PROXIMATE CAUSATION OF STABLE FLY (*STOMOXYS CALCITRANS* (L.)) HOST USE: THE INFLUENCE OF PHENOLOGY AND HOST BLOOD SUITABILITY

by

Kristina Marjorie Hale

A dissertation submitted in partial fulfillment
of the requirements for the degree

of

Doctor of Philosophy

in

Animal and Range Sciences

MONTANA STATE UNIVERSITY
Bozeman, Montana

April 2011

APPROVAL

of a dissertation submitted by

Kristina Marjorie Hale

This dissertation has been read by each member of the dissertation committee and has been found to be satisfactory regarding content, English usage, format, citation, bibliographic style, and consistency and is ready for submission to The Graduate School.

Gregory Johnson

Approved for the Department of Animal and Range Sciences

Glenn Duff

Approved for The Graduate School

Dr. Carl A. Fox

STATEMENT OF PERMISSION TO USE

In presenting this dissertation in partial fulfillment of the requirements for a doctoral degree at Montana State University, I agree that the Library shall make it available to borrowers under rules of the Library. I further agree that copying of this dissertation is allowable only for scholarly purposes, consistent with "fair use" as prescribed in the U.S. Copyright Law. Requests for extensive copying or reproduction of this dissertation should be referred to ProQuest Information and Learning, 300 North Zeeb Road, Ann Arbor, Michigan 48106, to whom I have granted "the exclusive right to reproduce and distribute my dissertation in and from microform along with the non-exclusive right to reproduce and distribute my abstract in any format in whole or in part."

Kristina Marjorie Hale

April 2011

ACKNOWLEDGEMENTS

I would like to thank my family and friends for their unending support and encouragement: Steve Hale, Judy and John Bowling, Christopher Hale, Carly and Steve Merriman, and Donna Hale. I would also like to thank past and present lab workers for their help both in the lab and in the field. Marni Rolston, our lab manager, has been the glue that has kept all elements together and has really made what could have been a rocky road as smooth as the streets in Naples. I thank Dr .Clayton Marlow for forcing me to think more about the physical properties of habitats and Drs. Tim Lysyk and Jerome Hogsette for providing invaluable advice on statistics and the behavioral ecology of arthropods, and for being patient with my 'enthusiasm'. Special thanks to Dr. Kevin O'Neill, who knows how to and has intentionally pushed my buttons these past six years to make me a better student and person, and for some of the most enlightening discussions and seminars in my college career. I would also like to thank all of the staff at Medicine Lake National Wildlife Refuge, especially the refuge manager, Jerry Rodriguez, for all of their incredible support and encouragement in the field. None of my graduate work would have happened if it weren't for their graciousness and trust. Penultimately, I would like to thank my advisor Dr. Gregory Johnson, for his tutelage and patience, for his work ethic that he has engrained in me, for showing me the value of both field and lab work, for exposing me to all of the other projects outside of my own, and for giving me a graduate experience that I know I'm lucky to have had. And, finally, I would like to thank all of the medical and veterinary pests of the world, without which, I might have just been a lawyer.

TABLE OF CONTENTS

TABLE OF CONTENTS - CONTINUED

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

The biting fly, *Stomoxys calcitrans* (L.) (Diptera: Muscidae) has become a cosmopolitan pest of livestock, companion animals, and humans. Both males and females require daily blood meals and will opportunistically feed on many terrestrial mammals. They have rarely been seen to feed on birds, despite the presence of many potential hosts. A unique feeding behavior was documented at a wildlife refuge in northeast Montana when stable flies were seen congregating on the heads and eyes of West Nile-infected American white pelicans. The objectives of this investigation were to describe adult phenology near the pelican colony and to determine daily and lifetime fecundity when fed on cattle, horse, or chicken blood. From 2008-2010, relative adult abundance was measured by placing white Coroplast® cards near the colony, around confinement lots, and along transects in pasture used by grazing cattle. Results showed that temporal dynamics varied by habitat type (2008: $F = 7.4$; df = 16, 191; $P < 0.001$; 2009: $F = 17.7$; df = 14, 270; $P < 0.001$) and that local dispersal occurred throughout the season. Weekly changes in abundance were explained by temperature, precipitation, and degree-days in 2008 ($F = 12.2$; $r^2 = 0.13$) and 2010 ($F = 13.8$; $r^2 = 0.27$) and by temperature and precipitation in 2009 ($F = 164.6$; $r^2 = 0.82$). Stable flies from a laboratory-maintained colony were provided with cattle, horse, or chicken blood and daily and lifetime fecundity rates were measured through F_2 adult emergence. Flies fed chicken blood laid more eggs per day than those fed cattle ($P = 0.008$) or horse blood ($P = 0.05$), but lifetime fecundity was similar between treatments ($\chi^2 = 3.4$; df = 2; $P = 0.2$) because of shorter oviposition periods in cohorts fed chicken blood. These results indicate that the nutritional composition of blood from these hosts does not explain the rarity of stable flies feeding on birds. Implications of host defenses as an explanation for stable fly behavior are discussed.

INTRODUCTION

Stable flies (*Stomoxys calcitrans* (L.) are blood-feeding Diptera that are cosmopolitan medical and veterinary pests (Hogsette and Farkas 2000). Both males and females require daily blood meals and feed on livestock, companion animals, humans, and, rarely, birds. Trapping, host identification, and fecundity studies have indicated that stable flies collected near birds such as chickens feed on other hosts, presumably because bird blood is nutritionally deficient for reproduction (Anderson and Tempelis 1970, Sutherland 1978). In northeast Montana, however, hundreds of stable flies were seen feeding on the crown of the heads and around the eyes of immobile West Nile virus-infected American white pelicans (*Pelecanus erythrorhynchos* Gmelin). These flies were aspirated and processed for detection of WNV and blood meal identification (Johnson et al. 2010a, Johnson et al. 2010b). Results conclusively showed that stable flies fed on pelicans and indicated that they may have a role in the WNV transmission cycle within the pelican colony. Thirty-nine pools of unengorged flies aspirated from sick juveniles were tested for WNV. An infection rate of 13 per 1,000 flies was estimated and was higher than the known primary vector in this area, *Culex tarsalis* Coquillett, which had an estimated infection rate of 11 females per 1,000. Furthermore, during the same period, the probability of human West Nile neuroinvasive disease increased five-fold in counties where WNV-associated pelican deaths occurred (Johnson et al. 2010b).

Stable fly populations in Montana have not been previously described. This is surprising because cattle are the most valuable agricultural commodity in Montana (USDA National Agriculture Statistics Service) and decreased weight gains in response

to stable fly attack has the potential to result in severe economic losses (Campbell et al. 2001). The objectives of this study, therefore, were to:

1. Describe adult stable fly seasonal abundance near the pelican colony in northeast Montana, and

2. Determine daily and lifetime fecundity rates of stable flies fed cattle, horse, and chicken blood.

LITERATURE REVIEW

Distribution

The genus *Stomoxys* is mostly afrotropical in distribution and includes at least 18 species (Zumpt 1973). *Stomoxys calcitrans* (L.), now considered to be cosmopolitan and the only *Stomoxys* spp. in North America, is palearactic in origin and was probably introduced into the Americas and Australia during the colonial era (Hennig 1964, Moon 2002). *Stomoxys niger* Marquart has also been described as a pest in parts of Africa (Gilles et al. 2005).

