STUDIES ON THE FIELD ECOLOGY, BREEDING BIOLOGY AND PARASITISM OF HOUSE FLIES, *MUSCA DOMESTICA* AND STABLE FLIES, *STOMOXYS CALCITRANS*, (DIPTERA: MUSCIDAE) TO IMPROVE INTEGRATED PEST MANAGEMENT FOR NORTH FLORIDA SMALL EQUESTRIAN FARMS

By

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To my wonderful family, Keith and Cameron

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House flies, *Musca domestica* L., and stable flies, *Stomoxys calcitrans* L.*,* (Diptera: Muscidae), are common pests on horse farms. Insecticides have been the primary method of fly control in most livestock facilities but increasing fly resistance to chemicals has prompted the need for alternative pest control options. In January 2010, a series of laboratory and field experiments were conducted with the goal of improving alternative pest control methods on small equestrian facilities in North and Central Florida.

A one-year field study began in July, 2010 to determined adult fly population levels and development sites on four small equestrian farms. Weekly surveillance showed differences in adult fly population levels between farms and between seasons. Additionally, breeding was not confirmed on two of the four small farms suggesting that subtle differences in husbandry on these sites may affect the presence immature flies. The greatest numbers of natural parasitoids collected were of the genus *Spalangia.*

Oviposition preferences and development of house flies and stable flies were assessed in the laboratory on six substrates commonly found on equine facilities. Substrates with fresh manure were preferred for oviposition by house flies. Stable flies

were considerably more variable in selecting substrates for oviposition, though they generally chose aged substrates over the fresh manure. Stable fly development was greatest in substrates with fresh manure.

Parasitism by *Spalangia cameroni* of house flies and stable fly pupae was assessed at two different host: parasitoid ratios. Six field collected equine substrates were used to determine if substrate had an effect on the attraction of female parasitoids and resulting pupal mortality. There were no effects on host species but substrate and host: parasitoid ratio did affect progeny production and total mortality. There was no difference in parasitoid progeny production between host: parasitoid ratios.

Equestrian farm and horse owners in North Florida were surveyed to determine their presentations and current control methods of pests affecting their horses. Ignorance of IPM practices was evident as was an over-reliance on chemical insecticides. The lack of knowledge provides an opportunity for educational outreach to facilities and horse owners on IPM systems.

CHAPTER 1
LITERATURE REVIEW OF THE HOUSE FLY, MUSCA DOMESTICA AND STOMOXYS CALCITRANS AND ASSOCIATED PROBLEMS ON EQUESTRIAN FARMS

Filth flies, such as house flies, *Musca domestica* Linneaus, and stable flies, *Stomoxys calcitrans* L., are major pests of livestock and humans. These flies frequently aggregate in great numbers at small equestrian farms in Florida and can cause pain and loss of condition in horses and transmit pathogens to animals and humans. Because homeowners generally live in close proximity to pastures and stables, filth fly infestations can impact them and their horses. Traps, insecticides, biological control, and cultural methods often are used to manage these fly populations but their effectiveness has not been documented on small equestrian farms. Thus, there is a great need by horse producers and hobbyists for information about Integrated Pest Management (IPM) use in Florida's small equestrian farms.

Several pest management challenges face owners of small equestrian farms in Florida. These farms are generally built in both rural and suburban areas where there is a mixture of single-family homes, small farms, relatively large livestock operations, and natural areas. Due to the proximity of homes to pastures and other livestock areas, interactions with filth-breeding flies are unavoidable. Moreover, higher concentrations of animals, the owner’s lack of knowledge of effective fly control practices and their limited time to effectively manage the environmental conditions on the small farm may make these small equestrian farms more prone to infestations than larger equestrian farms. The particular needs of small farms for IPM recommendations cannot be satisfied until the sources of these flies have been determined and natural biological control

organisms evaluated. Methods for managing immigrant flies would be different from those for flies that breed on-site.

Life History

House flies and stable flies can be found in close association with livestock. While the feeding habits of adult house flies and stable flies differ dramatically, their larval breeding habitats and developmental cycles are similar. House flies tend to have more rapid development and are more fecund than stable flies, but both species can proliferate quickly on sites with ample breeding habitats.

Life History of the House Fly

The house fly is found in close association with humans and livestock all over the world. House flies feed on a liquid diet containing predigested material, such as feces, sputum, and decaying organic matter. House flies are common in the summer months in Florida (LaBrecque et al. 1972).

Development from egg to adult is relatively quick in ideal conditions and fecundity is high. Most females require approximately six days from emergence at 21^{o}C to develop the first egg batch (Krafsur 1985). Adults seek appropriate medium for oviposition which may include fresh manure, decaying vegetation, spilled feed, manure piles, bedding in stalls, human waste and compost. A female house fly may produce up to 1000 eggs in her lifetime (LaBrecque et al. 1972), laying 120 to 150 eggs per clutch (James 1947). Temperature influences egg production (Elvin and Krafsur 1984) and, at 21^{o}C, four days are required between egg batches (Krafsur 1985). Under optimal conditions, hatch occurs in 12 to 18 hours after oviposition and development to the third instar requires approximately three to four days (Hogsette and Farkas 2000). Minimum development time from egg to adult is 6.92 days at 33.2^{o}C (Larsen and Thomsen 1940)

but it increases at lower temperatures. The life span of an adult house fly has been estimated to be 10 to 14 days in the field (Hogsette and Farkas 2000).

Life History of the Stable Fly

Stable flies are obligate blood feeders that occur wherever livestock are found (Skidmore 1985). Cattle blood seems to be preferred but many other mammalian species, including horses, goats, sheep, swine, dogs and humans also are suitable hosts (Anderson and Tempelis 1970). Sutherland (1978) concluded that the blood of herbivores, such as horses and donkeys, resulted in higher egg production and survival than that of omnivores. Five blood meals are necessary for the production of the first egg batch, with an additional three being required for a second (Chia et al. 1982).

Under favorable conditions in Florida, stable flies are usually active in the warmer periods of winter months (Simmons 1944). Stable flies tend to be difficult to find during the hot summer months when house flies are numerous, as their optimal development temperature is lower (Romero et al. 2010). Surprisingly, however, stable flies have been found in March through October on Florida horse farms (LaBrecque et al. 1972).

The typical stable fly life cycle has been widely studied in the laboratory. To begin the life cycle, females emerge and lay eggs after a minimum five day preoviposition period (Hogsette and Farkas 2000). Simmons (1944) found the mean preoviposition period to be 10.6 days in the early summer and 13.2 days in the winter. A single adult female can lay a clutch of 60 to 130 eggs with a lifetime maximum of 800 eggs (Hogsette and Farkas 2000). Eggs hatch approximately 12 to 24 hours after oviposition (Larsen and Thomsen 1940). Gilles et al. (2005) found that larval development required 13 and 71 days when air temperatures were 30 and 15^{o}C, respectively, demonstrating that development time is influenced significantly by temperature. Pupation requires 12

to 13 days at 27^{o}C (Larsen and Thomsen 1940). The majority of adult flies emerge 14 to 18 days after they pupate (Simmons 1944). Under similar conditions, Hogsette and Farkas (2000) determined that flies emerge at least seven days after pupation. Emergence occurred sooner in substrates containing a mixture of hay and manure than in pure manure (Talley et al. 2009). The average life span of a stable fly is most likely less than two weeks in the field (Killough and McKinstry 1965), though Simmons (1944) estimated up to three weeks.

In substrate choice tests, optimal development of stable fly larvae occurs within a narrow range of temperature, pH, and moisture (Moon et al. 2001); however, they can develop in breeding areas with a variable range of physical conditions (Rasmussen and Campbell 1981). Development temperature ranged from 21.10 to 25.25^{o}C, pH from 7.13 to 8.23, and moisture content from 22.9 to 65.02% in field investigations. Recent investigations have shown that bacterial symbionts may play an essential role in the survival of stable fly larvae (Talley et al. 2009).

Dispersal

House flies and stable flies normally disperse from pupation sites. Both species exhibit abilities for long range dispersal, potentially aided by weather systems. Dispersal from development sites is often prompted by density dependent factors, such as limited oviposition sites or feeding opportunities.

House Fly Dispersal

House flies can disperse to adjacent farms or undergo longer flights to locate oviposition and breeding sites. Adult house flies can disperse at least 20 km, in search of food and oviposition sites (Bishopp and Laake 1921). Quarterman et al. (1954) showed that flies dispersed at random over an area of eight to 16 km in diameter and

tended to fly to areas with favorable food and breeding sites. House flies were recovered approximately 8 km from release points in less than 24 hours by Quarterman et al. (1954). This finding was supported by Greenberg (1971) who reported 2.3 to 11.8 km flights in 24 hours. While house flies clearly have extensive flight ability, most are recovered within 2.3 miles of release sites (Schoof and Silverly 1954). Movement between breeding sites on different farms is a common phenomenon (Quarterman et al. 1954; Hanec 1956; Wada and Oda 1963; Morris and Hansen 1966; Pickens et al. 1967). Lysyk and Axtell (1986) found that the majority of flies remain at their breeding sites, but movement occurred between research sites. In areas with fewer breeding sites, house flies disperse at least 0.5 miles within three to eight hours. The same distance takes one to 14 days in areas with many breeding sites (Pickens et al. 1967). In England, Denholm et al. (1985) found that farms which had the earliest spring population increases of house flies serve as sources for other farms 2.5 km away. Hogsette (1981) determined that flies found on cleaner equine facilities most likely migrate from surrounding horse farms, as no breeding was confirmed at the clean sites. More recently and in Florida, Burrus (2010) found that house flies were able to disperse 3 km from a cattle facility to a nearby town.

Stable Fly Dispersal

Although short-range stable fly dispersal flights are common, long distance flight also occurs. Field dispersal studies by Bailey et al. (1973) showed that flies could travel at least 3.2 km in search of a blood meal. Hogsette et al. (1987) estimated that stable flies move 5 km or more in search of a blood meal. Based on genetic analyses, much longer flight distance is possible (Jones et al. 1991; Szalanski et al. 1996). Hogsette and Ruff (1985) caught wild stable flies more than 225 km from their marking sites in

Florida after the passage of a cold front. Dispersal patterns and flight distances appear to be affected by weather patterns, fly density, movement of potential hosts, such as horses (Gersabeck and Merritt 1983; Hogsette and Ruff 1985), and the availability of oviposition substrates.

Oviposition and Larval Development

Oviposition and larval development sites of the house fly and stable fly contain varying amounts of decomposing organic matter. Larvae of both flies develop in many kinds of decomposing organic wastes, including manure mixed with straw and other bedding materials (Schmidtmann 1988; Hogsette 1995), manure mounds (Skoda et al. 1991; Lysyk 1993), spilled feed (Meyer and Petersen 1983; Skoda and Thomas 1993), decaying vegetable matter (Bishopp 1913; Pinkus 1913; Bishopp et al. 1915; Cook et al. 1999;) and community garden compost piles (Skoda et al. 1993).

House flies prefer fresh cattle manure substrates for breeding (Broce and Haas 1999). In the early 1900's, horse manure was characterized as a preferred substrate for oviposition and development of the house fly (Bishopp et al. 1915). Coffey (1951) also found horse manure to be an important breeding substrate. Yates and Lindquist (1952) noted that house flies are attracted to the ammonia released from horse manure. Fermenting horse manure was determined to be the main substrate supporting efficient development of house fly larvae (Artigas 1994), and Ehmann (1997) specified that fermenting, fresh horse manure is a favorite breeding medium of the house fly. However, Leikina (1943) showed that house fly larvae develop poorly in horse manure. In Chile, Larrain and Salas (2008) demonstrated that adult flies reared in horse manure were significantly smaller than those reared in pig, poultry and milking cow manure. In

the absence of abundant manure, house flies can develop in sand containing less than 0.5% dairy manure solids and less than 5% moisture (Hogsette 1996).

Although house flies and stable flies develop in similar substrates, stable flies prefer substrates containing some decomposing plant material (Meyer and Petersen 1983; Schmidtmann et al. 1989; Schmidtmann 1991; Skoda et al. 1991; Skoda et al. 1993), as opposed to pure manure. In Florida, the stable fly occurs frequently in decaying vegetation and manure mixed with hay (Hogsette et al. 1987). Skoda et al. (1991) found that an area with mixtures of dung and bedding material that were not compacted was most suitable for development. Stable flies were collected more frequently from manure sources containing straw or hay (Meyer and Shultz 1990), and Hogsette et al. (2011) found that hay increased suitability for larval development. Recently, in Kansas, stable flies have been observed to develop and overwinter in round bales of hay located in pastures (Broce et al. 2005; Talley et al. 2008).

Economic and Health Importance

Although the economic impact of house flies and stable flies to the cattle, swine, and poultry industries has been investigated to a relatively large extent, it is difficult to determine the impact to the equine industry as it is hard to measure losses in horse production. However, the costs of fly control measures, including insecticide sprays, equine equipment (such as fly blankets, masks, and boots), biological control products, and traps, can be calculated. The filth fly impact on the health and well-being of cattle has been researched extensively but limited information is available on the impact to horses. Injuries to horses caused by persistent fly avoidance behavior can cause temporary or permanent performance losses and both house flies and stable flies mechanically transmit many pathogens to humans and horses.

Economic Importance of Filth Flies on Equestrian Farms

Horse owners spend millions of dollars on pest control every year. It is estimated that over $40 million was spent in the United States for ectoparasite control on horses in 1997 (Geden and Hogsette 2001); with the increase in available pesticides and non-chemical control methods, including biological control, the current total is likely much higher. Horses are important and rich sources of protein for stable flies (Keiper and Berger 1982). Organic material produced by horses and horse facilities, such as manure and bedding, provide breeding sites for both stable and house flies

Health Importance of Filth Flies on Equestrian Farms

House flies and stable flies have a negative impact on the health and welfare of horses. Persistent feeding by flies leads to pain and irritation, and elicits natural pest avoidance responses from horses, including tail swishing, head and neck movements, twitching, stamping and shelter seeking (McDonnell 2003). Stable flies feed primarily on the legs and abdomens of horses, causing pain and losses of condition (Foil and Foil 1988). House flies, while not blood feeders, feed on matter found around muzzles, mucous from the eyes, and blood and serum secreted around open wounds (Dougherty et al.1993). Feral horses pressured by high fly populations may alter grazing behavior, reduce grazing time, and lower forage intake (Keiper and Berger).

The physical effects of filth flies on cattle have been evaluated to a greater extent than on horses. Dougherty et al. (1993) found that the avoidance behaviors of grazing cattle reduced the available energy for growth, reproduction and maintenance. Physical changes can occur in cattle under intense stable fly pressure, such as increases in blood cortisol concentrations, heart and respiration rates, and rectal temperatures (Schwinghammer et al. 1986). Berry et al. (1983) estimated weight reductions of feedlot

cattle by 0.652% for each stable fly. Campbell et al. (2001) saw a 19% reduction in weight of yearling grazing cattle as compared to the control group which was protected with insecticides. It is likely that many of these same physical changes would be observed with equines as well, negatively affecting their health and well-being. In horses, self-mutilation, such as biting their flanks, abdomen, shoulder and chest, can be caused by intense and unrelieved physical discomfort (McDonnell 2008). Additionally, as with other ungulates, severe fly persistence may interfere with the growth and heath of foals by not permitting mares to maintain appropriate distances or remain stationary long enough for foals to nurse (Keiper and Berger 1982). Because nearly 70% of horses in Florida are involved in showing and recreation (Florida Department of Agriculture and Consumer Services 2004), constant stomping, movement and reduced grazing can have serious effects on the performance of pastured and show horses, including long or short-term injury and loss of condition.

Transmission of many organisms that infect horses has been demonstrated with stable and house flies. Souma, also referred to as nagana and caused by the protozoan *Trypanosoma vivax* (Gardiner and Wilson 1987), is transmitted to livestock by stable flies in some parts of the world (Bishopp 1913). Filth breeding flies are mechanical vectors of both *Cryptosporidum* and *Giardia*, protozoan pathogens which can affect horses, humans, other livestock and some wildlife species (Conn et al. 2007). Equine infectious anemia can result from house fly and stable fly feeding (Greenburg 1971) and equine stomach nematode worms, otherwise known as *Habronema*, can cause digestive disorders, diarrhea, progressive weight loss, ulcers, colic and skin disorders and are transmitted by both house flies and stable flies (Conn et al. 2007;

Naem 2007). Arthropod hypersensitivity and pruritis (itching) in horses has been linked to stable flies (Gortel 2008).

In North Florida, homes on small equestrian farms are generally located in close proximity to pastures, stalls or run-in sheds, manure piles and other fly breeding habitats. Because of this association, homeowners have a high risk of exposure to the multitude of organisms that can be transmitted by filth flies to humans (Förster et al. 2007). Over 100 pathogens associated with house flies cause disease in humans and animals (Malik et al. 2007), including shigellosis, cholera and poliomyelitis (Greenberg 1973; Grübel et al. 1997; Graczyk et al. 2001). Fecal and other pathogenic bacteria, such as *Escherichia coli* and *Salmonella*, acquired by flies can be transmitted to humans when the flies land on residents or household surfaces (Mian and Jacal 2002).

Monitoring and Control

Many house fly and stable fly monitoring and control options have been developed and improved over the past several decades. This is because exposure to these flies can cause horses to become poorly conditioned and contract disease and exposure to residents of small equestrian farms can increase health risks. Consequently, these pests must be controlled by the most effective means including a variety of cultural, physical and mechanical, biological and chemical methods. All these practices are important considerations in an IPM program for filth flies on equestrian farms.

Monitoring House and Stable Flies

Numerous methods are available for monitoring filth flies. Effective monitoring depends on the objectives, such as optimizing trap placement, capturing male or female flies or evaluating environmental factors. Spot cards, grid counts and jug traps have been used as house fly monitoring devices, with all three methods being correlated and

suitable for monitoring programs (Lysyk and Axtell 1985). Conversely, Beck and Turner (1985) found poor correlations between house fly numbers and grid counts. The Scudder grid (Murvosh and Thaggard 1966; Scudder 1998) has been used outdoors but captures decrease over time and its efficiency changes depending on environmental factors. Lysyk and Axtell (1985) found that fly catches in baited jug traps and spot card indices change proportionally with changes in house fly density and proposed that these methods be used to monitor house fly populations in poultry houses. The alsynite-based Williams trap and subsequent modifications catch large numbers of stable flies which use it as a resting location (Williams 1973; Ruff 1979; Patterson 1981; Gersabeck et al. 1982; Rugg 1982; Hogsette 1983; Broce 1988). Traps that are visually attractive to the flies have been used extensively for monitoring (Berry et al. 1981; Black and Krafsur 1985; Scholl et al. 1985; Tseng et al. 1986; Hogsette et al. 1987; Thomas et al. 1989; Hogsette and Ruff 1990; Pickens et al. 1973). Geden (2005) found that for monitoring house fly populations jug traps were effective up to four days in the outdoor field, spot cards were impractical for field situations and a trap that incorporated Quikstrike™ baited strips (Wellmark International, Schaumburg, IL) was found to be suitable.

Cultural Control

Purposefully modifying the environmental conditions is a critical component to filth fly management. Currently, there is a need to educate small equestrian farm owners on how best to manage facilities to minimize fly breeding. Cultural control is the most important method for on-site reductions of stable flies (Greene 1993). The reduction of breeding sites, prevention of contact between flies and animals and protection of people are all important considerations when designing a fly control program (Keiding 1986).

Kaufman et al. (2005) found lower house fly captures in farms with the best sanitation practices. Pickens et al. (1967) observed that daily removal of most of the fly breeding wastes from a dairy complex reduced the house fly adult population to two thirds that of the less sanitary facilities. While house flies were affected, stable fly populations were not influenced by sanitary practices in Nebraska (Muma and Hixson 1949); however, more recently in a study by Catangui et al. (1995), sanitation in a beef cattle feedlot reduced adult stable fly populations to one-third that of the unclean lots.

Manure management is one of the primary methods of maintaining a clean environment unfavorable for filth fly breeding. A manure storage area which is dry and well ventilated is critical to fly management, as manure moisture promotes fly breeding (Watson et al. 1998). However, in Florida, a year-round warm and humid climate in addition to high annual rainfall makes maintaining dry conditions difficult. Covering manure piles with burlap or treated tarps to increase the temperature was shown to prevent exposure to pests and made the medium unsuitable for development (Fay 1939). An alternative to stacking manure in a pit is spreading it over a pasture. Spreading a thin layer of poultry manure on agricultural fields encouraged drying and reduced fly development (Axtell 1984). Watson et al. (1998) mechanically incorporated poultry manure at various depths in soil and concluded that, while the machines killed many thousands of immature flies, no depth was significantly better at reducing fly populations than the surface treatment. Correctly composting manure is an effective means for eliminating breeding sites of filth flies. Composting increases the internal temperature of the waste and lowers moisture content, rendering the substrate unsuitable for filth fly breeding. In Chile, aged compost from swine manure did not

support development of the house fly, presumably due to the low moisture content and nutritive value (Larrain and Salas 2008). Although composting and agitation of compost lowers fly numbers, these practices also reduce natural predators.

Cultural control practices can provide economical and effective ways to prevent and begin to manage insect populations and generally result in a cleaner environment. This is because house flies and stable flies prefer to breed in organic matter mixed with excrement. Eliminating these substrates is necessary to break the breeding cycle of these flies. Stalls and other livestock congregation areas provide ample opportunities for filth fly immature development. Burning or stacking hay bales and hay waste, placing hay on a mobile wagon and frequently relocating round bales may reduce house fly and stable fly breeding medium (Broce et al. 2005). Schmidtmann (1991) found that, of the bedding tested in calf hutches, sawdust and gravel bedding were the most effective at reducing fly numbers. However, there currently are no effective cultural control programs for house flies and stable flies found on pastured horses (Bram 1995).

Trapping

A variety of trapping methods have been developed and modified for the control of house flies and stable flies in livestock operations. Many traps are limited by location or environmental factors. Some pheromone-based traps tend to have a male bias in capture.

Traps for controlling house flies

As many as fourteen, if not more, different types of baited traps were used to capture house flies between 1900 and 1995 (Pickens 1995). Traps are suitable for use at specific locations and temperatures and may selectively capture flies according to age or sex. Baited traps have been improved with respect to characteristics, such as

color, bait, design and location (Pickens 1995). Alsynite traps are attractive to house flies in Florida (Geden 2006). Pickens and Miller (1987) reported that baited traps and sticky pyramidal traps captured large numbers of house flies on dairy farms. The spider web trap, a high capacity sticky trap, catches high numbers of house flies (Kaufman et al. 2005). Geden et al. (2009) tested a variety of trap types in Florida and determined that Terminator® style jug traps (Wellmark International, Schaumburg, IL) collected about twice as many house flies as Flies Be Gone™ traps.

Traps for controlling stable flies

Traps for the control of stable flies are more limited in number than those developed for the house fly. However, the alsynite trap developed by Williams (1973) has been used frequently and tested in the field for capturing stable flies. Alsynite reflects ambient light in a UV wavelength that is very attractive to stable flies (Agee and Patterson 1983). This trap was further modified into the cylinder type developed by Broce (1988) and found by Hogsette and Ruff (1990) to be as effective when coated with Olson sticky stuff™ (Olson Products, Medina, OH). Hogsette and Ruff (1996) determined that permethrin-impregnanted yarn wrapped around the trap killed incoming flies but did not affect its attractiveness. Koehler and Patterson (1982) reported control of stable flies on dairy farms in Florida using permethrin coated yarn on alsynite traps.

Alternatives to alsynite-based traps have been investigated for controlling stable flies. While the alsynite trap has been widely used, testing of the Bite Free® and EZ Trap® products developed by Farnam (Phoenix, AZ) indicated that materials other than alsynite are attractive to stable flies (Taylor and Berkebile 2006). Visual attraction to traps with the use of blue and black colors has been shown to improve collections. Laveissière and Grebaut (1990) found that the Vavoua trap, used for sampling tsetse fly

in Africa, baited with octenol was as effective, if not more effective, than the Williams trap for capturing stable flies. The UK Trigger targets yielded significantly higher stable fly catches than did the alsynite cylinder traps (Foil and Younger 2006) and the Broce trap was significantly less efficient than the Vavoua, Nzi and Williams traps for capturing stable flies on Reunion Island (Gilles et al. 2007). Additionally, the Broce trap was the least specific to stable flies. Other materials used for trapping stable flies utilized adhesive-coated corrugated boards (Cilek 2003) and coroplast (Beresford and Sutcliffe 2006) in a variety of colors. Treated cloth targets (Foil and Younger 2006) are a more recent control method which is more effective than the standard alsynite sticky traps. Hogsette et al. (2008) recommended cloth targets treated with λ-cyhalothrin or zeta-cypermethrin for integrated stable fly control programs.

Biological control

Biological control of filth flies is becoming a popular component in filth fly IPM programs on equestrian farms. In the early half of the 20th century, research on biological control of filth flies focused on a range of natural enemies in areas where flies were a nuisance. Biological control proven not to be a sole method for fly control, but can be effective when coupled with cultural and mechanical control and the careful use of insecticides. The use of pathogens, predatory mites and entomopathogenic fungi has been investigated to some extent. More recently, research has centered on pupal parasitoid species primarily in the family Pteromalidae. Evaluations of life history and parasitization rates generally have been focused on the genera *Muscidifurax* and *Spalangia*. Given suitable habitats, hosts and locations, these parasitoids have the potential to suppress populations of filth flies (Morgan et al. 1975; Morgan and

Patterson 1977; Pickens et al. 1975; Morgan and Patterson 1977; Rutz and Axtell 1979; Propp and Morgan 1985; Pawson and Petersen 1988; Geden and Hogsette 2006).

Natural parasitism

Parasites, parasitoids and predators naturally contribute to regulation of filth fly populations (Geden et al. 1988; Rutz and Scoles 1989); however, due to inadequate cultural control and livestock management practices, these natural enemies are not able to suppress explosive filth fly populations. Other forms of filth fly biological control, such as pathogens, fungi, parasites and predators are common in nature and have been studied to some extent. The species composition of natural enemies appears to be influenced by local environmental factors, but is generally dominated by hymenopteran parasitoids in the family Pteromalidae. Butler et al. (1981) found *Spalangia cameroni* Perkins and *S. nigroaenea* Curtis to have the highest rates of natural house fly parasitism in Florida. To determine natural parasitism rates in the winter months, Greene et al. (1989) collected house fly and stable fly pupae from northwestern Florida dairies. *S. cameroni* was the dominant species, accounting for 58% of house fly and 76% of stable fly parasitism. Parasitism evaluations by Jones and Weinzierl (1997) and Meyer et al. (1990, 1991) determined that *Spalangia* spp. were most abundant in Illinois and California. Dominance by *Spalangia* spp. in Florida may be due to the higher humidity and temperature requirements of this genus relative to other parasitoid wasps (Ables and Shepard 1974).

Natural levels of parasitism by filth fly parasitoid wasps tend to be fairly low. Olbrich and King (2003) found 17% natural parasitism of house flies and 12% of stable flies in Illinois. Similar findings occurred in Nebraska where Petersen and Meyer (1985) saw 14.2% natural parasitism rates for house flies and 7.9% for stable flies. In their

study, *Muscidifurax* spp. made up 56% of the recovered parasitoid species. Skovgard and Jespersen (1999) found 12.7% overall parasitism from all species in Denmark. Romero et al. (2010) observed 26.9% natural house fly parasitism and 26.7% stable fly parasitism at the University of Florida Dairy Unit, with *Spalangia* spp. accounting for 85.7% of total parasitism. At that location, *S. endius* Walker attained 33.9% house fly parasitism and 27.3% stable fly parasitism, while *S. cameroni* had 27.9% and 40.6% parasitism levels for house and stable flies, respectively. *Spalangia endius* was most common during the winter months. On large equestrian farms in Florida, Pitzer et al. (2011b) found 5 to18% parasitism of house fly pupae and 7 to18% of stable fly pupae, overwhelmingly parasitized by *Spalangia* spp.

Augmentation of parasitism

Due to the development of resistance by house flies and stable flies to insecticides and the desire of owners and operators of small equestrian farms to rely less on chemical insecticides, augmentative biological control of filth flies has become a popular alternative for integrated pest management programs. Pupal parasitoids have been purchased and released on horse farms for many years but their effectiveness has been demonstrated on mostly swine, poultry and cattle facilities. The studies have yielded mixed results with suppression of fly populations by augmentative releases of parasitoids in some situations (Morgan and Patterson 1990; Geden et al. 1992; Petersen and Cawthra 1995; Crespo et al. 1998, 2002; Skovgard and Nachman 2004) and failures in others (Morgan et al. 1975; Morgan 1980; Meyer et al. 1990; Andress and Campbell 1994; Weinzierl and Jones 1998; McKay and Gallowway 1999; Kaufman et al. 2001). These confounding results in the use of hymenopteran pupal parasitoids may be partially attributed to environmental factors which affected their abundance and

distribution (Skovgard 2004). Site-specific environmental factors include sensitivity to insecticides, unfavorable hosts, low quality commercial colonies, microhabitat preferences and a lack of optimal timing and methods of release (Peterson and Meyer 1985; Patterson and Rutz 1986). Additionally, it is possible that existing active parasitoid populations may inhibit the establishment or function of a newly released species (Legner et al. 1990). Quarles (2006) suggested that the success of a biological control program using pupal parasitoids relies on matching the released species with the climate and habitat of the release location, generally by deploying endemic species. An in depth understanding of the ecology of the habitat of release is necessary to increase the likelihood that the released parasitoid will be effective in a given situation (Smith and Rutz 1991a, 1991 b; Jones and Weinzierl 1997). Greene et al. (1989) determined the rates of parasitism varied depending on habitat and substrate preference. Moisture content of the substrate was a primary factor in parasitism rates for several parasitoid species (Geden 1999), *Muscidifurax raptor* Girault and Sanders preferring 45% to 75% moisture content and three *Spalangia* spp. preferring 45% to 65%. Several species of *Spalangia* located pupae down to 2-cm below the surface of the substrate, while *M. raptor* parasitism rates where high with pupae located on or near the surface (Geden 2002). Thus, greater control may be achieved by releasing a variety of species.

