature, pH, and stable fly emergence values from each media were compared by ANOVA and means separated by Fisher's Least Significant Difference (LSD) (SAS Institute, 1999).

Laboratory Bioassays with Plastic Hay Surrogate. Bioassays were conducted to determine if the value of hay in enhancing hay/manure mixtures as larval medium is due to physical attributes of the hay. Hay varieties are variable in characteristics but the most common varieties utilized for hay production either have hollow straws or enclosed straws (prairie brome grass). For this then, plastic coffee stir straws (0.45 cm outer diam. X 12.5 cm length) (Clear Shield National Inc., Wheeling, IL) were cut into 2 - 5 cm lengths and treated with a 10% bleach solution, then autoclaved for sterilization. Since some hay varieties have stems that have enclosed stems another plastic surrogate was utilized in the form of plastic trimmer line (0.24 cm diam.) (Arnold Corporation, Shelby, OH) that was cut at similar lengths and sterilized in the

same manner as the plastic straw. Treatments consisted of: 1) Unsterilized chopped smooth brome hay, as previously described; 2) sterilized chopped smooth brome hay; 3) sterilized plastic straw; 4) sterilized plastic sticks; and 5) unsterilized manure with no hay. All treatments were mixed with manure at a 2:1 ratio hay:manure or plastic surrogate:manure. All substrates were seeded with 200 SF eggs then kept at 25 – 26° C, 70% RH, and 14:10 L:D photoperiod. Daily temperature and pH were monitored with a digital data logger on all days of observations (0-12 d). Stable fly emergence was recorded 28 days after inoculation of eggs into substrates and was subjected to ANOVA (SAS Institute, 1999) to determine if there were differences in fly emergence among substrate types. Cumulative temperature and pH values were compared among the different media by ANOVA (SAS Institute, 1999) and means from cumulative values were separated by Fisher's LSD with an α of 0.05.

Results

2004 Field Trial. Stable fly emergence was not significantly different among the three different zones during either high (5/17 - 6/3/2004) or low (7/26 - 8/12/2004) stable fly production periods but it was different between the two collection periods with the later collection being significantly lower than the first in all zones in the ring type feeder sites ($F = 8.27$; df = 5, 84; $P < 0.0001$; Table 3.1). Cone feeder sites had similar stable fly emergence as the ring feeder sites with emergence being significantly higher in the first collection period. However, the *intermediate zone* of the cone feeders showed a slight difference by exhibiting a statistically higher emergence in the first sampling period than the *inner and outer zones* ($F = 12.85$; df = 5, 84; $P < 0.0001$; Table 3.2).

Daily average temperatures of substrates were significantly higher in the latter collection period than the first collection period in all zones but each individual zone was not different from the other within the collection periods at the ring feeder sites ($F = 52.34$; df = 5, 204; $P < 0.0001$; Table 3.1). The cone feeder sites demonstrated the same trend as in the ring feeder sites with all zones exhibiting significantly higher daily average substrate temperatures in the latter sampling period than those observed in the first. No differences were observed in daily average substrate temperatures among the different zones within each sampling period ($F = 58.91$; df = 5, 204; $P < 0.0001$; Table 3.2). Daily minimum substrate temperatures were significantly lower in the first sampling period in all zones than those observed in the latter sampling period, each zone demonstrating similar minimum daily temperatures within each sampling period at the ring feeder sites ($F = 32.51$; df = 5, 204; $P < 0.0001$; Table 3.1). Daily minimum substrate temperatures at the cone feeder sites were significantly higher in the second sampling period than in the first. The *outer zone* exhibited a significantly higher minimum temperature than that

observed in the *inner zone*, but the *intermediate zone* exhibited a similar minimum substrate temperature as both the *inner and outer zones* during both sampling periods ($F = 52.74$; df = 5, 204; $P < 0.0001$; Table 3.2). Daily maximum substrate temperatures at the ring feeder sites demonstrated statistical differences between the three zones but were all within the development parameters (Lysyk 1998) for stable flies. ($F = 46.03$; df = 5, 204; $P < 0.0001$; Table 3.1). Similar results were exhibited at the cone feeder sites with certain zones being statistically different from others but still within the development parameters demonstrated by Lysyk (1998) for stable flies ($F = 52.96$; df = 5, 204; $P < 0.0001$; Table 3.2).

The *inner and intermediate zones* at the ring feeder sites were similar in moisture content with both exhibiting a significantly higher moisture level than that observed in the *outer zone* during both sampling periods. However, the *outer zone* in the first sampling period demonstrated to have significantly higher moisture level than that observed in that same exact zone during the second sampling period ($F = 16.53$; df = 5, 18; $P < 0.0001$; Table 3.1). At the cone feeder sites moisture content was similar as that observed in the ring feeder sites with the *outer and intermediate zones* exhibiting significantly higher moisture level during both sampling periods than that observed in the *outer zone* but the moisture content in the *outer zone* was no different between sampling periods ($F = 25.77$; df = 5, 18; $P < 0.0001$; Table 3.2).

Thickness of the hay/manure substrate was significantly different mainly between the *outer zone* and those observed in the other zones at the ring feeder sites ($F = 9.22$; df = 5, 18; $P = 0.0002$; Table 3.1). Similar results were observed at the cone feeder sites with the thickness being significantly different between the *outer zone* than that observed in the other two zones ($F = 15.34$; df = 5, 18; $P < 0.0001$; Table 3.2).

pH values at the ring feeder sites revealed significant differences among all zones but these differences were just marginal since the pH range was only from 6.55 to 7.03 ($F = 3.67$; df = 5, 18; $P = 0.0183$; Table 3.1). No differences were observed in pH from all *Zones* and between the first sampling period to the second sampling period at the cone feeder sites ($F = 1.69$; df = 5, 18; $P = 0.1885$; Table 3.2).

Concentration of fecal coliforms were similar in both the ring (Table 3.1) and the cone feeder sites (Table 3.2) with significantly higher fecal coliforms in all zones in the first sampling period than those observed in the second sampling period (Ring: $F = 36.96$; df = 5, 18; $P < 0.0001$; Table 3.1; Cone: $F = 32.86$; df = 5, 18; $P < 0.0001$; Table 3.2).

Laboratory Bioassays with Varying Ratios of Hay and Manure. Stable fly emergence from the four media with different hay:manure ratios were not significantly different from each other ($F = 3.14$; df = 3, 12; $P = 0.0654$; Fig. 3.2). Although emergence from the 2:1 and 1:1 media were the highest and approaching 100%, whereas the emergence from the manure only medium was 73%, all these values were not significantly different from each other (Fig. 3.2).

Daily temperatures followed a trend in the different ratio's of hay:manure media demonstrating an increase on Day 1 after egg inoculation, then progressively decreasing over time until termination of the larval period (Fig. 3.3). Also, daily substrate temperatures were higher in all media types with an increased amount of hay in the substrate; for instance, the 5:1 ratio medium was consistently higher than any other media with hay or the manure only substrate (0:1 ratio medium) (Fig. 3.3). Likewise, the 2:1 ratio medium demonstrated a higher substrate temperature than the 1:1 ratio medium and the 1:1 ratio medium temperature was higher than that observed in the 0:1 ratio medium throughout the larval development period (Fig.

3.3). This is also demonstrated when comparing the overall cumulative substrate temperatures among the different hay:manure ratio media. The substrate temperature in the 5:1 hay:manure ratio medium (27.4° C) was significantly higher than that observed in the 1:1 or 0:1 hay:manure ratio media (26.4 and 24.7° C, respectively). However, the 2:1 hay:manure ratio medium demonstrated similar substrate temperatures (26.9° C) to those observed in both the 5:1 and 1:1 hay:manure ratio media and the 0:1 hay:manure ratio medium exhibited the lowest substrate temperature than all other hay:manure ratio media ($F = 27.04$; df = 3, 188; $P < 0.0001$).

Daily substrate pH in all media containing hay showed a steady increase through Day 3. pH was lower in the manure-only medium (0:1 ratio) from Day 1 through Day 11 than that observed in all other hay:manure ratio media (Fig. 3.4). Interestingly, the daily substrate pH was higher with each additional increase in the amount of hay in the media throughout the larval development period (Fig. 3.4). To further demonstrate this point, when comparing the overall cumulative substrate pH between the different hay:manure ratio media the 5:1 medium exhibited a significantly higher substrate pH (8.24) than the other media with the 2:1 medium exhibiting a significantly higher substrate pH (7.93) than the 1:1 or 0:1 media (7.63 and 6.84, respectively). The 1:1 hay:manure ratio medium demonstrated a significantly higher substrate pH than that observed in the 0:1 hay:manure ratio medium that was significantly lower than that observed in all other media ($F = 100.18$; df = 3, 188; $P < 0.0001$).

Laboratory Bioassays with Plastic Hay Surrogate. Media containing unsterilized and sterilized hay exhibited the highest emergence rates (92.7 and 91.8%, respectively) and were significantly higher than those observed in the manure only (86.1%) and both media with plastic hay ($F = 1150.22$; df = 4, 15; $P < 0.0001$; Fig. 3.5). Stable fly emergence in the manure only substrate was slightly lower but significantly different than those observed in the unsterilized or

sterilized hay media (F =1150.22; df = 4, 15; P <0.0001; Fig. 3.5). The medium with the plastic straw showed a markedly lower emergence than those observed in media with unsterilized or sterilized hay and manure only substrates. No emergence was recorded in the medium with the plastic sticks.

Daily substrate temperature values were significantly different among media types during larval development (Days 0 -12) but after the initial two days of observations all substrate types demonstrated similar trends through day 12 (Fig. 3.6) when stable fly larvae reached the pupal stage in all substrate types, except in the plastic stick medium where no larval activity was observed after Day 3. Substrate temperature in both media containing hay as well as the manure only medium demonstrated a similar trend with all exhibiting higher temperature values than those observed in either plastic hay media from Day 1 to Day 12 of the larval development period (Fig. 3.6). Further illustrating this trend is when cumulative substrate temperatures were compared among the different media types' both media containing hay exhibited significantly higher substrate temperatures (25.5 and 25.3° C in unsterilized hay and sterilized hay media, respectively) than those observed in all other media. The manure only medium exhibited significantly higher substrate temperature (24.7° C) than those observed in either plastic hay media (23.7 and 23.5 in plastic straw and plastic stick media, respectively) with both plastic hay media exhibiting significantly lower substrate temperatures than any other medium (F =35.67; df = 4, 515; P <0.0001).