Morphology

Adults

Similar in size and color to the house fly, *Musca domestica* L., *S. calcitrans* has sometimes been referred to as the biting house fly (Moon 2002). The genus *Stomoxys* is distinguished from other muscids by its elbowed proboscis and short maxillary palps. Horn flies, *Haematobia irritans* (L.), are also similar in shape and color, but are smaller than stable flies and have maxillary palps that are as long as the proboscis. Adult stable flies are 4-7 mm in length and distinguished from other *Stomoxys* spp. by the relatively wide frons and the two lateral black round spots on the second and third abdominal segments. The male genitalia comprising the aedeagus, a pair of pre- and postgonites, and the horned sclerite, may also be used to distinguish *Stomoxys* spp. (Zumpt 1973, Masmeatathip et al. 2006). Antennae are aristate with dorsal setae on the arista.

Males and females may be distinguished by at least two characters. The frontal index, measured as the frontal space of vertex divided by the eye length, is typically smaller in males (average = 0.33 ± 0.03 mm) than in females (average = 0.55 ± 0.04 mm) (Masmeatathip et al. 2006). The easiest external character used to sex *S. calcitrans* is the presence (male) or absence (female) of an anal sclerite.

Stable fly ovaries are meroistic with each ovariole containing oocytes and nurse cells. The ovarian process is continuous because each oocyte is accompanied by its nurse cells as it develops. Scholl (1980) developed a standard to age-grade females according to ovarian physiological status. Previously, Detinova (1962) had proposed an eight-stage classification system originally proposed by Christophers (1911). According to Scholl, there are six recognized stages of ovarian development as determined by the morphology of primary oocytes. Follicles not separated from the germarium are classified as stage 0, while follicles that have separated and without yolk are at stage 1. Stage 2 occurs when follicles begin to elongate and yolk deposition is visible. The greatest growth occurs during stage 3 where follicles continue to elongate and the yolk occupies approximately 3/4 of the egg. The egg becomes almost its full length in stage 4 and nurse cells occupy less than 1/4 of the egg. The final stage is apparent by the hardening of the chorion and the hatching pleat.

Inferences about the age of stable fly populations are usually made from various physiological parameters of the females. In addition to ovarian age-grading, pterin accumulation, insemination, and parity may also be evaluated. Pterin is a fluorescent compound that accumulates in the eyes of flies as they age. Cumulative pterin

concentration is independent of the number of blood meals taken but that the oviposition rate changed with temperature and that degree-days were a predictor (Lysyk and Krafsur 1993). Because of this, pterin concentration provides additional information about the age of field populations and is recommended as an additional parameter that should be measured (Lysyk and Krafsur 1993). Insemination may be determined by bursting the paired spermatheca and looking for the presence of sperm with a contrast microscope. Flies may be classified as nulliparous, uniparous, or multiparous by combining staining procedures and identification of yellow bodies. Yellow bodies can be seen without staining in females that have oviposited twice or more, and are consequently indicative of older females. To determine insemination and parity status, flies must be killed immediately before dissection. Stored sperm and ovarian relics will rapidly degrade after death, even when stored in ethanol (Scholl 1980).

Eggs

Eggs are white, elliptical, and approximately 0.9-1.0 mm in length and 0.25-0.30 mm in width with a hatching pleat running laterally. The dorsal area between hatching pleats is a quarter as wide as the egg (Kano 1953). Muscidae eggs are typically covered with two to three layers of chorion consisting of an inner and outer layer with many small aeropyles and a middle layer with fewer larger aeropyles. Where there are holes in the chorion, the egg is more elastic, which is thought to enable the egg to withstand hydrostatic pressures (Zumpt 1973).

Larvae

The larval stage consists of three instars. First instars are 1.5-2.5 mm in length (Skidmore 1985). Second instars are approximately 4-6 mm in length and differ from first instars by the exception of having anterior spiracles with 4-7 lobes. Posterior spiracles on second instars are weakly pigmented and have two non-sinuous slits in each. This separates them from the larger third instar larvae (5-12 mm in length) which have three sinuous slits in each posterior spiracle. The characteristics of the posterior spiracles may be used to differentiate *Stomoxys* spp. from other Muscidae. Posterior spiracles of *Stomoxys calcitrans* are heavily sclerotized, widely spaced, and rounded-triangular in shape with a central scar surrounded by two or three sinuous slits. Also used for identification is the cephalopharyngeal skeleton which includes a sieving mechanism typical of filter-feeding muscid larvae as well as the mouth hook and dental, accessory, and pharyngeal sclerites.

Pupae

The puparium is the contracted skin of the third instar larva, and as such, retains some morphological features (Skidmore 1985). Atmospheric gas exchange occurs through pupal horns that protrude through the dorsolateral walls of the first abdominal segment. Posterior spiracular plates retain identical morphology of third instar larvae and may be used for identification in addition to the spiracular morphology on the abdominal welts. Puparia are approximately 10 mm in length and 2 mm in width. Pupal age is often estimated by color of the puparium, with pale white pupae being recently formed and

dark, brick red being older. About a day before the adult is ready to eclose, the pupa will turn almost black in color.

Larval Development and Distribution

Stable fly larvae are filter feeders, and, like house flies (Schmidtmann and Martin 1992), face flies, *Musca autumnalis* DeGeer, (Hollis et al. 1985), and horn flies (Haematobia irritans (L.)) (Perotti et al. 2001), require microorganisms for survival and successful development (Gingrich 1960, Romero et al. 2006, Mramba 2007). Although the role of microflora in larval development has not been fully defined, it is likely that larvae feed on the metabolic byproducts of the bacterial and fungal community rather than on the microorganisms (Lysyk et al. 1999). Given the need of an active microbial community, stable fly larvae are typically found in sites with fermenting vegetation, water, urine, and excrement. Therefore some stable fly larval development sites can be mostly ephemeral. For example, aquatic grasses were historically thought to be the main source of larval development along coastal shorelines. On the northwest coast of Florida, the two primary species of bay grasses where stable fly larvae were found to develop were shoal grass (*Halodule wrightii* Aschers) and turtle grass (*Thalassia testudinum* Koenig and Sims). Eel grass (*Vallisneria americana* Michx.) was shown to support development along the Mississippi and Alabama coasts where salinity was low (Simmons and Dove 1941). Elevated salinity prevents the fermentation needed for development. Larvae were found in a mixture of aquatic vegetation and mayfly corpses along the Kentucky Reservoir (Pickard 1968). In New Jersey, stable fly populations declined after

the local supply of eel grass was decimated by disease (Hansens 1951). Conservation efforts were made to restore this grass and within two years, the grass returned in abundance as did local stable fly populations. Stable flies can use fermenting aquatic grasses as larval substrates when available. However, because stable flies have been observed only sporadically along coastal areas following weather fronts, and the arrival of gravid adults has to coincide with the appropriate accumulation and fermentation levels of these grasses. Therefore, this type of vegetation does not routinely provide suitable sites for larval development (Hogsette et al. 1987).