Chemical Control

Historically, chemical control has been the primary method for managing filth flies in livestock operations. Temporary reductions in population levels may be observed; however, increasing costs of insecticide formulations and target insect resistance has reduced the effectiveness and applicability of chemical applications on these facilities.

Application

Management of large populations of filth flies has historically required the application of insecticides. Chemical insecticides have been developed in a variety of forms with varying modes of action. Typically, larvicides, residual treatment of resting-sites, impregnated strips or cords, toxic baits, space treatments and fumigation have been used with limited success. Chemical treatments may reduce flies to acceptable numbers temporarily on some farms; however, the increasing cost, awareness of toxicity to non-target species, environmental pollution and an increasing tolerance of flies to insecticides precludes their extended sole use (Pickens and Miller 1987; Cilek and Greene 1994; Kocisova et al. 2002; Marcon et al. 2003; Malik et al. 2007).

Resistance

Muscoid flies have been exposed to high levels of insecticides and extensive resistance has been documented for a variety of chemicals. Formulations of chemical insecticides and repellents are available which affect the insect nervous system, energy production, endocrine system, or water balance (Malik et al. 2007). Surveys of house fly populations have revealed widespread resistance to organophosphorous and carbamate insecticides (Harris et al. 1982; Chapman 1984; Chapman and Morgan 1992; Chapman et al. 1993). Pyrethroids are a common ingredient in fly repelling sprays for horses and are less toxic to animals and people than many alternatives. The overuse of permethrin in intensive animal units quickly led to high levels of pyrethroid resistance (Webb et al. 1989; Marcon et al. 2003). Because of observed resistance levels, application rates have been increased with ultimate loss of effectiveness (Malik et al. 2007). Resistant populations of house flies have been collected from dairy facilities, cattle feedlots, poultry facilities and pastured cattle (Schmidt et al. 1985;

Crosby et al. 1991; Cilek et al. 1991; Kaufman et al. 1999; Scott et al. 2000; Kaufman et al. 2001; Barros et al. 2000). Significant tolerance to beta-cyfluthrin, permethrin, imidacloprid and nithiazine was found in house flies in Florida dairies (Kaufman et al. 2010). Pitzer et al. (2011c) determined that field collected stable flies from equine facilities in Florida were resistant to permethrin. In Kansas, stable fly populations exhibited resistance to fly control products in facilities which had never used insecticides (Cilek and Greene 1994). This indicated that there was fly dispersal between farms, establishing the need for area wide control. The rise in resistance to virtually all insecticide formulations and continuous long term use of any product to control filth flies in intensive animal units eventually leads to failure (Sheppard et al. 1989; Kaufman et al. 2010).

Integrated Pest Management

Integrated Pest Management (IPM) includes a system of methods for prevention and control of insect pests including, detecting and identifying pests, monitoring their abundance, establishing threshold levels, selecting the best control methods and evaluating the results. The goal is to implement the pest combination of pest management strategies which reduce the economic, health and environmental risks while maximizing control.

An IPM program involving sanitation, mechanical control and biological control has the potential to significantly decrease reliance on chemical insecticides for control of filth flies. Sanitation was found to be the critical factor in on-site reduction of flies (Greene 1993) and can interrupt the life cycle of the pest flies by limiting oviposition or larval development. Traps and other mechanical control methods can decrease adult fly numbers and the use of parasitoids may reduce the survival of pupae. However, it is

necessary to understand house fly, stable fly and parasitoid bionomics because the effectiveness of the parasitoids depends on environmental and host factors. Even if a biological control program is successful initially, immigrating flies can rapidly increase fly populations (Quarles 2006), so an integrated approach to filth fly control is required. In some situations, chemical control is applicable; therefore an IPM program will not eliminate pesticides, but instead requires careful consideration of their use. An understanding of house fly and stable fly biology, monitoring and control methods is compulsory for small equestrian farm owners to effectively develop and implement an IPM program for filth flies.

Research Objectives

Research on the biology and management of filth flies in association with cattle is abundant; however, a large gap exists in knowledge of house flies or stable flies found in equine facilities, especially small farms. Additionally, while commercial parasitoids often are used as biological control agents for filth flies in equine facilities, little quantitative information exists on how they should be deployed and evaluated for effectiveness. My specific research objectives were to:

- Determine the number of adult house and stable flies present on small equestrian farms during the year, levels of fly breeding and occurrence and species composition of natural parasitoids.
- Quantify oviposition preferences and larval development of house flies and stable flies on substrates found commonly on equestrian farms.
- Evaluate the ability of the filth fly parasitoid, *Spalangia cameroni*, to locate and parasitize house fly and stable fly pupae in common substrates found on equestrian farms.
- Design and conduct a survey for small equestrian farm owners and operators on their perceptions, knowledge and management of pest problems and control in North and Central Florida.

CHAPTER 2
SEASONAL ABUNDANCE OF HOUSE FLIES AND STABLE FLIES AND THEIR PUPAL PARASITOIDS ON SMALL EQUESTRIAN FARMS IN NORTH FLORIDA

Introduction

House flies, (*Musca domestica* L.) and stable flies, (*Stomoxys calcitrans* L.) are major pests on equestrian facilities in Florida. These flies are not only a nuisance, but also are capable of mechanically transmitting many dangerous pathogens, such as *Escherichia coli* and the retrovirous responsible for equine infectious anemia (Malik et al. 2007, Greenburg 1971) to humans and horses. Many options are available for establishment of an integrated pest management (IPM) plan for filth flies on livestock operations, but the majority of these methods have not been evaluated on equestrian farms.

There is limited information on the status of filth fly population fluctuations and natural parasitism on equestrian farms in Central Florida. Pitzer et al. (2011a) determined that both house fly and stable fly adults were present year round on large equestrian farms (>200 acres) in Ocala, Florida, but significant differences were found in numbers of adult collections, even in these larger facilities. There are many contrasts in husbandry between large and small equestrian farms which could affect population levels and on-site breeding of filth flies, and therefore natural parasitism and success of augmentation programs. Because most of these small farms in North Florida are owner-operated, the variety of management methods may influence on-site fly breeding significantly. Differences in location, neighboring properties, habitat type and suitable substrates may exist between small equine facilities, which may affect the attractiveness of one facility verses another, the suitability for house fly or stable fly breeding and the parasitoid species composition or abundance.

Biological control is an important part of a comprehensive IPM system. Hymenopteran pupal parasitoids often are proposed for use for fly control and are becoming increasingly popular on equestrian farms (United State Department of Agriculture 2006; Chapter 5). However, applications of pupal parasitoids on livestock facilities have produced mixed results (Petersen et al. 1983; Meyer and Petterson 1990) and have not been evaluated on equestrian farms. The success of pupal parasitoids as biological control agents depends on accessibility of hosts, both temporally and spatially, and may be influenced by microhabitat associations (Greene et al.1989). Host availability for parasitism is limited by fly breeding in the available substrates on equestrian farms. Before augmentative control programs can be initiated with pupal parasitoids, an assessment of potential breeding and development habitats and the seasonality and species composition of natural parasitism is required.

House and stable flies routinely disperse from development sites in search of a suitable feeding and oviposition locations (Quarterman et al. 1954). Equestrian farms with insufficient sanitation may experience increasing fly pressure as flies immigrate to colonize suitable substrates. Conversely, farms with satisfactory cultural control practices that limit breeding areas (Kaufman et al. 2005), may experience increased fly infestations from adult populations arriving from off-site locations. Knowledge of the breeding locations and if, and to what degree, breeding is occurring on-site is crucial to the success of an augmentative biological control program using pupal parasitoids.

Developing socially, environmentally and economically feasible pest management strategies depends on understanding pest and parasitoid population fluctuations, weather effects and associated environmental conditions (Geden and

Hogsette 2001). However, a gap exists in knowledge about population levels and breeding habitats of filth flies on small equestrian facilities. The status of filth fly breeding on small equestrian farms is required to assess appropriate IPM control methods and determine if augmentation of natural parasitoids would be effective as hymenopteran parasitoids only attack the pupal stage of filth flies. A 1-year study was initiated in July, 2010, in Alachua County, Florida, to (1) assess adult and immature population fluctuations of house flies and stable flies, (2) identify suitable breeding substrates on small equestrian farms and (3) determine the species composition and seasonal distribution of parasitoids of house and stable flies on four comparable small equestrian farms.

Materials and Methods

Small equestrian farms. Four comparable small equestrian farms (sites) were located in Alachua County, Florida for this study. Small equestrian farms were defined as between 2 and 4 hectares in size with at least one horse and one pole barn or other structure for shade, open pasture and a single-family residence. Study farms were approximately 20 to 40 km west and northwest of Gainesville, Florida, and each farm was a minimum of 8 km from another study site. These farms were managed according to a set of prescribed protocols (Appendix A). Criteria included horse density (no more than 2 horses/0.4 hectares of pasture), pasture management (no fertilizer), run-in shed management (routine cleaning), horse management (stalled no more than 12 h per day), stall management (daily cleaning), fly trap placement and monitoring (by E.T.M. only), water trough management, manure management and hay and feed management. Each equestrian farm varied slightly in size and number of horses; site A: 4 hectares with 2 horses, site B: 4 hectares with 3 horses, site C: 2 hectares with 3

horses, Site D: 2 hectares with 3 horses. Only site A and B routinely stalled the horses for 12 hours a day or less in sand bedding. At site D, stalls were used infrequently for horse injury recovery or foaling of pregnant mares, and at site C the horses were allowed constant access to pasture. At sites A, B and D, waste was removed daily from stall or shed areas when in use and deposited into a manure pile. Grain concentrate and hay was fed at each site, though coastal Bermuda round bales were fed at site C. A timothy/alfalfa hay mix or perennial peanut by the flake was fed at the other sites. Each study site was within 1 km of a facility with cattle or horses, characteristic of small equestrian farms in North and Central Florida.

Adult fly surveillance. Adult fly activity and population levels were surveyed weekly for one year beginning in July 2010. An alsynite trap (Broce 1988) (Olson Products Inc., Medina, OH) as described by Hogsette and Ruff (1990) was placed at each of three fly congregation areas or other problem spots on each of the study sites to monitor stable flies. The cylindrical corrugated fiberglass traps (30-cm high x 20-cm diameter) each were mounted on a 5-cm wide x 5-cm deep x 120-cm high wooden stake placed 30 in the ground. The trap height was maintained at 90-cm from the ground (J.A. Hogsette, person. comm.) (Figure 2-1). The outer surface of the trap was covered with an adhesive on a clear polypropylene sleeve (Olson Products Inc., Medina, OH) attached to the fiberglass with metal clips. This sticky sleeve was replaced weekly and the used sleeves were returned to the laboratory for counting. In addition, three Captivator® jug traps (Farnum Companies Inc.,Phoenix, AZ) containing 30 mL Starbar® Fly Terminator Attractant (Farnum Companies Inc., Phoenix, AZ) and mixed with 1 L of tap water were placed near the sticky traps in fly congregation areas

to monitor house flies. If trap locations were in pastures, barriers were built to deter animals from interacting with the traps. On collection dates, Captivator® traps were emptied and captured flies were identified to species and counted in the field. The attractant and water mixture was replaced with fresh materials for the following week. Flies collected from all six traps at each farm were recorded by species, number, location and date and the average fly numbers for both adults and immatures was used for the analysis.

Immature fly and pupal parasitoid surveillance. Pupae were collected weekly on each farm. Fly development areas were identified from preliminary investigations prior to the initiation of the study; however, because development areas are often ephemeral, each property was surveyed again for active areas of immature development prior to each collection. Development sites were classified into the following 6 types, which were commonly found on all of the sites: (1) hay, aged + urine and manure from the apron surrounding a Bermudagrass hay round bale in the paddock, (2) pure manure (undetermined age) from various locations in paddocks, (3) pine shavings + urine and manure (<12 h old) from a stall, (4) pine shavings, aged manure pile + urine and manure (>72 h old), (5) soil, aged manure pile + urine and manure (>72 h old) (Figure 2-2) which was builders sand (site A) or natural soil (site B) and (6) soil, aged dirt lot + urine and manure (Figure 2-3) from an overgrazed paddock with manure waste of variable age (site C). The substrates labeled as containing soil were either naturally present ground soil or an artificially added builder's sand. Both were collectively termed soil for this study. Substrates were determined to fall in these categories by personal observation and interviews with property owners. These

substrates were not available consistently on all sites nor did all sites have every substrate. A record of the collection substrates was kept to determine differences in sanitation between habitats within farms and between farms. Collection was with a trowel as described by Hogsette et al. (2011) because the variety of substrates on each farm made flotation of pupae in water difficult. Regardless of availability, the search for pupae was terminated after ½ hour or when 100 newly formed (light brown) pupae were recovered per site (Romero et al. 2010, Pitzer et al. 2011a).

Collected pupae were held in a cooler with ice packs at approximately 10^{o}C and returned to the University of Florida IPM laboratory the same day of collection to be cleaned by flotation and air dried. Pupae that were damaged or had previous parasitoid emergence holes were discarded (Petersen and Meyer 1985). Once dried, pupae were placed individually in size #0 gelatin capsules and held at room temperature (22 to 25^{o}C) for emergence of a fly or a parasitoid (Morgan et al. 1989). Emerged parasitoids were identified to species using the keys of Rueda and Axtell (1985).

Environmental conditions. To account for weekly effects of temperature and precipitation on adult prevalence, larval development and parasitism, a rain gauge and maximum and minimum thermometer (Taylor Precision, Oak Brook, IL.) were installed at each site on 5-cm wide x 5-cm deep x 120 high-cm wooden stake placed 30-cm in the ground to maintain a height of 90-cm above the surface (Figure 2-4).

Statistical analysis. Data were normalized with a natural log transformation and subjected to analysis of variance (ANOVA) using JMP v.9 software (SAS Institute Inc., Cary, NC 2010). Back transformed data are presented in tables and figures. Means of adult fly and pupal collections from the four sites were compared between months using

Tukey's Honestly Different Separation Test at $P \leq 0.05$. Because many equestrian farm owners have difficulty identifying between fly species (Chapter 5), adult house fly and stable fly collection numbers were combined for analysis to provide more useful information on fly population abundance and fluctuations for farm owners (Table 2-1). Overall percent parasitism was calculated by dividing the number parasitoids emerged by total numbers of pupae collected and percent parasitism by species was calculated by dividing the number of parasitoids from a particular species by the total number of emerged parasitoids. Monthly population fluctuations of house flies and stable flies are presented graphically along with monthly precipitation totals and mean temperature.

Results

Adult fly surveillance. A total of 36,379 adult flies were captured on the small equestrian farms throughout the study; 30,745 house flies and 5,634 stable flies (Table 2-1). As expected, the main effects model was significant for month, site and month x site interactions ($F_{47,146}$=7.25; P=<0.0001). There were no significant differences in mean adult fly captures between farms for six months out of the 12-month study. Significantly fewer flies were captured at site C than the other sites during August and September, 2010. From April to June, 2011, significantly more flies were captured at site D than at the other three sites. The means of adult flies captured from April to June 2011 at site D were at least 4-fold higher than other monthly means.

Individual sites generally followed similar trends of adult fly captures with some divergence. Sites B and C had similar patterns of house fly population fluctuations (Figure 2-5), whereas site A had a sharp increase in house fly numbers in September and site D and increase in April, 2011. Stable fly captures at site C had a sharp peak in November, 2010 which was not similar to with the remaining sites (Figure 2-6).

However, the other sites followed months followed similar capture trends for the remaining months. Generally, stable fly captures were higher in May, 2011 through June, 2011 on all sites.

Pupal surveillance. A total of 1,807 pupae were collected throughout the study, but no pupae were recovered on sites A and B (Table 2-2). There were 1,282 pupae collected from site C with about half as many house flies as stable flies collected (*n*=407 and *n*=875, respectively). A total of 535 pupae were collected from site D, 322 house flies and 203 stable flies. On site C, average pupal collections were highest in March, 2011 (89.8) and lowest in September, 2010 (6.8), October, 2010 (9.0) and June, 2011 (45.3) (Figure 2-7). Collection averages on site D steadily increased from March, 2011 to a high in June, 2011 (65.3). Both sites exhibited high variability in average monthly pupal collections in April to June,

2011. As expected, there were significant effects of month on total pupae collections between sites C and D and a month x site interaction factor in the ANOVA. There was no significant difference between sites in total number of pupae collected (*P*=0.6337).

There were differences in pupal collections by substrate (Table 2-3). The highest percentage of pupae collected on both sites C and D was from the hay + urine and manure substrate (57.7%) though this substrate was dominated by stable fly pupae (81.5%). Only 1.2% of total collections were recovered from pure manure sources. A total of 56.7% of the house flies were recovered from the pine shavings + urine and manure (<12 h old), only 14.4% of total stable fly collections were from this substrate. The pine shavings, aged manure pile + urine and manure (>72 h old) had higher house

fly collection than stable fly (18.0% and 3.5%, respectively) but only accounted for 9.4% of total pupal collections. Soil-based substrates, soil, aged manure pile + urine and manure (>72 h old) and soil, aged dirt lot + urine and manure, had no recovered house fly pupae and minimal numbers of stable fly pupae (*n*=7), only equaling 0.3% of total collections.

Natural parasitism. A total of 115 total parasitoids were recovered from pupal collections representing five species (Table 2-2). Natural parasitism averaged 2.3% at site C and 16.4% at site D. *Spalangia cameroni* Perkins made up 86.1% of the overall parasitism on sites C and D combined, or 90.0% on site C and 83.7% on site D. Each additional parasitoid species was isolated to a single recovery and collection date; 8 *S. endius* recovered from house fly pupae on site D, 6 *Muscidifurax raptor* Girault and Saunders recovered from house fly pupae on site D and 2 *Aleochora* spp. (Coleoptera: Staphylinidae) recovered from stable fly pupae on site C. There was a greater percent parasitism in the pure manure (53.0%) than the remaining substrates (Table 2-3). Parasitism was 11.3% in the pine shavings + urine and manure (<12 h old) but very low parasitism was recorded in the remaining substrates. Overall, natural parasitism was 6.3% in all substrates.

Environmental conditions. There was considerable variation in weather by month and farm. Average monthly temperatures ranged from 10.7 to 33.5^{o}C (Figure 2-8) but sites were not statistically different from one another (*P*=0.9796). Total annual precipitation ranged from 94.6-cm (site B) to 101.1-cm (site A) and was highly variable by month between sites, with total precipitation in some months differing by >10-cm between farms (Figure 2-9).

Discussion

Adult surveillance. Adult flies were collected throughout the study and the number of house flies was greater than stable flies on all sites. Months of peak collection for each species differed with stable flies being more prominent in the spring months. This corroborated the results of Pitzer et al. (2011a) on larger equestrian facilities (>80 hectares) in Central Florida and Labrecque et al. (1972) who observed stable flies on equine facilities from March through October. The population peaks of both fly species differed by site and were not limited to farms with identified on-site fly breeding. No on-site development of immature flies was found on sites A and B, however there were no significant differences between the mean of total adult fly captures at all 4 sites during 6 of the 12 months of the study. The mean number of flies captured on site A in September 2010 was significantly higher than those captured at the other sites (Table 2-1). Therefore, there is a strong indication that the adult flies captured on sites A and B were dispersing from nearby cattle facilities to both sites A and B. Pitzer et al. (2011c) found that many stable flies captured on equestrian farms in Marion County, Florida had recently fed on bovine blood. Both house flies and stable flies have shown to move several miles from development sites (Pickens et al. 1967), so movement from pasture cattle to the small equestrian farms in the current study would not be unexpected. Furthermore, horse manure is a strong attractant for stable flies and preferred over cattle manure for breeding (Jeanbourquin and Guerin 2007). Small equestrian farm managers implementing cultural control practices may mistake the continued appearance of high numbers of flies around their horses as originating from their facility. This may give managers the impression that their efforts are ineffective or they may be more inclined to purchase pupal parasitoids for control, which will not be

effective if flies are immigrating to the sites. Off-site dispersal from bordering livestock facilities was strongly implied but not confirmed in this study,

Pupal surveillance. There was a dichotomy between farms in pupal abundance. There were no pupae collected on sites A and B throughout the study, but pupae were regularly found in substrates on sites C and D. This was in contrast to studies of large equestrian farms in Central Florida where Pitzer et al. (2011a) obtained pupae from each of the four study sites. Sanitation practices varied between study locations and may have significantly influenced fly breeding. Differences in sanitation practices including manure removal, bedding selection and hay feeding may account for these discrepancies. At sites A and B, manure from stalls and other accumulation areas was frequently removed and placed in large manure piles, a practice which was not employed at sites C and D, where horses were not consistently stalled. There was a low ratio of horses per acre on both sites A and B as well (approximately 1:5), unlike sites C and D which had a ratio of 3:5 acres, and this may have reduced manure accumulations in pasture areas for sites A and B. Both sites A and B utilized soil-based bedding and manure piles and, as seen in Chapter 3, soil-based substrates were not found to be effective for attracting oviposition of house flies and for the most part stable flies, nor for sustaining development of either species in the laboratory. Additionally, at site A or B horses were not fed with hay in round bales, the primary substrate from which stable flies were recovered on site C.

Pupal collections of both species differed by substrate. House fly and stable fly breeding was found in substrates on the study sites which generally corresponded to previously published literature and the results presented in Chapter 3. Fresh manure

has been reported to be a preferred substrate of house flies. Broce and Haas (1999) determined that house fly oviposition and development was most frequent in fresh cattle manure. The majority of house fly pupal collections in the current study were from the pine shavings + urine and manure (< 12 h old). This substrate was found to be preferred for oviposition by house flies in Chapter 3. An overwhelming majority of stable fly collections were from the hay-based substrate with smaller numbers from the pine shavings + urine and manure (<12 h old). The fresh pine shaving substrate was found to be a preferred for stable flies in Chapter 3 and the hay, aged + urine and manure was not statistically different from preferred substrates. In laboratory studies, substrates collected from the field were homogenized and the samples of hay were from a limited range around round bales. However, Talley et al. (2009) determined that there were zones of waste surrounding round bales and gravid stable fly females have the opportunity to select the most suitable substrates in the field on site C. Because hay round bales have recently been determined to be important overwintering habitats of stable flies in Florida (Broce et al. 2005), it is likely that this is an important breeding substrate on equestrian farms. Control of pest flies on equine facilities could be improved with future field practices and characterization of house fly and stable fly breeding. Sanitation could be improved on equestrian farms in Florida and areas evaluated for potential parasitoid release sites.

Even minor changes in use of the property and husbandry had large effects on both adult fly populations and breeding on these small equestrian farms. On site C, two hay round bales were placed in the paddock in October, 2010 and a subsequent spike of stable flies in November, 2011 and pupae in December, 2011 was recorded.

Similarly, a rise in the house fly population on site D in April, 2011 could be because of the use of two stalls with pine shaving bedding for a pregnant mare from March, 2011 until May, 2011. Subsequently pupal collections increased in April through June, 2011. Conversely, on site A where sanitation practices were more rigorous than sites C and D, these management changes did not affect breeding, only adult fly numbers. Site A exhibited a large peak in adult house fly populations in September, 2010 which corresponded to neighboring cattle being moved to an adjacent property for 4 weeks. Management decisions may have quick impacts on populations of adult and immature flies. Increases in fly numbers as a response to management changes supports the importance of sanitation on suppressing on-site production of filth flies, as previously observed by Pitzer et al. (2011a) and Kaufman et al. 2005. Further examination of how changes in management affect fly attraction to small equestrian farms and subsequent breeding could provide useful information on preemptive control to reduce these risks.

Natural parasitism. *Spalangia cameroni* accounted for the overwhelming majority of the emerged parasitoids collected on sites C and D. Other species collected were only present during the single sampling event at which they were recovered. The high recovery of *S. cameroni* is consistent with the results of Pitzer et al. (2011a) who recovered nearly 100% *Spalangia* spp. in the two year study on large equestrian farms in Central Florida. Greene et al. (1989) found 69% of parasitism was attributed to *S. cameroni* on dairy farms in the winter and spring months, however Romero et al. (2010) reported only 30.5% parasitism by *S. cameroni* on the University of Florida Dairy Research Unit. *Spalangia cameroni* prefers loose substrates (Smith and Rutz 1991b) and has demonstrated an ability to parasitize deeper in substrates, such as those on the

equestrian farms where pupae were collected in the present study. A field study in Nebraska found the majority of *S. cameroni* recovered from deeper substrates than were *Muscidifurax raptor* (Rueda and Axtell 1985), a finding which was supported in the laboratory by King (1997) and Geden (2002). In Chapter 4, I report that *S. cameroni* appears to be an appropriate biological control agent of house flies and stable flies in equestrian substrates. This corroborates the findings of Pitzer et al. (2011b) who tested *S. cameroni* in pine shaving waste from equestrian farms and found this species to be suitable and more effective than *M. raptor* at host seeking. The dominance of *S. cameroni* in this study and that of Pitzer et al. (2001b) supports the use of this species for augmentative releases on small equestrian farms.

Overall, the natural parasitism levels of house fly and stable fly pupae were low. This corresponded with Pitzer et al. (2011a) who found between 5 and 18% parasitism in house fly pupae and 7 to 18% parasitism in stable fly pupae on large equestrian farms, but this is low comparative to a natural parasitoid study conducted by Romero et al. 2010 on the University of Florida Dairy Research Unit where natural parasitism was > 26%. This discrepancy is possibly due to the long-term establishment of the substrates at the Dairy Research Unit versus the ephemeral substrates at the small equestrian farms. There was no consistency in peak months, seasons or habitats of parasitism between farms. Parasitism was highest in pupae from pure manure and the pine shavings + urine and manure (<12 h old), but there were too few samples with recovered parasitoids to elucidate if this pattern would continue in the field. It is likely that the temporary nature of suitable fly breeding substrates on small equestrian farms

does not support high levels of parasitoid reproduction, so targeted parasitoid releases may be beneficial for maintaining controlling levels.

Environmental conditions. Temperature and rainfall have been important factors influencing dispersal, development sites and adult fly prevalence (Elvin and Krafsur 1984, Krafsur et al. 1985, Hogsette and Farkas 2000, Larsen and Thompsen 1940). Additionally, development and oviposition of parasitoids has been determined at various optimal temperatures which are species dependent (Mann et al. 1990, Geden 1999). Temperature and rainfall did not differ in annual totals; however, there was variability between sites on collection dates. This is the first study to my knowledge to use site specific weather stations at each equestrian farm in North and Central Florida. Though sites were close to one another in Alachua County (8 to 15 km), monthly precipitation totals between sites differed by as much as 10-cm. However, pupal collections were too inconsistent between sites to determine if variations in environmental conditions between farms had significant influences on total fly captures and breeding. It is likely these site and monthly differences may affect breeding site suitability between farms, and even with ideal sanitation, adjacent properties produce high populations of flies (Taylor et al. 2007). This would increase the risk of colonization on neighboring small farms. Continuing evaluations of the breeding habitats on small equestrian farms for a longer duration than this study may provide the necessary data to make statistical connections between population levels and environmental conditions.

Management implications for small equestrian farms. Cultural control practices and the use of biological control have been successful at managing filth fly breeding populations on certain livestock facilities. However, assessments of the breeding status

and adult fly population levels and fluctuations on equestrian farms has been neglected and as such, it is unknown if the use of pupal parasitoids as biological control agents would be effective on these farms. In this study, variation was found between farms in filth fly pupal production, two sites being with and two without suitable breeding. Moreover, parasitism on the sites which produced pupae was low and dominated by *Spalangia cameroni.* This suggested that individual small farms differ in fly production and thus monitoring and management of pest flies will differ by facility.

Fly development was not found on sites using soil-based substrates as bedding, whereas pine shavings + urine and manure (<12 h old) was a frequent producer of pupae. The use of soil and other alternative bedding substrates has been found to be effective in the field in calf hutches (Schmidtmann 1991). The simple replacement of straw with sand resulted in a 76% reduction in house fly larvae and 100% reduction in stable flies. However, the contrast in husbandry and management between facilities where calves are held in constant confinement and horse facilities may not produce the same results. Based on results of the current study where breeding was not found on sites where horses were stalled on soil, bedding substitution warrants further investigation. Round bales produced high numbers of pupae. For sites where round bales were fed, management options to reduce fly breeding include frequent relocation of new round bales, feeding cones to limit waste and frequent manual removal of waste (Broce et al. 2005).

Though adult flies were captured in relatively similar levels between sites, flies were present on farms with no identifiable development sites. The discrepancy in adult fly numbers and breeding supports the need for equestrian farm owners on small farms

to monitor suitable breeding sites to confirm whether fly development is occurring on their property prior to purchases and release of biological control agents. If adult flies are immigrating to the equestrian farm, management practices should be focused on trapping and exclusion of adults, whereas biological control and further sanitation may be effective in situations where breeding is found on the property. Due to the low natural parasitism levels and domination of parasitism by *S. cameroni*, augmentative releases using this species may be the most effective. The use of pupal parasitoids on equestrian farms has not been evaluated for effectiveness in the field but certainly warrants investigation. Managers need an intimate understanding of breeding and fly dynamics on individual farms to integrate the principals of IPM and make knowledge-based decisions for filth fly management and control on equestrian farms.