Values of substrate pH were variable throughout the larval development period in all media with the manure only medium exhibiting a lower substrate pH than all other media types from Day 6 to Day 11 (Fig. 3.7). The sterilized hay medium exhibited a significantly higher substrate pH value (7.65) than all other media when comparing the cumulative substrate pH

among the different media throughout the larval development period. Media containing unsterilized hay and both plastic hay surrogates were similar with all exhibiting parallel substrate pH values (7.48, 7.47, 7.44 in unsterilized hay, plastic straw, and plastic stick media, respectively) that were significantly higher than those values observed in the manure only medium which was significantly lower (6.85) than any other media (F =24.87; df = 4, 515; P <0.0001).

Discussion

While there were no significant differences in stable fly emergence among the different concentric zones in the ring feeder sites, the *intermediate zone* of the cone feeder sites produced a numerically larger amount of stable flies (Table 3.2). Broce et al. (2005) demonstrated that hay feeding sites are viable developmental habitats for stable flies; results from this study agree with their findings but also demonstrate that certain zones within hay feeding sites are more suitable for larval development than others. This suggests that targeted control tactics should be utilized instead of treating the entire feeding area since hay-feeding sites can cover large areas (Broce et al. [2005] reported sites as large as 262 m^2). This study demonstrated that stable fly production decreases over time since both, the ring and cone feeder sites, exhibited higher stable fly production earlier in the fly season (5/17 - 6/3/2004) than later (7/26 - 8/12/2004). This could possibly suggest that the feeding sites themselves become less favorable as oviposition sites or the overall population has declined to a point where fewer stable flies are depositing their eggs at these hay-feeding sites.

Further studies into environmental parameters of different zones of developmental sites demonstrated some interesting results with differences in temperature and concentration of fecal coliforms between periods when stable fly immature development was fairly ubiquitous (5/17 - 6/3/2004) and when stable fly immature development was observed infrequently (7/26 - 8/12/2004). Average daily temperature increased from approximately 23° C (Table 3.1 & 3.2) in the first sampling period to 27° C (Table 3.1 & 3.2) in the second sampling period. Lysyk (1998) also observed higher survival of immature stable flies at temperatures that ranged from 20 to 23° C. However, temperatures in the second sampling period did not exceed the temperature range for immature development. Berry et al. (1976) demonstrated that the egg-larval survival

was greater than 80% at 30° C. This study suggests other factors may play a role in the decrease over time in stable fly development at these feeding sites.

Of interest was that in all zones in the first sampling period and at both feeder sites the concentration of fecal coliforms was considerably higher than that observed in the second sampling period of low stable fly production (Tables 3.1 & 3.2). Lenehan et al. (2005) reported that the concentration of fecal coliforms is greater in soil samples taken in months when cattle were present at the hay-feeding site continuously (winter months) than in soil samples taken in later months when cattle visited the hay-feeding site less frequently (summer months). It has been reported that the total daily fecal output of cattle ranges from 0.5 to 0.75% of their body weight on a dry-weight basis (Kronberg et al. 1986) and that on average, this fecal output contains approximately 3.8×10^{10} CFU/g fecal coliforms (Moore et al. 1988). If we consider that an average cow weighs 522 kg and that there were 10 head of cattle (a conservative estimate) feeding at a single hay feeder, then the total amount of fecal matter could reach as high as 40 kg of dry feces per day and with continued feeding at a single feeding site for a minimum of 3 months, common in midwestern U.S. cattle production, the total fecal output could possibly reach approximately over 3,600 kg dry weight at a single feeding site.

The influence of the concentration of fecal coliforms on stable flies developing at hay feeding sites has yet to be studied. Romero et al. (2006) recently demonstrated that gravid stable flies laid significantly more eggs on a natural substrate (a mixture of hay, soil and manure) with a live microbial community than on the sterilized natural substrate. Brookes & Fraenkel (1958) suggested that the nutritional components provided by bacteria, which include vitamins and sterols, are critical for larval survival. This is further supported by Lysyk et al. (1999) who demonstrated that stable fly larvae are unable to survive without live and active bacterial cells.

These previous studies, as well as the one herein reported, suggest that bacteria play a vital role in stable fly survival indicating that the concentration of cattle manure at these feeding sites rather than the amount of hay is an important factor contributing to favorable developmental sites for stable flies.

Although no difference in stable fly emergence was observed in the different hay:manure ratio media (Fig. 3.2), a noticeable trend was observed in the number of days required for adult emergence among the different media (Fig. 3.2). Days to adult emergence was lower in the media containing hay than that observed in the manure only medium. This coincides with the increases in temperature in all media containing hay as compared to the manure only media (Fig. 3.3). Lysyk's (1998) findings suggested that with increased temperatures of up to 31° C the results in a decrease in development time. The 2:1 and 1:1 hay:manure ratio media exhibited the highest emergence rates and similar days to adult emergence; this suggests that the minimal addition of hay to manure can influence the environment to the point of decreasing generation time (days). These two media also demonstrated similar temperatures (Fig. 3.3) as those reported by Lysyk (1998) who stated that greater reproduction and faster development times occurred when temperatures were between 25 and 30° C. The different hay:manure ratio media study demonstrated that development time decreases with the addition of hay due partly to increased temperature in these substrates.

The plastic hay surrogate study lead to further questions with the plastic media demonstrating significantly lower emergence than that observed in the manure only substrate. In fact, the plastic stick media were detrimental as a substrate to stable fly immatures (Fig. 3.5) with no biological effect on temperature or pH dynamics since all values were within acceptable ranges for stable fly development (Rasmussen & Campbell 1981; Broce & Haas 1999; Figs. 6 &

7 of this study). The two media containing hay (unsterilized and sterilized hay) were similar in stable fly emergence suggesting that microbial associations with the hay are limited in regards to enhancing stable fly immature development since the manure of the substrate was not sterilized. This study also suggests that microbial communities associated with manure could be improved with the addition of hay since both hay substrates demonstrated significantly higher emergence than that observed in the manure only media. This trial also demonstrated contrasting results to those of Meyer & Shultz (1990) who found the highest numbers of stable flies in manure only media. However, it is in agreement with studies in Florida that suggest that stable flies prefer to develop in decaying vegetation or decaying vegetation mixed with manure (Williams et al. 1980; Hogsette et al. 1987).

This study identified specific immature developmental sites within hay feeding areas that could be utilized in targeted control efforts. Greene (1993) suggested that sanitation is the most important technique for the reduction of stable fly populations in CAFOs; this could be accomplished with limited labor inputs in pastures if only certain areas of the feeding site are targeted instead of attempting to clean an entire hay feeding area. This study suggests further that targeted insecticide application could be utilized instead of treating the entire feeding area which could be beneficial not only to producers in reduced costs but could also reduce the probability of off-site contamination such as near-by streams. Targeted insecticide applications could also reduce any negative impact of the pesticide on arthropods that play the role of natural control agents of stable fly immatures. It is evident that further studies are needed to identify the role hay plays, whether it is physical or biological, in contributing to successful stable fly immature development. Moreover, further investigation is needed to assess microbial

communities associated with hay:manure media to clearly identify cues for stable fly development.

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Table 3.1 Stable fly emergence, environmental, and physical factors in three different zones of hay/manure accumulation during high stable fly production (5/17 - 6/3/2004) and low stable fly production (7/26 - 8/12/2004) at hay ring feeder sites

	First Sampling Period (5/17 - 6/3/2004)			Second Sampling Period (7/26 - 8/12/2004)		
	Zones (SE)			Zones (SE)		
	Inner	*Intermediate*	*Outer*	*Inner*	*Intermediate*	*Outer*
SF/trap/day*	5.24a (1.27)	5.95a (1.62)	3.31a (1.16)	0.05b (0.03)	0.02b (0.01)	0.02b (0.01)
Temp. (C) Avg.*	23.69b (0.34)	23.28b (0.35)	22.94b (0.36)	27.99a (0.35)	27.92a (0.34)	27.85a (0.36)
Min.*	22.38b (0.42)	21.64b (0.31)	21.47b (0.37)	26.01a (0.48)	25.37a (0.29)	25.56a (0.33)
Max.*	25.14a (0.32)	24.89b (0.45)	24.27b (0.54)	29.88a (0.31)	30.03a (0.43)	30.09a (0.46)
Moisture (%)*	70.55a (1.02)	65.54a (2.96)	48.18b (7.72)	72.55a (1.14)	70.88a (0.60)	35.95c (3.37)
Depth of Hay:Manure Substrate (cm) *	23.50a (1.22)	19.69ab (3.30)	10.48cd (2.46)	17.15abc (3.18)	13.34bc (1.84)	3.81d (0.52)
pH*	6.68bcd (0.13)	6.62cd (0.15)	7.03a (0.11)	6.55d (0.08)	6.93abc (0.09)	6.99ab (0.08)
Fecal Coliforms (CFU/g) *	1.91×10^4a (0.02×10^4)	5.19×10^1a (0.02×10^4)	4.07×10^4a (0.02×10^4)	<10b	0.09×10^2b (0.04×10^4)	0.03×10^2b (0.03×10^4)

*Read within row. Means followed by the same letter are not significantly different (P = 0.05, LSMeans test [SAS Institute 1999]).

Table 3.2 Stable fly emergence, environmental, and physical factors in three different zones of hay/manure accumulation during high stable fly production (5/17 - 6/3/2004) and low stable fly production (7/26 - 8/12/2004) at hay cone feeder sites

	First Sampling Period (6/3/2004)			Second Sampling Period (8/12/2004)		
	Zones (SE)			Zones (SE)		
	Inner	*Intermediate*	*Outer*	*Inner*	*Intermediate*	*Outer*
SF/trap/day*	3.31b (0.70)	6.01a (1.33)	2.38b (0.56)	0.06c (0.03)	0.21c (0.12)	0.24c (0.14)
Temp. (˚C) Avg.*	22.92b (0.42)	23.17b (0.26)	23.74b (0.27)	27.37a (0.40)	27.60a (0.25)	28.13a (0.29)
Min.*	21.12d (0.39)	21.97cd (0.29)	22.54c (0.28)	25.50b (0.44)	26.12ab (0.27)	26.79a (0.30)
Max.*	24.32c (0.45)	24.42bc (0.28)	25.28b (0.33)	28.94a (0.38)	29.10a (0.27)	29.55a (0.31)
Moisture (%)*	70.38a (2.92)	65.63a (3.46)	30.43b (5.98)	74.35a (0.95)	71.48a (0.99)	40.55b (4.71)
Depth of Hay:Manure Substrate (cm) *	21.59a (2.20)	22.54a (1.31)	8.57c (2.10)	18.10ab (2.23)	15.24b (1.56)	4.76c (1.31)
pH	6.38 (0.34)	6.60 (0.36)	6.90 (0.14)	5.92 (0.17)	6.55 (0.33)	6.82 (0.21)
Fecal Coliforms (CFU/g) *	2.14×10^4a (0.02×10^2)	2.29×10^4a (0.05×10^2)	4.15×10^3a (0.03×10^2)	<10b	<10b	<10b

*Read within row. Means followed by the same letter are not significantly different (P = 0.05, LSMeans test [SAS Institute 1999]).