In feedlots, suitability of sites for larval development varies widely according to geographical location and microhabitat parameters (Rasmussen and Campbell 1981). In Nebraska, larval abundance measured in 16 sites showed that open silage, spilled feed and manure in drainage ditches and under fence lines consistently contained larvae (Meyer and Petersen 1983). In another study in eastern Nebraska feedlots, however, 80% of the larvae came from the soil-to-concrete interface of the feed apron (Skoda et al. 1991). Animal pen litter and animal excrement produced the most stable flies in southwest Georgia (Haines 1955).

Outdoor calf hutches on a central Maryland dairy were heavily exploited by both house flies and stable flies with both species appearing and rapidly increasing in abundance one to three weeks after the introduction of calves to pens (Schmidtmann 1988). Decomposing silage and green chop (freshly cut sorghum or corn) were identified as primary sites of larval development in northwestern Florida (Williams et al. 1980). In Alberta, larvae were generally found in highest abundance in manure mounds, general

lots, and residual manure and bedding near the indoors of dairies (Lysyk 1993b). A significant site-farm interaction in this study provided additional evidence for the opportunistic nature of stable flies and the subsequent importance of microhabitat parameters for the behavioral ecology of this species.

Stable flies overwinter as quiescent larvae that exhibit a reduction in metabolism and development. Quiescence is terminated immediately following a change back to favorable climactic conditions. There is controversy surrounding the exact mechanism of overwintering due primarily to the variance of behavior of this fly at different locations. While it was once thought that adults may survive the winter in heated barns and warm locations, it is now widely accepted that stable flies overwinter as quiescent larvae in substrate that provides adequate moisture and warmth for survival such as open silage storage systems (Scholl et al. 1981). In northwest Florida, peanut litter was thought to provide overwintering sites (Simmons and Dove 1941), however it is questionable whether stable flies overwinter in Florida based on the presence of large adult populations in winter (J Hogsette, unpublished data).

Adult Behavior and Physiology

The effects of temperature and precipitation on growth rates of localized stable fly populations appear to vary according to geographic region (Lysyk 1993a). In the high plains (i.e., Nebraska and Kansas) and central and southern California, population peaks have been positively correlated with moisture levels rather than temperature (Greene et al. 1989, Mullens and Petersen 2005, Taylor et al. 2007). In contrast, peak activity in

Alberta has been attributed to temperature (Lysyk 1993a). The national trend that emerges in North America is that growth rates of local populations are explained more by moisture in warmer climates and by temperature in cooler climates.

In California, spring rains wet potential oviposition sites and explain peaks seen between April and June (Mullens and Peterson 2005). Populations begin to decline soon afterwards, probably as a result of both elevated temperatures and reduced rainfall. Lab studies have shown that fecundity and pupal survival decrease dramatically at 30°C or higher, indicating the sensitivity of stable flies to high ambient temperatures (Lysyk 1998). This is reflected by populations in Kansas and Nebraska where peaks are observed in June and late September followed by a smaller peak in late September / early October (Broce et al. 2005, Taylor et al. 2007). In the cooler climate found in Alberta, populations do not peak until late August and mid-September (Lysyk 1993a).

Stable flies are diurnal feeders that must take at least one blood meal a day and will feed on hosts between one and ten minutes when not disturbed (Mitzmain 1913, Simmons 1944, Lee and Davies 1979). The average amount of blood ingested is about 10 mg (Smith and Hansens 1975). In Michigan, *S. calcitrans* was active within 30 minutes of sunrise until 30 minutes before sunset (Voegtline et al. 1965). Flies tended to become active later in the mornings that were preceded by relatively cool nights. No relationship was found between sky cover and biting activity, but, in contrast to Mitzmain (1913), feeding was greater at lower relative humidities. Stable flies need internal temperatures to reach 15°C to fly (Buschman and Patterson 1981). Biting activity was positively correlated with temperature up to 32°C, possibly due to either an increase in

metabolism or water loss that would require the flies to take another blood meal. In the high plains, peak activity was recorded to occur between 1300 and 1630 h (Berry and Campbell 1985). Because stable flies are primarily a pest of cattle, they are often counted on the legs of cattle to estimate biting activity.

The level of local or long distance dispersal of stable flies is an issue that remains unclear. Support for long distance dispersal comes from a flight mill study that demonstrated that stable flies were capable of flying up to 29 km in a day as well as studies in Florida (Bailey et al. 1973, Hogsette and Ruff 1985). The research area in Florida used by Hogsette and Ruff (1985) included an agricultural zone in the north consisting primarily of dairies and a Sand Hill zone to the south where no stable fly hosts or habitat existed. A beach was the third zone that borders the Sand Hills and the Gulf of Mexico. At least one self-marked, wild stable fly (Hogsette 1983) dispersed 225 km from its self-marking area in the agricultural zone to its collection point along the coastal zone after the passage of a weather front. Furthermore, dissections of the females (unmarked) collected on the coastal zone revealed that nearly 60% were nulliparous with a catch average of 70% females. This was in contrast to the sex ratio of one male per female found by the same authors using the same traps on dairies. Johnson et al. (1966) asserted that many migrating insect populations may consist mostly of pre-reproductive females.

Evidence for local dispersion has been accumulated in studies conducted in Nebraska, Ontario, and Oregon. Eddy et al. (1962) showed local dispersal when 25,000 fluorescently marked lab-reared and 750 marked wild stable flies were released in

Oregon (along with horn flies and three species of mosquitoes). Of these, a total of 51 flies were recovered one and five miles from the release site. Beresford and Sutcliffe (2009) found evidence for local dispersion when they modeled population dynamics at refuge and non-refuge farms in late spring and early summer in Ontario. In this study, local dispersion occurred early in the season and was either an inverse function of stable fly density or was density-independent. In Nebraska, a dispersal study conducted in late June suggested that stable flies followed a passive, short-distance diffusion model (Taylor et al. 2010).

Nutrition and Reproduction

In stable flies, the immediate destinations of sucrose and blood are the crop and the midgut, respectively. Although male and female stable flies require blood meals, they also will feed on nectar. Sucrose solutions are directed to the midgut, but may be retained in the crop and gradually released into the midgut to fuel flight (Venkatesh and Morrison 1980). The crop is a membranous blind sac that is normally found in the abdomen and is connected to the junction between the esophagus and the proventriculus via a long duct. Lee and Davies (1979) demonstrated that in flies two to three days old, blood does not enter the crop, but the crop will fill with sucrose solution. Digestion begins at the posterior end of the midgut approximately two hours following ingestion of a blood meal. Generally, flies less than 12 h old will refuse a blood meal, but will readily take one after a day old, theoretically because sensory receptors on the mouthparts and legs are not fully functional and because the mouthparts have not adequately hardened.

Air bubbles tend to be present in flies 10 h old or younger. As the flies age, the air bubbles disappear and the extent of blood in the midgut advances from just the anterior portion. There is no difference in males or females in the destination of sucrose or blood.

Stable flies will exhibit pre-diuresis (Mitzmain 1913). More sugar-fed flies were found in urban rather than rural environments in Nebraska (Taylor and Berkebile 2008). In the rural habitats, more sugar-positive flies were collected in pastures than in croplands or near feedlots, indicating that stable flies are opportunistic nectar feeders. Regardless of urban or rural classification, sugar feeding rates in Nebraska appeared to be highest when more flowers were in bloom in the spring and dropping throughout the summer as the abundance of flowering plants decreased.