Table 2-1. Monthly comparison of the combined number of adult house flies and stable flies collected on four small equestrian farms in Alachua County, Florida[1].

Month	Site A Adult Flies Captured ($\bar{X}$ ± SEM)	Site B Adult Flies Captured ($\bar{X}$ ± SEM)	Site C Adult Flies Captured ($\bar{X}$ ± SEM)	Site D Adult Flies Captured ($\bar{X}$ ± SEM)
Jul 2010	90.0 ± 32.9[a]	29.3 ± 11.3[a]	18.3 ± 7.7[a]	13.5 ± 4.6[a]
Aug 2010	51.3 ± 14.7[ab]	17.5 ± 8.5[b]	3.0 ± 0.6[c]	88.8 ± 18.4[a]
Sep 2010	219.8 ± 64.4[a]	17.8 ± 4.2[b]	2.6 ± 1.2[c]	43.2 ±8.9[b]
Oct 2010	60.3 ± 26.1[a]	35.5 ± 14.0[a]	16.0 ± 6.0[a]	120.5 ± 47.4[a]
Nov 2010	143.3 ± 42.2[a]	226.5 ± 78.3[a]	188.8 ± 83.9[a]	220.5 ± 105.1[a]
Dec 2010	41.5 ± 4.8[b]	130.5 ± 10.2[a]	61.5 ± 35.7[ab]	54.3 ± 42.8[ab]
Jan 2011	156.5 ± 54.7[a]	55.3 ± 14.3[a]	110.3 ± 29.9[a]	37.3 ± 14.5[a]
Feb 2011	22.0 ± 8.0[a]	21.5 ± 9.7[a]	14.8 ± 2.6[a]	16.0 ± 4.6[a]
Mar 2011	133.4 ± 93.7[a]	156.0 ± 71.2[a]	149.4 ± 44.6[a]	98.8 ± 51.0[a]
Apr 2011	158.3 ± 26.5[b]	162.0 ± 16.6[b]	114.3 ± 19.6[b]	790.5 ± 222.4[a]
May 2011	105.3 ± 17.0[b]	114.8 ± 9.2[b]	97.0 ± 21.9[b]	2,592.8 ± 543.7[a]
Jun 2011	62.0 ± 14.5[b]	139.0 ± 29.8[b]	38.3 ± 6.0[b]	1,724.0 ± 1,054.7[a]

[1]Means in a row followed by the same letter are not significantly different (Tukey's HSD test, α =0.05).

Table 2-2. Total number of house fly and stable fly adults and pupae from four small equestrian farms in Alachua County, Florida between July 2010 anad June 2011. Total numbers of pupal parasitoids and percent parasitism are presented.

				No. Parasitoid Species (% Parasitism)			
Site	Adults	Pupae	No. Parasitoids (% Parasitism)[2]	*S. cameroni*	*S. endius*	*M. raptor*	*Aleochora* Spp.
A							
House Fly	4,050	0	*	*	*	*	*
Stable Fly	1,203	0	*	*	*	*	*
A Total	5,253	0	*	*	*	*	*
B							
House Fly	2,803	0	*	*	*	*	*
Stable Fly	1,543	0	*	*	*	*	*
B Total	4,346	0	*	*	*	*	*
C							
House Fly	2,086	407	5(1.2)	5(100.0)	0	0	0
Stable Fly	1,322	875	24(2.7)	22(88.0)	0	0	2(8.0)
C Total	3,408	1,282	29(2.3)	27(90.0)	0	0	2(6.7)
D							
House Fly	21,806	322	32(9.9)	18(56.3)	8(25.0)	6(18.7)	0
Stable Fly	1,566	203	54(26.6)	54(100.0)	0	0	0
D Total	23,372	525	86(16.4)	72(83.7)	8 (9.3)	6 (7.0)	0
Study Total	36,379	1,807	115(6.3)	99(86.1)	8(7.0)	6(5.2)	2(1.7)

[1] Percent parasitism was calculated by the number of emerged parasitoids recovered from intact pupae divided by the total number of pupae collected.

[2] There were no pupae recovered from Farms A and B in any month and therefore mean percent parasitism could not be calculated as denoted by asterisks.

Table 2-3. Total numbers of house fly and stable fly pupae collected by substrate on four small equestrian farms in Alachua County, Florida and the number and percent parasitism of Intact pupae in these substrates.

Substrate[1]	No. Pupae Collected (% of total species collection)			No. Parasitoids (% Parasitism)[2]
	House Fly	Stable Fly	Total	
Hay, aged + urine and manure	169 (23.1)	875 (81.2)	1,044 (57.7)	30 (2.9)
Fresh manure (<12 h old)	16 (2.2)	3 (0.3)	19 (1.2)	10 (53.0)
Pine shavings + urine and manure (<12 h old)	413 (56.7)	155 (14.4)	568 (31.4)	64 (11.3)
Pine shavings, aged manure pile + urine and manure (>72 h old)	131(18.0)	38 (3.5)	169 (9.4)	12 (7.0)
Soil + aged manure pile + urine and manure (>72 h old)	0 (0.0)	0 (0.0)	0 (0.0)	* (*)
Soil, aged dirt lot + urine and manure	0 (0.0)	7 (0.6)	7 (0.3)	0 (0.0)
Total	729 (100.0)	1,078 (100.0)	1,807 (100.00)	116 (6.4)

[1]Substrates were determined to fall in these age categories based on the farm management practices of each facility and a personal interview with the owner/manager.
[2]Percent parasitism was calculated by dividing the total number of parasitoids that emerged from pupae collected from each substrate by the total pupae collected from the substrate.

Figure 2-1. Alsynite fiberglass trap mounted 90-cm from ground level. *Photo courtesy of Erika T. Machtinger.*

Figure 2-2. A soil-based manure pile, augmented by manure removal from stalls with soil bedding, was found on sties A and B. *Photo courtesy of Erika T. Machtinger.*

Figure 2-3. High concentrations of horses in small acreages often results in dirt-based paddocks. *Photo courtesy of Erika T. Machtinger.*

Figure 2-4. A rain gauge and maximum/minimum thermometer mounted on a wooden stake that was placed at each study site. *Photo courtesy of Erika T. Machtinger.*

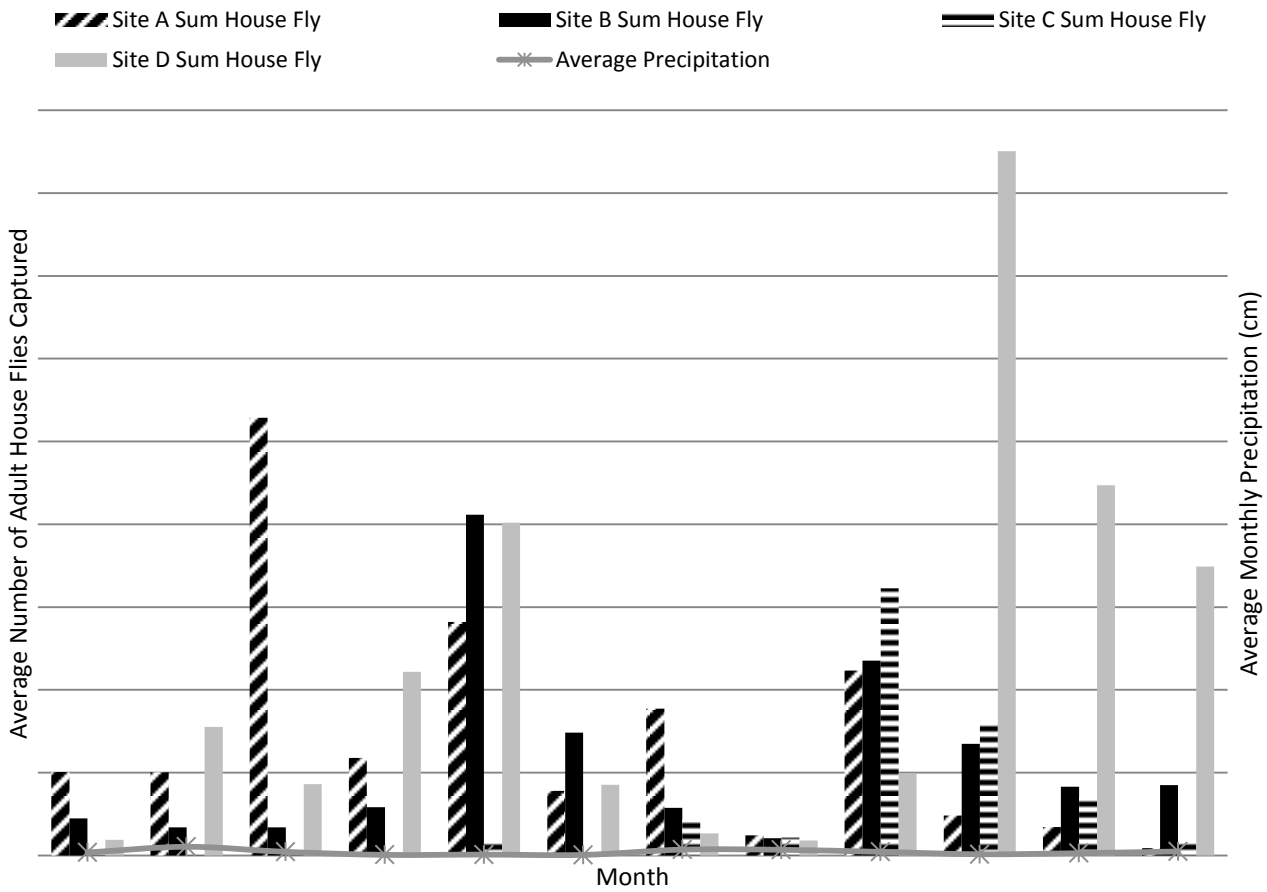


Figure 2-5. Average number of house flies captured at each month at four small equestrian farms in Alachua County, Florida, plotted against average monthly precipitation across all sites.

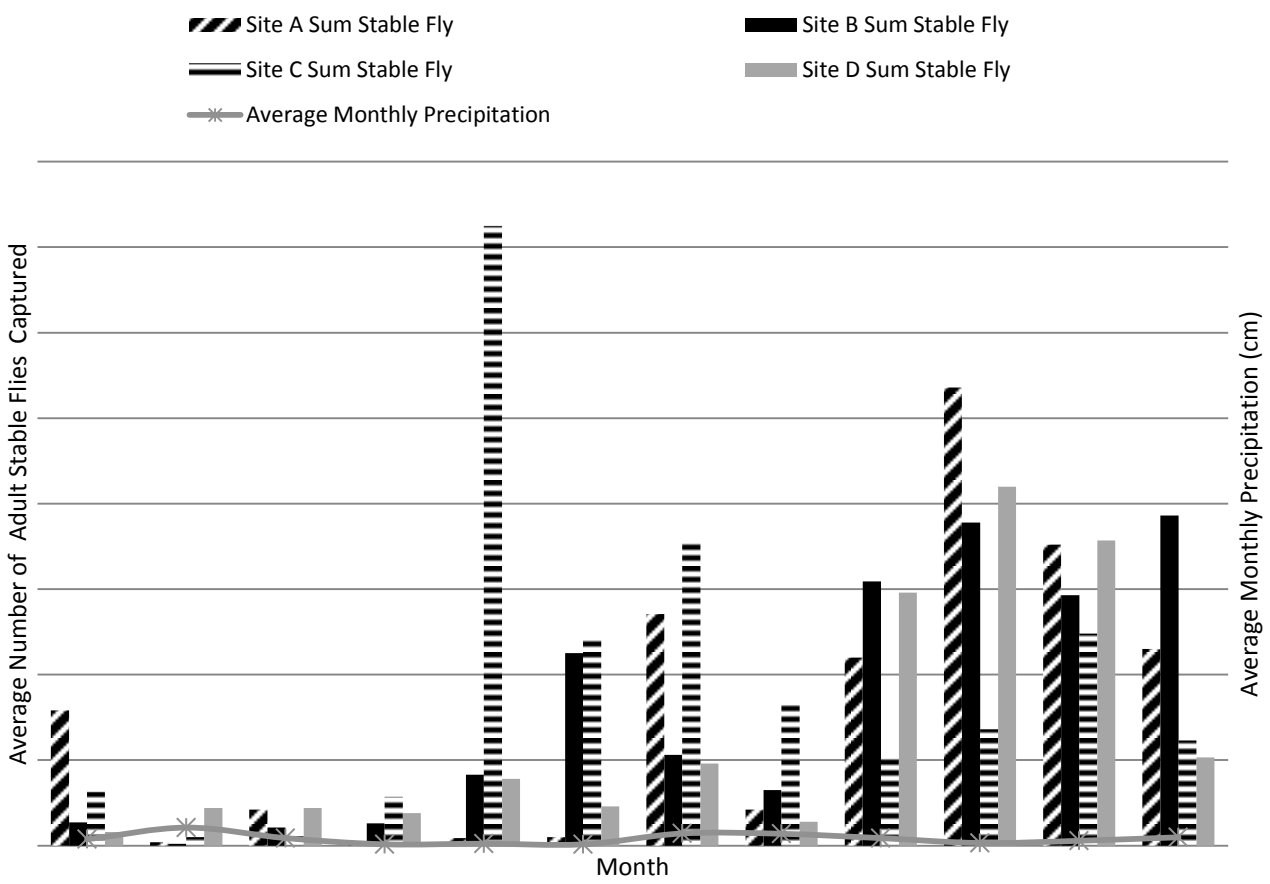


Figure 2-6. Average number of stable flies captured at each month at four small equestrian farms in Alachua County, Florida, plotted against average monthly precipitation across all sites.

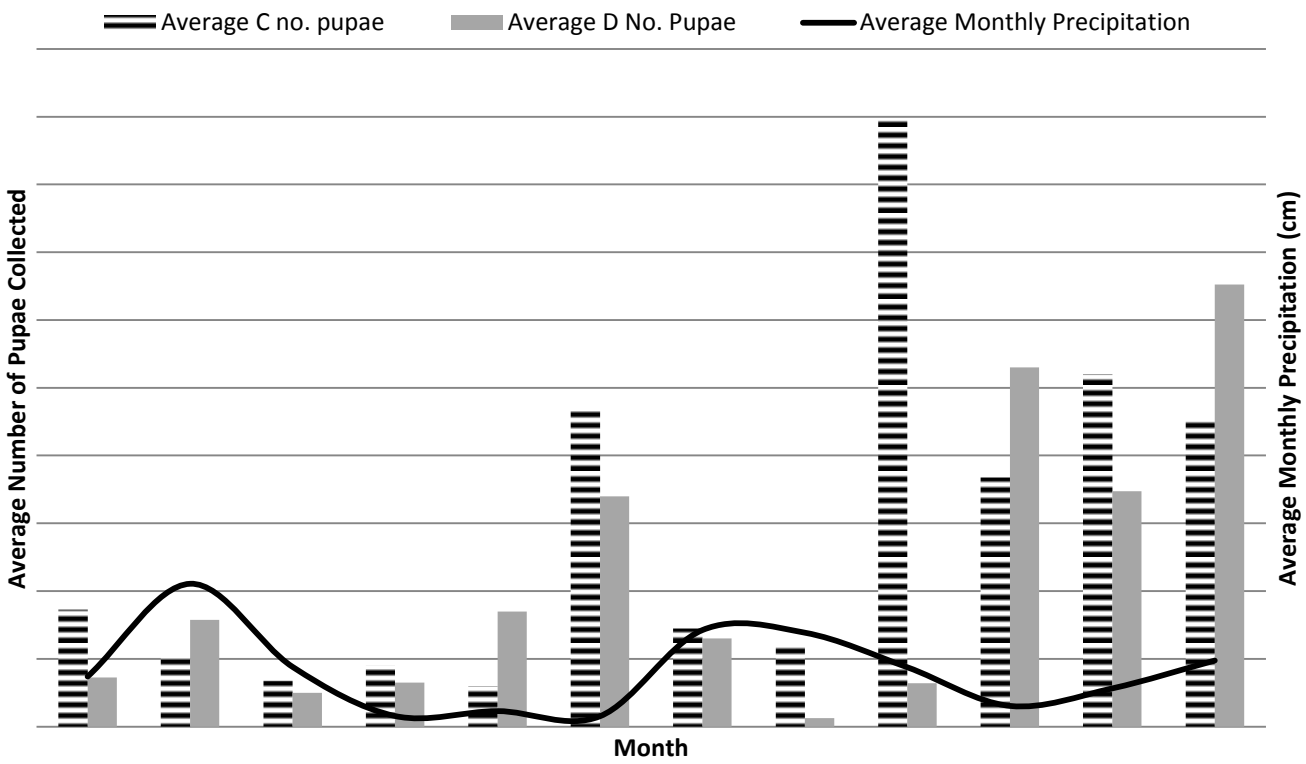


Figure 2-7. Monthly comparison of the average number of pupae collected at sites C and D. There were no pupae recovered from sites A and B.

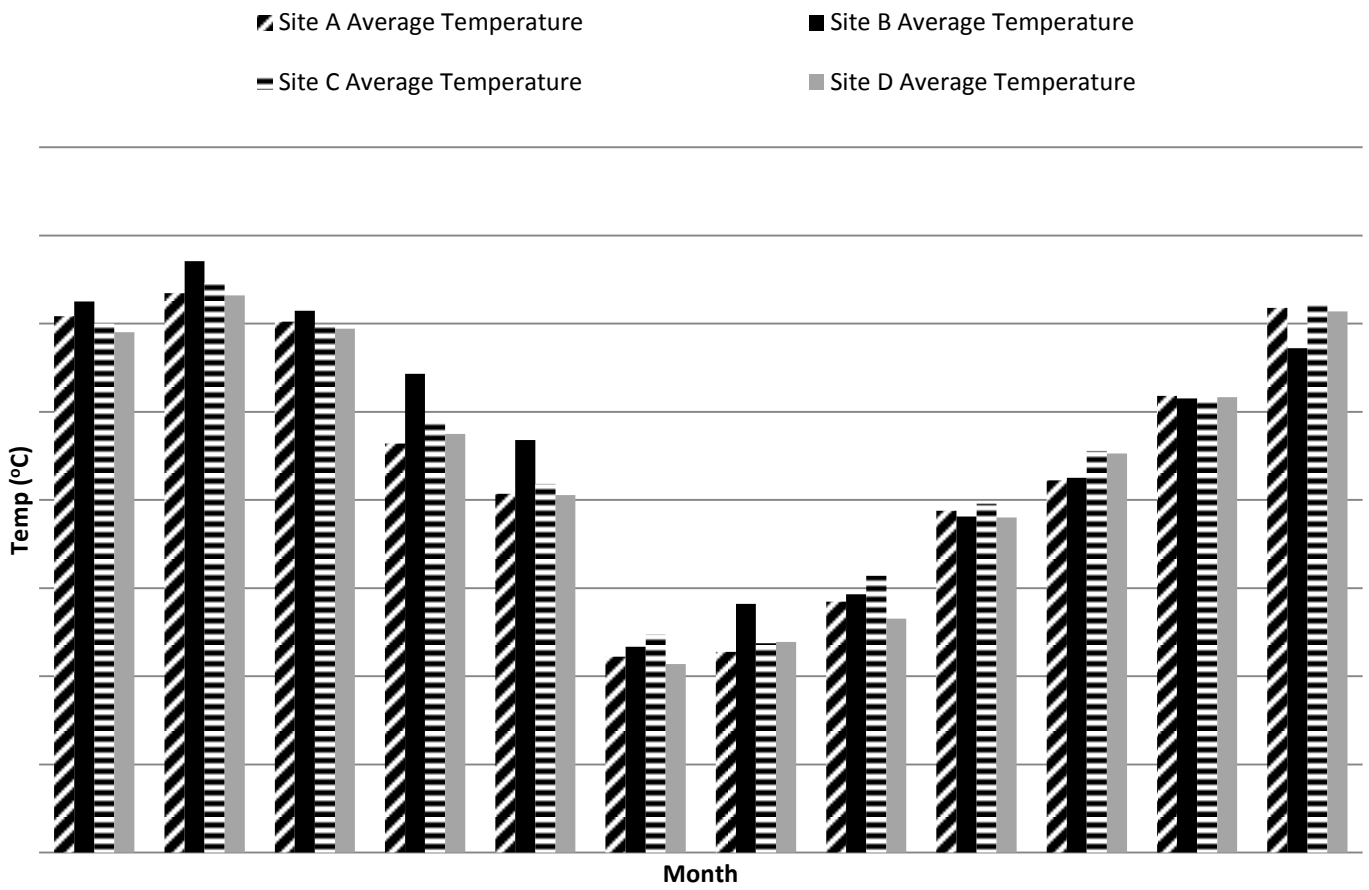


Figure 2-8. Mean monthly temperatures from July 2010 to July 2011 collected from weather stations installed on four small equestrian farms in Alachua County, Florida.

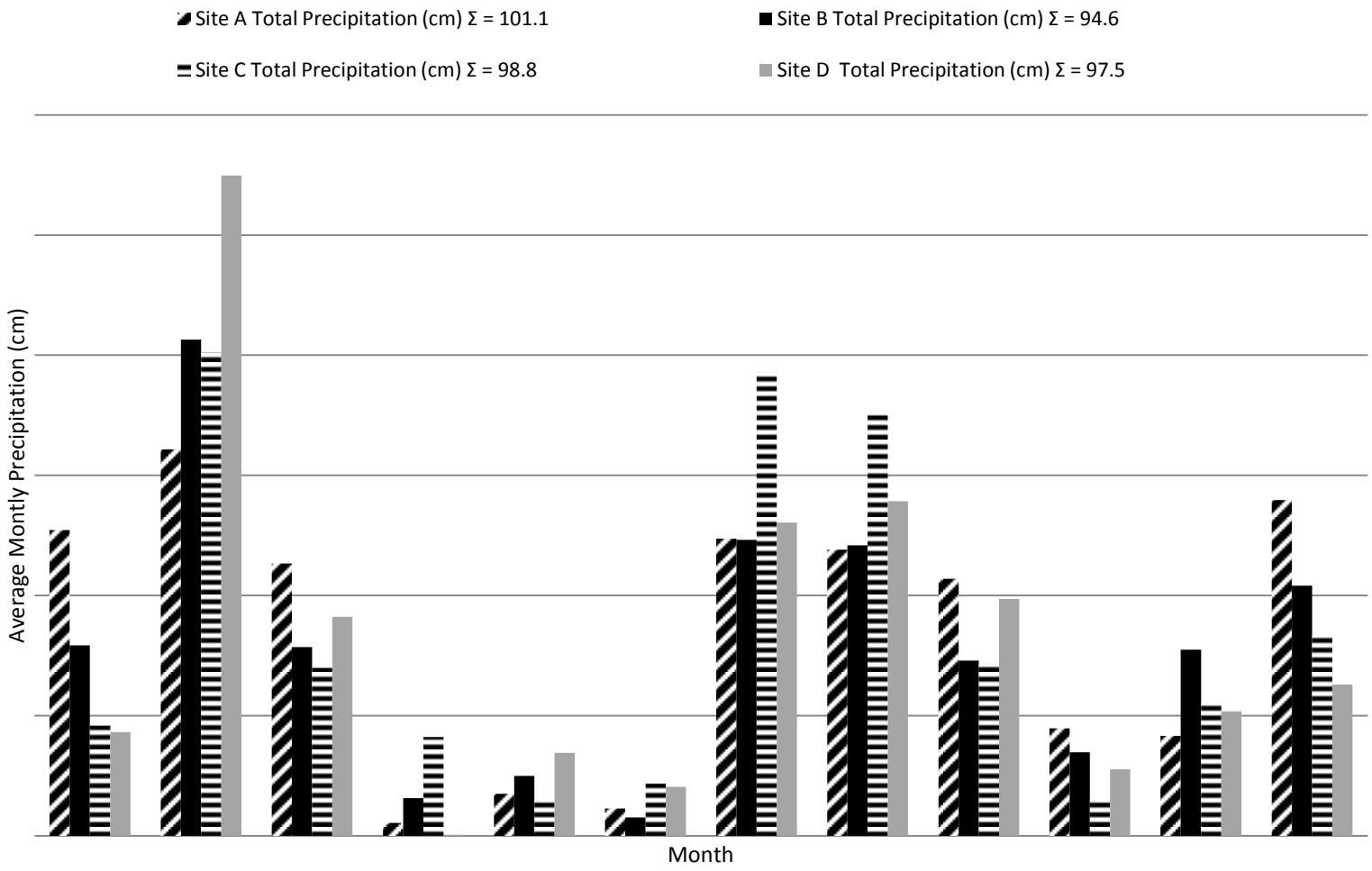


Figure 2-9. Total monthly precipitation for each small equestrian farm in Alachua County, Florida between July 2010 and July 2011 from installed weather stations on each property.

CHAPTER 3
OVIPOSITION PREFERENCE AND DEVELOPMENT OF HOUSE FLIES AND STABLE FLIES IN SIX COMMON EQUINE FACILITY SUBSTRATES

Introduction

To be a reproductively successful adult, a gravid female fly must select suitable development habitats for her offspring. Once she has deposited her eggs, the development of the offspring, including time, eventual size and future fecundity is a function of many factors and can significantly influence future population levels. House and stable fly oviposition and development occur in a wide range of substrates but preferences differ somewhat in the field. Although house flies oviposit and immatures develop in many organic mixtures (Bishopp et al. 1915), they develop more consistently in pure manure (Meyer and Shultz 1990; Broce and Haas 1999; Larrain and Salas 2008). Stable flies prefer decomposing plant matter mixed with animal wastes (Hall et al. 1982; Broce et al. 2005; Meyer and Peterson 1983; Meyer and Shutlz 1990; Skoda and Thomas 1993). Sites for house and stable fly oviposition are influenced by biotic and abiotic characteristics of the substrate, including bacterial composition (Lysyk et al. 1999; Romero et al. 2006; Talley et al. 2009; Lam et al. 2007), age and level of decomposition (Broce and Haas 1999), nutritional value (Lee and Toyama 1990; Larrain and Salas 2008), moisture level (Hogsette 1996; Moon et al. 2001), pH (Rasmussen and Campbell 1981; Sweeney et al. 2000a; Calvo et al. 2010) and chemical composition (Crumb and Lyon 1921).

Data on filth fly oviposition preference and development on equestrian farms is lacking. It has been assumed that the ecology of these flies in breeding sites found at other livestock facilities would be similar on horse farms; however, equine management and husbandry differ significantly from other livestock. Furthermore, the composition

and nutritional value of equine manure waste differs from that of ruminants or omnivores, such as swine (Lawrence et al. 2003; Larrain and Salas 2008). As seen in Chapter 2, though suitable substrates for fly development exist on equestrian farms in Florida, the attributes of these substrates may be more or less attractive to gravid females for oviposition.

Fly control could be improved with increased knowledge of the breeding biology of filth flies on equestrian farms. Preferred breeding sites must be characterized along with their influence on larval growth and development in order for pest management to be effective. Equine property owners generally clean areas of manure, such as stalls and paddock sheds and place the accumulation in larger piles (United States Department of Agriculture 2006). However, sanitation may not be sufficient to prevent the production of filth flies, as breeding still may occur in remaining waste or in the new manure mounds. Alternative types of bedding or manure management may be necessary to reduce fly production.

Natural populations of filth fly pupal parasitoids are found at varying levels in Florida livestock operations, including horse farms (Pitzer et al. 2011a). Parasitoids are available commercially and increasingly popular with operators. Even with this increased application of biological control, limited data exists on the implementation of these parasitoids. The spatial distribution of hosts may affect the ability of parasitoids to find and destroy hosts (Rueda and Axtell 1985; Meyer et al. 1991; Smith and Rutz 1991b; Pitzer et al. 2011b). Also, parasitoids may have microhabitat preferences (Smith and Rutz 1991b; Geden 1999). Knowledge of the most attractive sites for filth fly

breeding may help equestrian farm managers improve the effectiveness of biological control by releasing parasitoids in the most suitable areas.

The purpose of this research was to increase information on the breeding biology of filth flies associated with common substrates found in equine facilities. With more knowledge on preferred breeding sites, equestrian farm managers will be able to improve sanitation and target the release of parasitoids. The objectives of this study were to determine (1) preferences for oviposition by gravid females and (2) the development and survival of stable and house fly larvae in six substrates commonly found in equine facilities.

Materials and Methods

House and stable flies. The University of Florida stable fly colony, established in February, 2007 from the University of Florida Dairy Research Unit in Hague, FL (UFSF colony), was used for the oviposition and development experiments. Citrated bovine blood was provided daily on a saturated cotton roll in a 120 mL plastic container (blood cup). To provide a sugar and electrolyte source, Gatorade® was provided *ad-libitum* in a 120 mL covered cup fitted with a wick. Eggs were collected by washing gently with water from blood cups and added to the larval substrates adapted from Hogsette (1992). The diet contained 2500 mL of fly diet mix, (50% wheat bran, 30% alfalfa meal and 20% fine corn meal) and 5000 mL of water. A modification to the Hogsette (1992) diet was made where 2500 mL of vermiculite (expanded mica) was added in lieu of pelleted peanut hulls. After approximately 12 days, pupae were removed from the diet by flotation in tap water.