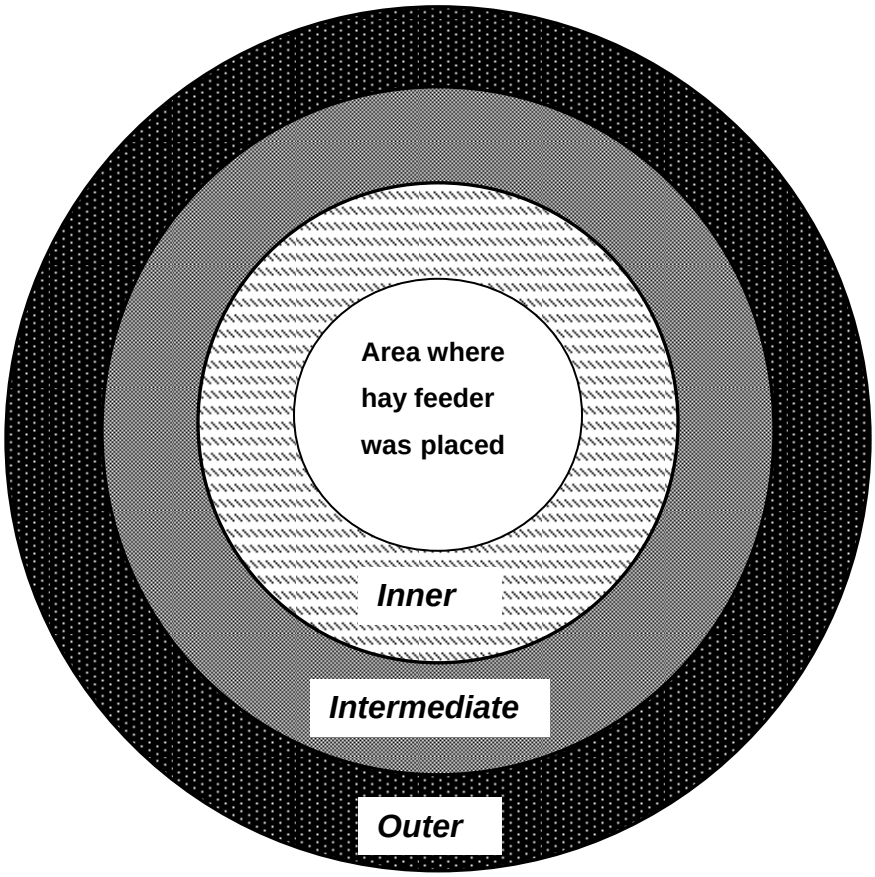

Fig. 3.1 Diagram of the different zones of hay/manure accumulations and their relationship to location of the hay feeder

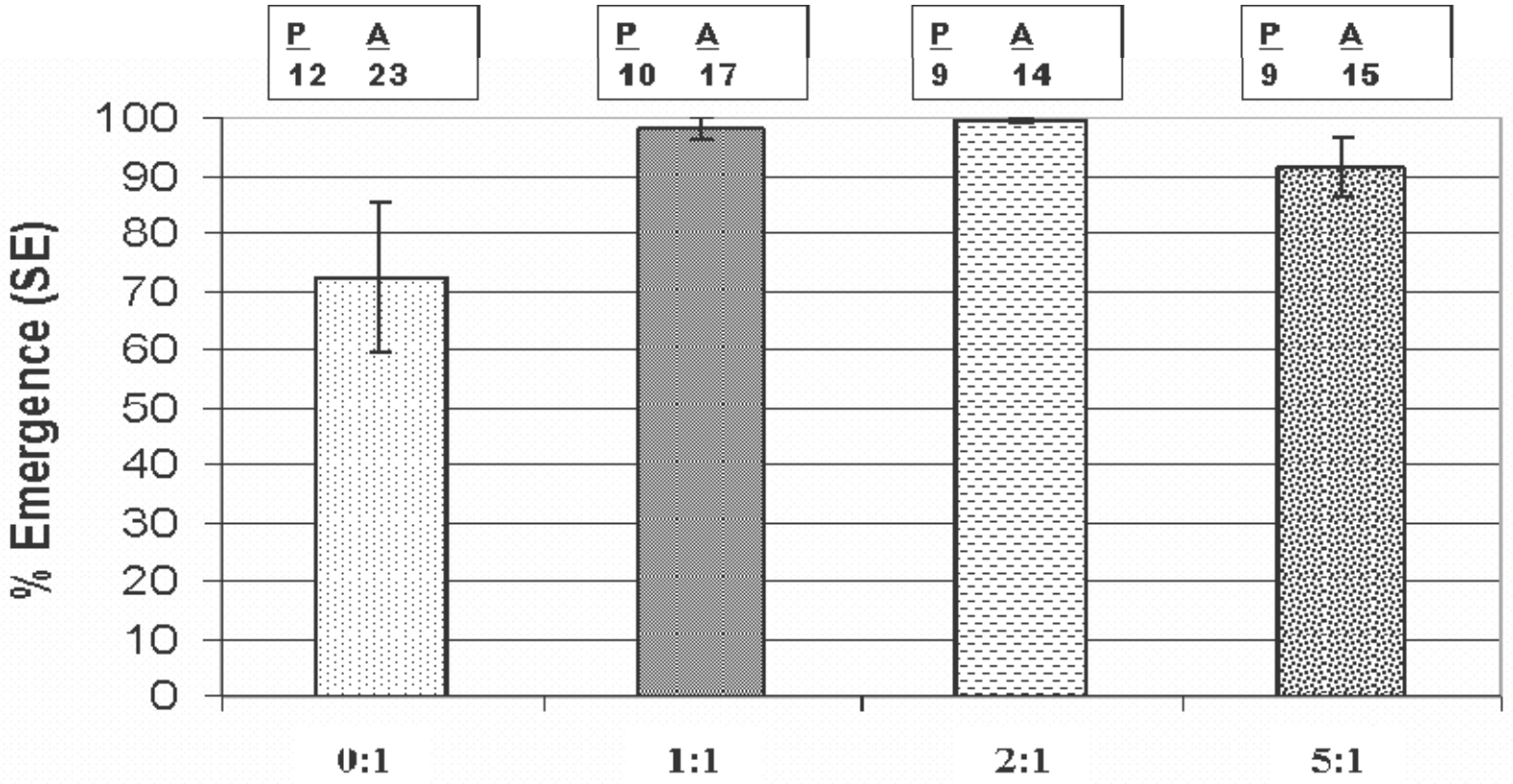

Fig. 3.2 Stable fly emergence,(days to pupation P and days to adult emergence A), from media with different hay:manure ratios

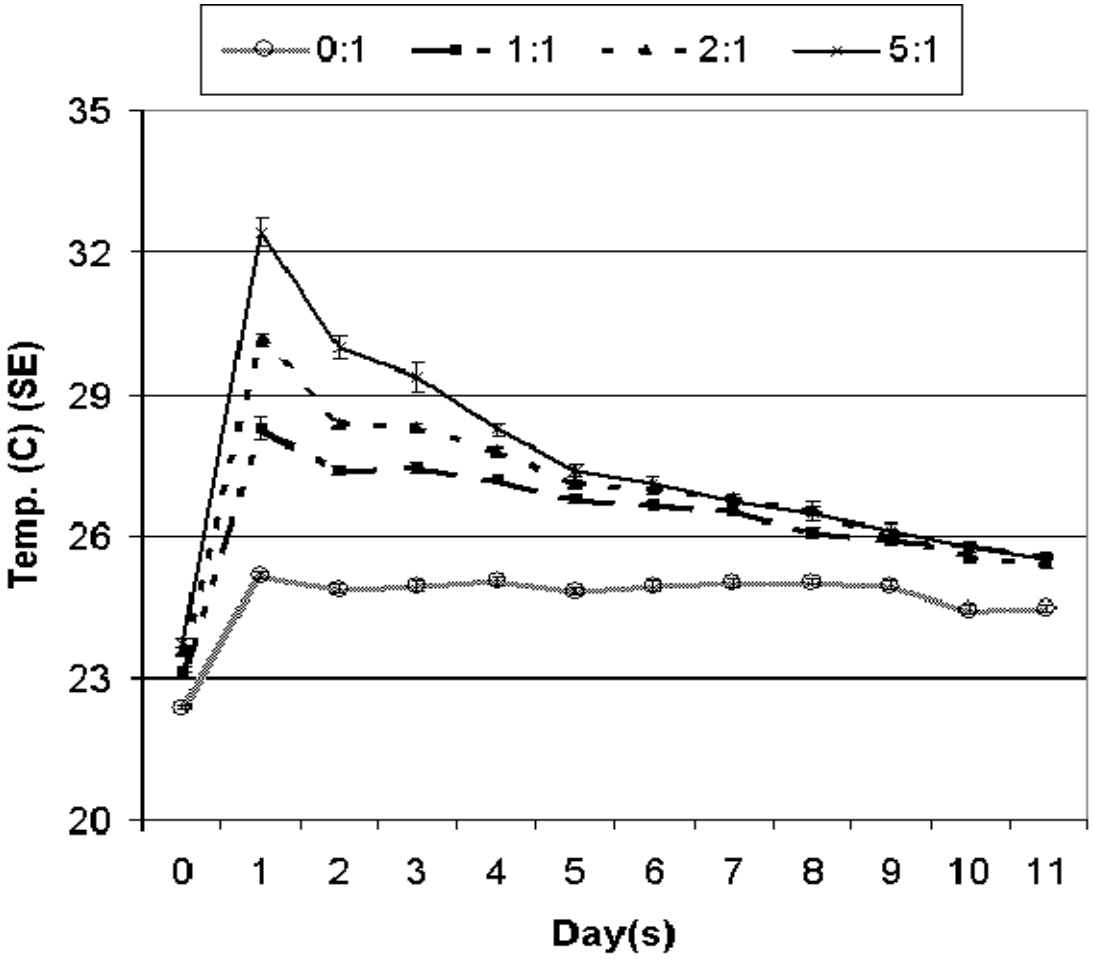

Fig. 3.3 Average daily substrate temperature in the different hay:manure ratio media