Both sperm transfer and egg development will not occur unless stable flies are provided a blood meal (Harris et al. 1966). If they are not given a blood meal shortly after emergence, their ability to reproduce may not be repaired (Jones et al. 1985). Male accessory glands double in size within 24 h of emergence and reach full size within three days (Morrison et al. 1981).

The process of spermatogenesis includes the formation of three flagellated cell types. Fusiform cells are the early spermatids in which the tail appears and are classified as either fat fusiform with nuclei lengths of 13.0 μm and widths of 4.0 μm or elongate fusiform with nuclei lengths of 18.0 μm and width of 2.6 μm. When the fusiform spermatid lengthens and constricts, the spermatozoa, which is thin and elongate, is formed and has nuclei lengths of 26.0 μm and width of 1.3 μm. Like the house fly, the stable fly lacks a distinct male accessory gland, but uses the median ejaculatory duct

instead. Spermatogenesis was similar in males fed either a sucrose solution or blood, and males on both diets also induced oviposition in the female. The male accessory gland prevents subsequent insemination in females by other males, but a blood meal is necessary for the accessory gland to fully express receptivity-inhibition. And, although sucrose fed males are able to produce sperm, they will not transfer sperm to females (Jones et al. 1985).

Diet rather than aging induces the secretion of sex pheromone which may be found as both saturated and unsaturated hydrocarbons on the female cuticle (Uebel et al. 1975, Meola et al. 1977). When Gatorade®-fed males were paired with blood-fed females, or when Gatorade®-fed females were paired with blood-fed males, mating did not occur. However, when sex pheromone was applied to Gatorade-fed females, blood-fed males exhibited mating behavior. This indicated that sex pheromone is produced as a cuticular hydrocarbon after blood feeding. Males touch the heads of females with their mouthparts before copulation (Buschman and Patterson 1981).

Females deprived of a blood meal until three days post emergence will produce smaller, fewer eggs (Sutcliffe et al. 1993). In this study, fat bodies of deprived females were smaller and required an extra blood meal to reach maximum size. In a study that specifically addressed the relationship between ingested blood and follicular growth, it was found that yolk deposition began after two days of blood feeding when follicles were a quarter of their final length (Chia et al. 1982). Females fed every 24 h required five blood meals to complete the first egg maturation and three blood meals for the second. Larval fat body disappeared after two days of emergence. Thus, females must build up

the protein reserves necessary for oogenesis. Fat bodies in adult females increase in weight following a blood meal with peak weight occurring two days after ingestion of the first meal.

Daily and lifetime fecundity of stable flies may be a function of temperature, age, oviposition substrate, and host source. Approximately 90 eggs mature in the ovarioles, and, on average, batches of 20 are oviposited at a time and may be dependent on temperature (Lysyk 1998, Gilles and Duvallet 2005). Given citrated bovine blood, maximum lifetime fecundity has been calculated to be up to 632 (Mitzmain 1913), 376 (Parr 1962), and 3,614 (Sutherland 1978) eggs per female. Lysyk (1998) found that lifetime fecundity ranged from less than 30 eggs per female at 15°C to more than 700 at 25°C. Berry and Kunz (1978) reported that oviposition may be greater and will begin at younger ages when females are exposed to higher temperatures.

Host type may also affect fecundity (Spates and DeLoach 1985). Sutherland (1978) allowed stable flies to feed on chicken, dog, cattle, donkey, goat, horse, sheep and pig blood. Of these, the maximum reproductive potential was highest from flies fed on cattle, sheep, and donkey blood. When fed chicken blood, 100% mortality was recorded before eggs were laid. Differences in fecundity based on host type have been attributed to nutritional castration. Ovaries in females fed chicken blood might have reached a stage where the follicles resorbed the yolk, which has previously been seen in starved insects and insects deprived of a specific nutritional element. In addition to possible effects of type of blood ingested, odors released by the microbial community may also affect the number of eggs deposited (Romero et al. 2006). Field-collected media elicited

the oviposition of approximately 400 eggs per female, but when sterilized, almost no eggs were deposited.

Oviposition sites are partially determined by olfactory cues. In one study, horse dung was found to be preferred to cow dung (Jeanbourquin and Guerin 2007). Stimulants identified were dimethyltrisulphide (from methionine degradation, released from fermented grasses, cheese, human skin, and livestock wastes), carboxylic acids, aliphatic C_7-C_8 alcohols and oct-1-en-3-ol (a product of the oxidation of linoleic acid). They also identified the terpenes citronelle, D-limonene, β-caryophyllene, β-cyclocitral, and α-humulene as stimulants. In general, terpenes are suspected as being used by flies to locate flowers for nectar feeding, but given that common oviposition media includes fermenting plant material, the terpenes identified by Jeanbourquin and Guerin (2007) might also be used to identify oviposition sites. Depending on temperature, eggs will hatch 12-80 h after deposition.

Collection of Adults

Evaluating different trap designs for stable flies has had two primary objectives: 1) to determine demographic-biases that may be inherent in trap design, and thus be able to more accurately interpret population trends, and 2) to identify potential candidates for fly control. Traps for stable flies have been developed based on observations that stable flies prefer to fly near ground level and will utilize open paths such as those created by transects of fire towers and power lines in undeveloped areas (Williams and Rogers 1976). Alsynite® panels or cylinders is the two main type of material used to collect

stable flies 20-30 cm above ground. The first trap designed to target stable fly adults was the Williams trap which comprised two pieces of Alsynite® fiberglass panels placed as an intersecting 4 x 4 on a wooden stake approximately 140 cm above ground (Williams 1973). Panels were coated with Tack trap® and placed next to box traps containing inverted plastic cones along beaches, dairies, and an open path in the woods. Williams traps collected seven times as many adults as the box traps which only worked when placed on white, sandy beaches (Williams 1973). When white, red, and black colors were evaluated, the white, translucent panels collected more stable flies. The attractiveness of stable flies to Alsynite® is thought to be due to the sudden shift of the ratio of short to long wavelength photons from 0.17 to 0.77 when viewed at a 15 degree angle from the normal (Zachs and Loew 1989). Theoretically, *Stomoxys* spp. might prefer to feed on the lower portions of animals where shadows, which are richer in short wavelength photons, are more likely to occur. Agee and Patterson (1983) also found a visual preference to surfaces that have a 5-20% reflectance in the UV range from 350 to 420 nm, which is the generally accepted theory.

Williams traps have since been modified in two major ways. One modification turned the traps into self-marking devices by coating some with fluorescent dusts of various colors instead of adhesive. Marked flies were detected with a UV light (Hogsette, 1983, Hogsette and Ruff 1990). Another modification improved the Williams trap by using cylindrical pieces of Alsynite® covered with sleeves coated in adhesive. The cylindrical traps collected more flies per surface area (Hogsette and Ruff 1990) and took less time to process (Broce 1988). Accumulation of dust and dead flies on the

surfaces of the Williams trap reduced trap efficiency. Adhesive sleeves allowed investigators to maintain the integrity of the trap. The efficiency of five trap types (Olson, Broce, Farnam Bite Free®, Farnam EZ®, and Nzi) was compared in eastern Nebraska. The BiteFree® traps collected the most stable flies with an average of 214 flies per trap per day. All traps collected a sex ratio of approximately two males per female, contrasting with the 1 male: 1 female ratio collected on Alsynite® cylinder traps and 1 male: 2 females ratio on Williams traps in Florida (Hogsette and Ruff 1990). In Kansas, however, Alsynite® traps tended to collect two males per female (Guo et al. 1998).