House flies used for the experiments were from the USDA, ARS-CMAVE wild type colony (WTHF colony) which was established in 2010 using flies from a dairy in Gilchrist

County. Adult flies were provided water *ad-libitum* in a 1-gallon bucket with foam chips. Flies were fed a fresh mixture of 67% granulated sugar, 27% powdered milk and 6% powdered egg yolk three times per week. Eggs were collected as needed from the flies by creating an artificial collection device for oviposition. A moistened black cloth was wrapped tightly around an 8- to 10-cm ball of aging rearing medium. These collection devices were placed in a shallow cup and cups placed in adult fly cages. Eggs were gently washed off this cloth and added to larval rearing diet as described by Hogsette (1992). This diet consisted of 5000 mL of fly diet mix and 3800 mL of water. After 7 days, pupae were floated from the diet.

Approximately 3000 pupae for each species were dried separately in a blower following flotation, placed in separate 250-cm^3 plastic cups and transferred to individual 40 x 40 x 50-cm screen rearing cages. The UFSF and WTHF colonies were maintained in these separate cages at 26.7^oC, constant light and 60% RH. Females and males of each respective species were held together and allowed to mate freely. Adults used for the oviposition experiment were aspirated from the rearing cages and counted while anesthetized with CO_2.

Test substrates. Six substrates commonly found on equestrian farms (Figure 3-1), each collected from one of the study sites listed in Chapter 2 in Alachua County, Florida were evaluated. These substrates included: (1) hay, aged + urine and manure from around a coastal Bermudagrass round bale (site C), (2) fresh manure (<12 h old) collected from a thoroughbred mare fed perennial peanut hay and concentrated feed (site D), (3) 0.1 to 0.3-cm long fresh pine shavings + urine and manure (<12 h old) plus small amounts of waste perennial peanut hay from a thoroughbred mare (site D), (4)

pine shavings, aged manure pile + urine and manure (>72 h old) containing small amounts of waste perennial peanut hay (site D), (5) soil, aged manure pile + urine and manure (>72 h old) containing small amounts of waste hay from an Arabian mare fed coastal Bermudagrass hay and concentrated feed (site A) and (6) soil, aged dirt lot + urine and manure (>72 h old) (site D) from a dirt paddock. Substrates were collected from accumulations from the surface down to a maximum depth of 10-cm and from study sites with no insect growth regulator or area wide pesticide use. Samples were collected with a trowel, placed in a plastic bag and immediately put in a plastic cooler held at ambient air temperature. A subsample of each substrate was weighed and dried to determine the field moisture level and samples of each substrate were analyzed for composition and moisture by the University of Florida Institute of Food and Agricultural Sciences Extension Soil Testing Laboratory in Gainesville, Florida (Appendix B). The remaining substrate was double bagged in freezer bags and frozen at 4^{o}C for at a minimum of one week to kill any existing arthropods. To ensure homogeneity, individual substrates collected on different days were mixed thoroughly while frozen. Substrates were placed in the cups used during the experiment and weighed when the cups were approximately ¾ full. Because densities differed by substrate, so did the weights. However, a standardized amount of each substrate was used for the experiments based on the initial weights which were; 50 g of hay, aged + urine and manure, 100 g of fresh manure (<12 h old), 50 g pine shavings + urine and manure (<12 h old), 50 g of pine shavings, aged manure pile + urine and manure (>72 h old), 100 g of soil, aged manure pile + urine and manure (>72 h old) and 100 g of soil, aged dirt lot + urine and manure.

Oviposition experiment. Three 60 x 60 x 60-cm clear arenas covered with nylon netting were used for each trial (Figure 3-2) (MegaView Science, Taiwan). Each substrate was placed in a 250-cm^3 plastic cup and adjusted to the standardized weight. Substrates were hydrated to 65% moisture using deionized water based on previously determined field moisture levels using a spray bottle and then the substrate was mixed gently to ensure uniform moisture content. One cup of each of the six field substrates was positioned randomly in an array in each trial arena. Randomization was achieved by assigning each substrate a number (1 through 6) and each position in the trial arena a number (1 through 6). A random number generator was used to output six numbers which became the order position of each substrate starting with substrate number 1. Adult females between 7 and 10 days old, which were visually determined to be gravid, were aspirated from the rearing cage, counted while anaesthetized with CO_2 and placed in 60-cm^3 cups with perforated lids. Adults were released in the center of each trial arena. Thirty gravid house flies or 50 gravid stable flies were used for their respective trials, and had free access to the substrates for 6 hours from midmorning to afternoon. A cotton ball saturated with citrated bovine blood in a 150-cm^3 plastic cup was placed in the center of each release arena during the stable fly oviposition trials.

After the 6 hour exposure period, the substrate in each cup was placed in an individual 1-gallon plastic bucket filled with three – quarts of the respective fly rearing medium for each species. To prevent desiccation of eggs, the added substrate from the trial cups was covered with fly rearing medium until the added substrate was lightly covered (Figure 3-3). The buckets were covered with a cloth fabric secured with a rubber band then held at 22^oC. Buckets were inspected after 4 days for overcrowding.

If high numbers of larvae were present, the content of the bucket was divided to prevent longer development intervals or unintentional mortality from resource limitation. Pupae were extracted from the medium in the buckets by flotation a minimum of 24 hours after pupation and no further larvae were observed (Figure 3-4) (Lee and Toyama 1990) and frozen for future counting. Total numbers of recovered pupae were counted for each substrate. For each species, three buckets containing only the laboratory medium used to maintain each colony were held under the same conditions as those that had the trial substrates as a control.

Development experiment. All six field substrates were evaluated along with the laboratory rearing medium of each species. Eggs were collected in the morning from flies. Eggs were suspended in deionized water, pipetted onto a moistened black cloth and counted using a binocular microscope set at 8 x magnifications. Groups of 100 eggs were added to the surface of each substrate by rinsing them gently off the cloth with a maximum of 30 mL of deionized water to ensure that the moisture content of each substrate did not exceed 65%. The black cloth was placed lightly on the substrate and, along with the washed eggs, was covered lightly (until the cloth was just covered) with substrate to prevent desiccation (Figure 3-5). The moisture content of each substrate in the trial cups was then adjusted to 65%. Cups were weighed daily to determine if moisture was needed to maintain this level. Since each substrate differed in water retention, a spray bottle was used to adjust moisture levels uniformly at a slow rate to avoid pooling and runoff.

Each cup was inspected daily for larval development and pupation. To avoid unintentional larval mortality, inspections began 4 days after establishment. Pupae were

removed from each substrate daily when they started to tan and rinsed to remove rearing medium. Pupae were allowed to air dry overnight before being weighed to ensure that weight was not affected by moisture from the substrate or rinsing. All pupae were weighed if the daily recovery was <10. If daily samples were over >10, a subsample of at least 50% were weighed. Pupae were weighed individually on a digital scale and then held in 60-cm^3 emergence cups covered with perforated lids then marked with the trial, species, substrate and replicate in a growth chamber at 25^oC, L:D 24:0, and approximately 80% RH and checked for eclosion daily. Adults were chilled for less than one minute to inhibit movement and allow for removal from the cups holding the pupae and then counted. Each development cup was checked daily and maintained at the correct moisture content until there was no larval activity in the cup. Emergence cups were inspected daily for adult eclosion for a minimum of two weeks after the last recorded eclosion date of each individual cup.

Statistical analysis. For both the oviposition and development experiments, three trials were conducted on the same day and the trials were replicated three times on different dates using different cohorts of adult flies. Numerical and percentage data were normalized by log and arcsine transformation, respectively, and then subjected to a separate one-way Analysis of Variance (ANOVA) for each species. Means were separated with Tukey's Honestly Different Separation test for comparison ($\alpha = 0.05$). Values in the text and tables are back transformed data. All statistical tests were run on JMP software (JMP v.9, SAS Institute Cary, NC, 2010).

Results

House fly oviposition and development. A total of 24,356 house fly pupae were recovered from the six field-collected oviposition substrates (Table 3-1). The pine shavings + urine and manure (<12 h old) and the fresh manure (<12 h old) were not statistically different. Three of the substrates produced fewer, but varying, numbers of pupae: hay, aged + urine and manure; soil, aged manure pile + urine and manure (>72 h old); and soil, aged dirt lot + urine and manure. Although the fresh manure had numerically over 30 times more recovered pupae than the soil, aged manure pile + urine and manure (>72 h old) and soil, aged dirt lot + urine and manure (*n*=739.00, *n*=24.11, *n*=6.11 respectively) variation was high and these substrate did not differ statistically from one another. No pupae were recovered from the pine shavings, aged manure pile + urine and manure (>72 h old).

When eggs were added to each of the six field-collected oviposition substrates and to laboratory rearing medium in the laboratory, the percentage of eggs which developed to pupae was highest with the fresher substrates; fresh manure (<12 h old) and the pine shavings + urine and manure (<12 h old) and the rearing medium (Table 3-2). Pupation occurred in an average of 6.9 to 7.6 days and was not significantly different by substrate, including the rearing medium. The rearing medium produced pupae that were significantly heavier and nearly twice the weight of those from the other substrates, an average of 20.7 mg versus between 5 and 10 mg. The average weights of pupae from the fresh manure (<12 h old) and pine shavings + urine and manure (<12 h old) were not significantly different (10.4 and 10.2 mg, respectively). Pupae produced in the from the pine shavings, aged manure pile + urine and manure (>72 h old) were

significantly smaller, averaging 5 mg, from those substrates produced in the other substrates. Development was not completed in the hay, aged + urine and manure; soil, aged manure pile + urine and manure (>72 h old); or soil, aged dirt lot + urine and manure.

Stable fly oviposition and development. A total of 12,648 stable fly pupae were recovered from the oviposition trials (Table 3-1). No significant differences were observed between the number of pupae recovered from the hay, aged + urine and manure, pine shavings + urine and manure (<12 h), pine shavings, aged manure pile + urine and manure (>72 h old) and soil, aged manure pile + urine and manure (>72 h old). There were no significant differences between the hay, aged + urine and manure, fresh manure (<12 h old) and soil, aged dirt lot + urine and manure. The pine shavings + urine and manure (<12 h old), pine shavings, aged manure pile + urine and manure (<72 h old) and soil + aged manure pile + urine and manure (>72 h old) were significantly different from the soil, aged dirt lot + urine and manure. Though not significant from each other, the mean of the pine shavings + urine and manure (<12 h old) was twice that of the pine shavings, aged manure pile + urine and manure (> 72 h old) and soil, aged manure pile + urine and manure, potentially due to high variation in the pupal recovery (n=600.2, n=299.7, n=244.7, respectively). The soil, aged dirt lot + urine and manure averaged significantly fewer pupae (n=2.44).

All six field substrates and the rearing medium produced stable fly pupae when eggs were added to the substrates (Table 3-3). The greatest average percentage of development to pupation was in the pine shavings + urine and manure (<12 h old) (60.2%) though this substrate was not significantly different from the fresh manure

(<12h old) or rearing medium. Average time to pupation ranged from 7.5 to 27.1 days with the quickest development occurring in the pine shavings + urine and manure (<12h old) and the rearing medium. Development to the pupal stage was significantly faster with the fresh manure (<12 h old) and rearing medium. Eggs added to the soil, aged dirt lot + urine and manure, developed the slowest to the pupal stage. The fresh manure (<12 h old) and rearing medium produced the heaviest pupae (11.0 mg and 11.5 mg, respectively) which was statistically different from the remaining field-collected substrates. The fresh manure (<12h old) and the pine shavings + urine and manure (<12h old) had the greatest average percentage of adult fly emergence. The percentage of adult emergence was the only parameter measured where the laboratory rearing medium was significantly less successful than any of the field substrates.

Chemical analysis of substrates. Each of the six field collected substrates and the laboratory rearing medium had a unique chemical composition (Table 3-4). Nitrogen content was the highest in the rearing medium (1.04%), hay, aged + urine and manure (0.51%) and the fresh manure (<12 h old) (0.48%). The pine shavings, aged manure pile + urine and manure (> 72 h old) had the lowest nitrogen at 0.04%. The pH was much more acidic in the rearing medium (4.6) than the remaining substrates which ranged from 7.3 to 8.9. The moisture levels at collection were variable. The fresh manure (<12 h old) was the wettest at 78% moisture and the soil, aged dirt lot + urine and manure was the driest with 22% moisture. Total ash also was variable. The rearing medium and fresh manure (<12 h old) had a very low total ash percentage (2.7% and 4.7%, respectively) while the soil, aged dirt lot + urine and manure (47.1%),

pine shavings + urine and manure (<12 h old) (27.7%) and soil, aged manure pile + urine and manure (>72 h old) (23.5%) were much higher.

Discussion

These experiments clearly demonstrated that different equine substrates affect oviposition preferences of both species and influences larval development and pupation, at least under laboratory conditions. Habitat alteration can be an effective and sustainable approach to pest management (Hokkanen 1991; Foster and Harris 1997) and the results presented here can be used to improve management of small equestrian farms in Florida by improving sanitation and strategic releases of parasitoids.

Substrate preferences for oviposition of both house flies and stable flies were demonstrated by both flies when females were given a choice of oviposition sites. Female house flies had a strong preference for fresher substrates, preferring pine shavings + urine and manure (<12 h old) and to a lesser extent, fresh manure (<12 h old) over the remaining substrates. Fewer pupae were recovered in all soil-based substrates and the hay, aged + urine and manure, and none were recovered from the pine shavings, aged manure pile + urine and manure substrate. Stable flies shared the preference for fresh pine shavings + urine and manure (<12 h old) with house flies but did not significantly discriminate between this substrate and the hay, aged + urine and manure; pine shavings, aged manure pile + urine and manure (> 72 h old) or soil, aged manure pile + urine and manure (> 72 h old) demonstrating a greater range in oviposition preference than house flies. Though percent of egg hatch was not calculated for the oviposition experiments which may have had an influence on total numbers of recovered pupae, it was assumed that this variable would be likely similar across each substrate and the large difference between the preferred substrates.

Development was dependent on substrate for both species of fly. House fly larval development corresponded well with oviposition results, the greatest number of pupae being produced on the fresh manure (<12 h old) and pine shavings + urine and manure (<12 h old). Few house fly pupae were recovered from aged substrates with a soil base in the oviposition trials and none were produced from known numbers of eggs in the development experiments. Additionally, larvae did not reach the pupal stage in the hay, aged + urine and manure substrate. Historically, house flies have been considered opportunistic breeders with a capacity to develop in substrates with only 0.47% dairy cow manure solids (Hogsette 1996). Interestingly, in the current study, house flies showed little flexibility in developing on substrates collected from equine facilities. House fly pupae were never collected from soil- based substrates in the field, as described in Chapter 2, and these laboratory studies support the limited suitability of these substrates for house fly breeding. Field based substrates are not static in location or content on most equine farms, however, and field conditions may be affected by environment or management, increasing or decreasing substrate suitability on individual farms. Though no pupae were recovered from the pine shavings, aged manure pile + urine and manure (>72 h old) in the trials, immature flies did develop in this substrate when eggs were added artificially. These results suggest that eggs deposited on fresh manure cleaned from stalls with pine shavings as bedding may still be viable if added to a larger manure pile, a common practice on equestrian farms throughout the country (United States Department of Agriculture 2006). Additional management of these aged manure piles or alternative waste disposal may be necessary to prevent development in these areas.

Unlike the house fly, stable fly development data did not correspond well with oviposition trials. Stable flies did not have a strong preference to oviposit in fresh manure (<12 h old) (Table 3-1). However, this substrate produced high numbers of pupae and adults in development trials. Unlike the fresh manure (<12 h old), the pine shavings + urine and manure (<12 h old) substrate was a preferred for oviposition but had lower pupal weights and longer development times comparative to the fresh manure (<12 h old). Stable flies had significantly longer development times, lower pupal weights and fewer adult flies emerging on the two soil-based substrates (Table 3-3).

House fly oviposition preferences and high levels of larval development are known to occur on substrates with fresh manure. However, house flies also develop in a range of decomposing animal manures (Bishopp et al. 1915; Pont 1973; Amano 1985; Larrain and Salas 2008) and decaying vegetation (Bishopp et al. 1915). Broce and Haas (1999) observed that house fly colonization of cattle manure was more consistent in the fresher substrates. House flies were not only highly attracted to equine substrates with fresh manure in this study, but also properties of the fresh manure were apparently necessary for high levels of house fly larval development.

The oviposition behavior of stable flies was consistent with previous literature as the majority of the deposition was in substrates containing some form of decaying vegetation. Oviposition on the hay, aged + urine and manure substrate was not significantly different from preferred substrates and larval development time was moderate. Waste surrounding hay round bale sites has been found to be an important overwintering habitat that produces large numbers of stable flies (Hall et al. 1982; Broce et al. 2005; Talley et al. 2009). Field collections of stable fly pupae presented in

Chapter 2 support this finding, as the majority were located in hay waste and associated manure. As the laboratory substrates were homogenized field samples, wild stable flies may be more selective in ovipositing on more natural hay waste from a more suitable zone surrounding the bale (Talley et al. 2009) and this would improve reproductive success from what was demonstrated in the current study. With the results from these laboratory experiments supporting field observations of stable fly oviposition and development in waste hay, it is probable that hay surrounding round bales is a desirable oviposition and development site for stable flies on equestrian farms. In some cases, results of the development experiments were contradictory to previous work on stable flies. Stable fly surveys in Florida have indicated that pure manure was an unsuitable breeding medium for stable flies (Williams and Westby 1980; Hogsette et al. 1987) and animal feces are less attractive to stable flies when not mixed with decomposing plant material (Hafez and Gamal-Eddin 1959; Skoda and Thomas 1993). Fresh manure was not a preferred oviposition substrate for stable flies but larvae demonstrated a high capability for development in this substrate. Previous work with cattle manure demonstrated that stable fly development was limited to cattle manure that had undergone an advanced ageing process (Broce and Haas 1999). Cook et al. (1999) confirmed age discrepancies between house and stable flies in poultry litter and observed that stable flies clearly preferred litter over 4 days old. It is interesting to note that less than 4 day old pine shavings + urine and manure (<12 h old), pine shavings , aged + urine and manure (> 72 h old) and soil, + aged urine and manure (> 72 h old), were equally preferred stable fly egg laying sites. The most productive development substrates for stable flies, fresh manure (<12 h old) and pine shavings + urine and

manure (<12 h old), were under a day (1 d) old. Horse manure has been demonstrated to be a preferred oviposition substrate over cow dung (Jeanbourquin and Guerin 2007). It is possible that, due to the non-ruminant digestion of horses and the fibrous structure of horse manure, degradation may not follow the same pattern as in cattle or other livestock manure. The physical characteristics and attractiveness of horse manure to stable flies may occur at an earlier stage of decomposition. In contrast, the high preference and low variability of house flies to oviposit in pine shavings + urine and manure (<12 h old) and fresh manure (<12 h old) substrates may be generated from an intraspecific cue. Intraspecific pheromones have been researched in house flies and found to elicit behavioral changes encouraging egg clustering on substrates as a result of semiochemicals originating from the ovaries of adult females (Jiang et al. 2002). Once a substrate is selected as suitable by a pioneer fly, other females may gravitate towards that cue to deposit their eggs, even if other suitable substrates are available. Egg laying may shift to additional substrates if fly populations are high and flies receive an oviposition inhibition cue for that particular substrate from egg laying females (Lam et al. 2007). It was clear in the current study that the pine shavings + urine and manure (<12 h old) was initially preferred by house flies, followed by fresh manure (<12 h old); however, the short period of time allowed for oviposition in the laboratory may have prohibited further house fly investigation of the other substrates and critical egg mass levels and inhibition cues may not have been triggered.

Oviposition behavior appears to differ between house and stable flies. Stable flies do not tend to cluster eggs, opting to disperse oviposition both spatially and temporally. It may be that chemical cues from mass numbers of house fly eggs or developing larvae

discourages oviposition and subsequent development of stable flies in fresher substrates, though this requires further investigation. These semiochemical cues may account for the previous observations of stable fly oviposition restraint on fresher substrates in the field, potentially a response to resource competition. The restriction of oviposition by stable flies to aging substrates, and allocation of eggs to many substrates may be an evolved trait to reduce interspecific competition between stable flies and house flies.

The acceptance of substrates for oviposition and ability for house flies and stable flies to develop in each substrate may be related to the physical or chemical properties of each. Analysis of the substrates did not reveal deficiencies between treatments which would explain the performance differences of these two flies. There was no consistency in the substrate analysis in the measured parameters between substrates which were successful for oviposition and development and those that were not. It is likely that differences in odor, micronutrients or bacterial composition (Lam et al. 2007; Talley et al. 2008) were more influential for than the tested properties for female attraction and larval development. Nutrient composition of dung is affected by feeding programs. Disparity in equine feeding programs and digestion affecting the nutritional composition of the substrate might be a factor in attracting gravid females of both species. House flies are more attracted to the manure of grain fed over grass fed cattle (Mau 1978) and similar results were found with other muscid species *(*Lee and Toyama 1990). The physical properties of manure can be altered by species, age, productivity and diet (Lawrence et al. 2003) and changes to any of these categories may make substrates more or less attractive on individual farms.

Filth fly management implications for equestrian farms in Florida. Substrate preferences were exhibited by both species for oviposition and development in the laboratory studies. Though these results need to be confirmed in the field, It is clear that house flies have limited flexibility to oviposit in a variety of substrates and develop in common equine substrates in the laboratory. Conversely, stable flies demonstrated a broader range of tolerances. These fly preferences and abilities to develop in these common equine substrates have control implications on equestrian farms in Florida.

Development time, adult production and body size are important fitness factors that influence future fly populations on farms. Larger flies have a greater fecundity than smaller females (Black and Krafsur 1987) and those that develop faster into adults can accelerate the production of future generations. Additionally, higher numbers of individual adult flies clearly presents an opportunity for more females to produce progeny. Substrates containing fresh manure attract significantly more gravid females for oviposition and quickly produced larger pupae for both species of fly. These results underscore the need for management of fresh manure as a key element in sanitation.

Alterations to sanitation practices, such as increased frequency of waste removal or substituting soil for pine shavings in bedding choice could further reduce breeding of filth flies (Schmidtmann et al. 1989) by removing or degrading fresh manure sources in barn areas. To reduce the availability of fresh manure in horse congregation areas within pastures, frequent dragging to expedite desiccation of manure waste (Watson et al. 1998) or hand removal could be integrated as part of an improved sanitation program. The mineral acid, sodium bisulfate, could be added to substrates suitable for filth fly breeding (pH 7.3 to 8.9) to reduce the pH of bedding. The lower pH reduces

available larval food by creating an environment which is not suitable for bacterial development (Calvo et al. 2010). This technique has proven safe and effective at significantly reducing larval fly populations in horse stalls (Sweeney et al. 2000a, 2000b) and calf bedding (Calvo et al. 2010) and may be an alternative to more frequent manure removal. Hay waste was a moderately important substrate for stable fly oviposition and development in the laboratory and has been found to harbor overwintering populations of stable flies in the field in Florida (Broce et al. 2005). Further manipulation of round bale feeding sites, such as frequent relocation of bales and alternatives to standard hay rings (Broce et al. 2005) may discourage breeding and overwintering of stable flies. Substrates which were less attractive and productive to stable and house flies in the laboratory should remain part of a monitoring program as environmental conditions or other factors may alter their suitability. Additionally, covering manure piles with tarps or plastic can make the substrate unsuitable for development and physically exclude egg laying adults (Fay 1939).

If monitoring reveals that suitable breeding substrates are present on a site, applications of pupal parasitoids can be applied directly to these sites to facilitate high rates of parasitism. The egg clustering tendency of house flies and pupal aggregation of both species has the potential to increase the effectiveness of pupal parasitoids for house fly control as well. If parasitoids are released in these areas, egg clusters and subsequent concentration of immature flies may reduce the searching time of the parasitic wasps and may increase their efficiency and warrants future investigation.

Differences in on-site management in Florida equine facilities, manure waste composition and seasonality of flies pose unique challenges for managing stable and

house fly populations. There is evidence to suggest that the breeding biology of stable flies on equestrian farms may differ from other livestock operations. Further research specific to equestrian facilities is necessary to develop the most appropriate management techniques for these facilities. Studies on field attraction of stable and house flies to these substrates on equestrian farms could continue to increase the effectiveness of cultural control methods and improve the use of pupal parasitoids in locations where breeding is known to occur. These IPM practices could improve filth fly management on equestrian facilities.

Table 3-1. House fly and stable fly pupae recovered from oviposition substrates[1].

Substrate	House fly		Stable fly	
	$\bar{X}$ ± SEM[1]	N (%)	$\bar{X}$ ± SEM	N (%)
Hay, aged + urine and manure	18.8 ± 18.0[c]	169 (0.7)	201.9 ± 117.9[ab]	1,817 (14.4)
Fresh manure (<12 h old)	739.0 ± 134.3[ab]	6,651 (27.3)	22.7 ± 19.8[ab]	204 (1.6)
Pine shavings + urine and manure (<12 h old)	1,918.2 ± 458.1[a]	17,264 (70.9)	600.2 ± 533.2[a]	5,704 (45.1)
Pine shavings, aged manure pile + urine and manure (>72 h old)	0 ± 0.0[d]	0 (0.0)	299.8 ± 131.6[a]	2,698 (21.3)
Soil + aged manure pile + urine and manure (>72 h old)	24.1 ± 14.3[bc]	217 (0.9)	244.8 ± 60.9[a]	2,203 (17.4)
Soil, aged dirt lot + urine and manure	6.1 ± 6.1[bc]	55 (0.2)	2.4 ± 1.8[b]	22 (0.2)
Total recovered pupae		24,356 (100.0)		12,648 (100.0)

[1]Means in a column followed by the same letter are not significantly different (Tukey's HSD test, α =0.05).

Table 3-2. House fly development on oviposition substrates[1].

Substrate[2]	Developed to Pupae (%)	Time to Pupation (days)	Pupal Weight (mg)	Adult Emergence (%)
Hay, aged + urine and manure	*	*	*	*
Fresh manure (<12 h old)	82.4 ± 12.3[a]	6.9 ± 0.2[a]	10.4 ± 0.7[b]	74.0 ± 3.8[a]
Pine shavings + urine and manure (<12 h old)	75.9 ± 4.1[a]	6.9 ± 0.2[a]	10.2 ± 5.2[b]	62.6 ± 5.4[a]
Pine shavings, aged manure pile + urine and manure (>72 h old)	33.0 ± 4.2[b]	7.6 ± 0.4[a]	5.0 ± 0.2[c]	27.0 ± 4.7[b]
Soil + aged manure pile + urine and manure (>72 h old)	*	*	*	*
Soil, aged dirt lot + urine and manure	*	*	*	*
Rearing medium	85.0 ± 3.8[a]	7.3 ± 0.3[a]	20.7 ± 0.4[a]	63.0 ± 6.7[a]

[1]Means in a column followed by the same letter are not significantly different (Tukey's HSD test, α =0.05).
[2] House flies did not develop to pupation on substrates indicated by an asterisk.

Table 3-3. Stable fly development on oviposition substrates[1].

Substrate	Developed to Pupae (%)	Time to Pupation (days)	Pupal Weight (mg)	Adult Emergence (%)
Hay, aged + urine and manure	40.9 ± 4.4^{bc}	10.4 ± 0.4^{bc}	6.4 ± 0.5^{bc}	17.7 ± 3.5^{bc}
Fresh manure (<12 h old)	57.0 ± 6.9^{ab}	7.5 ± 0.2^{a}	11.0 ± 0.5^{a}	45.2 ± 4.9^{a}
Pine shavings + urine and manure (<12 h old)	60.2 ± 2.0^{a}	8.8 ± 0.3^{b}	8.0 ± 0.5^{b}	49.9 ± 3.1^{a}
Pine shavings, aged manure pile + urine and manure (>72 h old)	35.4 ± 15.6^{c}	10.3 ± 0.2^{bc}	6.1 ± 0.4^{bc}	16.9 ± 2.5^{bc}
Soil + aged manure pile + urine and manure (>72 h old)	41.1 ± 9.0^{bc}	14.0 ± 0.5^{c}	5.7 ± 0.3^{c}	16.3 ± 2.0^{bc}
Soil, aged dirt lot + urine and manure	2.7 ± 0.9^{d}	27.1 ± 5.8^{d}	3.5 ± 0.8^{d}	1.0 ± 0.6^{c}
Rearing medium	44.3 ± 6.5^{abc}	7.8 ± 0.2^{a}	11.5 ± 0.5^{a}	27.9 ± 5.1^{b}

[1]Means in a column followed by the same letter are not significantly different (Tukey's HSD test, $\alpha = 0.05$).

Table 3-4. Laboratory analysis of each equine substrate and the rearing medium.