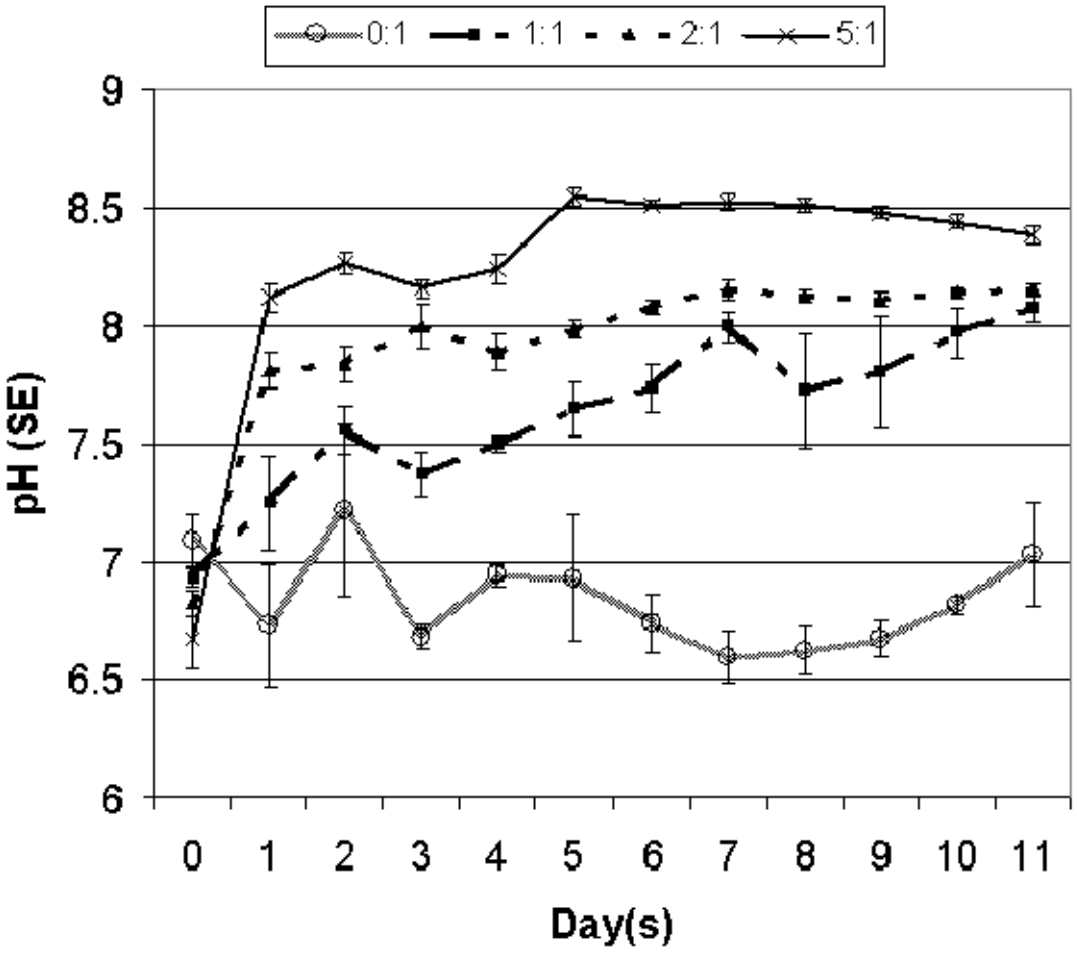

Fig. 3.4 Daily pH average in the different hay:manure ratio media

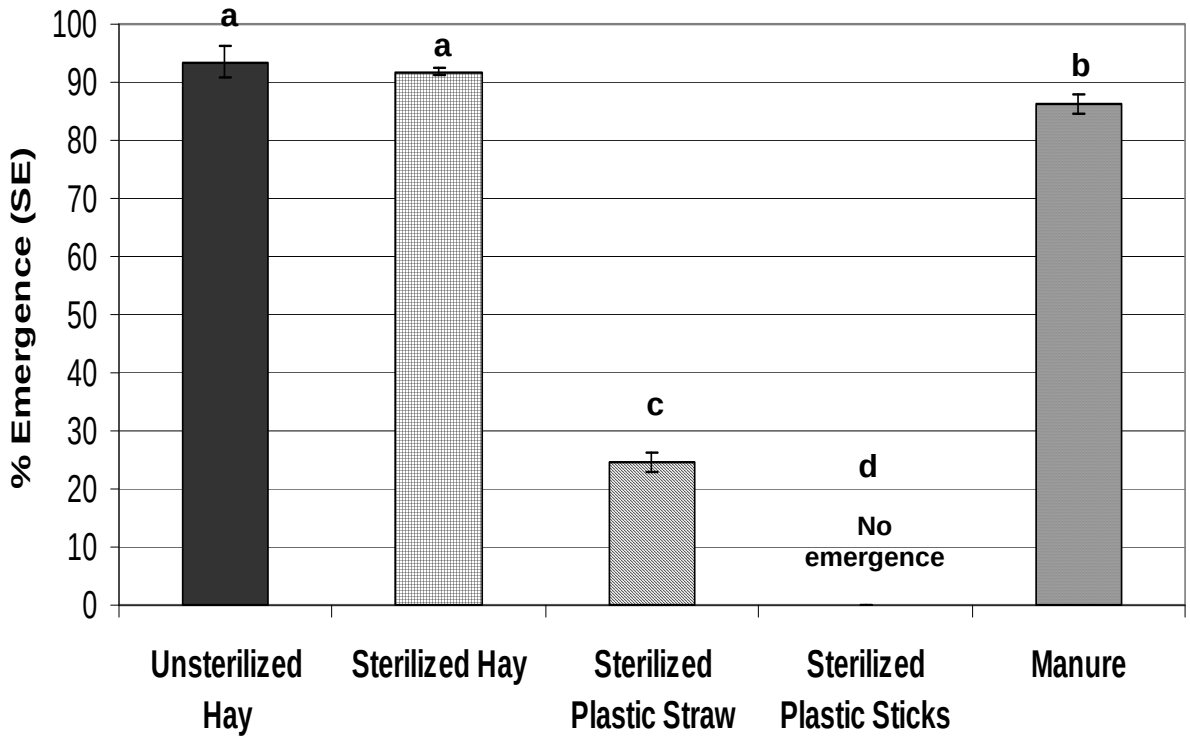

Fig. 3.5 Stable fly emergence from media with different physical characteristics . Bars followed by the same letter are not significantly different (P = 0.05, LSMeans test [SAS Institute 1999]).

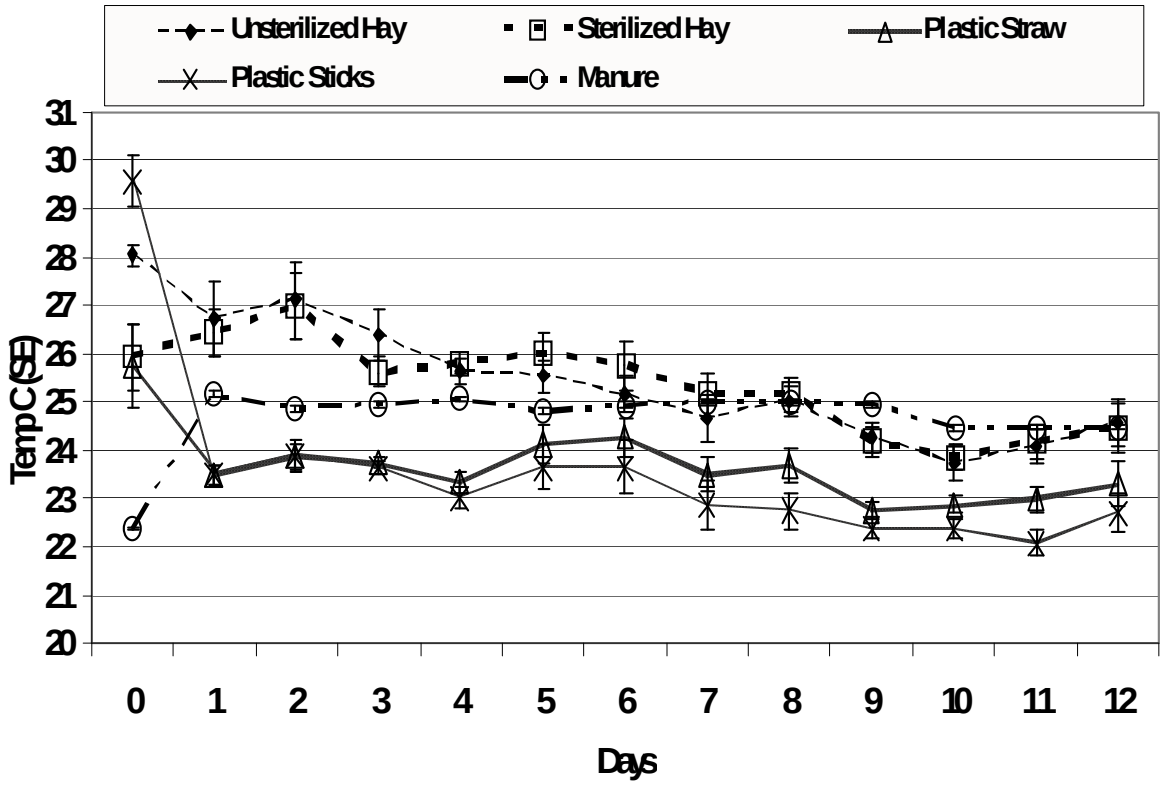

Fig. 3.6 Average daily temperature of different media simulating the physical characteristics of hay