Sweep net collections provide different sex ratios according to microhabitat. Approximately two males per female were hand-netted along a Florida beach while one male per female was collected at nearby dairies (Jones et al. 1985). In Mauritius, sweep-net collections were almost always female-biased with yearly averages of 1.4 and 1.6 females per male.

Recently, Coroplast® cards have been also been evaluated (Beresford and Sutcliffe 2006). When compared to Alsynite® traps, Coroplast® cards collected more stable flies, likely due to a higher abundance of males and nulliparous females. For accurate population data, Coroplast® cards should be placed on wooden stakes so that the distance between the lower edge of the card is 20-24 cm above underlying vegetation (Beresford and Sutcliffe 2008). There was a positive correlation between hourly Coroplast® card catches and leg counts on cattle. When white, grey, red, green, yellow, blue, and black cards were used to investigate color effects, white cards were the most effective, probably due to different reflectance of the Coroplast® cards, Alsynite®

cylinders, and cloth. In contrast to Hogsette et al. (2008), black and blue colored cards were the least effective.

Cilek (2002) evaluated the effects of color and contrasting colors in Florida by painting beach balls painted either black, white, black with white circles, or plain (kept colors used by manufacturer and were configured such that blue, yellow, and red diamond-shaped panels were separated by white panels of similar size and shape) and coating them with Tangle trap®. Of these schemes, the plain balls attracted the most stable flies. No difference in abundance was detected between the black and white balls. In agreement with observations of Broce et al. (1991) the author concluded that the balls may have served as waiting stations where males wait for females to fly by, as a place of thermoregulation, or as a refuge from the wind. Male stable flies in Gainesville, FL preferred locations that were light colored vertical surfaces near livestock for both thermoregulation as well as waiting stations (Buschman and Patterson 1981). Because females of all reproductive stages were collected on the waiting stations, and because females only mate once, Buschman and Patterson (1981) postulated that the vertical surfaces must serve another purpose other than mating sites. In the same study, internal stable fly temperature increased from 6°C when in shade to 28°C when basking in the sun and with ambient air temperature only 6°C. In Alberta, resting sites varied on a farm-by-farm basis, but in general, adults preferred to rest on surfaces for both thermoregulation and wind shelter (Lysyk 1993b).

Carbon dioxide and octenol are the two odors most commonly evaluated for their effectiveness in attracting stable flies. Differences in attractiveness to these odors may be

dependent on the level of starvation and age of the fly, proximity of baits to hosts, and rate of release. Warnes and Finlayson (1985b) found that CO_2 attracts stable flies. High doses may have the effect of habituation sooner than lower doses which will activate the flies at a slower rate, but them keep them active longer. Additionally, the level of response of stable flies to carbon dioxide was a function of degree of starvation and the emission rate of carbon dioxide (Warnes and Finlayson 1985a). Male flies respond less to CO_2 as they age (Beresford and Sutcliffe 2008). In wind tunnel experiments, 49% of the males three days old or younger moved upwind towards the CO_2 plume vs. only 4.5% of the males older than three days. Torr et al. (2006) suggest that differences in individual cow attractiveness are probably due to variability in rates of production of carbon dioxide. At rates between 0.0001 and 0.038 liter s^{-1}, activation, orientation, and probing were dependent on concentration, but attraction was not (Alzogaray and Carlson 2000). Cilek (1999) collected 25 times more flies when Alsynite® cylinder traps were baited with dry ice. He also showed a six-fold increase in catches when traps were baited with a 4:1:8 mixture of octenol, prophylphenol, and methylphenol. In an earlier study, there was no increase in collections when octenol was released at a rate of 0.6 mg/hr, but this may have been a result of traps being placed too close to cattle (Mullens et al. 1995). Mihok et al. (1995) found that 1-octen-3-ol released at 0.2-2.0 mg/hr increased catches of *Stomoxys* spp. up to 3.7 fold in Vavoua traps, but only when used by itself. In contrast to what Cilek (1999) found, octenol was ineffective when used in conjunction with phenols or urine.

Medical/Veterinary Importance

Adult stable flies are opportunistic and attack most terrestrial mammals. Cattle are the preferred host, but dogs, humans, and other mammals are often attacked in the absence of cattle. Attacks on the ears of dogs can be so numerous that stable flies are sometimes referred to as 'dog flies' in the Florida panhandle. Stable flies will also occasionally feed on birds. In Nigeria, *Stomoxys niger* and *Stomoxys calcitrans* frequently fed from the combs of Rhode Island Red cockerels (Golding 1946). During an outbreak of West Nile virus, stable flies were also shown to feed on American white pelicans in a colony located in northeast Montana (Johnson et al. 2010a). These reports, though, may be a reflection of the opportunistic nature of the adults rather than feeding preferences. Anderson and Tempelis (1970) identified the hosts of 158 engorged stable flies collected on poultry ranches and found that 155 had fed on cattle, two on horses, and one on a dog. Most research on stable fly host interactions has focused on the responses of cattle since they are the primary host with measurable economic value. In several studies, peak leg counts on cattle range between approximately 3 to 25 flies per leg (Lysyk 1993a, Thomas et al. 1989, Mullens and Meyer 1987). An economic threshold of two flies per leg has been estimated for confined cattle in feedlots (Campbell et al. 1987) and three flies per leg in pastured calves (Campbell et al. 2001).

In Zimbabwe, most *Stomoxys* spp. were observed to leave cattle before feeding to repletion (approximately 83%) (Schofield and Torr 2002). Only 24% of these flies responded to host defensive behavior but 44% were disturbed by other flies. The authors made an interesting comparison of this relatively risky behavior to that of tsetse flies,

which were more responsive to host defensive behaviors. Stable flies have a shorter life span and their oviposition substrates are mostly ephemeral (in contrast to the larviparous tsetse fly), therefore they will take greater risks to obtain a blood meal (Schofield and Torr 2002).

Stable flies are aggressive, painful biters that will even drive cattle into any body of water so that the legs, which are the part of the animal within the horizontal flight range of the stable fly, are protected. In an open field environment, stable fly attacks can be so persistent that cattle will eventually become habituated to pain (Mullens et al. 2006). Cow defensive behaviors, defined as head throws, stamps, skin twitches, and tail flicks, decreased as stable fly abundance increased. Moorhouse (1972) postulated that restriction of stable flies to the lower extremities might be due to this defensive behavior. He notes that the head toss keeps the neck free of flies while tail swishing keeps them from the rear. But, stamping will not necessarily dislodge flies from the knees, and if it does, the fly will return to the same feeding site. Cutaneous lesions formed during intense stable fly attacks may allow for secondary infection to ensue (Moorhouse 1972).