	Laboratory Results						
Substrate	Kjeldahl Nitrogen (N) Mg/kg (%)	Phosphorus (P_2O_5) Mg/kg (%)	Potassium (K_2O) Mg/kg (%)	pH	Natural moisture (%)	Total solids (%)	Total ash (%)
Hay, aged + urine and manure	5,052 (0.51)	1,101 (0.11)	5,568 (0.56)	8.9	68.6	31.4	8.2
Fresh manure (<12 h old)	4,814 (0.48)	2,013 (0.20)	3,310 (0.33)	7.3	78.2	21.8	4.7
Pine shavings + urine and manure (<12 h old)	1,094 (0.11)	1,853 (0.19)	4,259 (0.43)	8.8	35.2	64.8	27.7
Pine shavings, aged manure pile + urine and manure (>72 h old)	449 (0.04)	1,147 (0.11)	5,223 (0.52)	8.8	54.9	45.1	13.9
Soil + aged manure pile + urine and manure (>72 h old)	1,116 (0.11)	1,271 (0.13)	2,301 (0.23)	8.3	59.7	40.3	23.5
Soil, aged dirt lot + urine and manure	3,890 (0.39)	1,351 (0.14)	817 (0.08)	7.7	22.6	77.4	47.1
Rearing medium	10,352 (1.04)	3,571 (0.36)	5,721 (0.57)	4.6	63.3	36.7	2.7

Figure 3-1. Six substrates collected from small equestrian farms (right to left, top to bottom): (a) hay, aged + urine and manure; (b) fresh manure (<12 h old); (c) pine shavings + urine and manure (<12 h old); (d) pine shavings, aged manure pile + urine and manure (> 72 h old); (e) soil, aged manure pile + urine and manure (>72 h old); (f) soil, aged dirt lot + urine and manure; (g) laboratory rearing medium. *Photo courtesy of Erika T. Machtinger.*

Figure 3-2. Trial arenas used for oviposition experiments with random circular placement of hydrated substrates in 120 mL cups. *Photo courtesy of Erika T. Machtinger.*

Figure 3-3. One gallon buckets filled with laboratory rearing medium and used to rear deposited eggs from the oviposition trials. *Photo courtesy of Erika T. Machtinger.*

Figure 3-4. Black cloth placed on hydrated substrate after placement of counted eggs. *Photo courtesy of Erika T. Machtinger.*

Figure 3-5. Pupae floated from the one gallon containers prior to being thoroughly washed and counted. *Photo courtesy of Erika T. Machtinger.*

CHAPTER 4
THE PREFERENCE OF SPALANGIA CAMERONI (HYMENOPTERA: PTEROMALIDAE) FOR HOST SEEKING IN COMMON SUBSTRATES FOUND ON EQUESTRIAN FARMS

Introduction

Many options for filth fly control are available for use in equine facilities; however, historically the use of insecticides been used as the primary method of fly control. According to a USDA, APHIS Animal Health Monitoring System census (2006), over 69% of equestrian farm managers in the southern region use insect repellents. Over 95% of respondents to the Perceptions of Pests Problems and Control Practices on Equestrian Facilities in North and Central Florida (Chapter 5) used fly spray or other forms of chemical insecticides to manage pests. Consistent use of chemicals has led to widespread resistance to many common insecticides in both stable and house flies (Meyer et al. 1987; Scott et al. 1989; Cilek and Greene 1994; Marcon et al. 1997; Keiding 1999; Kaufman et al. 2010; Pitzer et al. 2011b). In some cases, insecticide application rates have been increased with ultimate loss of effectiveness (Malik et al. 2007).

The use of hymneopteran pupal parasitoids of the family Pteromalidae is becoming a popular biological control method and alternative to chemical control for management of house flies and stable flies on equine operations in the United States. Pupal parasitoids have been used throughout the country but dominant genera have been found to differ by region and production system. In Florida, the genus *Spalangia* has been observed to be the most common on livestock facilities, specifically *Spalangia cameroni* Perkins (Butler et al. 1981; Greene et al. 1989; Pitzer et al. 2011a; Romero et al. 2010). *Spalangia cameroni* is most frequently observed in the spring and summer

months on Florida livestock operations (Greene et al. 1989; Pitzer et al. 2011a; Romero et al. 2010), the seasons in which over 95% of respondents to my survey (Chapter 5) perceive they experience the greatest pest pressure on their horse farms.

Biological control offers many advantages over insecticide applications; however the success of augmentative releases of parasitoids has been inconsistent. Increased parasitism of filth flies has been observed after releases in some cases (Morgan and Patterson 1977; Rutz and Axtell 1979; Morgan 1980; Morgan and Patterson 1990; Weinzierl and Jones 1998; Geden and Hogsette 2006). Conversely, in some cases releases have not resulted in significant differences in fly control (Rutz and Axtell 1980; Petersen et al. 1983; Andress and Campbell 1994). Differences in the success of release programs may be the result of substrate and microhabitat preferences of the released species which could influence hose attack rates. Field and laboratory studies have demonstrated that parasitism levels vary by substrate, potentially influenced by many factors including substrate moisture (Legner 1977; Smith and Rutz 1991b; Geden 1999), light (Smith and Rutz 1991b), temperature (Pawson and Petersen 1990) and dispersal of hosts within the habitat and accessibility of hosts (Legner 1977; Rueda and Axtell 1985; Geden 2002; Pitzer et al. 2011b).

There is surprisingly little known about microhabitat preferences and the effects of release rates on habitat preference for parasitism by *S. cameroni*, one of the most common pupal parasitoids on Florida equine facilities (Pitzer et al. 2011). *Spalangia cameroni* is commonly offered through commercial insectaries, but the use of this species in an augmentative parasitoid release program may be affected by microhabitat preferences and influences on equestrian farms. A better understanding of the behavior

and preferences of parasitoids may lead to more refined protocols for use as biological control agents on equine operations that may differ by facility type and geographic location.

My focus in the present study was to examine the preferences of *S.cameroni* in the laboratory among six different common substrates found at equestrian facilities with two different hosts, house flies and stable flies, at two different host: parasitoid ratios. The objectives were to (1) determine if host attack preferences differed by substrate, (2) determine if preference for attack differed by host and (3) determine if host: parasitoid ratio influenced attack rate and future parasitoid production. This knowledge could improve the use of *S. cameroni* as a pupal parasitoid of filth flies by giving equine operators insight in to the habitats where parasites are most effective when augmentative releases are initiated and assess differences in parasitoid preferences when populations are high.

Materials and Methods

Test substrates. Test substrates were the same six common equine substrates and managed as described in Chapter 3. Briefly, these substrates included: (1) hay, aged + urine and manure from around a coastal Bermudagrass round bale, (2) fresh manure (<12 h old) collected from a thoroughbred mare fed perennial peanut hay and concentrated feed, (3) 0.1 to 0.3-cm fresh pine shavings + urine and manure (<12 h old), and small amounts of waste perennial peanut hay from a thoroughbred mare, (4) pine shavings, aged manure pile + urine and manure (>72 h old), containing small amounts of waste perennial peanut hay, (5) soil, aged manure pile + urine and manure, (>72 h old) containing small amounts of waste hay from an Arabian mare fed coastal Bermudagrass hay and concentrated feed and (6) soil, dirt lot + aged urine and aged

manure. Substrates were partially dried in an oven at 45^{o}C to achieve a moisture level of 45%, within the range of substrate moisture preference of *Spalangia cameroni* (Geden 1999). If a substrate had natural moisture level less than 45%, deionized water was added to the substrate to reach the desired hydration. Prior to the experiment, three samples of each substrate were measured to trial weights and hydration and allowed to air dry for five days. Weights were recorded daily on a balance to determine the rate of moisture loss. As cups could not be removed from the cages during the trials, these previously determined rates of moisture loss were used to add moisture to trial cups daily during the experiment.

House flies, stable flies and parasitoids. Third instar house and stable fly larvae were obtained from the USDA-ARS, Center for Medical, Agriculture and Veterinary Entomology (CMAVE) susceptible colony one day prior to expected pupation. Larvae were reared on the diets described in Hogsette (1992) and further detailed in Chapter 3. Larvae were extracted from the rearing diet by placing them in a #10 sieve in a plastic pan. The larvae migrated through the sieve to the pan and then the remaining medium was replaced in the rearing tray. The larvae were place on top of the sieve again and the procedure was repeated three times. For each trial and species, 100 larvae were counted and placed on the surface of a 250-cm^{3} plastic substrate cup containing one of the six substrates and a control cup that contained 50 g of moistened vermiculite. Cups were covered with fabric and secured with plastic lid rims. Larvae were held at 27^{o}C, 80% RH and 24 h light and allowed to pupate naturally in each cup.

Spalangia cameroni females were obtained from a 2010 colony maintained by C.J. Geden at the USDA-ARS, CMAVE in Gainesville, FL. This colony was originally

obtained from a dairy in Gilchrist County, FL in November 2010. Weekly maintenance consisted of providing parasitoids with 2-day-old house fly pupae at a host: parasitoid ratio of 5:1 in 32.5 x 32.5 x 32.5-cm cages (MegaView Science, Taiwan) and held at 25^{o}C, 80% RH under constant darkness. For each experiment, parasitoids were anesthetized by chilling on a cooling table to identify females. Two host: parasitoid ratios were tested for each host, a 5:1 and 20:1 ratio (120 and 30 individual females, respectively, with 600 total fly pupae in each trial).

Bioassay. Release arenas (60 x 60 x 60-cm) with two side panels of polyester netting (72 x 26 mesh) for ventilation (MegaView Science, Taiwan) were used for this study. When 95% of the larvae in the control cups were observed to have pupated, cups were placed randomly in a circle array around a central release point in each of the trial arenas. Parasitoids were released in the center of the array and allowed 72 hours to seek hosts (Kaufman et al. 2001, Pitzer et al. 2011b) (Figure 4-1). After three days, the cups were removed from the chambers, the substrate emptied into containers with water to float and recover the pupae (Lee and Toyama 1990) which were then air dried. Recovered pupae were held in covered 60-cm^3 plastic cups at 25^{o}C and approximately 80% RH with 24 h light for adult fly eclosion. Adult flies were counted after 7d. Adult flies and empty puparia were removed and the remaining pupae were held for parasitoid emergence. After one week without further parasitoid emergence (ca. 6 weeks after test set up), pupae were examined for the presence of emergence holes under a binocular microscope to determine the number that produced adult parasitoids.

Statistical analysis. Three arenas (trials) were used per test date for each species and host: parasitoid ratio and the entire experiment was replicated on three

occasions using different cohorts of parasitoids and flies. Data were subjected to a full factorial analysis of variance (ANOVA) using JMP v. 9 (SAS Institute Inc., Cary, NC 2010) to assess substrate, species and host: parasitoid ratio as main effects for three evaluation parameters. These parameters were (1) percent total host mortality, labeled as "attack," or pupae that did not produce an emerged adult fly divided by total pupae recovered from each cup; (2) percent progeny production, or pupae that produced an adult parasitoid divided by the total recovered pupae recovered from each cup; and (3) the percent of residual host mortality, or the difference between percent pupae recovered and the sum of successful parasitism and adult fly emergence.

Additional ANOVAs were conducted for house flies and stable flies separately with substrate and host: parasitoid ratio as main effects. Means were calculated for host: parasitoid ratios with and without species separation and presented means were separated with Tukey's HSD test for comparison (α=0.05). Percentage data were normalized before ANOVA by an arcsine transformation, but untransformed values were used to present results in tables and text.

Results

Percent total host mortality. Substrate and host: parasitoid ratio were significant main effects on the influence of *Spalangia cameroni* to induce pupal mortality in the whole model ANOVA and there was a significant species x substrate interaction term (Table 4-1). When host: parasitoid ratios were analyzed separately, incorporating both host species, there were no significant differences among substrates in host mortality at the 5:1 host: parasitoid ratio (Table 4-2). However, the percent of pupal mortality was high for all substrates, ranging from 82.7 to 97.7%. Total percent pupal mortality in each substrate ranged from 62.2 to 84.6% at the 20:1 ratio. *Spalangia cameroni*

frequently killed pupae in the substrate composed of pine shavings, aged manure pile + urine and manure (>72 h old) (84.6%).

Comparison of host: parasitoid ratio between host species revealed that attack by *S. cameroni* was influenced by substrate for the percent pupae killed of both house fly and stable fly hosts and host: parasitoid ratio had significant effect on stable fly hosts (Table 4-3). There were no significant differences between means of percent house fly host mortality for any substrate at the 5:1 ratio, which ranged from 76.3 to 99.2% (Table 4-4). House fly pupal mortality was lower for the 20:1 host: parasitoid ratio than the 5:1 host: parasitoid ratio. In the 20:1 ratio, the hay, aged + urine and manure (80.0%), pine shavings, aged manure pile + urine and manure (>72 h old) (78.0%), fresh manure (83.3%) and soil, aged dirt lot + urine and manure (75.0%) had significantly higher pupal mortality than the remaining substrates. The fresher pine shavings + urine and manure (<12 h old) had the lowest host mortality for both host ratios (45.5%). There were no significant differences in means of percent pupal mortality of stable fly hosts (Table 4-5) at either host: parasitoid ratio. However, when *S. cameroni* had access to fewer hosts, percent mortality ranged from 96.6 to 99.7%. There was a broader range of percent mortality in the 20:1 ratio which ranged from 67.7% to 91.2%.

Percent progeny production. In the full model ANOVA, there was a significant substrate effect and interaction terms of species x substrate and substrate x host: parasitoid ratio on *S. cameroni* progeny production (Table 4-1). The pine shavings, aged manure pile + urine and manure (>72 h old) and hay, aged + urine and manure were significantly preferred by *S. cameroni* for attack and progeny production (average 65.6 and 63.2%, respectively) ($F_{5,228}$= 5.01; P=0.0002) and pine shavings + urine and

manure (<12 h old) was the least favorable (48.3% production). Though there was no overall significant effect of host: parasitoid ratio on progeny production in the whole model, *S. cameroni* showed significantly higher reproductive success in the hay, aged + urine and manure and pine shavings, aged manure pile + urine and manure (>72 h old) (66.9 and 67.4%, respectively) when hosts were limited at the 5:1 host: parasitoid ratio. *Spalangia cameroni* produced significantly fewer progeny in fresh manure (45.1%). There were no significant differences in the percentage of progeny produced at the 20:1 host: parasitoid ratio, which ranged from 44.0 to 64.6%.

When intraspecific differences were analyzed, there was a significant substrate and substrate x host: parasitoid interaction on progeny production with house fly hosts, but no significant main effects with stable flies (Table 4-3). *Spalangia cameroni* did not demonstrate substrate discrimination for progeny production at the 5:1 host: parasitoid ratio with house flies on all substrates and there were no significant differences between means (Table 4-4). There were more distinct substrate preferences when more house fly hosts were available. *Spalangia cameroni* parasitized significantly more pupae in the soil, aged manure pile + urine and manure (>72 h old) (57.7%) and soil, aged dirt lot + urine and manure(56.6%), fresh manure (<12h old) (55.7%) and hay, aged + urine and manure (54.8%). Two-fold more progeny were produced in these substrates than the pine shavings + urine and manure (<12 h old) substrate (25.6%) (Table 4-4). In the ANOVA, there were no significant main effects influencing progeny production of *S. cameroni* with stable fly hosts and *S. cameroni* was not selective in any substrate at any host: parasitoid ratio. At the 5:1 host: parasitoid ratio, progeny production ranged from 38.7 to 63.35% and at the 20:1 ratio , production ranged from 48.1 to 76.1% (Table 4-5).

Percent residual host mortality. Substrate, host: parasitoid ratio and the interaction of substrate x host: parasitoid ratio had significant influences on the percent of residual host mortality in the three-way ANOVA (Table 4-1). There were no significant differences between means of percent residual host mortality at the 5:1 ratio on all hosts (Table 4-2). However, at the 20:1 host: parasitoid ratio significantly higher percent residual host mortality was observed in fresh manure (<12 h old) (27.2%). The pine shavings + urine and manure (<12 h old) (18.6%) and soil, aged manure pile + urine and manure (>12 h old) (16.2%) and soil, aged dirt lot + urine and manure (19.0%) had the lowest percent residual host mortality at the 20:1 host: parasitoid ratio.

When analyzed by host species, there were significant substrate, host: parasitoid ratio and substrate x host: parasitoid interactions on the percent residual house fly mortality in the ANOVA. The host: parasitoid ratio had a significant influence on percent residual stable fly mortality (Table 4-3). At the 5:1 host: parasitoid ratio, there was no separation in the means of percent residual mortality on house fly hosts. However, significant differences were observed at the 20:1 host: parasitoid ratio with hay, aged + urine and manure (25.2%) fresh manure (<12 h old) (27.6%) and pine shavings, aged manure pile + urine and manure (>72 h old) (27.5%) having the highest percent residual host mortality (Table 4-4). The lowest percent residual mortality for house fly hosts at this ratio was in the soil, aged manure pile + urine and manure (>72 h old) (14.2%). There were no significant differences in the means of percent residual mortality of pupae at either host: parasitoid ratios for stable flies. The percent residual host mortality ranged from 32.2 to 57.9% at the 5:1 ratio and 15.1 to 26.8% at the 20:1 host: parasitoid ratio (Table 4-5).

Discussion

Microhabitat has been shown to be influential in the host attack preferences of several species of hymenopteran pupal parasitoids (Petersen and Meyer 1983; Rueda and Axtell 1985; Smith and Rutz 1991a, 1991b; Geden 1999; Geden 2002). The results of the present study support the importance of microhabitat associations on the effectiveness of *Spalangia cameroni* to induce mortality of filth fly hosts at low densities and further suggest that greater parasitoid population levels diminish habitat discrimination by S. *cameroni,* inducing greater total mortality of hosts in common equine substrates. As seen in Chapter 2 and detailed by Pitzer et al. (2011a), *S. cameroni* is an important pupal parasitoid on equestrian farms and host habitat associations and the influence of host density are important factors for improving augmentative release programs for control of filth flies on these facilities.

Substrate had a significant effect on total mortality of hosts and on parasitoid progeny production. In a field survey, Smith and Rutz (1991a) determined that *S. cameroni* preferred loose substrates and Geden (2002) found that induced mortality by all tested species of parasitic wasps in the laboratory was hindered by the sandy loam soil treatment. The results presented here support these generalizations in terms of the overall preference of *S. cameroni* for looser substrates, such as pine shavings and hay. However substrate density did not appear to be a factor in overall attack success. The soil-based substrates in the current study were not significantly preferred or avoided by *S. cameroni* and did not have significant influences on progeny production or overall host mortality. It does not appear as though substrate texture or host accessibility influenced the preferences of substrate as a combination of both dense and loose substrates were preferred and avoided. Overall, *S. cameroni* had significant

preferences for the less dense pine shavings, aged manure pile + urine and manure (>72 h old) and the hay, aged + urine and manure but were not as successful in the looser fresh pine shavings + urine and manure (<12 h old) from the stall or the more compact fresh manure (<12 h old). Considering this, chemical cues may have a greater influence on habitat selection than the physical characteristics of the substrate. Carbon dioxide (CO_2) emissions increase as horse manure decomposes (Jeanbourquin and Guerin 2007). As the fresher substrates were less preferred by *S. cameroni*, this increased CO_2 emission, or potentially other chemicals, may serve as semiochemical cues for attracting *S. cameroni* oviposition in the aging equine substrates. Further investigations with a lower ratio of parasitoids to hosts may further refine substrate effects on total mortality.

Though total mortality was higher with the 5:1 host: parasitoid ratio, no difference was exhibited between host: parasitoid ratios in percent progeny production, contrary to the findings of Legner (1967) who saw increased progeny production with increased numbers of female *S. cameroni*. However, progeny production was influenced by substrate with increasing conspecific competition for hosts. In the 5:1 host: parasitoid ratio, the pine shavings, aged manure pile + urine and manure (>72 h old) and hay, aged + urine and manure were significantly preferred by *S. cameroni* for reproduction. Progeny production was similar throughout all substrates at the 20:1 host: parasitoid ratio, but there were total mortality differences by substrate. Significantly more total host mortality was documented at the 5:1 ratio than when fewer parasitoids were allowed many hosts. Furthermore, there was substantially more residual mortality at the 5:1 host: parasitoid ratio than the higher host: parasitoid ratio of 20:1. Geden (1999)

observed more frequent residual mortality of hosts with *S. cameroni* at less preferred moisture levels. Similarly, residual mortality differed in the current study when more hosts were available but was not different at the 5:1 host: parasitoid ratio. *Spalangia cameroni* is a solitary parasitoid which avoids superparasitism (Legner 1967; Wylie 1971; 1972). Propp and Morgan (1983) determined that *Spalangia endius* Walker, another solitary parasitoid, could discriminate and avoid ovipositing in previously parasitized pupae, but only after drilling into the puparia. This behavior may be shared with *S. cameroni* and under population pressure, the assessment and subsequent drilling by the parasitoid to avoid superparasitism could be potentially fatal to the developing fly or newly laid wasp egg, increasing overall host mortality and decreasing successful parasitism. Alternatively, *S. cameroni* females may determine pupae suitable for host feeding in less desirable substrates out of necessity when hosts are limited but may refrain from proceeding with oviposition, as suggested by Geden (1999). Differences in percentage of residual host mortality and progeny production between host: parasitoid ratios may be a result of parasitoid feeding on hosts prior to oviposition, making hosts unsuitable for increased progeny production at the 5:1 ratio and supporting previous findings.

There were no overall differences in host mortality or progeny production between house fly and stable fly hosts, and this observation is consistent with previous literature (Petersen and Meyer 1983). However, there were differences in the effectiveness of *S. cameroni* on the various equine substrates between host species. With house fly hosts, substrate influenced total pupal mortality, progeny production and the residual mortality at the 20:1 host: parasitoid ratio. When host numbers were reduced, there was a

behavior change and preferences were balanced throughout all six substrates. *Spalangia cameroni* demonstrated ability to adjust to population pressure and attack hosts in a range of substrates, potentially as a mechanism to avoid superparasitism (Geden 1999). Interestingly, there were no differences in substrate preference in either host: parasitoid ratio when *S. cameroni* attacked stable fly pupae. Differences were certainly found between host: parasitoid ratios. However, as expected, there was greater percentage of total host mortality at the 5:1 host: parasitoid ratio than the 20:1 ratio with both house fly and stable fly hosts. House fly and stable fly pupae are often found in similar habitats on equestrian farms, increasing the likelihood of *S. cameroni* encountering suitable hosts of both species simultaneously. However, as described in Chapter 2, peak population levels of filth flies differ seasonally in Florida equestrian farms (Pitzer et al. 2011a). These moderate differences in substrate preferences between species may influence seasonal parasitism between hosts at natural levels; however differences appear to be eliminated with greater numbers of parasitoids as would be present with an augmentative release program.

A disparity between house fly host oviposition preferences and parasite attack is apparent. As seen in Chapter 3, house fly hosts had preferences for oviposition in fresh manure based substrates, whereas stable flies preferred the aged substrates. These preferences have also been noted under field conditions (Broce and Haas 1999; Cook et al. 1999). King (1997) determined that *S. cameroni* produced more progeny on younger pupae when given a choice and younger stable fly pupae are likely to be found in aging substrates on equestrian farms. In the current study, the lack of preference for stable fly hosts and the occurrence of stable fly immature development in aged

substrates in the field may initially bias natural parasitoid seeking of stable fly hosts. However, house fly pupal development time in the equine generated substrates tested was a minimum of 6 days (Chapter 3) and maximum parasitoid attack may occur before emergence and after the pupation substrate has reached an attractive level for fly oviposition. Though there was no effect of individual species on total percent killed pupae or progeny production, the interaction and effect of host competition was not evaluated in this experiment and could be assessed in future trials.

Field assessments and laboratory experiments, including the present study, have shown initial habitat preferences of *S. cameroni*. However, though larvae were allowed to pupate naturally in the cups used for the experiment, natural pupation is generally not so limited and larvae may leave the development site and move to more suitable areas to avoid parasitism within substrates in the field. Furthermore, natural parasitoids, and even those in some release programs, experience spatial and dispersal challenges to identify where hosts may be located. This laboratory experiment allowed for free access to hosts without impediment of determining host and habitat location and subsequent dispersal and may have influenced host discrimination by *S. cameroni*. Future studies on dispersal range and the effect of semiochemical cues on dispersal and host habitat identification and preference of *S. cameroni* could provide more insight in to the behavior of this species to locate and attack hosts on equestrian farms.

In summary, *S. cameroni* has been evaluated in multiple lab and field based studies for host location ability, effect of host density, and to a limited degree, the effect of substrate on parasitism. However, this is the first study to my knowledge to evaluate the effect of multiple equine generated substrate choices at varying host: parasitoid

ratios on two different hosts. Progeny production did not increase with greater numbers of female parasitoids in this experiment and thus further research on the effect of increased parasitoid releases on the reproductive behavior of Florida collected *S. cameroni* in equine generated substrates are needed to determine the effect on augmentative release programs. This study demonstrated the selectivity of *S. cameroni* to attack pupae found in the aging equine substrates which was mitigated at higher release numbers. Not only is *S. cameroni* already an important natural parasite of filth flies on equestrian farms in Florida, but the ability of *S. cameroni* to exhibit flexibility in host attack between substrates when released in innundative levels makes this species highly suitable for augmentative control programs. Further experimentation to evaluate interspecific effects of competitive parasitoids on the behavior and effectiveness of *S. cameroni* in equine generated substrates and ability to moderate filth fly populations in a field release program is necessary to fully evaluate the potential of this species for IPM systems on equestrian farms.

Table 4-1. Full factorial ANOVAs for effects of the host species, host: parasitoid ratio and substrate on the rates of host attack by *Spalangia cameroni* and killed hosts.

	Percent Total Host Mortality		Percent Parasitoid Progeny Production		Percent Residual Host Mortality	
Variable	*F*	*P*	*F*	*P*	*F*	*P*
Fly species	0.47	0.3113NS	0.88	0.3485NS	0.01	0.9356NS
Substrate[1]	5.9	<0.0001**	4.26	0.0010**	2.27	0.0486**
Host: parasitoid ratio[2]	4.99	0.0266**	0.77	0.3824NS	4.17	0.0424**
Species x substrate	3.99	0.0018**	3.65	0.0035**	1.25	0.2863NS
Species x H:P ratio	0.11	0.7387NS	0.00	0.9882NS	0.63	0.4274NS
Substrate x H:P ratio	2.19	0.0570NS	2.36	0.0410**	2.65	0.0238**
Species x substrate x H:P ratio	1.85	0.1042NS	2.09	0.0683NS	1.18	0.3185NS

[1] Pupae in substrates of hay, aged + urine and manure; fresh manure (<12 h old); pine shavings + urine and manure (<12 h old); pine shavings, aged manure pile + urine and manure (>72 h old); soil, aged manure pile + urine and manure (>72 h old); and soil, aged dirt lot + urine and manure.
[2] Host: parasitoid ratios of 5:1 and 20:1
**, $P \leq 0.05$; NS, $P > 0.05$.

Table 4-2. Influence of host: parasitoid ratio on pupae killed, *Spalangia cameroni* progeny production and residual mortality by test substrates[2].

Ratio	Substrate	Total Host Mortality ($\bar{X}$ % ± SEM)	Parasitoid Progeny Production ($\bar{X}$ % ± SEM)	Residual Host Mortality ($\bar{X}$ % ± SEM)
5:1	Hay, aged + urine and manure	94.9 ± 1.2^{a}	66.9 ± 2.9^{a}	28.2 ± 2.1^{a}
	Fresh manure (<12 h old)	88.0 ± 5.6^{a}	45.1 ± 6.1^{b}	42.9 ± 5.5^{a}
	Pine shavings + urine and manure (<12 h old)	82.7± 5.3^{a}	52.1 ± 6.7^{ab}	33.4 ± 5.0^{a}
	Pine shavings, aged manure pile + urine and manure (>72 h old)	97.7 ± 0.7^{a}	67.4 ± 3.4^{a}	30.4 ± 3.0^{a}
	Soil + aged manure pile + urine and manure (>72 h old)	94.9 ± 2.0^{a}	55.8 ± 3.1^{ab}	39.1 ± 3.3^{a}
	Soil, aged dirt lot + urine and manure	93.6 ± 2.6^{a}	61.4 ± 3.5^{ab}	32.2 ± 2.8^{a}
20:1	Hay, aged + urine and manure	81.2 ± 3.9^{ab}	58.8 ± 4.9^{a}	22.4 ± 1.8^{ab}
	Fresh manure (<12 h old)	81.5 ± 5.2^{ab}	54.5 ± 5.7^{a}	27.2 ± 1.9^{a}
	Pine shavings + urine and manure (<12 h old)	62.6 ± 5.9^{b}	44.0 ± 6.6^{a}	18.6 ± 2.7^{b}
	Pine shavings, aged manure pile + urine and manure (>72 h old)	84.6 ± 3.9^{a}	63.5 ± 5.2^{a}	21.1 ± 2.1^{ab}
	Soil + aged manure pile + urine and manure (>72 h old)	80.8 ± 4.7^{ab}	64.6 ± 4.2^{a}	16.2 ± 1.2^{b}
	Soil, aged dirt lot + urine and manure	71.3 ± 4.7^{ab}	52.4 ± 5.3^{a}	19.0 ± 2.2^{b}

[1]Both stable fly and house fly pupae were included in the host: parasitoid ratio analysis

[2]Means in a column followed by the same letter are not significantly different (Tukey's HSD test,α =0.05).

Table 4-3. ANOVAs for comparative effects of test substrate and host: parasitoid ratio for stable flies and house flies.

		Percent Total Host Mortality		Percent Parasitoid Progeny Production		Percent Residual Host Mortality	
Species	Variable	*F*	*P*	*F*	*P*	*F*	*P*
House fly	Substrate[1]	4.34	0.0012**	4.22	0.0015**	2.51	0.0337**
	Host: parasitiod ratio[2]	3.69	0.0574NS	0.76	0.3856NS	4.61	0.0338**
	Substrate x H:P ratio	1.62	0.1615NS	2.34	0.0460**	2.94	0.0157**
Stable fly	Substrate	4.31	0.0091**	2.08	0.0738NS	1.09	0.3734NS
	Host: parasitiod ratio	11.17	0.0012**	0.71	0.4008NS	8.07	0.0055**
	Substrate x H:P ratio	1.14	0.3470NS	0.99	0.4286NS	0.47	0.7998NS

[1] Pupae in substrates of hay, aged + urine and manure; fresh manure (<12 h old); pine shavings + urine and manure (<12 h old); pine shavings, aged manure pile + urine and manure (>72 h old); soil, aged manure pile + urine and manure (>72 h old); and soil, aged dirt lot + urine and manure.
[2]Host: parasitoid ratios of 5:1 and 20:1
**, $P \leq 0.05$; NS, $P > 0.05$.