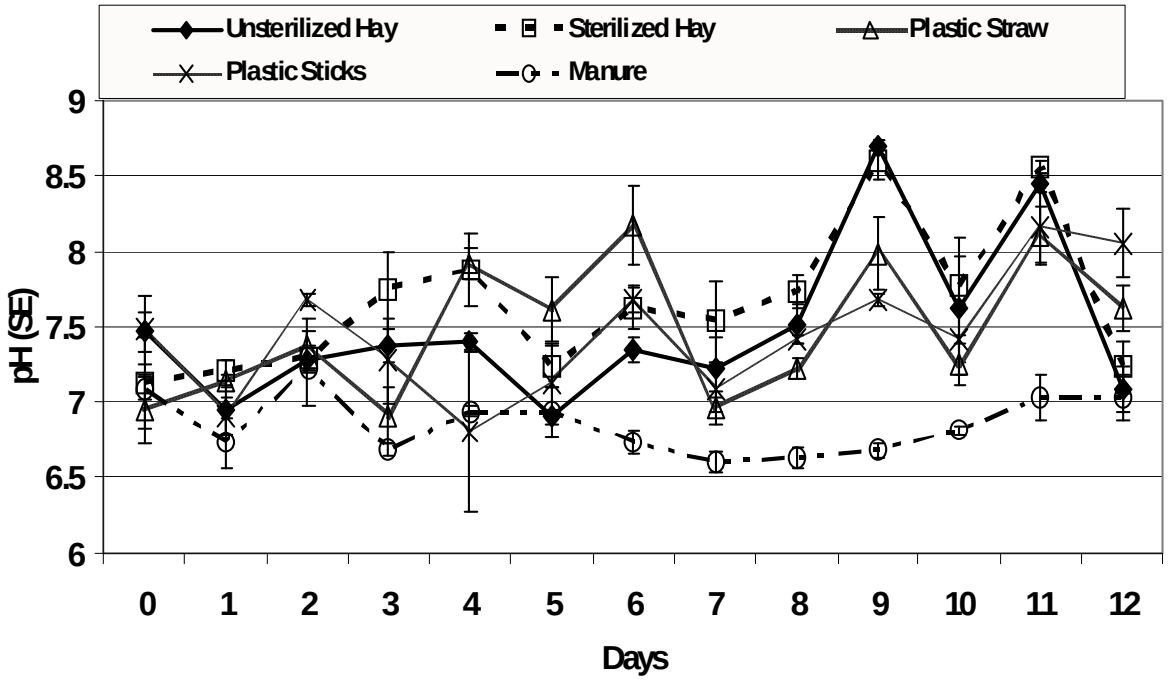

Fig. 3.7 Average daily pH of different media simulating the physical characteristics of hay

CHAPTER 4 - Evaluation of potential insecticides for the control of stable flies (Diptera: Muscidae) developing in round bale feeding sites

ABSTRACT

Stable flies, *Stomoxys calcitrans* (L.), have been identified as serious pests of cattle in pasture ecosystems. Research has identified round hay bale feeding sites as viable larval developmental sites for stable flies within pastures. Identification of potential insecticides that are effective in controlling stable flies developing at these hay-feeding sites was carried out in this study. Boric acid dust was tested at two different rates (22.7 and 45.4 g/m^2) on substrates prepared from a 1:2 (by volume) manure to hay mixture to simulate a natural hay-feeding site. Boric acid demonstrated promising results with STABLE FLY mortality reaching as high as 98.97% at the higher rate (45.4 g/m^2) and 79.04% at the lower rate (22.7 g/m^2). *Metarhizium anisopliae* (Metchnikoff) Sorokin (Deuteromycota: Hyphomycetes) an entomopathogenic fungus was also tested at two different rates (0.40g/m^2 [8.96 X 10^9 conidia] and 0.04g/m^2 [8.96 X 10^8 conidia]) but did not provide sufficient stable fly control. Even though boric acid demonstrated favorable results as a dust formulation it was inhibited from the same mortality levels due to a hay layer that ranges from 2-5 cm thick. Considering the hay layer boric acid was treated to substrates that include a hay layer and those that do not as an aqueous solution at 0.4L of 45.4 g boric acid solution/ m^2. Stable fly mortality was considerably higher in substrates treated with the aqueous boric acid solution in substrates that did not include a layer of dry hay (64.25%) than those substrates with a hay layer (19.74%). Commercially available tetrachlorvinphos, an organophosphate insecticide marketed for muscoid control on livestock premises, was tested on substrates with a hay layer and without a hay layer. Bioassays containing a hay layer exhibited significantly lower stable fly mortality (76.94%) at the labeled application rate than bioassays

with no hay layer (99.12%). Reasons for application of these insecticides as potential control agents against stable flies developing at round hay bale feeding sites are discussed.

Key Words: stable fly, *Stomoxys calcitrans*, hay, round bale, *Metarhizium anisopliae,* boric acid, tetrachlorvinphos

Stable flies, *Stomoxys calcitrans* (L.), have been identified as a major pest of cattle in pastures due to their development at winter feeding sites of hay in round bales (Broce et al. 2005). Since most hay feeding sites are located in pastures near streams or high precipitation runoff areas, it is vital to identify environmentally-safe control strategies that are effective in suppressing stable fly development. The efficacy of insecticide sprays on pastured cattle's legs and underside for control of stable flies is short-lived given that residues are quickly removed by the wet vegetation from early morning dew (Campbell and Hermanussen 1971). While many organic insecticides are available to ranchers/producers, the ongoing challenge of insecticide resistance development and environmental contamination can limit the number of product choices in the future.

Boric acid (H_2BO_3) has been used to control insects, primarily cockroaches, since the middle of the 19th century (Ebling 1995). It has a very good safety record for mammals; it does not volatilize (in contrast to organic insecticides); and its absorption through unbroken skin is negligible (Ebling 1995). In addition, it is inexpensive and no case of insect resistance to boric acid has been reported. An interest in boric acid as a control agent for certain pest species has been renewed in the search for more environmentally-friendly pesticides (Hogsette and Koehler 1992; Strong et al. 1993; Klotz et al. 1994; Zurek et al. 2002).

Metarhizium anisopliae (Metchnikoff) Sorokin (Deuteromycota: Hyphomycetes) is an entomopathogenic fungus with a wide host range (Gunner et al. 1991). As of late, ***M. anisopliae*** has been utilized as a synergist with several insecticides (Pachamutu et al. 1999; Quintela and McCoy 1997; Zurek et al. 2002). ***M. anisopliae*** efficacy as a pre-emergence agent has yet to be proven on stable fly larvae.

Historically, stable fly management has been directed at adult populations and has relied heavily on commercially available chemical products (Campbell 1993) such as organophosphate insecticides which have been useful tools in controlling stable flies. However, the specter of insecticide resistance coupled to environmental safety issues, have put such products under scrutiny. Tetrachlorvinphos is a commercially available product (Rabon®) that commonly is formulated as an oral larvicide and fed as a supplement to cattle for fly control. Kaufman et al. (2001) reported that the highest level of resistance in house flies in New York dairies was to tetrachlorvinphos. Reported cases of tetrachlorvinphos resistance have been well documented (Kaufman et al. 2001; Marcon et al. 2003; Marcon et al. 1997). Stable fly resistance to organophosphates in Kansas feedlots was first reported by Cilek and Greene (1994). Marcon et al. (1997) stressed the need for ongoing surveys on stable fly susceptibility to currently available products to monitor for resistance in stable flies. The concerns for organophosphate resistance in stable flies have lead to research on alternatives, such as boric acid and *M. anisopliae*. Thus the goal of this study was to evaluate the efficacy of these products on stable flies developing in larval substrates at hay feeding sites.

Materials and Methods

Laboratory Bioassays. Boric acid was evaluated for its potential to control STABLE FLY larvae developing in manure/hay habitats. Manure pats were obtained from cows at the KSU Purebred Beef Teaching Research Center (KSU-PBU) and which had not been treated with any pesticide recently. Manure was frozen (−20°C) to kill any existing arthropods. Chopped alfalfa hay was obtained from the KSU feedlot and sieved through a No. 4 sieve to eliminate the smaller dust but retain the larger alfalfa stems. Natural media for the bioassays was made from a 1:2 ratio (volumetrically) of cattle manure:chopped alfalfa hay with appropriate moisture levels added for stable fly larval development. Manure and hay were thoroughly mixed and then put into plastic containers (20 x 20 x 25 cm) each with two screened emergence cups and set aside for at least two days for the media to age (Broce and Haas 1999). For each treatment and control group, 500 stable fly eggs from the KSU laboratory-reared colony were seeded into the media. To evaluate the efficacy of boric acid in controlling stable fly larvae developing in this medium, the surface of the medium in the plastic containers was treated with boric acid dust (Acros Organics, Pittsburg, PA) in two concentrations of 22.7 and 45.4 g/m^2. All containers were kept at 25-26°C, 70% R.H., and 14:10 L:D period. Total numbers of emerged stable flies were recorded 28 days after treatment application and corrected by Abbott's correction formula for mortality (Abbott 1925). Corrected mortality among treatments was compared by PROC GLM and separated by Fisher's Least Significant Difference (LSD) (SAS Institute, 1999).

Efficacy of *M. anisopliae* was also assessed by application of two fungal spore concentrations (0.40g/m^2 [8.96 X 10^9 conidia] and 0.04g/m^2 [8.96 X 10^8 conidia]) (Zurek et al. 2002) to the top of the media surface. The rest of the evaluation procedures followed those described above for boric acid evaluation.

Boric Acid Field Trial. A field trial was conducted in the spring 2004 at the KSU cow/calf unit (~ 3.2 km northwest of Manhattan, KS) on round bale feeding sites, using a water solution of 45.4 g boric acid /m^2 intended for penetrating the top layer of dry hay. Three feeding sites which had used the conventional ring type feeder were utilized in this trial, separated by ~ 15m from each other and each having significant hay residues to support stable fly development (areas of 12 - 16 m^2 covered with hay residues). Boric acid treatment was applied with a backpack sprayer to four 0.25 m^2 areas within each feeding site and equal numbers of control areas were sprayed with water. Emergence traps, as described by Broce and Haas (1999), were placed over the treated surfaces to capture emerging adults. Stable fly emergence in treated and control sites on days after treatment (DAT) 0, 3, 7, 14, and 21 were compared by PROC GLM (SAS Institute, 1999).