Stable flies have not been implicated as a primary, biological vector of any organism, but they have been shown to serve occasionally as mechanical vectors. The tendency of stable flies to feed on multiple hosts before repletion is probably the major factor allowing this to occur. *Stomoxys calcitrans* failed to mechanically transmit lumpy skin disease, a viral disease of cattle, and Potomac horse fever, but have been shown capable of mechanically transmitting bovine leukemia virus, a retrovirus that infects the B-lymphocytes of cattle (Buxton et al. 1985, Burg et al. 1994, Chihota et al. 2003). The

volume of blood and viral titers that stable fly proboscis can hold indicates that transmission of bovine leukemia virus is probably limited to occurrences when stable fly populations are elevated (Weber et al. 1988). Stable flies have been shown to transmit African swine fever virus, capripox virus, Rift Valley fever virus, and possibly West Nile virus, as well as anthrax, and vesicular stomatitis (Ferris et al. 1955, Potgieter et al. 1981, Mellor et al. 1987, Turrell and Knudson 1987, Johnson et al. 2010b, Turrell et al. 2010). Mramba et al. (2007) demonstrated that *Enterobacter sakazakii*, a gram-negative bacillus that causes meningitis, enterocolitis, and sepsis, supported stable fly larval development and was maintained and colonized in the guts of the flies through pupation and the emergence of adults.

Control

Chemical

Chemical control of stable flies has so far been largely ineffective, but current research has offered some new possibilities. The most efficient use of chemical control is for elimination of larval development sites. Even when adults emerge at sites that include mates, hosts, and oviposition substrate, they disperse. Insecticide-impregnated ear tags on the primary host, cattle, are of limited use due to both the preferred feeding site on the host, the amount of time spent on the host, and natural levels of insecticide resistance. Stable flies tend to feed on the lower extremities of cattle which is the area that receives the least amount of insecticide. Additionally, unlike horn flies which spend the majority of their lives on the host, leaving only to deposit eggs and when dead, stable

flies may require only a few minutes a day to obtain a blood meal and disperse to resting locations afterwards. Exposure, therefore, to the amount of insecticide that does make it to the lower extremities of the host is relatively brief.

Because stable flies cannot be effectively managed by application of pesticides to cattle, an alternative currently being pursued is to use pesticide-treated resting sites. Hogsette et al. (2008) evaluated four types of blue fabric impregnated with various concentrations of cypermethrin, permethrin, ζ-cypermethrin, or λ-cyhalothrin. Treated fabrics were placed inside buildings and outside to assess effects of weather. Insecticide-impregnated blue cloth was chosen because in Africa, tsetse flies were attracted to blue/black cloth targets (Vale 1993) impregnated with deltamethrin, which remained active in the field for more than four months (Torr 1992). Because stable flies have previously been shown to land on blue/black fabric and remain on it for at least 30 s (Foil and Younger 2006, Mihok 2002, Mihok et al. 1995), the authors modified this method of control to target *S. calcitrans*. Results indicated that trigger fabric impregnated with either ζ-cypermethrin or λ-cyhalothrin at 0.1% showed potential (Hogsette et al. 2008). Another method currently being pursued is a push-pull method to repel stable flies from confined cattle and to attract them to alternate sites (Zhu et al. 2009). Catnip oil was found to substantially deter feeding, but the future of this repellent in stable fly control is still uncertain because of side effects when applied topically and the short half-life of the compound.

Chemical control of larvae has centered around treatment of development sites. But, because development sites can be ephemeral, targeting larvae is a challenge. Meyer

and Petersen (1983) suggested that larval sites in eastern Nebraska that are inaccessible for mechanical control, such as drainage ditches, should be targeted early in the season with either chemical larvicides or growth regulators. Lysyk (1993b) also recommends mechanical reduction of sources, but suggests using residual insecticides in late July and early August in Alberta, or before populations sizes peak. In the 1950's, 0.5% DDT emulsion was sprayed every two weeks over decaying vegetation along New Jersey Bay Shores and was estimated to reduce adult populations by 90-95% (Hansens 1951). Chamberlain and Matter (1986) found that a calcium cyanamide fertlizer applied over larval sites resulted in effective stable fly control.

Intensive use of pyrethroids for control of other muscids commonly associated with stable flies, e.g. *Haematobia irritans* (L.) (horn flies) and *Musca domestica* L. (house flies), has contributed to permethrin resistance in some stable fly populations (Pitzer et al. 2010, Olafson et al. 2011). Pitzer et al. (2010) found that under heavy selection pressure, a colony of stable flies that had not been exposed to pesticides for 30 yr were capable of developing five-fold resistance within five generations. Mullens et al. (1995) tested 0.03% λ-cyhalothrin and 0.1% cyfluthrin as an insecticide impregnated on bright blue cloth and failed to see even a knockdown effect in central and southern California populations. Because of known levels of pyrethroid resistance in horn fly populations, Hogsette and Ruff (1986) recommended using insecticide-impregnated ear tags and ear tape for horn and stable fly control only in areas that did not have heavy insecticide use.

Biological

Most research in biological control has focused around hymenopteran parasitoids, but has also included nematodes, fungi, and bacteria. Simmons and Dove (1941) observed ants, one species being the fire ant (*Solenopsis geminata* (F.)), carrying away the eggs of stable flies and even feeding on them. They also reported seeing a predaceous wasp, *Vespula squamosa* (Drury), *Stictia carolina* (F.), and a predaceous bee, *Xylocopa micans* (Lepeletier) capture or destroy eggs or adults. Petersen (1989) reviewed several studies that demonstrate the efficacy of nematodes when applied in high concentrations to larvae in Petri plates. But once applied to the field, real atmospheric conditions seem to negatively impact nematode survival and efficacy and render them useless. Stable fly eggs are also susceptible to the entomopathogenic fungus *Metarhizium anisopliae* (Metchnikoff) Sorokin, but the larvae and pupae are not (Moraes et al. 2008). The potential of *Serratia marcescens*, a facultative pathogen, to be useful as a biological control agent in the field was not favorable because of low mortality rates in the lab (Watson and Peterson 1981).

Pteromalid wasps are indigenous pupal parasites of both stable flies and house flies. As Petersen reports (1989), measuring natural incidence in the field may be difficult and populations appear to vary throughout the season. In northwest Florida, stable fly and house fly puparia were collected weekly November through April to estimate the incidence of parasitism in open-barn dairies (Greene et al. 1989). In agreement with work done in Nebraska by Petersen and Meyer (1983), Greene et al. (1989) found that the rate of parasitism decreased as the winter progressed. During this

study, an average of 36% of stable fly pupae (3,403 collected) and 24% of house fly pupae (1,545 collected) were parasitized. *Spalangia cameroni* Perkins accounted for 76% of the parasites that attacked stable fly pupae. Other parasites included *Spalangia nigroaenea* Curtis, *Spalangia endius* Walker, and *Muscidifurax* spp. Significantly higher parasitism (61%) occurred in silage when compared to hay and manure. Rates of parasitism in various habitats might reflect the longevity of those habitats for larval development, with older sites having higher rates of parasitism. Because the species composition of the pteromalid population at the dairy differed from reports from a swine facility, the authors suggest that if pteromalids are considered an option for biological control, it might be prudent to first determine which species are capable of surviving in the specific habitat to be targeted. Other field-based pteromalid assessments have supported this recommendation (Petersen and Pawson 1988, Jones and Weinzierl 1997, and Geden and Moon 2009).