Table 4-4. Influence of test substrate with house fly hosts on killed pupae, *Spalangia cameroni* progeny production and residual mortality per trial[1].

	Total Host Mortality ($\bar{X}$ % ± SEM)		Parasitoid Progeny Production ($\bar{X}$ % ± SEM)		Residual Host Mortality ($\bar{X}$ % ± SEM)	
Substrate	5:1	20:1	5:1	20:1	5:1	20:1
Hay, aged + urine and manure	97.4 ± 1.2^{a}	80.0 ± 4.9^{a}	72.3 ± 1.8^{a}	54.8 ± 6.5^{a}	25.1 ± 8.0^{a}	25.2 ± 2.3^{a}
Fresh manure (<12 h old)	81.5 ± 9.4^{a}	83.3 ± 5.6^{a}	49.9 ± 5.7^{a}	55.7 ± 7.7^{a}	31.6 ± 19.9^{a}	27.6 ± 5.7^{a}
Pine shavings + urine and manure (<12 h old)	76.3 ± 8.8^{a}	45.5 ± 7.8^{b}	56.3 ± 4.1^{a}	25.6 ± 7.5^{b}	24.6 ± 14.3^{a}	19.8 ± 4.1^{ab}
Pine shavings, aged manure pile + urine and manure (>72 h old)	99.2 ± 0.3^{a}	78.0 ± 6.8^{a}	70.3 ± 3.8^{a}	50.9 ± 7.7^{ab}	29.0 ± 13.1^{a}	27.1 ± 3.8^{a}
Soil + aged manure pile + urine and manure (>72 h old)	93.7± 3.5^{a}	71.9 ± 8.5^{ab}	56.4 ± 4.6^{a}	57.7 ± 7.7^{a}	37.3 ± 15.8^{a}	14.2 ± 4.6^{b}
Soil, aged dirt lot + urine and manure	95.8 ± 1.9^{a}	75.0 ± 4.4^{a}	66.3 ± 4.5^{a}	56.6 ± 6.6^{a}	29.5 ± 15.7^{a}	18.4 ± 4.5^{ab}

[1]Means in a column followed by the same letter are not significantly different (Tukey's HSD test,α =0.05).

Table 4-5. Influence of test substrate with stable fly hosts on killed pupae, *Spalangia cameroni* progeny production and residual mortality per trial[1].

	Total Host Mortality ($\bar{X}$ % ± SEM)		Parasitoid Progeny Production ($\bar{X}$ % ± SEM)		Residual Host Mortality ($\bar{X}$ % ± SEM)	
Substrate	5:1	20:1	5:1	20:1	5:1	20:1
Hay, aged + urine and manure	91.7 ± 2.2[a]	82.4 ± 6.3[a]	60.0 ± 5.6[a]	62.9 ± 7.4[a]	32.3 ± 3.3[a]	19.6 ± 2.2[a]
Fresh manure (<12 h old)	96.6 ± 1.2[a]	79.7 ± 9.2[a]	38.7 ± 8.0[a]	53.2 ± 9.7[a]	57.9 ± 8.0[a]	26.8 ± 2.9[a]
Pine shavings + urine and manure (<12 h old)	91.2 ± 2.8[a]	79.8 ± 3.8[a]	46.4 ± 12.0[a]	62.3 ± 6.7[a]	45.0 ± 9.3[a]	17.5 ± 3.6[a]
Pine shavings, aged manure pile + urine and manure (>72 h old)	95.7 ± 1.3[a]	91.2 ± 2.3[a]	63.5 ± 6.0[a]	76.1 ± 3.7[a]	32.2 ± 5.1[a]	15.1 ± 2.3[a]
Soil + aged manure pile + urine and manure (>72 h old)	96.4 ± 0.8[a]	89.7 ± 1.7[a]	54.9 ± 5.6[a]	71.5 ± 2.3[a]	41.6 ± 5.0[a]	18.2 ± 1.6[a]
Soil, aged dirt lot + urine and manure	90.7 ± 5.7[a]	67.7 ± 8.7[a]	54.9 ± 5.5[a]	48.1 ± 8.4[a]	35.8 ± 2.5[a]	19.6 ± 3.4[a]

[1]Means in a column followed by the same letter are not significantly different (Tukey's HSD test,α =0.05)

Figure 4-1. Array of 250-cm3 plastic cups in the trial arena with a different test equine substrates and released parasitoids in the center of the array. *Photo courtesy of Erika T. Machtinger.*

CHAPTER 5
SURVEY ON PERCEPTIONS OF PEST PROBLEMS AND CONTROL PRACTICES ON EQUINE FACILITIES IN NORTH AND CENTRAL FLORIDA

Introduction

Integrated pest management for equestrian farms requires identification of pest problems and coordinated use of science-based decisions for management. The goal is to implement pest management strategies which reduce economic, health and environmental risks while maximizing control. This approach to managing pests is described in the National IPM Road Map (Coble and Ortman 2009).

The practices of many equine farms are often excluded from survey at the national level. The USDA, APHIS Animal Health Monitoring System census (2006), which details management practices on equestrian farms nationwide, did not include properties with pastures and fewer than five horses, and service facilities, such as boarding barns and racetracks. This restriction potentially eliminated approximately half of the properties in the United States which house horses, and certainly the small equestrian farms and presents an inaccurate analysis of the needs and practices of the equine industry.

There are many options for managing pests affecting horses. An abundance of pest control products currently are available and marketed to horse and equestrian facility owners, including biological control agents, chemical sprays, personal protection devices (fly masks, wraps and blankets) and traps. However, there is insufficient information on the efficacy of many of these products. It is estimated that horse owners spent over $40 million in the United States for ectoparasite control on their animals in 1997 (Hogsette and Geden 2001) and the current total is likely much higher due to the increase in available pesticides and non-chemical control methods, including biological

control. A significant amount of this expense may be unjustified because current methods of pest control on these facilities may be unnecessary or ineffective. A lack of understanding of endoparasite control was found in a survey of horse owners in Tennessee where contamination and strategic dosing was common (Reinmeyer and Rohrbach 1990). Lendal et al. (1998) concluded that there was evidence of a gap in the knowledge of horse owners on how to control parasites to avoid resistance development.

The overuse of insecticides and other chemicals creates a risk of environmental contamination and pest resistance. In a survey of Pony Club members in the United Kingdom, Biggin et al. (1999) found the majority of endo- and ecto-parasite control measures were chemical based. Endoparasite resistance to antihelminthic drugs has been reported recently in equines (Kaplan et al. 2004; Brady and Nichols 2009) due to the over and habitual use of these chemicals. In Florida, insecticide resistance has been documented in common equine pests, such as the stable fly, house fly and horn fly (Schmidt et al. 1985; Kaufman et al. 2010; Pitzer et al. 2010). Additionally, the excessive use of insecticides may have negative effects on natural enemies (Wills et al. 1990; Geden et al. 1992).

To develop an effective IPM system, the current perceptions of pest problems and management techniques of facility and horse owners needs to be identified. Many practices of an IPM system currently may be over or under utilized by North and Central Florida equestrians. This might be attributed to the lack of available information and training on developing IPM systems for equestrian farms.

The purpose of this survey was to gain knowledge about IPM on equestrian farms in North and Central Florida and use it to guide research and Extension activities. The objectives of this survey were to determine the (1) perceived pest problems on equestrian farms, (2) pest management practices used on equestrian farms and (3) research and Extension needs of horse and facility owners in North and Central Florida.

Methods

A survey was conducted from 15 November, 2010 to 20 June, 2011 through Survey Monkey (www.surveymonkey.com) to collect and analyze responses from anonymous respondents. The link was sent to 16 of the major equestrian organizations in North and Central Florida (including regional shows, clubs and associations) in the counties listed in Appendix C, with the request that it be distributed to memberships and mailing lists. It also was provided in the Central Florida Equestrian Magazine and to county Extension agents. Respondents could have accessed the anonymous survey from any of these sources.

The survey had three sections. The first section asked the respondents to detail pest problems and pest control practices on their facilities. The second sought opinions on research and Extension materials. The final section asked for basic demographic information of the participants, including discipline, property acreage and number of horses. Discipline was divided into pleasure riders, performance (or show) horses and other.

Cumulative data were presented in frequency tables and histogram form. Many of the respondents did not answer all of the questions and several questions had multiple answers. Consequently, percent response for each question was calculated by dividing the number of respondents who answered the question by the total number of

respondents who completed the survey. Percentage data was used for comparing totals of responses. Acreage was divided into 1 to 10 acres and greater than 10 acres. Horse numbers were grouped into 1 to 5 horses and over 6 horses, the latter being included in the USDA, APHIS Animal Health Monitoring System census. Responses were divided in these demographic groups for inclusion in a Chi-Square analysis; however, responses by demographic groups were not found to be significant and thus are not seperated in these results.

Results

The survey yielded 299 responses, however not every respondent answered every question. A relatively low number of responses often occurred for a second question which followed a base question. The number of responses to questions ranged from 274 to 299.

Demographics. Respondents represented a diverse population of the equestrian community (Table 5-1) from every county in North and Central Florida except Baker, Hamilton and Madison Counties. The survey population consisted of a high percentage of pleasure riders (49.1%, 139/283). Horses were used for performance riding by 41.7% of the survey population (118/283).

The majority of the respondents were facility owners (84.5%, 240/284) and 56.4% (159/282) of the facilities were 10 acres or less. Most of the facilities had between one and five horses (63.7%, 181/294).

Perceived pest problems. To identify what month pest problems were perceived as the greatest, survey takers were asked when they experienced the highest level of pest pressure. Overwhelmingly, the summer months (June, July and August) were perceived as the most troublesome (86.1%, 254/295). Respondents were asked which

pests give them the most problems and were allowed to choose multiple answers. Sixty-three percent (188/298) found stable flies to be a problem on their horses and 61.6% (182/298) responded with gnats (Figure 5-1). For the purpose of this survey, the term “gnats” was defined as any very small fly around the horse. Mosquitoes comprised 59.4% (177/298) of the answers and horse flies and deer flies were about 46.3% (138/298). About 33% (99/298) found house flies to be a problem. Respondents were allowed to write in other pests which were problems on their facilities (*n*=32). *Culicoides* spp. midges were the most popular write-in answer (*n*=5). To follow-up, survey takers were asked what pests they could accurately identify and common responses were mosquitoes (93.6%, 280/299), stable flies (61%, 184/299) and house flies (75%, 227/299). Eighty percent (241/299) responded that they could identify horse and deer flies accurately. “Yellow flies” was the most common write-in response for additional pests that could be identified by the survey takers (*n*=5). Respondents were asked if they monitored for pests on their property. Of the 298 respondents, 76.8% (229/298) did not monitor pest populations during the year, many because they lack knowledge about the required methods and materials (59.1%, 133/225).

Pest management practices and opinions. Survey takers were asked multiple questions about property and pest management practices on their equestrian farms. Thresholds for pest activity around respondent’s animals was low, 61.7% (177/287) responding that less than 10 pests would be tolerated around the animal or sticky trap. Only 1.7% (5/287) of the survey population would tolerate over 50 pests on their animal or sticky trap.

Manure management is one of the most important factors in IPM of filth fly pests and some form was practiced by virtually all the respondents (Table 5-2). The most common method was the use of a manure spreader (49.0%, 144/294), followed by dragging pastures (22.1%, 65/294), or mechanically breaking up manure piles and spreading across the field, placing manure in a pile (24.8%, 73/294) and active composting (25.9%, 76/294). However, approximately two-thirds did not think they have enough time to properly manage manure on their property (66.1%, 193/292). Additionally, only two-thirds (61.0%, 203/294) think they are able to recognize where pests breed.

The predominant methods used to manage pests were insecticides (95.9%, 284/299) and physical exclusion with fly masks (66.2%, 19/296), although respondents also used traps (37.8%, 112/296), biological control (22.0%, 65/296), fly sheets (21.3%, 63/296) and feed through control (14.5%, 43/296) (Table 5-3). Feed through control was defined as the use of insect growth regulators in a form which can be fed to the animal and excreted in feces. Between two and four different types of control methods were used by over 76% of the responding population (Table 5-4). Less than three quarters of the respondents thought their pest control products were somewhat effective (72.3%, 214/296) and only 9.6% (27/296) found them to be very effective. More than 55% of respondents (165/297) spent in excess of $100 on pest control annually, but 51% (148/288) indicated that the pest control products they purchased were not cost effective.

Research and extension requests. About half of the survey population (54.3%,152/280) thought that not enough research and Extension support was being

provided to meet their needs. To obtain feedback as to what would meet their needs, respondents were asked what topics they would like to have emphasized (Table 5-5). The population was allowed to choose multiple options. Biological control was the most popular selection with 62.1% (172/277) wanting more Extension materials on this subject. The use of chemicals was selected by 40.4% (112/277) of respondents and cultural control methods by 38.3% (106/277). When asked what kinds of research they would like to have increased, the majority requested biological control (80.2%, 231/276); cultural control methods were next (48.6%, 140/276) followed by the use of insecticides. (40.0%,121/276).

Discussion

The results presented here show that horse owners in North and Central Florida attempt to manage their pest insects with a variety of measures, many of which are components of an IPM program. However, based on survey responses, many opportunities are available for research and Extension programming on the fundamentals of IPM to reduce the prevalence of pests on equestrian farms.

The equine industry in North and Central Florida represents a diverse sector of agriculture. There were many differences in the acreage and number of horses at the farms and over 21 horse uses were cited. More than 90% of the survey respondents were pleasure riders or engaged in performance activities, which was significantly higher than the 2005 American Horse Council survey results that found 60% of Florida horses were used in showing and recreation. However, equestrians participating in showing and recreational activities are likely to be affiliated with at least one organization or group that had access to the recent survey. Therefore, it was expected that higher numbers might respond. Owners and managers of small facilities (those

under 10 acres) contributed most of the answers. A small farms survey conducted by Gaul et al. (2009) found that 64% of all farms in Florida and encompassing all livestock were less than 50 acres, and horses were the second most common small farm enterprise following beef cattle. Based on these results it would seem advisable to encourage research and Extension efforts in support of the small equestrian farm sector.

Many insect pests were seen as problems on horse farms by the survey population. The most common pest species were mosquitoes, stable flies and gnats with many write-in responses for yellow fly (*Diachlorus ferrugatus* Fabricius*)*, the so-called "cow fly" that respondents may have applied to the horn fly (*Haematobia irritans* L.) , stable fly or even house fly. However, the colloquial term “cow fly” typically refers to the horn flies most often found on the backs of cows. *Culicoides* spp., or midges, were also responses that, along with yellow fly and “cow fly,” were options in the base question. This was interesting as it demonstrated that many respondents may not be certain in what group their pests belong or may use colloquial terminology. This was seen again in the follow-up question on which pests survey takers could accurately identify. While 63.1% answered that stable flies were a problem, only 61.5% thought they could identify stable flies accurately. Several answers were written in for yellow fly or horse fly as well which was one of the available answers. It is surprising that so many felt that stable flies were a problem as they are not seen frequently feeding on horses. In a field study conducted by Pitzer et al. (2011c), stable flies in Ocala, Florida were found to have fed primarily on cattle. Though stable flies can be found on horses, they prefer to feed on the forelegs (Mullens et al. 1988) and do not linger, preferring to

rest on nearby walls or fence lines (Gerry et al. 2007; Mullens et al. 1988), making it unlikely that horse owners would observe them frequently. Although respondents reported most pest pressure in the summer, stable flies are most abundant in late winter and spring on horse farms in Florida (Pitzer et al. 2011a). Respondents may be observing horn flies or house flies and mistaking them for stable flies. Based on responses, the ability of the survey population to accurately identify pest species is questionable. This survey demonstrates a gap in the knowledge of equestrian facility owners and an educational opportunity for Extension agents and others to educate property owners on pest biology and identification. This knowledge will improve the use of species-specific pest control methods.

Monitoring and action thresholds are necessary to determine when control measures should be applied in an IPM program. Over 76% in this survey answered that they did not monitor pests on their property and cited lack of knowledge and time as the primary reasons. As equestrian farms are often owned by families which have full-time employment elsewhere or are boarding and training facilities which engage in riding lessons and other services, a lack of time not only to learn and implement monitoring methods but also gather the required information is understandable. When asked to describe monitoring techniques in the comment box, many of the responses listed some form of control (such as biological control, traps or insecticides). It is clear that monitoring in the context of IPM, regular observations on the status of pests on a property, is not widely understood by horse owners. Respondents are aware of their lack of knowledge of pest monitoring and this could provide an opportunity for Extension agents to provide training. It is clear that increasing the knowledge base of this clientele

is critical for establishing IPM systems on equestrian facilities. Monitoring techniques will have to be time-efficient and easy to implement.

Action (also known as economic or damage) thresholds are the basis for decision making in an IPM system (Higley and Pedigo 1996). Respondents were asked in this survey how many pests they would tolerate around their animals and the majority responded less than 10 pests. Unfortunately, action thresholds have not been determined for horses as the economic or damage level is difficult to quantitatively assess in terms of losses. Action thresholds for horn flies on cattle have been found to be 50 to 200 flies (Hogsette et al. 1991). The overall tolerance for flies on horses is apparently lower and multiple pest species might accumulate on any one animal. However, in our survey, it might have been difficult for owners to assign numbers of pests around their animals into the groupings we provided, so they answered 10 or lower wishing to convey a lower, but not necessarily an accurate, number. Another opportunity for Extension may be presented in the education of horse owners in establishing personalized action thresholds based on previous monitoring and observed activity of pests on and around their animals. Action thresholds are important and should be included in future studies on IPM for equestrian farms. Cultural control and sanitation is a vital component of an IPM program (Greene 1993; Keiding 1986). All but three of the 272 respondents (99%) had some practice of manipulating manure waste, suggesting that horse owners are aware that manure waste and bedding are a source of pest production or attraction. The use of a spreader was the most common method for manure disposal, though 89.1% used multiple methods. Similarly, 63% of facility owners nationally applied waste to pastures and this was the most common disposal

method in the southern region (United States Department of Agriculture 2006). Allowing waste to accumulate or leaving it to nature was popular as well (34.7%). The USDA survey did not specify if leaving manure to nature or allowing manure to accumulate included a manure pile or compost area. Additionally, it was not clear if applying manure to fields included the use of a drag or manure spreader. Lloyd et al. (2000) found that pasture hygiene in the United Kingdom was common on equestrian facilities and 49% removed manure from pastures at least once a week. As a whole, it appears as though equine managers are attuned to manure management, most likely as a means to maintain the health and welfare of their animals. This may reduce the opportunities for filth fly breeding in carefully managed facilities.

The most common control method for pests was spraying insecticides. Over 95% of horse owners responded that they use of some form of insecticide. The use of insecticides as the primary control method was seen by Biggin et al. (1999) in the United Kingdom where over 45% used chemical repellents. Overuse of chemicals is common in systems that do not practice IPM. Insecticides are commonly marketed for the equine industry as horses do not tolerate external pests well, and neither do horse owners according to the responses of threshold levels in this survey. The high numbers of respondents using insecticides suggests an over-reliance on chemical control or a habitual use. A general trend through this survey was a seeming lack of understanding of the components of IPM. Owners should be educated on IPM practices which maximize pest control using methods that reduce reliance on chemical applications and minimize the potential adverse affects to the environment and beneficial insects.

Pest tolerance around animals was low, however, the majority of respondents did not monitor pests on their property. Regardless, many used more than one pest control method to attempt to reduce pest numbers. Additionally, most of the survey population spent over $100 annually on control products. Even with multiple methods and high annual costs, an overwhelming majority found products to be only somewhat effective. Because of a lack of monitoring, respondents were uninformed on the status of pests on their property yet were eager to apply control products to and around their horses and were willing to spend money to do so with no basis for evaluating efficacy. With education on thresholds, pest monitoring and choosing and applying the most appropriate control methods for their facility, the effectiveness of the control products used may increase and costs decrease. The implementation of IPM reduces reliance on insecticides (Burrows 1983) which not only reduces environmental risks but also may significantly reduce pest control costs.

Based on the high percentage of pesticide use, it might be assumed that the survey population had a positive attitude about chemical control. However, over 80% of respondents wanted to see more research conducted on biological control and the most popular write-in answer was for non-toxic solutions. Additionally, half of the respondents did not think research was being conducted which met their needs. When asked which sources they most frequently use to obtain information on pest control, the most common answer was through the internet. Respondents also consulted with veterinarians, employees at feed stores, friends and Extension agents in similar frequencies. Garforth et al. (2006) stated that, in farm animal production, veterinarians are influential and important sources of information. Earle et al. (2002) found that 42%

of the survey population sought animal care advice from their veterinarians. This survey was conducted online and, though the internet was the primary source of information to facility owners in this survey, it is likely that the survey population was predisposed to internet browsing and may have a tendency to be more proactive in research on the internet. It is possible that respondents did not think veterinarians are properly trained in pest control methods or levels of annoyance caused by pests were not sufficiently high to warrant professional consultation. However, this need for pest management information presents an opportunity for increasing production and electronic dissemination of Extension educational materials for both equestrian farm owners and large animal veterinarians.

The concluding result of this survey is that many property owners have insufficient knowledge of pest identification, biology and presence on their property to develop a successful IPM program. Consequently, they continue to rely on insecticides without an adequate understanding of available alternative control methods. This deficiency in knowledge is consistent with many studies on horse owner control of internal parasites (Reinmeyer and Rohrbach 1990; Lendal et al. 1998). Though a comprehensive understanding of IPM was not demonstrated by survey takers, a willingness to protect themselves and animals was apparent. Given the lack of knowledge yet willingness to control pests, research on pest management for equestrian facilities and associated Extension support is warranted. A concerted effort should be made to educate equestrian facility owners on the benefits of carefully planned IPM programs to manage external pests of horses.

Table 5-1. Demographics of survey respondents.

Demographic Parameter	Respondents N	Respondents %
Primary discipline (*n*=283)		
Pleasure riding	139	49.1
Performance horses	118	41.7
Other	26	9.2
Property status (*n*=284)		
Barn/facility owner	240	84.5
Boarder at a facility	36	12.7
Rider at a facility	3	1.1
Other	5	1.8
Property acreage (*n*=282)		
1-10 acres	159	56.4
11+	123	43.6
Number of horses (*n*=294)		
1-5 horses	181	63.7
6+	103	36.3

Table 5-2. Manure management options at equestrian farms in North and Central Florida[1]

Manure Management Method	Respondents N	Respondents%
Drag	65	22.1
Manure pile (not active management)	73	24.8
Spreader	144	49.0
Compost (active management)	76	25.9
Other	38	12.9

[1]Respondents were allowed to select multiple methods of manure management.

Table 5-3. Types of pest control methods used on equestrian farms in North and Central Florida[1].

Pest Control Methods	Respondent N	Respondent %
Fly Spray	283	95.9
Fly Masks	116	39.3
Fly Sheets	63	21.4
Traps	112	38.0
Biological Control	65	22.0
Other Insecticidal Control	53	18.0
Feed Through Control	43	14.6
Other	45	15.3

[1]Respondents were allowed to select multiple types of pest control methods.

Table 5-4. Number of alternative pest control methods used on equestrian farms in North and Central Florida.

Number of Methods	Respondents N	Respondents %
1 product	35	11.9
2	97	32.9
3	82	27.8
4	45	15.3
5	19	6.4
6	7	2.4
7	0	0.0

Table 5-5. Types of research and Extension information requested by the respondents[1].

Type of Research or Information	Respondent N	Respondent %
Research Applicability (*n*=280)		
No	152	54.3
Yes	128	45.7
Research Requests (*n*=276)		
Biological control	231	80.2
Cultural control methods	140	48.6
Insecticides	121	40.0
Trapping options	1	21.2
Other	11	3.8
Physical barriers	23	8.0
Extension Requests (*n*=277)		
Biological control	172	62.1
Pesticide use	112	40.4
Cultural control methods	106	38.3
Trapping options	83	30.0
Identification	62	22.4
Sampling methods	61	22.0

[1]Respondents were allowed to select multiple requests for Extension and research.

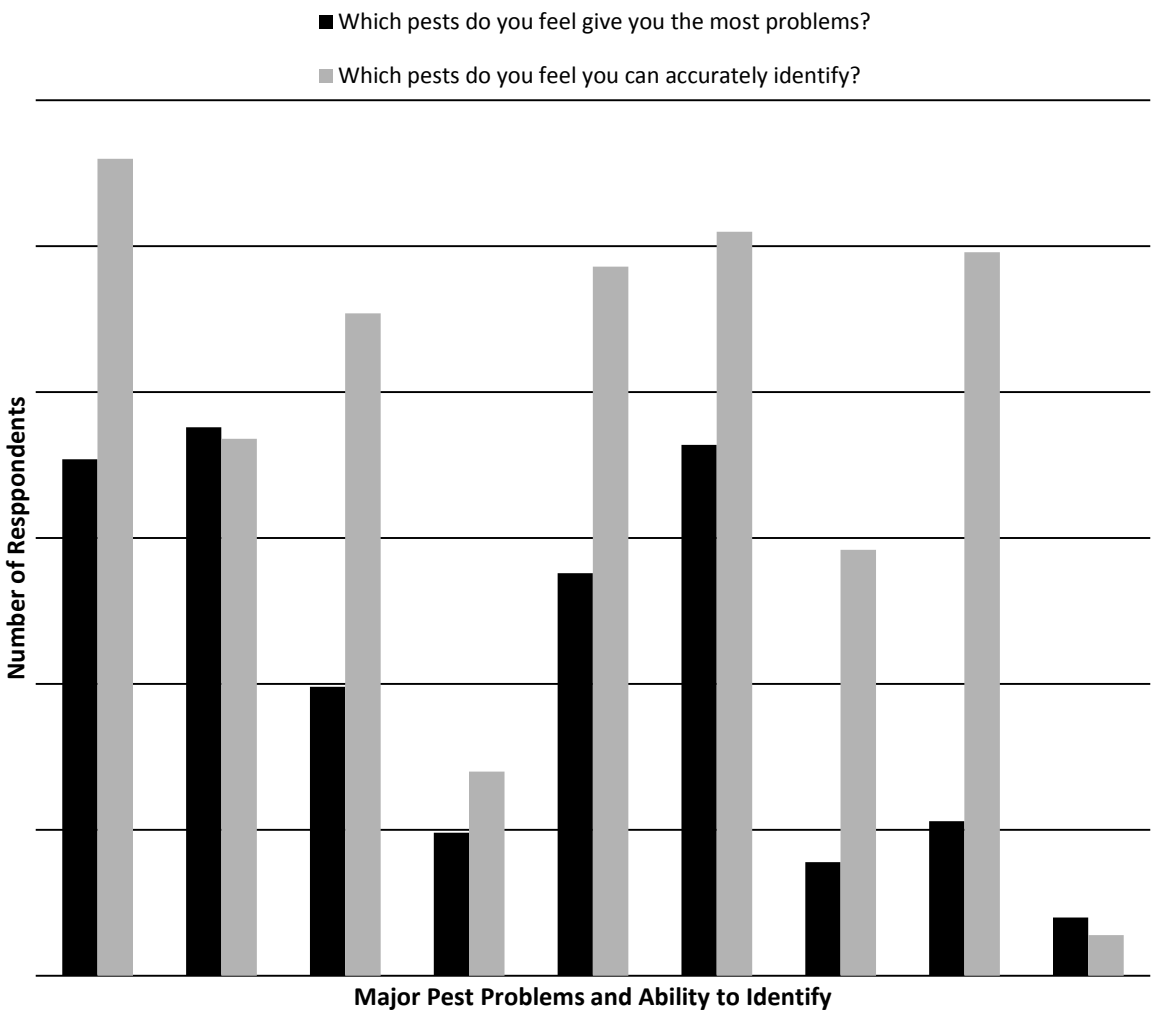


Figure 5-1. Comparison of perceived pest problems and ability to identify pests. Respondents were allowed to select multiple pests.

CHAPTER 6
DISCUSSION OF FINDINGS AND FUTURE RESEARCH DIRECTIONS FOR INTEGRATED PEST MANAGEMENT OF HOUSE FLIES AND STABLE FLIES ON SMALL EQUESTRIAN FARMS IN NORTH AND CENTRAL FLORIDA

Applications of synthetic chemical insecticides have historically been the sole method of filth fly pest control on livestock facilities, including horse farms. It is not surprising therefore that the vast majority of respondents to the 2006 USDA Changes in the Equine Industry survey, as well as the survey detailed in Chapter 5, continue the habitual use of insecticides on or around their horses. However, continuing concerns over the effects of toxicity and overuse of chemical applications to the environment, livestock and humans has generated interest in research on alternative pest control measures. Outside the risks to health and wildlife, the overuse of chemicals has led to high levels of insect resistance. Resistance to chemical applications have been observed in both house flies and stable flies on equestrian farms and other livestock facilities (Kaufman et al. 2010; Pitzer et al. 2011c), even farms with no recorded use of insecticides (Cilek and Greene 1994). The long-term implication of resistance in filth flies is the necessity for development and successful application of IPM programs for equestrian facilities to reduce the application of chemical treatments.