Laboratory Bioassays on Substrates with and without a Hay Layer. Upon further examination of the larval habitat at hay feeding sites, the high incidence of a dry hay layer became apparent. Because of the possibility of such layer affecting the efficacy of tested chemicals by retaining their water solutions, it was determined that further bioassays would include a dry hay layer. Boric acid was further tested by using a liquid formulation applied at 0.4L of 45.4 g boric acid solution/m^2. Rearing pots containing the 1:2 manure:hay medium were seeded with 200 eggs and kept at 25–26°C and 70% RH for 2 d, after which treatments were applied to media with and without a 2.54 cm layer of dry hay. When stable flies pupated all rearing pots were transferred into the previously described plastic containers with emergence cups. Total numbers of emerged stable flies were recorded 28 days after treatment application and corrected by Abbott's formula. Corrected mortality of the various treatments were compared by PROC GLM and separated by Fisher's LSD (SAS Institute, 1999).

The commercially available insecticide tetrachlorvinphos (Rabon 50 WP®; KMG-Bernuth Inc., Houston, TX) was tested for its efficacy against stable flies developing in the manure:hay substrate. Three concentrations of tetrachlorvinphos were tested initially on substrates with and without a 2.54 cm layer of dry hay. The three concentrations of tetrachlorvinphos were, half the labeled rate (0.4L of 0.5 % Rabon 50 WP® solution/m^2), the labeled rate (0.4L of 1 % Rabon 50 WP® solution/m^2), and twice the labeled rate (0.4L of 2 % Rabon 50 WP® solution/m^2). Rearing containers with the manure:hay medium were seeded with 200 eggs each and kept at 25–26°C and 70% RH for 2 d, after which treatments were applied to the media. Total number of emerged stable flies was recorded 28 days after treatment application. Further comparison of the two higher concentrations (0.4L of 1 % Rabon 50 WP® solution/m^2 and 0.4L of 2 % Rabon 50 WP® solution/m^2) were tested in the same manner but only on the substrates that had the dry hay layer on top of the manure:hay medium. Control groups were treated with 0.4L of distilled water/m^2. All treatments and controls were replicated 3 times. Total numbers of emerged stable flies were recorded 28 days after treatment application and corrected by Abbott's formula. Corrected mortality in the various treatments were compared by PROC GLM and separated by Fisher's LSD (SAS Institute, 1999).

Results

Laboratory Bioassays. Stable fly immature mortality due to boric acid was significantly higher (98.97 versus 79.04%) in substrates treated with the higher (45.4 versus 22.7 g dust/m^2) than in the lower concentration and in both of these treatments immature mortality was significantly higher than in the control (22.73%; $F = 44.78$; df = 2, 24, $P = 0.0001$; Fig. 4.1). Immature mortality was variable in the *M. anisopliae* treatments with no significant difference observed between the control or either of the treatment groups (0.40g/m^2 [8.96 X 10^9 conidia] and 0.04g/m^2 [8.96 X 10^8 conidia]) of *M. anisopliae* ($F = 0.37$; df = 2, 24, $P = 0.7058$). A negligible trend was observed with the higher rate of *M. anisopliae* (0.40g/m^2 [8.96 X 10^9 conidia]) exhibiting the highest immature mortality (37.33%)(Fig. 4.2).

Boric Acid Field Trial. No differences were observed in stable fly emergence between areas treated with a solution of 45.4 g boric acid /m^2 and control groups (Table 4.1). A dramatic decrease in stable fly emergence was observed between DAT 0 and 3 indicating other factors than the boric acid were involved in influencing stable fly emergence during this time period (Table 4.1). This may be attributed to an abnormal daily maximum temperature change from 29°C on Day 0 to 35°C on Day 3; in addition, precipitation was also limited.

Laboratory Bioassays on Substrates with and without a Hay Layer. Stable fly immature mortality was significantly higher in substrates without a hay layer and treated with a solution of 45.4 g boric acid /m^2 (64.25 versus 19.74%) than in substrates with a hay layer; and also significantly higher than in control groups with and without hay layers (8.83 and 11.75%, respectively; $F = 70.46$; df = 3, 20, $P < 0.0001$; Fig. 4.3). Stable fly mortality was lower in substrates treated with the solution of 45.4 g boric acid /m^2 that contained a hay layer (19.74%) and was not significantly different from the control group that did not have a hay layer (11.75%)

but was statistically different from the control group that included a hay layer (8.83%)(F = 70.46; df = 3, 20, P < 0.0001; Fig. 4.3).

All three concentrations of tetrachlorvinphos applied to substrates without a hay layer had significantly higher stable fly mortality (93.20, 99.12, 100.00% in 0.5, 1.0, 2.0% solutions, respectively) than the control group with no hay layer (13.50%)(F = 217.14; df = 7, 40, P < 0.0001; Fig. 4.4). However, only the highest (2.0%) tetrachlorvinphos formulation applied to substrates with a hay layer exhibited similar stable fly mortality (93.47%) to the tetrachlorvinphos treatments applied to substrates containing no hay layer. The 1% tetrachlorvinphos solution applied to substrates with a hay layer exhibited significantly higher mortality (76.94%) than the 0.5% tetrachlorvinphos solution (45.47%) and the control group with a hay layer (17.25%)(F = 217.14; df = 7, 40, P < 0.0001; Fig. 4.4). The tetrachlorvinphos solution applied to substrates with a hay layer had significantly higher stable fly mortality (45.47%) than both control groups, but was significantly lower than any other tetrachlorvinphos treatment (F = 217.14; df = 7, 40, P < 0.0001; Fig. 4.4). Stable fly immature mortality was not statistically different in the control group with a hay layer (17.25%) or in the control group without a hay layer (13.50%)(F = 217.14; df = 7, 40, P < 0.0001; Fig. 4.4).

Further examination of the immature mortality in the two higher concentrations of tetrachlorvinphos (1 & 2%) applied to substrates containing a dry layer of hay revealed that stable fly mortality in both concentrations were significantly higher than the control group with a hay layer (F = 37.88; df = 2, 15, P = 0.0001; Fig. 4.5). Even though mortality was higher in the 2.0% tetrachlorvinphos treatment, (92.42%) it was not significantly different from that in the 1.0% concentration (76.33%)(F = 37.88; df = 2, 15, P = 0.0001; Fig. 4.5).

Discussion

Boric acid demonstrated effective control of immature stable flies in manure:hay substrates in laboratory bioassays (Fig. 4.1) suggesting that boric acid could be an environmentally-safe alternative. These findings agree with previous research that demonstrated effective control of muscoid flies in compost piles (Lal and Srivastava 1950) and manure (Midgley et al. 1943). However, the presence of a 3 – 5 cm dry layer of hay on the top of the substrate likely represents a barrier to effective control. While results from the field trial were inconclusive due to increased temperatures during lower than normal precipitation it is still plausible to suggest from further bioassays (Fig. 4.3) that only a limited amount of the 45.4 g boric acid aqueous solution was reaching the target area of stable fly development. The volume of the boric acid solution was identical to the commercial tetrachlorvinphos recommendation (Rabon 50 WP®) application of $0.4L/m^2$. Further investigation into varying volumes should be conducted to clearly determine whether this concentration of boric acid is ineffective.

Another alternative of utilizing boric acid that should be investigated is combining a limited cultural control practice such as disturbing the layers of hay residues with the subsequent application of boric acid dust. Testing this integrated approach would have to include a cost-benefit analysis to account for the added labor and time to disrupt the integrity of the larval substrate.

While *M. anisopliae* has been proven as an effective synergist in speeding up the action of several insecticides (Pachamutu et al. 1999; Quintela and McCoy 1997; Zurek et al. 2002), it was ineffective in controlling stable fly immatures, at least at the rates used in this study (Fig. 4.2). Further comparison of *M. anisopliae* applied with boric acid as a synergist should be

investigated to determine efficacy on stable flies developing in substrates that contain manure and hay.

Testing the commercially available insecticide tetrachlorvinphos (Rabon 50 WP®) gave promising results in controlling stable flies developing at hay feeding sites. Bioassays also demonstrated that the efficacy of tetrachlorvinphos can be negatively affected by the presence of the dry hay layer (Fig. 4.4) by retaining a portion of the chemical; thus not allowing it to reach the stable fly larval sites. Reducing the labeled rate by half (0.5%) gave good stable fly control in substrates not containing a hay layer, but were ineffective on substrates containing such layer (Fig. 4.4). While the highest rate of tetrachlorvinphos (2.0%) provided excellent control in substrates with a dry hay layer, it would be more environmentally-safe not to use this high rate since most hay feeding sites are located near streams or on areas with high precipitation runoff. Even though the 2% rate of tetrachlorvinphos resulted in higher stable fly mortality than the labeled rate, this study suggests that if producers are looking for a quick reduction in stable fly larvae, the labeled rate (1%) would be satisfactory in controlling stable flies developing at hay feeding sites (Fig. 4.5). As with all organophosphate insecticides, resistance is a major concern especially to already available tetrachlorvinphos products such as Rabon 50 WP® since resistance has been previously reported in other muscoid flies (Kaufman et al. 2001). Stable fly resistance to organophosphate insecticides has been previously reported in Kansas feedlots (Cilek and Greene 1994) and this could present possible problems due to their ability to travel intermediate distances (Hogsette and Ruff 1985). Further testing should be carried out in field trials to properly assess tetrachlorvinphos (Rabon 50 WP®) effect on stable flies developing at hay feeding sites. As in the case of the boric acid trials within this study further examination of differing volumes should be tested to determine an optimal volume to apply insecticides for

controlling stable flies developing in substrates that contain a possible restrictive boundary of dry hay that the products would have to penetrate.

In conclusion, this study demonstrated that boric acid can be effective in controlling stable flies (Fig. 4.1) but further studies are needed to assess the effect of the dry hay layer on the amount of boric acid penetrating to reach the targeted stable fly larval microhabitats. Also, the commercially available insecticide tetrachlorvinphos demonstrated effective control of stable flies developing in substrates that included a dry hay layer (Fig. 4.4) suggesting this insecticide can be used as an effective control agent on stable fly larval sites within hay feeding areas in pastures. This study also indicates that hay may act as a restrictive layer for insecticide penetration and presents problems in obtaining proper control.

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Table 4.1 Number of stable flies emerging from areas within hay feeding sites treated with a 45.4 g boric acid solution compared to emergence from control areas.