Mechanical

Cleaning potential sites of larval development is probably the most effective form of stable fly control. Skoda and Thomas (1993) stated that in agricultural settings, "...sanitation is the primary factor in reducing fly breeding sources." Three types of management were evaluated in eastern Nebraska for larval and adult population dynamics in beef feedlots: minimum (cleaned once or less annually), intermediate (cleaned once annually with insecticides applied occasionally), and intense (cleaned as needed and insecticides used on a regular schedule). Intensity of management negatively correlated with degree of adult stable fly attack. These findings were in agreement with

what Burg et al. (1991) found on horse farms in Kentucky. Campbell and McNeal (1979) and Meyer and Petersen (1983) suggested that on large feedlots in eastern Nebraska, material under fence lines, which was one of the primary development sites identified, should be pushed into the open of the pen to allow for trampling by cattle. Open silage, one of the most consistent and prolific breeding sites in eastern Nebraska (Scholl et al. 1981) could be managed by placing plastic covers or tarps over the top. In Alberta, mechanical reduction of manure mounds, general lots, indoors, and along fences is recommended before July (Lysyk 1993b). Adequate drainage and protection from rainfall near silage mounds is also suggested.

STABLE FLY (*STOMOXYS CALCITRANS* (L.)) DISTRIBUTION AND PHYSIOLOGY ON A LENTIC WETLAND IN NORTHEAST MONTANA

Abstract

The stable fly, *Stomoxys calcitrans* (L.), is a cosmopolitan species of blood-feeding Muscidae and an important pest of confined and pastured cattle. Although cattle represents the largest agricultural commodity in Montana, no published record exists in this area detailing stable fly populations. Coroplast® cards used to monitor populations in three microhabitats (peninsula, pasture, and cattle pens) in northeast Montana 2008-2010 indicated that temporal dynamics differed by site and that local dispersal occurred throughout the season. Temperature was the most influential weather variable correlated with weekly changes in population abundance. White, white/black, multi colored, and black beach balls coated with Tangle trap® were also used to collect stable flies in 2009. White beach balls collected the most stable flies. All colors were male biased (2 males per female) and tended to collect young females (ovarian stage one). The average number of stable flies attacking pastured cattle ranged from 0.5-6.5 per leg. Also discussed are implications for overwintering and long-distance migration.

Keywords: Muscidae, Coroplast® cards, dispersal, overwinter, migration

Introduction

The stable fly is a hematophagous muscid whose impact on the cattle industry has been well documented (Bishopp 1913, Campbell et al. 2001, Mullens et al. 2006). Despite intensive efforts, aspects of the behavior and ecology of stable flies remain

ambiguous, probably because of the opportunistic nature of this cosmopolitan species. For instance, discrepancies have been documented in methods of dispersal, sites of larval development, as well as the effects of weather variables on population growth. Although stable flies have been shown to migrate up to 225 km in north Florida (Hogsette and Ruff 1985), local dispersion has been described as the primary method of movement in Nebraska and Ontario (Taylor et al. 2007, Beresford and Sutcliffe 2009). Suitability of sites for larval development is dependent on availability of moisture and, as such, is ephemeral. As a result, sites of larval development may vary widely according to both geographical location and microhabitats within smaller areas (Rasmussen and Campbell 1981, Lysyk 1993a). Population peaks of stable flies in Alberta during late August and mid-September have been attributed mainly to temperature (Lysyk 1993b). Alternatively, Broce et al. (2005) and Mullens and Petersen (2005) presented evidence to suggest that precipitation is the primary factor associated with population peaks typically seen between April and June in Kansas and central and southern California.

In Montana, the cattle industry accounts for about half of the state's yearly income from agricultural commodities, and in 2009 revenue approached $1.1 billion from cattle, calves, and dairy. The total monetary worth of the approximately 2.55 million head of cattle and calves in Montana in 2010 was estimated to be $2.4 billion. (USDA National Agriculture Statistic Service).

Even though there is a high potential for severe economic losses from stable fly attacks, the population dynamics of this species have not been described in Montana. The objective of this study was to monitor adult stable fly populations in northeast Montana

to determine their seasonal abundance and physiology at three microhabitats. Effects of weather on population growth and implications of dispersal were also investigated.

Materials and Methods

Site Description

Medicine Lake National Wildlife Refuge (MLNWR), located in the prairie pothole region of northeast Montana, (48º 27'N, 104º23'W, elevation 597.1 m) is a wetland formed by glacial recession. Established in 1935 in Sheridan Co. as a waterfowl refuge, MLNWR comprises 12,727 ha, including Medicine Lake (3,320 ha) and Homestead Lake (518 ha), and serves as a stopover and nesting grounds for approximately 125 species of migratory waterfowl and shorebirds. The surrounding area is composed of rural towns with populations less than 2,000 and is used primarily for small grain, hay, and cattle production. In an effort to control invasive weeds and introduced grass species, refuge personnel have initiated an aggressive weed management program including burning and cattle grazing in confined areas of the refuge. Given the cattle grazing and close proximity of confined cattle to the lake, MLNWR provides an opportunity to study stable fly biology with respect to different habitat types. Adult stable flies were therefore collected at three different sites representing three habitat types (Figure 1.1).

Site 1, a peninsula extending into Medicine Lake, is the primary roosting location of approximately 10,000 American white pelicans (*Pelecanus erythrorhynchos* Gmelin). Access to the peninsula is restricted by a predator exclosure fence. Three sections of

wind breaks formed by green ash (*Fraxinus pennsylvanica* Marsh), chokecherry (*Prunus virginiana* L.), willow (*Salix* spp.), and elm (*Ulmus* spp.) are surrounded by a mixture of grasses, shrubs, and forbs including crested wheatgrass (*Agropyron cristatum* (L.) Gaertn.), quack grass (*Agropyron repens* (L.)), ragweed (*Ambrosia* spp.), fringed sage (*Artemisia frigida* Willd.), showy milkweed (*Asclepias speciosa* Torr.), smooth brome (*Bromus inermis* Leyss.), prairie sandreed (*Calamovilfa longifolia* (Hook) Scribn.), Canada thistle (*Cirsium arvense* (L.) Scop.), Canada wild rye (*Elymus canadensis* L.), wild licorice (*Glycyrrhiza lepidota* Pursh), Maximilian sunflower *(Helianthus maximilianii* Schrad), hairy golden aster (*Heterotheca villosa* (Pursh) Nutt), foxtail barley (*Hordeum jubatum* L.), dotted blazing star(*Liatris punctata* Hook), reed canary grass (*Phalarus arundinacea* L.), Kentucky bluegrass (*Poa pratensis* L.), curly dock (*Rumex crispus* L.), Russian thistle (*Salsola tragus* (L.)), Missouri goldenrod (*Solidago missouriensis* (Nutt.)), prairie cordgrass (*Spartina pectinata* Bosc ex Link), and snowberry (*Symphoricarpos albus* (L.) Blake).