According to the IPM roadmap, a successful IPM program will incorporate appropriate techniques to manage flies on individual properties in the most economical means possible while minimizing the risks to people, livestock and the environment (Coble and Ortman 2009). The underlying challenge to achieving this goal on equestrian farms is that studies on improving IPM for filth flies on livestock operations have been heavily biased towards commercial food animals, such as cattle and poultry, or directed towards large production facilities, leaving pest control questions on

equestrian facilities largely unanswered. My studies presented here have demonstrated the uniqueness of small equestrian farms in Florida. Behavior assays on equine substrates have elucidated potential differences in breeding habitat use of house flies and stable flies in the field on equestrian farms and other livestock facilities due to unique animal husbandry techniques and effects of these substrates on host seeking behavior of the hymenopteran pupal parasitoid *Spalangia cameroni.* Furthermore, a survey of the North and Central Florida equestrian community has revealed gaps in the knowledge of IPM techniques and applications on equine facilities but a willingness to improve on current methods.

Population data collected demonstrated the dichotomy between small equestrian farms and the individuality of each farm. Both house flies and stable flies were found at varying degrees throughout the year on all farms, regardless of whether breeding was observed on-site. Even on larger equine operations, fly production and management was found to differ significantly (Pitzer et al. 2011c). As only four comparable farms were used in the study, there is a high likelihood of further differences in filth fly levels on equestrian farms exist. The discrepancy in adult fly collections between farms with fewer sanitation practices and breeding populations of flies and those farms with greater sanitation and no observable development of immature flies is likely related to dispersal from neighboring development sites (Pickens et al. 1967). The odors associated with equine husbandry may serve as a strong attractant for adult flies, regardless of whether breeding sites are available. Further research is needed on the dispersal patterns and attraction of filth flies to equestrian farms to develop methods to prevent or reduce the

effect of off-site attraction and identify control methods suitable for those facilitates that attract but do not produce flies.

Monitoring of adult and immature populations of house flies and stable flies is required to determine the most effective IPM measures. Current monitoring methods for house flies and stable flies are generally well established, presenting a variety of options for horse owners to evaluate relative fly abundance over time. However, these methods are limited to the adult fly. Methods for larval monitoring are more labor intensive and require a greater degree of knowledge to interpret appropriately. Nonetheless, horse owners would benefit if they could reliably assess the extent of on-site breeding. Additionally, commercial parasitoids purchased for a biological control program attack the pupal stage of the fly and releases are futile if breeding is not occurring on-site. Therefore, research to innovate or improve effective and time efficient methods of monitoring larval activity on horse farms is necessary to equip equestrian farm owners with the skills required to make educated control decisions based on fly activity on their site.

My laboratory study found the initial breeding preferences of house and stable flies on equestrian based substrates to be similar. However, house flies exhibited greater fidelity in oviposition site selection and rigidity in development ability in equine substrates than stable flies. Conversely, the observed flexibility in stable fly oviposition and development coupled with the discrepancies between stable fly oviposition site selection and development ability suggest that breeding behavior may be influenced by interspecies avoidance. Thus, research on the competitive effects of house flies and stable flies in the field and the potential competition avoidance behavior of stable flies is

warranted. Additionally, fly behavior of both species in the field is a logical direction for future research to determine oviposition choice by house flies and stable flies on equestrian farms. The resulting findings could lead to improvements in cultural control practices to reduce on-site breeding and attraction of off-site flies.

The influence of substrate on the effects of natural parasitoids of house flies and stable flies has biological control implications. *Spalangia cameroni* appears to be compatible with common equine substrates. It was more effective at higher release numbers in the laboratory, although there was not a clear dose-response effect to the substrate. The data I present provide support for the use of this species for biological control programs on equine facilities; however, subsequent testing of a release program on horse farms is a logical next step to establishing and evaluating biological control programs for filth flies on these operations. Coupled with a release program, studies on the cues associated with dispersal to host habitats of *S. cameroni* are prudent to establish protocols for release providing the greatest coverage and effectiveness to reduce fly populations.

The necessity for the advancement of IPM and pest control education of horse and equine facility owners is evident. Based on survey responses, facility owners use a variety of methods for pest control on and around their animals, yet primary methods are heavily reliant on insecticides. The survey population had high annual expenditures for pest control, yet was unaware of monitoring techniques to determine population levels. There was also a desire to see further research on non-chemical based control methods and to increase their knowledge of control methods. This presents a potential and substantial opportunity for Extension agents and scientists to

provide applicable research and extension materials to an important and underserved community.

Each experiment presented has been in an effort to improve the use of IPM on small equestrian farms. My studies have shown that differences may exist in population levels, attraction and oviposition and natural parasitism, not only on other livestock facilities, but between horse farms. These studies represent a beginning to understanding the ecology of filth flies on equestrian farms. Further research is necessary to fully comprehend the intricacies of house and stable fly ecology and control to improve IPM recommendations for management of these important pests.

APPENDIX A
EQUINE FACILITY PROTOCOLS

Thank you for agreeing to be a volunteer in this study on the biological control of filth flies in North and Central Florida. The following is a detailed description of basic protocol that must be followed to participate in this project. Each of these requirements has been established to maintain as much uniformity as possible between research locations. If you have any questions, please feel free to contact Erika Machtinger at irishtangerine@ufl.edu or at (904) 294-2247.

Horse density

There must be 3 or less horses per acre of pasture (*i.e.,* 3 or less in a 1 acre paddock, 6 or less in a two acre paddock, etc.). Horses should remain on study pasture (*i.e.,* not rotated though different pastures) throughout the course of the study. There are circumstances where pastures must be rotated (small pasture size, overgrazing), so please let the researcher know what the planned rotation schedule is. It is understood that horse density may change due to illness, death, sales, or acquisition. Please inform the researcher if the density changes.

Field management

Fields should not be fertilized during the study period.

Run-in Shed Management

Run-in sheds should be picked out in constant schedule determined by the property owner. Fans should not be used in run-in sheds.

Horse Management

Horses should be stalled no more than 12 hours out of the day. Pasture turnout should be 12-24 hours a day.

Stall Management

Stalls should be cleaned daily and bedding added as necessary. Fans should not be used in stalls.

Fly Traps

Study fly traps should not be manipulated, shaken, or otherwise moved during study. No other method of fly control (including strips or baits) can be used throughout the study, only those provided by researcher.

Insecticides and other fly control

Horses can be sprayed with topical insecticides as applicable; however, insecticides mists, sprays, or granules, other than those specifically to kill or manage fire ants, grubs, or mole crickets should not be applied to fields or misted through the barns or run in sheds.
Parasitoid wasps should not be used during the study.

Water Trough Management

Water troughs should not be bleached more than once a week. There are no restrictions on where excess water can be dumped.

Manure management

Manure should be put in the manure or compost pile or spread on fields, please inform researcher which method you choose. The manure or compost pile should not be turned or manipulated during the study and should not be decreased in size in anyway (*i.e.,* taken off-site or moved). There is no minimum or maximum size for a manure or compost pile.

Hay and Feed Management

Hay should be either given as flakes on the ground or in hay rings. Loose hay should not be manipulated (hay that has fallen to the ground) until the next hay roll arrives. Feed should be stored in bins or bags, not left out in the open.

APPENDIX B
LIVESTOCK WASTE TESTING RESULTS

UNIVERSITY OF FLORIDA

Institute of Food and Agricultural Sciences
Analytical Services Laboratories
Livestock Waste Testing Laboratory, Gainesville, FL

Livestock Waste Testing Lab
631 Wallace Building
Gainesville, FL 32622
(352) 392-1950
FAX (352) 392-1960

Livestock Waste Analysis Grower Report

Erika Machtinger
19308 NW CR 236
High Springs, FL 32643

PHONE:

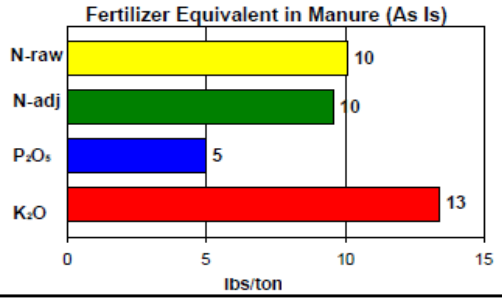
Lab #	7281
Sample Label	Hay
Date Collected	April 1, 2011
Date Delivered	
Date of Report	June 8, 2011
County of Sampl	Alachua
Collected By	0

Sample Type:	Other composted material.
Crop or Use:	#N/A
Application Equipment:	Applied by manure spreader
Incorporation	#N/A
Previous Applications	#N/A

***Nutrient Content in Manure as Delivered to Laboratory

Nutrient Constituent	Raw Sample	Adjusted For Application Losses of N	Units
Nitrogen (N):	10	10	lbs/ton
Phosphorus (P2O5):	5	5	lbs/ton
Potassium (K2O):	13	13	lbs/ton

pH as Sampled	8.9
Moisture Content:	68.6%
Total Solids:	31.4%
Total Ash:	8.2%



***Total Nutrient Requirement for:	lbs. N/acre	lbs P_2O_5/acre	lbs K_2O/acre
#N/A	#N/A	#N/A	#N/A
Totals	#N/A	#N/A	#N/A

Nitrogen Recommendation Base

***Manure application rate (As Is) to supply crop N requirement:
#N/A tons/acre

By supplying the crop N requirement at the rate shown above, the following total nutrients will be applied:
#N/A lbs. N/acre
#N/A lbs P2O5/acre
#N/A lbs K2O/acre

Supplemental nutrients needed:
#N/A lbs. N/acre
#N/A lbs P2O5/acre
#N/A lbs K2O/acre

***Economic value of manure at the rate shown above:
N #N/A per acre
P2O5 #N/A per acre
K2O #N/A per acre

***Cost of additional nutrients needed:
#N/A N per Acre
#N/A P2O5 per acre
#N/A K2O per acre

Phosphorus Recommendation Base

***Manure application rate (As Is) to supply crop P requirement:
#N/A tons/acre

By supplying the crop P requirement at the rate shown above, the following total nutrients will be applied:
#N/A lbs. N/acre
#N/A lbs P2O5/acre
#N/A lbs K2O/acre
#N/A

Supplemental nutrients needed:
#N/A lbs. N/acre
#N/A lbs P2O5/acre
#N/A lbs K2O/acre

***Economic value of manure at the rate shown above:
N #N/A per acre
P2O5 #N/A per acre
K2O #N/A per acre

***Cost of additional nutrients needed:
#N/A N per Acre
#N/A P2O5 per acre
#N/A K2O per acre

*** Assumptions are shown in footnotes on Page 2. Prices Updated on: 1/23/2009
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(352)392-1950

Livestock Waste Analysis Grower Report

Erika Machtinger
19308 NW CR 236
High Springs, FL 32643

PHONE:

Lab #	**7281**
Sample Label	**Hay**
Date Collected	**April 1, 2011**
Date Delivered	
Date of Report	**June 8, 2011**
County	**Alachua**
Collected By	**0**

Sample Type:	**Other composted material**
Crop or Use:	**#N/A**
Application Equipment:	Applied by manure spreader
Incorporation:	#N/A
Previous Applications:	#N/A

Laboratory Results (All weights are based on sample weight as received)

Total Solids:	313900 mg/kg	31.4%	628 lbs/ton
Total Ash:	81900 mg/kg	8.2%	164 lbs/ton
Total Kjeldahl N*:	5052 mg/kg	0.51%	10.1 lbs/ton
Ammonia Nitrogen:	221 mg/kg	0.02%	0.4 lbs/ton
Total Elemental P:	1101 mg/kg	0.11%	2.2 lbs/ton
Total Elemental K:	5568 mg/kg	0.56%	11.1 lbs/ton
Moisture:	68.61%		
pH:	8.9		

* Total Kjeldahl Nitrogen is equivalent to Total N for manure and high organic samples

Estimated Nitrogen Losses:

N-Content of Sample as Tested:			10.1 lbs/ton
***N-losses during application:	5%	-	0.5 lbs
***N-losses while awaiting incorporation:	0%	-	0.0 lbs
***Other N-Losses:	0%	-	0.0 lbs
Estimated Available N:	**95.0%**		**9.6 lbs/ton**

Footnotes:

Fertilizer Equivalent in Manure - The nitrogen value is an estimate based on inherent losses from using animal manures.

Total Nutrient Requirement For - This is the total N-P2O5-K2O recommended for the crop for a growing season assuming low P2O5 and K2O sc tests. Split applications of N and K2O result in more efficient nutrient use. For assistance in determing individual application rates, see your County Extension Agent, nutrient management specialist or Soil and Water Conservation District Technician.

Manure application rate - The maximum application rate that should be applied if it is split applied at least three times during this crop, and t amount applied in each application adjusted to crop intake. If single applications are used, then manure should be applied at 50% of the above rate with the remaining N requirement being met by supplemental fertilization. Sprayfields with frequent applications may also need an adjusted rate.

Economic Value This is by nature a rough approximation meant for comparative purposes only. Since the value of N and P2O5 are by far the most important in determining economic value of manure, only these are considered in the calculations. The commercial values of N and P2O5 are estimated using ammonium nitrate at $580/ton, concentrated superphosphate (0-46-0) at $1120/ton, and potassium chloride (0-0-60) at $800/ton.

N-Losses during applicatior - A loss of 25% is assumed for liquid samples with a pH above 7 and for situations where sprinklers are used t application. A standard loss of 5% is assumed for all other materials and situations.

N-Losses while awaiting incorporatio - It is assumed there will be no N loss to volitilization if solid or slurry manures are incorporated within hours and a 25% loss if they are not. Liquid applications are considered to have an additional 25% volatilization loss before stabilization in soil.

Other N-Losses - A 50% reduction in N availability is calculated whenever a manure having an ammonia to organic nitrogen ratio less than or equ 1 is applied to a field where manure was not applied the previous year.

Regular soil testing is recommended where manures are applied ofte

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Livestock Waste Analysis Grower Report

Erika Machtinger
19308 NW CR 236
High Springs, FL 32643

PHONE:

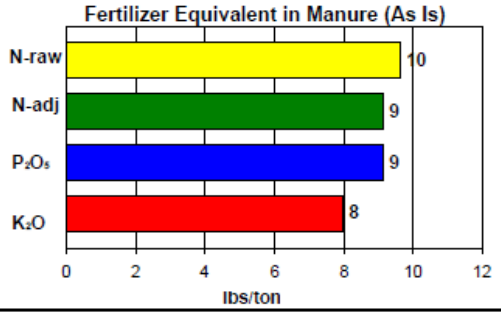
Lab #	7282
Sample Label	Man
Date Collected	April 1, 2011
Date Delivered	
Date of Report	June 8, 2011
County of Sampl	Alachua
Collected By	0

Sample Type:	Other composted material.
Crop or Use:	#N/A
Application Equipment:	Applied by manure spreader
Incorporation	#N/A
Previous Applications	#N/A

***Nutrient Content in Manure as Delivered to Laboratory

Nutrient Constituent	Raw Sample	Adjusted For Application Losses of N	Units
Nitrogen (N):	10	9	lbs/ton
Phosphorus (P2O5):	9	9	lbs/ton
Potassium (K2O):	8	8	lbs/ton

pH as Sampled	7.3
Moisture Content:	78.2%
Total Solids:	21.8%
Total Ash:	4.7%



***Total Nutrient Requirement for:		lbs. N/acre	lbs P_2O_5/acre	lbs K_2O/acre
#N/A		#N/A	#N/A	#N/A
	Totals	#N/A	#N/A	#N/A

Nitrogen Recommendation Base

***Manure application rate (As Is) to supply crop N requirement:
#N/A tons/acre

By supplying the crop N requirement at the rate shown above, the following total nutrients will be applied:

#N/A	lbs. N/acre
#N/A	lbs P2O5/acre
#N/A	lbs K2O/acre

Supplemental nutrients needed:

#N/A	lbs. N/acre
#N/A	lbs P2O5/acre
#N/A	lbs K2O/acre

***Economic value of manure at the rate shown above:

N	#N/A per acre
P2O5	#N/A per acre
K2O	#N/A per acre

***Cost of additional nutrients needed:

#N/A	N per Acre
#N/A	P2O5 per acre
#N/A	K2O per acre

Phosphorus Recommendation Base

***Manure application rate (As Is) to supply crop P requirement:
#N/A tons/acre

By supplying the crop P requirement at the rate shown above, the following total nutrients will be applied:

#N/A	lbs. N/acre
#N/A	lbs P2O5/acre
#N/A	lbs K2O/acre

#N/A

Supplemental nutrients needed:

#N/A	lbs. N/acre
#N/A	lbs P2O5/acre
#N/A	lbs K2O/acre

***Economic value of manure at the rate shown above:

N	#N/A per acre
P2O5	#N/A per acre
K2O	#N/A per acre

***Cost of additional nutrients needed:

#N/A	N per Acre
#N/A	P2O5 per acre
#N/A	K2O per acre

*** Assumptions are shown in footnotes on Page 2. Prices Updated on: 1/23/2009
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Livestock Waste Analysis Grower Report

Erika Machtinger
19308 NW CR 236
High Springs, FL 32643

PHONE:

Lab #	**7282**
Sample Label	**Man**
Date Collected	**April 1, 2011**
Date Delivered	
Date of Report	**June 8, 2011**
County	**Alachua**
Collected By	**0**

Sample Type:	**Other composted material**
Crop or Use:	**#N/A**
Application Equipment:	Applied by manure spreader
Incorporation:	#N/A
Previous Applications:	#N/A

Laboratory Results (All weights are based on sample weight as received)

Total Solids:	217900 mg/kg	21.8%	436 lbs/ton
Total Ash:	46500 mg/kg	4.7%	93 lbs/ton
Total Kjeldahl N*:	4814 mg/kg	0.48%	9.6 lbs/ton
Ammonia Nitrogen:	140 mg/kg	0.01%	0.3 lbs/ton
Total Elemental P:	2013 mg/kg	0.20%	4.0 lbs/ton
Total Elemental K:	3310 mg/kg	0.33%	6.6 lbs/ton
Moisture:	78.21%		
pH:	7.3		

* Total Kjeldahl Nitrogen is equivalent to Total N for manure and high organic samples

Estimated Nitrogen Losses:

N-Content of Sample as Tested:			9.6 lbs/ton
***N-losses during application:	5%	-	0.5 lbs
***N-losses while awaiting incorporation:	0%	-	0.0 lbs
***Other N-Losses:	0%	-	0.0 lbs
Estimated Available N:	**95.0%**		**9.1 lbs/ton**

Footnotes:

Fertilizer Equivalent in Manure - The nitrogen value is an estimate based on inherent losses from using animal manures.

Total Nutrient Requirement For - This is the total N-P2O5-K2O recommended for the crop for a growing season assuming low P2O5 and K2O sc tests. Split applications of N and K2O result in more efficient nutrient use. For assistance in determing individual application rates, see your County Extension Agent, nutrient management specialist or Soil and Water Conservation District Technician.

Manure application rate - The maximum application rate that should be applied if it is split applied at least three times during this crop, and t amount applied in each application adjusted to crop intake. If single applications are used, then manure should be applied at 50% of the above rate with the remaining N requirement being met by supplemental fertilization. Sprayfields with frequent applications may also need an adjusted rate.

Economic Value This is by nature a rough approximation meant for comparative purposes only. Since the value of N and P2O5 are by far the most important in determining economic value of manure, only these are considered in the calculations. The commercial values of N and P2O5 are estimated using ammonium nitrate at $580/ton, concentrated superphosphate (0-46-0) at $1120/ton, and potassium chloride (0-0-60) at $800/ton.

N-Losses during applicatior - A loss of 25% is assumed for liquid samples with a pH above 7 and for situations where sprinklers are used t application. A standard loss of 5% is assumed for all other materials and situations.

N-Losses while awaiting incorporatio - It is assumed there will be no N loss to volitilization if solid or slurry manures are incorporated within hours and a 25% loss if they are not. Liquid applications are considered to have an additional 25% volatilization loss before stabilization in soil.

Other N-Losses - A 50% reduction in N availability is calculated whenever a manure having an ammonia to organic nitrogen ratio less than or equ 1 is applied to a field where manure was not applied the previous year.

Regular soil testing is recommended where manures are applied ofte

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Livestock Waste Analysis Grower Report

Erika Machtinger
19308 NW CR 236
High Springs, FL 32643

PHONE:

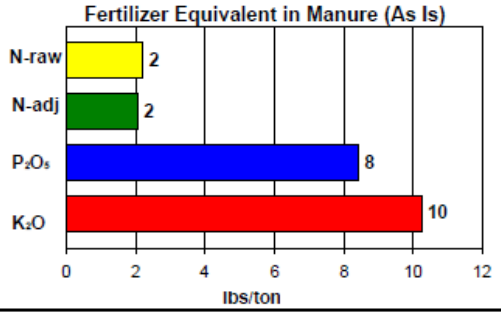
Lab #	7284
Sample Label	Shav
Date Collected	April 1, 2011
Date Delivered	
Date of Report	June 8, 2011
County of Sampl	Alachua
Collected By	0

Sample Type:	Other composted material.
Crop or Use:	#N/A
Application Equipment:	Applied by manure spreader
Incorporation	#N/A
Previous Applications	#N/A

***Nutrient Content in Manure as Delivered to Laboratory

Nutrient Constituent	Raw Sample	Adjusted For Application Losses of N	Units
Nitrogen (N):	2	2	lbs/ton
Phosphorus (P2O5):	8	8	lbs/ton
Potassium (K2O):	10	10	lbs/ton

pH as Sampled	8.8
Moisture Content:	35.2%
Total Solids:	64.8%
Total Ash:	27.7%



***Total Nutrient Requirement for:		lbs. N/acre	lbs P_2O_5/acre	lbs K_2O/acre
#N/A		#N/A	#N/A	#N/A
	Totals	#N/A	#N/A	#N/A

Nitrogen Recommendation Base

***Manure application rate (As Is) to supply crop N requirement:
#N/A tons/acre

By supplying the crop N requirement at the rate shown above, the following total nutrients will be applied:
#N/A lbs. N/acre
#N/A lbs P2O5/acre
#N/A lbs K2O/acre

Supplemental nutrients needed:
#N/A lbs. N/acre
#N/A lbs P2O5/acre
#N/A lbs K2O/acre

***Economic value of manure at the rate shown above:
N #N/A per acre
P2O5 #N/A per acre
K2O #N/A per acre

***Cost of additional nutrients needed:
#N/A N per Acre
#N/A P2O5 per acre
#N/A K2O per acre

Phosphorus Recommendation Base

***Manure application rate (As Is) to supply crop P requirement:
#N/A tons/acre

By supplying the crop P requirement at the rate shown above, the following total nutrients will be applied:
#N/A lbs. N/acre
#N/A lbs P2O5/acre
#N/A lbs K2O/acre
#N/A

Supplemental nutrients needed:
#N/A lbs. N/acre
#N/A lbs P2O5/acre
#N/A lbs K2O/acre

***Economic value of manure at the rate shown above:
N #N/A per acre
P2O5 #N/A per acre
K2O #N/A per acre

***Cost of additional nutrients needed:
#N/A N per Acre
#N/A P2O5 per acre
#N/A K2O per acre

*** Assumptions are shown in footnotes on Page 2. Prices Updated on: 1/23/2009
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Livestock Waste Analysis Grower Report

Erika Machtinger
19308 NW CR 236
High Springs, FL 32643

PHONE:

Lab #	**7284**
Sample Label	**Shav**
Date Collected	**April 1, 2011**
Date Delivered	
Date of Report	**June 8, 2011**
County	**Alachua**
Collected By	**0**

Sample Type:	**Other composted material**
Crop or Use:	**#N/A**
Application Equipment:	Applied by manure spreader
Incorporation:	#N/A
Previous Applications:	#N/A

Laboratory Results (All weights are based on sample weight as received)

Total Solids:	648100 mg/kg	64.8%	1296 lbs/ton
Total Ash:	276600 mg/kg	27.7%	553 lbs/ton
Total Kjeldahl N*:	1094 mg/kg	0.11%	2.2 lbs/ton
Ammonia Nitrogen:	1046 mg/kg	0.10%	2.1 lbs/ton
Total Elemental P:	1853 mg/kg	0.19%	3.7 lbs/ton
Total Elemental K:	4259 mg/kg	0.43%	8.5 lbs/ton
Moisture:	35.19%		
pH:	8.8		

* Total Kjeldahl Nitrogen is equivalent to Total N for manure and high organic samples

Estimated Nitrogen Losses:

N-Content of Sample as Tested:			2.2 lbs/ton
***N-losses during application:	5%	-	0.1 lbs
***N-losses while awaiting incorporation:	0%	-	0.0 lbs
***Other N-Losses:	0%	-	0.0 lbs
Estimated Available N:	**95.0%**		**2.1 lbs/ton**

Footnotes:

Fertilizer Equivalent in Manure - The nitrogen value is an estimate based on inherent losses from using animal manures.

Total Nutrient Requirement For - This is the total N-P2O5-K2O recommended for the crop for a growing season assuming low P2O5 and K2O sc tests. Split applications of N and K2O result in more efficient nutrient use. For assistance in determing individual application rates, see your County Extension Agent, nutrient management specialist or Soil and Water Conservation District Technician.

Manure application rate - The maximum application rate that should be applied if it is split applied at least three times during this crop, and t amount applied in each application adjusted to crop intake. If single applications are used, then manure should be applied at 50% of the above rate with the remaining N requirement being met by supplemental fertilization. Sprayfields with frequent applications may also need an adjusted rate.

Economic Value This is by nature a rough approximation meant for comparative purposes only. Since the value of N and P2O5 are by far the most important in determining economic value of manure, only these are considered in the calculations. The commercial values of N and P2O5 are estimated using ammonium nitrate at $580/ton, concentrated superphosphate (0-46-0) at $1120/ton, and potassium chloride (0-0-60) at $800/ton.

N-Losses during applicatior - A loss of 25% is assumed for liquid samples with a pH above 7 and for situations where sprinklers are used t application. A standard loss of 5% is assumed for all other materials and situations.

N-Losses while awaiting incorporatio - It is assumed there will be no N loss to volitilization if solid or slurry manures are incorporated within hours and a 25% loss if they are not. Liquid applications are considered to have an additional 25% volatilization loss before stabilization in soil.

Other N-Losses - A 50% reduction in N availability is calculated whenever a manure having an ammonia to organic nitrogen ratio less than or equ 1 is applied to a field where manure was not applied the previous year.

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Livestock Waste Analysis Grower Report

Erika Machtinger
19308 NW CR 236
High Springs, FL 32643

PHONE:

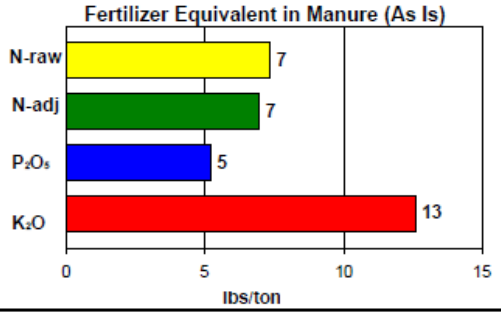
Lab #	7283
Sample Label	MPS
Date Collected	April 1, 2011
Date Delivered	
Date of Report	June 8, 2011
County of Sampl	Alachua
Collected By	0

Sample Type:	Other composted material.
Crop or Use:	#N/A
Application Equipment:	Applied by manure spreader
Incorporation	#N/A
Previous Applications	#N/A

***Nutrient Content in Manure as Delivered to Laboratory

Nutrient Constituent	Raw Sample	Adjusted For Application Losses of N	Units
Nitrogen (N):	7	7	lbs/ton
Phosphorus (P2O5):	5	5	lbs/ton
Potassium (K2O):	13	13	lbs/ton

pH as Sampled	8.8
Moisture Content:	54.9%
Total Solids:	45.1%
Total Ash:	13.9%



***Total Nutrient Requirement for:		lbs. N/acre	lbs P_2O_5/acre	lbs K_2O/acre
#N/A		#N/A	#N/A	#N/A
	Totals	#N/A	#N/A	#N/A

Nitrogen Recommendation Base

***Manure application rate (As Is) to supply crop N requirement:
#N/A tons/acre

By supplying the crop N requirement at the rate shown above, the following total nutrients will be applied:

#N/A	lbs. N/acre
#N/A	lbs P2O5/acre
#N/A	lbs K2O/acre

Supplemental nutrients needed:

#N/A	lbs. N/acre
#N/A	lbs P2O5/acre
#N/A	lbs K2O/acre

***Economic value of manure at the rate shown above:

N	#N/A per acre
P2O5	#N/A per acre
K2O	#N/A per acre

***Cost of additional nutrients needed:

#N/A	N per Acre
#N/A	P2O5 per acre
#N/A	K2O per acre

Phosphorus Recommendation Base

***Manure application rate (As Is) to supply crop P requirement:
#N/A tons/acre

By supplying the crop P requirement at the rate shown above, the following total nutrients will be applied:

#N/A	lbs. N/acre
#N/A	lbs P2O5/acre
#N/A	lbs K2O/acre

#N/A

Supplemental nutrients needed:

#N/A	lbs. N/acre
#N/A	lbs P2O5/acre
#N/A	lbs K2O/acre

***Economic value of manure at the rate shown above:

N	#N/A per acre
P2O5	#N/A per acre
K2O	#N/A per acre

***Cost of additional nutrients needed:

#N/A	N per Acre
#N/A	P2O5 per acre
#N/A	K2O per acre

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Livestock Waste Analysis Grower Report

Erika Machtinger
19308 NW CR 236
High Springs, FL 32643

PHONE:

Lab #	**7283**
Sample Label	**MPS**
Date Collected	**April 1, 2011**
Date Delivered	
Date of Report	**June 8, 2011**
County	**Alachua**
Collected By	**0**

Sample Type:	**Other composted material.**
Crop or Use:	**#N/A**
Application Equipment:	Applied by manure spreader
Incorporation:	#N/A
Previous Applications:	#N/A

Laboratory Results (All weights are based on sample weight as received)

Total Solids:	451400 mg/kg	45.1%	903 lbs/ton
Total Ash:	139200 mg/kg	13.9%	278 lbs/ton
Total Kjeldahl N*:	3674 mg/kg	0.37%	7.3 lbs/ton
Ammonia Nitrogen:	449 mg/kg	0.04%	0.9 lbs/ton
Total Elemental P:	1147 mg/kg	0.11%	2.3 lbs/ton
Total Elemental K:	5223 mg/kg	0.52%	10.4 lbs/ton
Moisture:	54.86%		
pH:	8.8		

* Total Kjeldahl Nitrogen is equivalent to Total N for manure and high organic samples

Estimated Nitrogen Losses:

N-Content of Sample as Tested:			7.3 lbs/ton
***N-losses during application:	5%	-	0.4 lbs
***N-losses while awaiting incorporation:	0%	-	0.0 lbs
***Other N-Losses:	0%	-	0.0 lbs
Estimated Available N:	**95.0%**		**7.0 lbs/ton**

Footnotes:

Fertilizer Equivalent in Manure - The nitrogen value is an estimate based on inherent losses from using animal manures.