	Days after treatment (DAT)				
Trt Group	0	3	7	14	21
		Total SF Emergence[1]			
0.4L of 45.4 g boric acid solution/m^2	263.33 (81.72)	9.00 (3.22)	21.67 (6.13)	100.00 (31.68)	27.67 (10.18)
Control	321.00 (125.89)	12.00 (5.57)	33.00 (2.52)	94.33 (44.62)	17.67 (6.75)
		SF/Trap/Day[2]			
0.4L of 45.4 g boric acid solution/m^2	7.83 (1.80)	0.36 (0.14)	0.75 (0.13)	2.24 (0.80)	1.01 (0.43)
Control	9.37 (3.10)	0.50 (0.24)	1.21 (0.13)	1.83 (0.68)	0.64 (0.28)
		SF/ m^2/Day[3]			
0.4L of 45.4 g boric acid solution/m^2	31.33 (7.20)	1.44 (0.60)	3.00 (0.51)	8.97 (3.18)	4.06 (1.72)
Control	37.46 (12.41)	1.98 (0.95)	4.83 (0.54)	7.32 (2.73)	2.57 (1.12)

[1] Averaged from three different hay feeding sites

[2] Averaged from three different hay feeding sites with 4 emergence traps per feeding site within ea. treatment group

[3] Calculated from a 0.25 m^2 emergence traps and averaged in the same manner as SF/Trap/Day

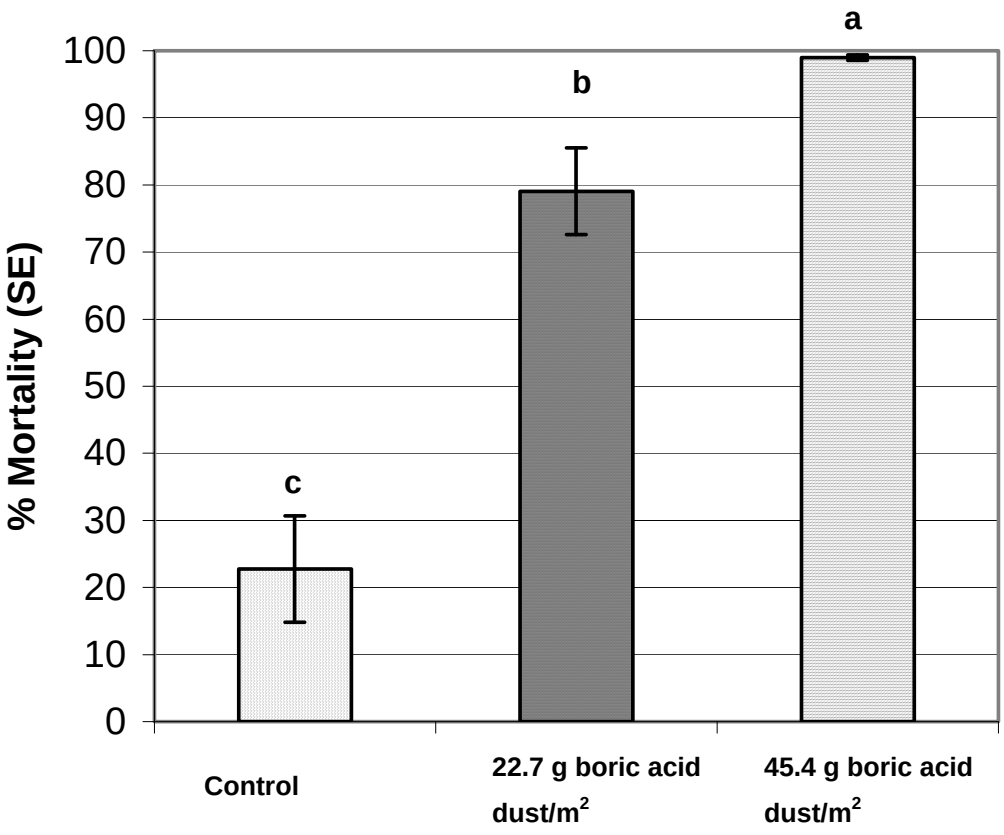

Fig. 4.1 Stably fly mortality after two different rates of boric acid dust (22.7 and 45.4 g/m^2) treatment. Bars followed by the same letter are not significantly different (P = 0.05, LSMeans test [SAS Institute 1999]).

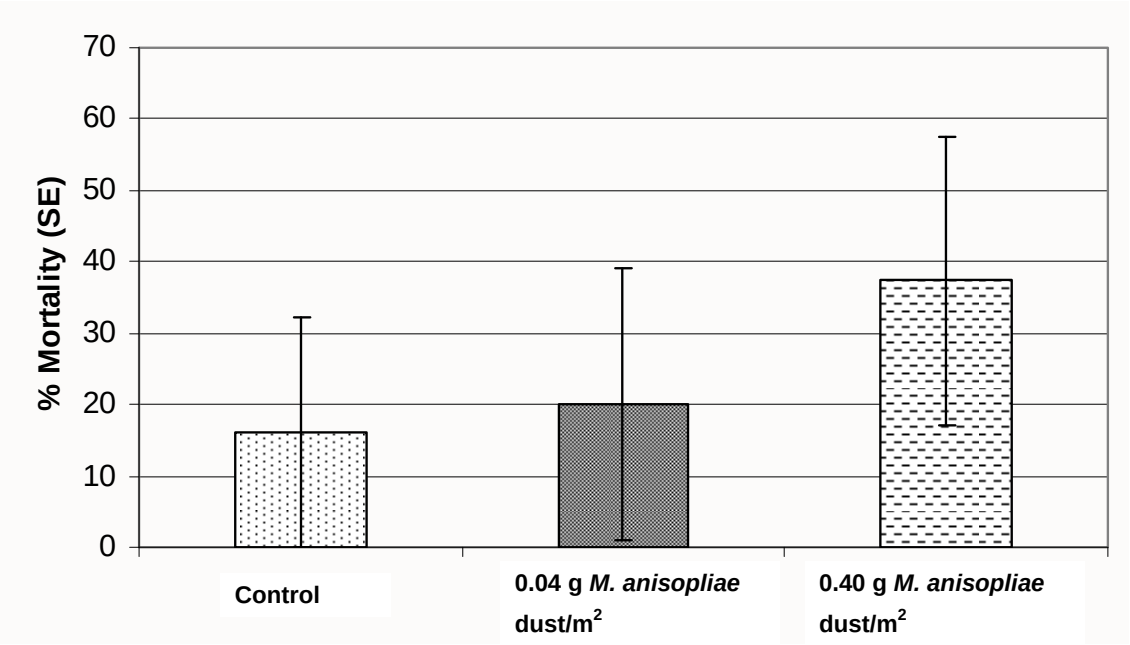

Fig. 4.2 Mortality of stable flies treated with two different rates of *Metarhizium anisopliae* dust (0.04g/m^2 [8.96 X 10^8 conidia] and 0.40g/m^2 [8.96 X 10^9 conidia]).

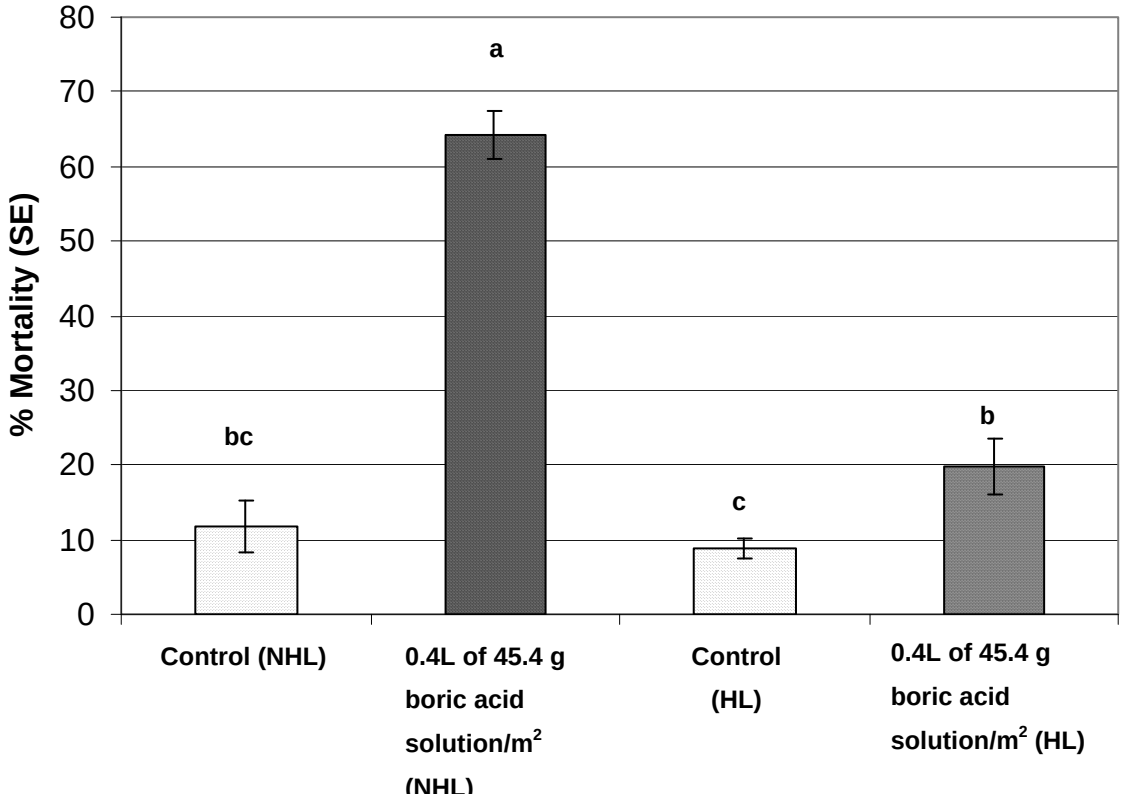

Fig. 4.3 Mortality of stable fly immatures from manure/hay media with (HL) and without (NHL) a hay layer and treated with 0.4L of 45.4 g boric acid solution/m^2. Bars followed by the same letter are not significantly different (P = 0.05, LSMeans test [SAS Institute 1999]).

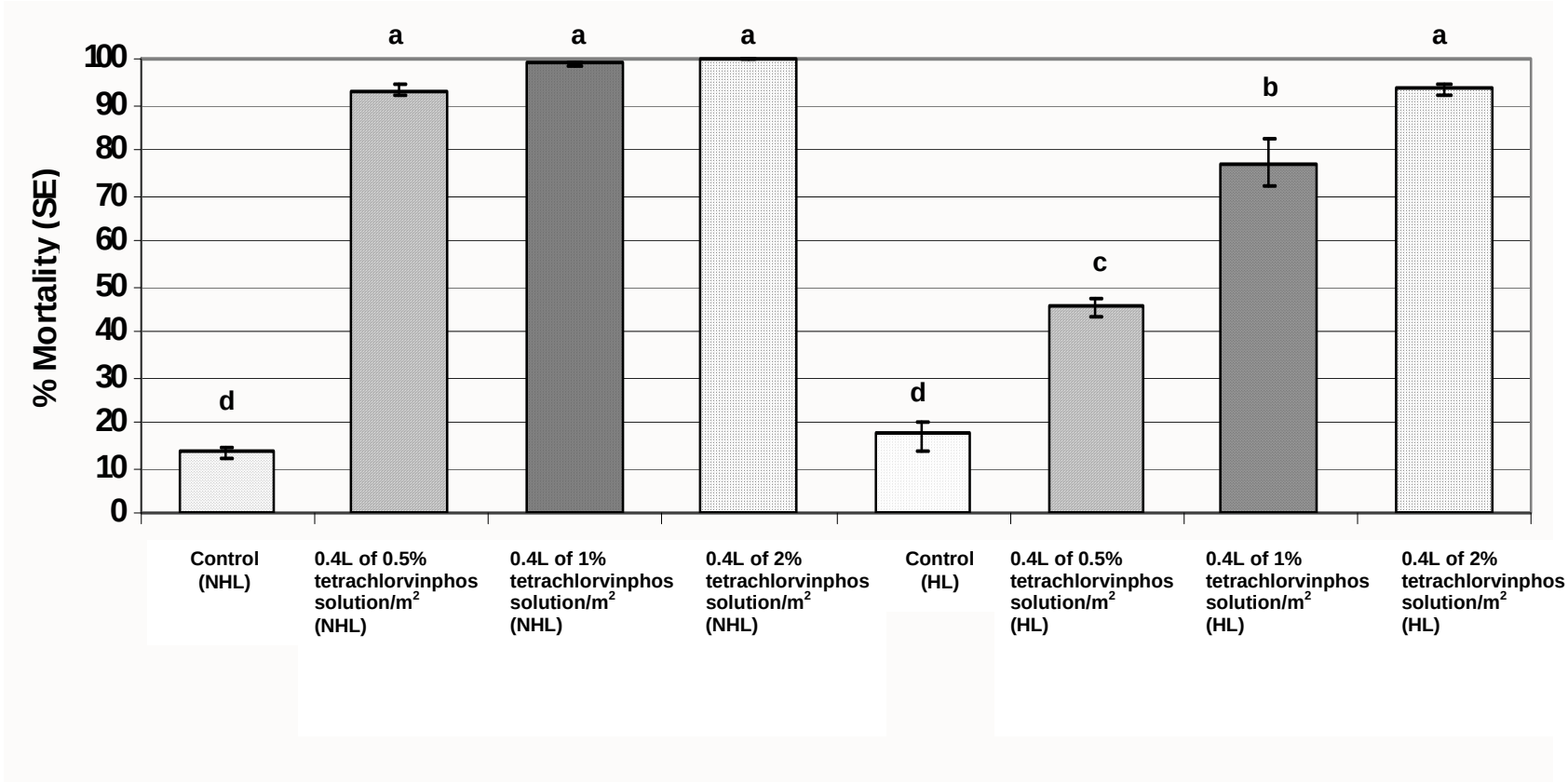

Fig. 4.4 Mortality of stable fly immatures in manure/hay larval substrates with (HL) and without (NHL) a layer of dry hay and treated with one of three concentrations of tetrachlorvinphos (0.4L of 0.5, 1.0, or 2.0% of tetrachlorvinphos/m^2). Bars followed by the same letter are not significantly different (P = 0.05, LSMeans test [SAS Institute 1999]).

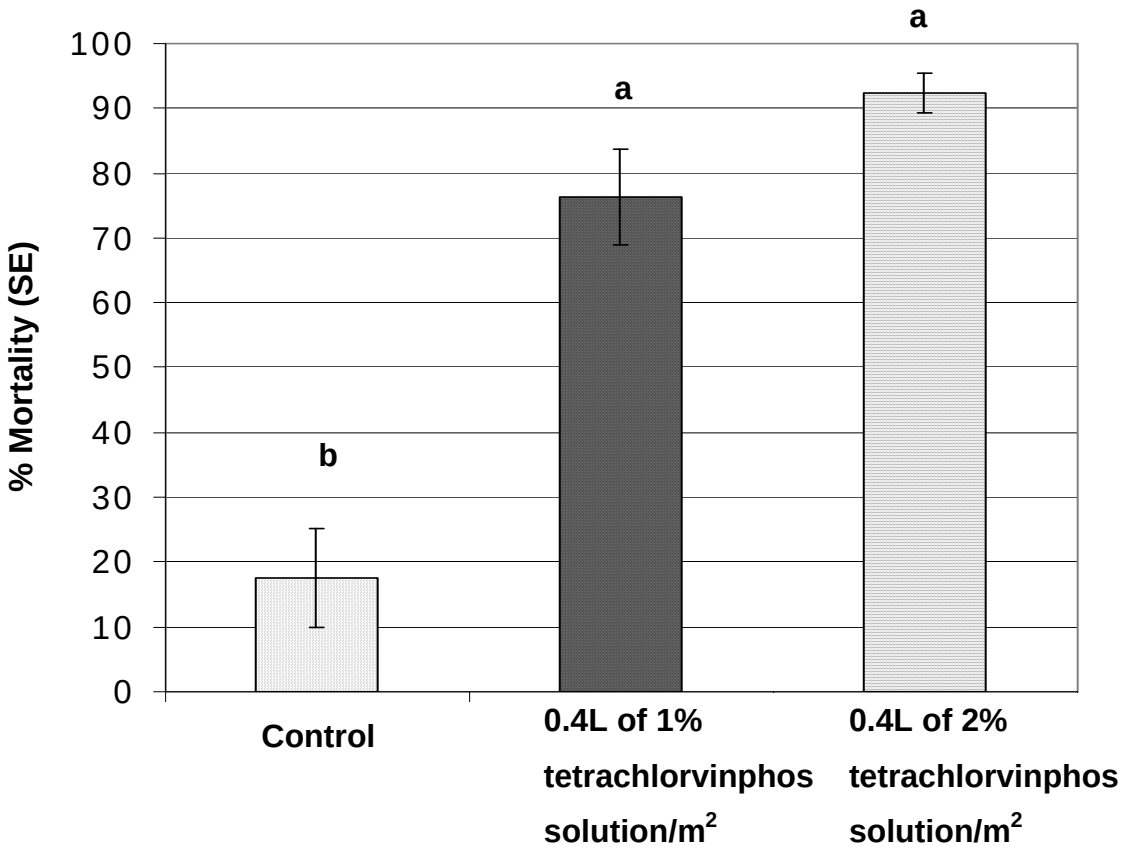

Fig. 4.5 Mortality of stable fly immatures in manure:hay larval substrates with a hay layer and treated with one of two concentrations of tetrachlorvinphos (0.4L of 1.0 or 2.0% of tetrachlorvinphos/m^2). Bars followed by the same letter are not significantly different (P = 0.05, LSMeans test [SAS Institute 1999]).

CHAPTER 5 – SUMMARY

Stable flies have demonstrated the capability of developing in hay feeding areas. The previously mentioned studies have identified specifics of certain management strategies that affect development. The facts gained from this study illustrated the role of concentrating the manure at hay feeding sites influences the potential for stable fly development more than the amount of hay. For example, when comparing the cone and ring feeders there was a significant difference in the amount of wasted hay, but no difference in stable fly emergence (Chapter 2). Cost of the cone feeder is higher (~$600) than the ring feeder (~$100) and the savings would have to come from less hay waste rather than stable fly control. Interestingly, the method that provided no stable fly development was unrolling the bales which require no specialized feeder. Most Midwestern cattle producers use hay haulers attached to the back of their truck to unroll bales. This method of unrolling the bales requires less labor to feed the hay and spreads the cattle over a larger area. When spreading the cattle over a larger area the manure is also spread and decreases the favorability of the habitat for stable flies. The most economic manner for producers to alter the stable fly habitat is to unroll the hay bales over different areas since this method did not provide stable fly development and did not reduce the amount of available forages (Chapter 2).

Characterizing the hay feeding habitat for stable flies demonstrated that certain zones exist at the hay feeding site (Chapter 3). The zones represented different compositions of hay and manure with each zone being delineated by the manner in which the cattle feed. All parameters that were measured demonstrated little difference and were within developmental ranges of the stable fly. The only difference that was noted were the amount of fecal coliforms at the hay feeding site during high stable fly production versus those found during low stable fly production. The measuring period for the high stable fly production was earlier in the summer and the second measuring period was later this signifies that the habitat becomes less favorable for stable flies due to the decrease in the amount of bacteria at the site. This decrease in bacteria over time could indicate a potential area of future research. Specific bacteria were not identified during this study but it was noted that certain areas of the hay feeding site provided a larger number of stable flies even though no statistical differences were noted in stable fly emergence from the different zones. Also, within the study to characterize the stable fly habitat an artificial

media was tested. The artificial media was constructed from plastic and while it was a material that closely imitated hay it was also inert and provided little to no stable fly development. Imitating the physical attributes of hay was the main goal of using plastic straws and sticks but further research will be needed to determine if certain physical properties of hay contribute to stable fly development.

Studies to determine if certain insecticides could be utilized to control stable flies at hay feeding sites noted that a restrictive dry hay layer exists. This restrictive hay layer is approximately 8-15 cm in depth and decreased the efficacy of the insecticides applied to the substrates. Boric acid was tested as a dust form initially but it was later determined that an aqueous solution was needed to pass through the restrictive hay layer for the product to reach the areas of larval development. Boric acid demonstrated promising results (>90% mortality) when used as a dust but was less effective (no difference from control) in the field as an aqueous solution. The commercially available tetrachlrovinphos (Rabon®) was effective at controlling stable flies at the labeled rate of 1% tetrachlrovinphos applied at 0.4 L/m^2 even with a restrictive hay layer.

While there are several basic questions about the larval habitats of stable flies developing in hay substrates still to be answered, the premise of this research was to find alternative control practices. Through the findings of this project some simple management alterations such as unrolling the bales instead of placing the hay in ring feeders can reduce the favorability of these sites for stable fly development. Also, the determination of certain parameters of the hay substrate led to a better understanding of the larval development sites.