Total Nutrient Requirement For - This is the total N-P2O5-K2O recommended for the crop for a growing season assuming low P2O5 and K2O sc tests. Split applications of N and K2O result in more efficient nutrient use. For assistance in determing individual application rates, see your County Extension Agent, nutrient management specialist or Soil and Water Conservation District Technician.

Manure application rate - The maximum application rate that should be applied if it is split applied at least three times during this crop, and t amount applied in each application adjusted to crop intake. If single applications are used, then manure should be applied at 50% of the above rate with the remaining N requirement being met by supplemental fertilization. Sprayfields with frequent applications may also need an adjusted rate.

Economic Value This is by nature a rough approximation meant for comparative purposes only. Since the value of N and P2O5 are by far the most important in determining economic value of manure, only these are considered in the calculations. The commercial values of N and P2O5 are estimated using ammonium nitrate at $580/ton, concentrated superphosphate (0-46-0) at $1120/ton, and potassium chloride (0-0-60) at $800/ton.

N-Losses during applicatior - A loss of 25% is assumed for liquid samples with a pH above 7 and for situations where sprinklers are used t application. A standard loss of 5% is assumed for all other materials and situations.

N-Losses while awaiting incorporatio - It is assumed there will be no N loss to volitilization if solid or slurry manures are incorporated within hours and a 25% loss if they are not. Liquid applications are considered to have an additional 25% volatilization loss before stabilization in soil.

Other N-Losses - A 50% reduction in N availability is calculated whenever a manure having an ammonia to organic nitrogen ratio less than or equ 1 is applied to a field where manure was not applied the previous year.

Regular soil testing is recommended where manures are applied ofte

Revised October 2008.

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Gainesville, FL 32622
(352) 392-1950
FAX (352) 392-1960

Livestock Waste Analysis Grower Report

Erika Machtinger
19308 NW CR 236
High Springs, FL 32643

PHONE:

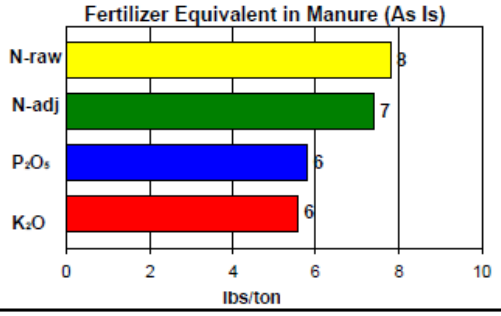
Lab #	7285
Sample Label	MPD
Date Collected	April 1, 2011
Date Delivered	
Date of Report	June 8, 2011
County of Sampl	Alachua
Collected By	0

Sample Type:	Other composted material.
Crop or Use:	#N/A
Application Equipment:	Applied by manure spreader
Incorporation	#N/A
Previous Applications	#N/A

***Nutrient Content in Manure as Delivered to Laboratory

Nutrient Constituent	Raw Sample	Adjusted For Application Losses of N	Units
Nitrogen (N):	8	7	lbs/ton
Phosphorus (P2O5):	6	6	lbs/ton
Potassium (K2O):	6	6	lbs/ton

pH as Sampled	8.3
Moisture Content:	59.7%
Total Solids:	40.3%
Total Ash:	23.5%



***Total Nutrient Requirement for:		lbs. N/acre	lbs P_2O_5/acre	lbs K_2O/acre
#N/A		#N/A	#N/A	#N/A
	Totals	#N/A	#N/A	#N/A

Nitrogen Recommendation Base

***Manure application rate (As Is) to supply crop N requirement:
#N/A tons/acre

By supplying the crop N requirement at the rate shown above, the following total nutrients will be applied:

#N/A	lbs. N/acre
#N/A	lbs P2O5/acre
#N/A	lbs K2O/acre

Supplemental nutrients needed:

#N/A	lbs. N/acre
#N/A	lbs P2O5/acre
#N/A	lbs K2O/acre

***Economic value of manure at the rate shown above:

N	#N/A per acre
P2O5	#N/A per acre
K2O	#N/A per acre

***Cost of additional nutrients needed:

#N/A	N per Acre
#N/A	P2O5 per acre
#N/A	K2O per acre

Phosphorus Recommendation Base

***Manure application rate (As Is) to supply crop P requirement:
#N/A tons/acre

By supplying the crop P requirement at the rate shown above, the following total nutrients will be applied:

#N/A	lbs. N/acre
#N/A	lbs P2O5/acre
#N/A	lbs K2O/acre

#N/A

Supplemental nutrients needed:

#N/A	lbs. N/acre
#N/A	lbs P2O5/acre
#N/A	lbs K2O/acre

***Economic value of manure at the rate shown above:

N	#N/A per acre
P2O5	#N/A per acre
K2O	#N/A per acre

***Cost of additional nutrients needed:

#N/A	N per Acre
#N/A	P2O5 per acre
#N/A	K2O per acre

*** Assumptions are shown in footnotes on Page 2. Prices Updated on: 1/23/2009
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Livestock Waste Analysis Grower Report

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PHONE:

Lab #	**7285**
Sample Label	**MPD**
Date Collected	**April 1, 2011**
Date Delivered	
Date of Report	**June 8, 2011**
County	**Alachua**
Collected By	**0**

Sample Type:	**Other composted material**
Crop or Use:	**#N/A**
Application Equipment:	Applied by manure spreader
Incorporation:	#N/A
Previous Applications:	#N/A

Laboratory Results (All weights are based on sample weight as received)

Total Solids:	402600 mg/kg	40.3%	805 lbs/ton
Total Ash:	234800 mg/kg	23.5%	470 lbs/ton
Total Kjeldahl N*:	3898 mg/kg	0.39%	7.8 lbs/ton
Ammonia Nitrogen:	1116 mg/kg	0.11%	2.2 lbs/ton
Total Elemental P:	1271 mg/kg	0.13%	2.5 lbs/ton
Total Elemental K:	2301 mg/kg	0.23%	4.6 lbs/ton
Moisture:	59.74%		
pH:	8.3		

* Total Kjeldahl Nitrogen is equivalent to Total N for manure and high organic samples

Estimated Nitrogen Losses:

N-Content of Sample as Tested:			7.8 lbs/ton
***N-losses during application:	5%	-	0.4 lbs
***N-losses while awaiting incorporation:	0%	-	0.0 lbs
***Other N-Losses:	0%	-	0.0 lbs
Estimated Available N:	**95.0%**		**7.4 lbs/ton**

Footnotes:

Fertilizer Equivalent in Manure - The nitrogen value is an estimate based on inherent losses from using animal manures.

Total Nutrient Requirement For - This is the total N-P2O5-K2O recommended for the crop for a growing season assuming low P2O5 and K2O sc tests. Split applications of N and K2O result in more efficient nutrient use. For assistance in determing individual application rates, see your County Extension Agent, nutrient management specialist or Soil and Water Conservation District Technician.

Manure application rate - The maximum application rate that should be applied if it is split applied at least three times during this crop, and t amount applied in each application adjusted to crop intake. If single applications are used, then manure should be applied at 50% of the above rate with the remaining N requirement being met by supplemental fertilization. Sprayfields with frequent applications may also need an adjusted rate.

Economic Value This is by nature a rough approximation meant for comparative purposes only. Since the value of N and P2O5 are by far the most important in determining economic value of manure, only these are considered in the calculations. The commercial values of N and P2O5 are estimated using ammonium nitrate at $580/ton, concentrated superphosphate (0-46-0) at $1120/ton, and potassium chloride (0-0-60) at $800/ton.

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Livestock Waste Analysis Grower Report

Erika Machtinger
19308 NW CR 236
High Springs, FL 32643

PHONE:

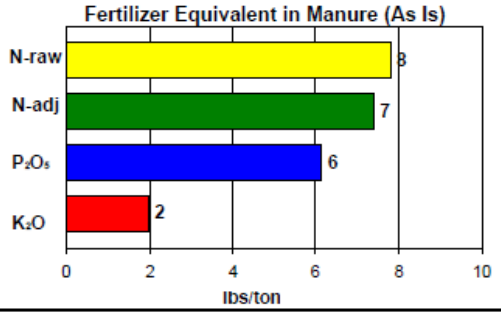
Lab #	7286
Sample Label	DL
Date Collected	April 1, 2011
Date Delivered	
Date of Report	June 8, 2011
County of Sampl	Alachua
Collected By	0

Sample Type:	Other composted material.
Crop or Use:	#N/A
Application Equipment:	Applied by manure spreader
Incorporation	#N/A
Previous Applications	#N/A

***Nutrient Content in Manure as Delivered to Laboratory

Nutrient Constituent	Raw Sample	Adjusted For Application Losses of N	Units
Nitrogen (N):	8	7	lbs/ton
Phosphorus (P2O5):	6	6	lbs/ton
Potassium (K2O):	2	2	lbs/ton

pH as Sampled	7.7
Moisture Content:	22.6%
Total Solids:	77.4%
Total Ash:	47.1%



***Total Nutrient Requirement for:	lbs. N/acre	lbs P_2O_5/acre	lbs K_2O/acre
#N/A	#N/A	#N/A	#N/A
Totals	#N/A	#N/A	#N/A

Nitrogen Recommendation Base

***Manure application rate (As Is) to supply crop N requirement:
#N/A tons/acre

By supplying the crop N requirement at the rate shown above, the following total nutrients will be applied:
#N/A lbs. N/acre
#N/A lbs P2O5/acre
#N/A lbs K2O/acre

Supplemental nutrients needed:
#N/A lbs. N/acre
#N/A lbs P2O5/acre
#N/A lbs K2O/acre

***Economic value of manure at the rate shown above:
N #N/A per acre
P2O5 #N/A per acre
K2O #N/A per acre

***Cost of additional nutrients needed:
#N/A N per Acre
#N/A P2O5 per acre
#N/A K2O per acre

Phosphorus Recommendation Base

***Manure application rate (As Is) to supply crop P requirement:
#N/A tons/acre

By supplying the crop P requirement at the rate shown above, the following total nutrients will be applied:
#N/A lbs. N/acre
#N/A lbs P2O5/acre
#N/A lbs K2O/acre
#N/A

Supplemental nutrients needed:
#N/A lbs. N/acre
#N/A lbs P2O5/acre
#N/A lbs K2O/acre

***Economic value of manure at the rate shown above:
N #N/A per acre
P2O5 #N/A per acre
K2O #N/A per acre

***Cost of additional nutrients needed:
#N/A N per Acre
#N/A P2O5 per acre
#N/A K2O per acre

*** Assumptions are shown in footnotes on Page 2. Prices Updated on: 1/23/2009
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Livestock Waste Analysis Grower Report

Erika Machtinger
19308 NW CR 236
High Springs, FL 32643

PHONE:

Lab #	**7286**
Sample Label	**DL**
Date Collected	**April 1, 2011**
Date Delivered	
Date of Report	**June 8, 2011**
County	**Alachua**
Collected By	**0**

Sample Type:	**Other composted material**
Crop or Use:	**#N/A**
Application Equipment:	Applied by manure spreader
Incorporation:	#N/A
Previous Applications:	#N/A

Laboratory Results (All weights are based on sample weight as received)

Total Solids:	774100 mg/kg	77.4%	1548 lbs/ton
Total Ash:	470900 mg/kg	47.1%	942 lbs/ton
Total Kjeldahl N*:	3890 mg/kg	0.39%	7.8 lbs/ton
Ammonia Nitrogen:	30 mg/kg	0.00%	0.1 lbs/ton
Total Elemental P:	1351 mg/kg	0.14%	2.7 lbs/ton
Total Elemental K:	817 mg/kg	0.08%	1.6 lbs/ton
Moisture:	22.59%		
pH:	7.7		

* Total Kjeldahl Nitrogen is equivalent to Total N for manure and high organic samples

Estimated Nitrogen Losses:

N-Content of Sample as Tested:			7.8 lbs/ton
***N-losses during application:	5%	-	0.4 lbs
***N-losses while awaiting incorporation:	0%	-	0.0 lbs
***Other N-Losses:	0%	-	0.0 lbs
Estimated Available N:	**95.0%**		**7.4 lbs/ton**

Footnotes:

Fertilizer Equivalent in Manure - The nitrogen value is an estimate based on inherent losses from using animal manures.

Total Nutrient Requirement For - This is the total N-P2O5-K2O recommended for the crop for a growing season assuming low P2O5 and K2O sc tests. Split applications of N and K2O result in more efficient nutrient use. For assistance in determing individual application rates, see your County Extension Agent, nutrient management specialist or Soil and Water Conservation District Technician.

Manure application rate - The maximum application rate that should be applied if it is split applied at least three times during this crop, and t amount applied in each application adjusted to crop intake. If single applications are used, then manure should be applied at 50% of the above rate with the remaining N requirement being met by supplemental fertilization. Sprayfields with frequent applications may also need an adjusted rate.

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Livestock Waste Analysis Grower Report

Erika Machtinger
19308 NW CR 236
High Springs, FL 32643

PHONE:

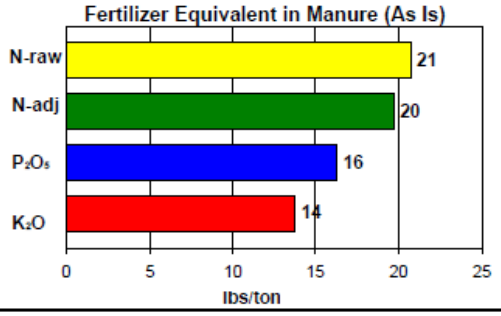
Lab #	7287
Sample Label	Diet
Date Collected	April 1, 2011
Date Delivered	
Date of Report	June 8, 2011
County of Sampl	Alachua
Collected By	0

Sample Type:	Other composted material.
Crop or Use:	#N/A
Application Equipment:	Applied by manure spreader
Incorporation	#N/A
Previous Applications	#N/A

***Nutrient Content in Manure as Delivered to Laboratory

Nutrient Constituent	Raw Sample	Adjusted For Application Losses of N	Units
Nitrogen (N):	21	20	lbs/ton
Phosphorus (P2O5):	16	16	lbs/ton
Potassium (K2O):	14	14	lbs/ton

pH as Sampled	4.6
Moisture Content:	63.3%
Total Solids:	36.7%
Total Ash:	2.7%



***Total Nutrient Requirement for:	lbs. N/acre	lbs P_2O_5/acre	lbs K_2O/acre
#N/A	#N/A	#N/A	#N/A
Totals	#N/A	#N/A	#N/A

Nitrogen Recommendation Base

***Manure application rate (As Is) to supply crop N requirement:
#N/A tons/acre

By supplying the crop N requirement at the rate shown above, the following total nutrients will be applied:
#N/A lbs. N/acre
#N/A lbs P2O5/acre
#N/A lbs K2O/acre

Supplemental nutrients needed:
#N/A lbs. N/acre
#N/A lbs P2O5/acre
#N/A lbs K2O/acre

***Economic value of manure at the rate shown above:
N #N/A per acre
P2O5 #N/A per acre
K2O #N/A per acre

***Cost of additional nutrients needed:
#N/A N per Acre
#N/A P2O5 per acre
#N/A K2O per acre

Phosphorus Recommendation Base

***Manure application rate (As Is) to supply crop P requirement:
#N/A tons/acre

By supplying the crop P requirement at the rate shown above, the following total nutrients will be applied:
#N/A lbs. N/acre
#N/A lbs P2O5/acre
#N/A lbs K2O/acre
#N/A

Supplemental nutrients needed:
#N/A lbs. N/acre
#N/A lbs P2O5/acre
#N/A lbs K2O/acre

***Economic value of manure at the rate shown above:
N #N/A per acre
P2O5 #N/A per acre
K2O #N/A per acre

***Cost of additional nutrients needed:
#N/A N per Acre
#N/A P2O5 per acre
#N/A K2O per acre

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Livestock Waste Analysis Grower Report

Erika Machtinger
19308 NW CR 236
High Springs, FL 32643

PHONE:

Lab #	**7287**
Sample Label	**Diet**
Date Collected	**April 1, 2011**
Date Delivered	
Date of Report	**June 8, 2011**
County	**Alachua**
Collected By	**0**

Sample Type:	**Other composted material.**
Crop or Use:	**#N/A**
Application Equipment:	Applied by manure spreader
Incorporation:	#N/A
Previous Applications:	#N/A

Laboratory Results (All weights are based on sample weight as received)

Total Solids:	366700 mg/kg	36.7%	733 lbs/ton
Total Ash:	26800 mg/kg	2.7%	54 lbs/ton
Total Kjeldahl N*:	10352 mg/kg	1.04%	20.7 lbs/ton
Ammonia Nitrogen:	357 mg/kg	0.04%	0.7 lbs/ton
Total Elemental P:	3571 mg/kg	0.36%	7.1 lbs/ton
Total Elemental K:	5721 mg/kg	0.57%	11.4 lbs/ton
Moisture:	63.33%		
pH:	4.6		

* Total Kjeldahl Nitrogen is equivalent to Total N for manure and high organic samples

Estimated Nitrogen Losses:

N-Content of Sample as Tested:			20.7 lbs/ton
***N-losses during application:	5%	-	1.0 lbs
***N-losses while awaiting incorporation:	0%	-	0.0 lbs
***Other N-Losses:	0%	-	0.0 lbs
Estimated Available N:	**95.0%**		**19.7 lbs/ton**

Footnotes:

Fertilizer Equivalent in Manure - The nitrogen value is an estimate based on inherent losses from using animal manures.

Total Nutrient Requirement For - This is the total N-P2O5-K2O recommended for the crop for a growing season assuming low P2O5 and K2O sc tests. Split applications of N and K2O result in more efficient nutrient use. For assistance in determing individual application rates, see your County Extension Agent, nutrient management specialist or Soil and Water Conservation District Technician.

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APPENDIX C
SURVEY RESULTS SUMMARY

Perceptions of Pests and Control on Equestrian Facilities

SurveyMonkey

1. When do you experience the most pressure from pests? (Please choose one)

	Response Percent	Response Count
Winter (December - February)	0.3%	1
Spring (March - May)	9.2%	27
Summer (June-August)	86.1%	254
Fall (September - November)	4.4%	13
answered question		295
skipped question		5

2. Which pests do you feel give you the most problems? (Answer all that apply)

	Response Percent	Response Count
Mosquitoes	59.4%	177
Stable Flies	**63.1%**	**188**
House Flies	33.2%	99
Horn Flies	16.4%	49
Horse and Deer Flies	46.3%	138
Gnats	61.1%	182
Bot Flies	13.1%	39
Ticks	17.8%	53
Other	6.7%	20
	If other, please specify.	32
	answered question	**298**
	skipped question	**2**

3. Which of these pests do you feel you can accurately identify? (Check all that apply)

	Response Percent	Response Count
Mosquitoes	**93.6%**	**280**
Stable Flies	61.5%	184
House Flies	75.9%	227
Horn Flies	23.4%	70
Gnats	85.3%	255
Bot Flies	48.8%	146
Ticks	82.9%	248
Horse and Deer Flies	80.6%	241
Other	4.7%	14
If other, please specify.		22
answered question		**299**
skipped question		**1**

4. Do you monitor pest populations on your property?

	Response Percent	Response Count
Yes	23.2%	69
No	**76.8%**	**229**
If you answered yes, please provide a brief description of how (product, how often, etc.) This helps us help you!		68
answered question		**298**
skipped question		**2**

5. If you answered No to pest monitoring, why? (Answer all that apply)

	Response Percent	Response Count
Lack of Time	22.2%	50
Lack of Knowledge	**59.1%**	**133**
Lack of Control Options	18.2%	41
Do Not Feel it is Necessary	20.0%	45
Other	6.7%	15
	If other, please specify.	23
	answered question	**225**
	skipped question	**75**

6. What level of pests are you willing to tolerate around your animals? (Please choose one)

	Response Percent	Response Count
Less than 10 Pests on Animal or Sticky Trap	**61.7%**	**177**
11-25 Pests on Animal or Sticky Trap	31.7%	91
26-50 Pests on Animal or Sticky Trap	4.9%	14
Greater than 50 Pests on Animal or Sticky Trap	1.7%	5
	answered question	**287**
	skipped question	**13**

7. What products do you use most often for pest control? (Answer all that apply)

	Response Percent	Response Count
Other	3.0%	9
Fly Masks	66.2%	196
Fly Sheets	21.3%	63
Feed Through Control	14.5%	43
Fly Spray	**95.9%**	**284**
Other Insecticidal Control	18.2%	54
Biological Control (Such as Parasitoids)	22.0%	65
Traps	37.8%	112
Other (please specify)		45
answered question		**296**
skipped question		**4**

8. Do you feel pest control products are effective in reducing pest problems?

	Response Percent	Response Count
Very effective	9.1%	27
Somewhat effective	**72.3%**	**214**
Somewhat ineffective	14.5%	43
Very ineffective	4.1%	12
Comments on control effectiveness, how do you think they could be improved?		67
answered question		**296**
skipped question		**4**

9. Do you feel pest control products are cost effective?

		Response Percent	Response Count
Yes		48.6%	140
No		**51.4%**	**148**
		If no, please specify why.	67
		answered question	**288**
		skipped question	**12**

10. Which describes your manure management?

		Response Percent	Response Count
Drag		22.1%	65
Manure pile (no active management)		24.8%	73
Spreader		**49.0%**	**144**
Compost (active management including turning, etc.)		25.9%	76
Other		12.9%	38
		If other, please specify.	48
		answered question	**294**
		skipped question	**6**

11. Do you feed hay round bales?

	Response Percent	Response Count
Yes	31.0%	61
No	69.0%	136
If yes, please elaborate on your hay management (do you move the hay bales to different locations, clean up waste before adding a new round bale, etc.)		51
answered question		197
skipped question		103

12. Do you feel you have time to properly manage your facility to reduce pest numbers and breeding?

	Response Percent	Response Count
Yes	66.1%	193
No	33.9%	99
Please provide any additional information you feel is appropriate.		28
answered question		292
skipped question		8

13. Are you able to recognize where pests breed?

	Response Percent	Response Count
Yes	69.0%	203
No	31.0%	91
Please provide any additional information/questions on pest breeding areas		26
answered question		294
skipped question		6

14. Do you feel your neighbors do enough to suppress pests?

	Response Percent	Response Count
Yes	36.1%	99
No	63.9%	175
If no, what do you feel they could improve on?		60
answered question		274
skipped question		26

15. Annually, how much do you spend on fly control products (including physical barriers, traps, insecticides, and biological control)

	Response Percent	Response Count
$0	0.3%	1
Less than $50 Annually	8.4%	25
$50-$100 Annually	17.2%	51
$100-$150 Annually	22.6%	67
$150-$200 Annually	18.5%	55
Greater than $200 Annually	**33.0%**	**98**
	answered question	**297**
	skipped question	**3**

16. Do you feel research is being applied towards your concerns regarding pests?

	Response Percent	Response Count
Yes	45.7%	128
No	**54.3%**	**152**
Where/who do you go for answers to your pest questions (ex. feed store, seminars, extension agents, friends/family, trainer/barn owner, product companies, trade shows, etc.)?		109
	answered question	**280**
	skipped question	**20**

17. What focus area would you like to see more research on? (Answer all that apply)

	Response Percent	Response Count
Biological Control	**80.2%**	**231**
Insecticides	42.0%	121
Physical Barriers (fly masks, fly sheets, etc.)	8.0%	23
Traps	21.2%	61
Manure Management and Sanitation (Cultural Control)	48.6%	140
Other	3.8%	11
	If other, please specify.	20
	answered question	**288**
	skipped question	**12**

18. What would you like to know more about (pamphlets, seminars, other educational material? (Please choose one)

	Response Percent	Response Count
Sampling methods	22.0%	61
Identification	22.4%	62
Trapping options	30.0%	83
Pesticide use	40.4%	112
Biological control	**62.1%**	**172**
Cultural control methods (composting, sanitation, etc.)	38.3%	106
	answered question	**277**
	skipped question	**23**

19. What county are you from?

	Response Percent	Response Count
Alachua	27.8%	68
Baker	0.0%	0
Bradford	0.4%	1
Brevard	2.9%	7
Citrus	2.9%	7
Clay	4.5%	11
Columbia	3.3%	8
Dixie	1.6%	4
Duval	2.0%	5
Flagler	0.8%	2
Gilchrist	2.9%	7
Hamilton	0.0%	0
Hernando	2.4%	6
Hillsborough	4.1%	10
LaFayette	0.0%	0
Lake	3.7%	9
Levy	3.7%	9
Madison	0.0%	0
Marion	18.4%	45
Nassau	0.8%	2
Oceola	0.8%	2
Orange	1.2%	3
Pasco	1.2%	3

Pinellas	3.3%	8
Polk	2.4%	6
Putnam	0.8%	2
Seminole	0.4%	1
St. Johns	3.7%	9
Sumter	0.4%	1
Suwanee	0.8%	2
Taylor	0.8%	2
Union	0.8%	2
Volusia	1.2%	3
	answered question	245
	skipped question	55

20. How would you describe yourself?

	Response Percent	Response Count
Barn/facility Owner	84.5%	240
Boarder at a Facility	12.7%	36
Rider at a Facility	1.1%	3
Other	1.8%	5
	If other, please specify.	11
	answered question	284
	skipped question	16

21. What is your primary discipline? (Please select one)

	Response Percent	Response Count
Western	3.9%	11
Fox Hunting	2.1%	6
Hunter/Jumpers	13.4%	38
Eventing	9.5%	27
Dressage	13.4%	38
Pleasure Riding/Trail Riding	**39.6%**	**112**
Rodeo	0.7%	2
Roping/Team Penning	1.1%	3
Barrel Racing	1.4%	4
Horse Racing	0.7%	2
Gaited Horses	2.5%	7
Reining	2.5%	7
Other	9.2%	26
	If other, please specify.	41
	answered question	**283**
	skipped question	**17**

22. How many acres is your property?

	Response Count
	283
answered question	**283**
skipped question	**17**

23. How many horses (on average) are on the property?

	Response Count
	285
answered question	285
skipped question	15

24. We are always looking for potential volunteers for research projects in central Florida. If you are interested in being volunteer for future research projects we would sincerely appreciate your contact information.

	Response Percent	Response Count
Name:	96.5%	136
Address:	85.8%	121
Address 2:	8.5%	12
City/Town:	94.3%	133
State:	99.3%	140
ZIP:	87.2%	123
Email Address:	97.9%	138
Phone Number:	79.4%	112
answered question		141
skipped question		159

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BIOGRAPHICAL SKETCH

Erika Machtinger was born in the small coastal town of Blue Hill, Maine. Her passion for horses began at four years old when her mother started her riding at a local barn. She started competing in the Olympic sport of Three-day Eventing and quickly was competing on a national level. Once her sights turned to international competition, she put together a committee at her high school to complete graduation requirements in three, instead of four, years in order to become a working student for Olympian Bruce Davidson prior to college.

Erika began her undergraduate degree at the University of Delaware in the Wildlife Conservation and Ecology program. The program at Delaware was heavily influenced by the Entomology Department and Erika quickly found a passion for insects. She molded her degree to incorporate many courses with an emphasis on entomology.

While completing her degree, Erika gained practical experience rearing, monitoring, and researching arthropods as a Research Assistant at the USDA, ARS, Beneficial Insect introduction Research Laboratory. Post graduation, Erika worked for the Wildlife Habitat Council in Washington D.C. as a Wildlife Biologist and NRCS Extension Coordinator to develop a series of grant-sponsored extension materials. Desiring a more permanent position, Erika relocated to Northeast Florida after accepting a position with an Environmental Permitting company.

Completing graduate studies was always a goal of Erika's. She applied and was accepted in to the M.S. non-thesis program in Entomology at the University of Florida. During her first semester, she successfully located a Major Professor and secured funding to work in the field of Integrated Pest Management and Veterinary Entomology while incorporating her love for horses.

Erika has presented the findings of her research at various scientific conferences such as the Florida Entomological Society and the Society of Vector Ecology. She has mentored two undergraduate students wishing to gain more research experience. Her ultimate goal is to pursue a PhD at the University of Florida and earn a position as a research scientist at the university level while continuing to ride and compete.