Site 2 had two grazing areas cattle, either to the south side of the lake, in the area leading directly up to the predator exclosure fence, or north of the lake. In 2008, 230 cow/calf pairs grazed in 585 ha on the south portion of Medicine Lake. In 2009, 140 cow/calf pairs and 148 yearlings also grazed in 303 ha along the south shore between 18 May and 1 July. These animals were later moved to 463 ha on or near site one between 1 July and 28 October.

The refuge straddles two potential native vegetation associations which differ on the north and south shores of Medicine Lake (Ross and Harold 1976). The silty-loam

north shore soil is predominantly a Mollisol soil, an order in the USDA soil taxonomy defined as typical of grassland ecosystems and including wind-blown sand. Climax vegetation is characterized by the cool season grasses thickspike wheatgrass (*Agropyron dasystachyum* (Hook.) Scribn.), needle-and-thread (*Hesperostipa comata* (Trin. & Rupr.) Barkworth), and green needlegrass (*Nassella viridula* (Trin.) Barkworth). Silty soils on the south shore are composed of an Alfisol, which is a clay-enriched subsoil that gets part of its name from the inclusion of aluminum and iron. This soil supports sand bluestem (*Andropogon hallii* Hack.), prairie sandreed, needle-and-thread, and Indian ricegrass (*Achnatherum hymenoides* (Roemer & J.A. Shultes) Barkworth).

In early-season grazing, cattle probably consume young crested wheatgrass that is still soft and palatable. As crested wheatgrass hardens around June, cattle shift to green needlegrass, little bluestem, and eat thread-and-needle last. By August, prairie sandreed , needle-and-thread, Kentucky bluegrass, and little bluestem (*Schizachyrium scoparium* (Michx.) Nash) are probably the main grasses being consumed (C. Marlow, personal communication, Taylor and Lacey 1994). Thus during my study, cattle were rotated to different summer pastures as determined by forage availability. Nevertheless, cattle were always grazing less than two km from the peninsula.

Site 3 was located southeast of the lake, on a small farm that had pastured and confined cattle. Several confinement lots of variable sizes were located on the property, but one, measuring 43.5 x 60 m, contained a round hay bale and sanitation was minimally managed. Most of the enclosed area had a hay-manure-soil mixture at least 61 cm deep.

Cattle were infrequently held in open-environment lots throughout the season, and were usually held there only for a few days before shipment for processing.

Immature Populations

Sites 1-3 were macroscopically examined for media containing muscid larvae or pupae with a trowel one to two hours per week in 2008 and 2009. In 2010, a larger confinement lot approximately 30 km northwest of the refuge was identified as another potential site for immature stable fly development and was sampled between one to five hours per week.

Materials potentially containing immature stable flies were collected and further processed in the laboratory. Within one to two days of collection, pupae were removed either by floatation or by hand. Posterior spiracles were used for identification of stable flies (Greenberg 1971). *Stomoxys calcitrans* pupae were pooled in small cups and placed into screened cages fitted with tubular stockinet sleeves. Emerging adults were aspirated, euthanized, and species identification was confirmed. After removal of visible pupae, remaining materials were placed into plastic containers of various sizes with white cloth mesh secured over the top. If flies emerged, they were immobilized at -17°C for five to ten minutes (depending on size of container and amount of material), then removed and euthanized by freezing. Material was classified either as containing stable flies or not (binomial model).

Adult Stable Flies

Stable fly populations were monitored weekly in July and August and once or twice per month during April-June and September-October in 2008-2010 with white 20.3 by 30.5 cm Coroplast® cards (Coroplast, Great Pacific Interprises Inc., Granby, Quebec, Canada and Dallas, TX) coated with Tangle trap® adhesive (Contech Enterprises Inc., Victoria, British Columbia, Canada). Cards were stapled to wooden stakes and placed 20 cm above underlying vegetation. Because vegetation grew taller as the season progressed, the distance from the ground to the bottom of the cards varied by week.

At Site 1, cards were placed along the length of the peninsula (15 cards in 2008 and 16 cards in 2009) and spanned a distance of approximately 0.6 km. At Site 2 cards were placed along four transects in the pasture with varying distances from grazing cattle (16 cards in 2008 and 2009). Each transect included four cards that were placed perpendicular to Medicine Lake and separated from each other by 4.5 to 30 m. At Site 3, cards were placed along the fence perimeter of a confinement lot (six cards in 2008 and 2009). In 2010, adults were monitored at Site 1on the peninsula (15 cards) and at Site 2 along one transect on the north shore (5 cards) and one transect on the south shore (5 cards). Because access to site 3 was restricted in 2010, it was excluded from the study.

After exposure in the field for approximately 24 h, cards were removed, placed into bins to keep them from sticking together, transported to Montana State University, and placed in a refrigerator (3.1°C) until processing. Flies were removed from the cards using paint thinner, washed in ethanol, then sorted according to site, collection date, and

sex. Stable flies were counted and stored for up to 8 months in labeled vials containing 70% ethanol until physiological age grading could be accomplished.

Physiological Age Grading

Physiological age of females removed from Coroplast® cards was determined by observation of the ovaries and placing each fly into one of six stages of ovarian development using the following characters (Scholl 1980); Stage 0 representing females that have never laid eggs (just emerged) or just laid eggs; Stage 1 having follicles that have separated from the germarium; Stage 2 having follicles that begin to elongate and visibly contain yolk; Stage 3 having follicles with the yolk occupying approximately 3/4 of the egg; Stage 4 follicles have eggs that are almost full length and are have nurse cells occupying less than 1/4 of their volume; Stage 5 eggs have a hardened chorion and a hatching pleat, representing the last stages of ovarian development just prior to oviposition. Because flies were stored in 70% ethanol for up to eight months before processing, insemination status and parity were not determined (sperm rapidly degrade in the spermatheca after death, even when stored in ethanol).

In stable flies, maturation of eggs is staggered so when the first egg completely matures (Stage 5), a second one is also developing and will reach stage 2 or 3 as the first egg is oviposited. Thus, stable flies classified as stage 0 or 1 are newly emerged flies and stable flies classified as stage 2 or 3 could be nulliparious, biparious or multiparious.

After age grading was completed, the mean ovarian stage of groups of females, or score, was calculated by $\Sigma \text{ovarian stage}_{\text{individual}}/N$ where N is the total number of females

dissected per group. Mean sex rations (± SE) of groups were also calculated and signficant differences in sex ratio by site were determined by Z-tests.

Beach Balls

Stable flies were previously observed feeding on American white pelicans on the peninsula (Johnson et al. 2010a), and it was hypothesized that in addition to olfactory cues, the shape or color of an object might attract stable flies. To test this hypothesis, glossy panel beach balls (51 cm deflated diameter, Intex Recreation, Long Beach, California) were sprayed with glossy enamel spray paint 24 h before deployment into the field. Inflated balls were painted all black, all white, half black and half white, or with a clear coat. Balls that were painted clear had blue, red, and yellow panel, each separated by a white panel. Beach balls were hung on inverted L poles approximately 20 cm above underlying vegetation and 12 m from each other. Balls were wrapped with a single layer of plastic saran wrap (The Glad Products Company, Oakland, CA) on which Tangle Trap® was then painted . After 24 h, stable flies were collected from individual beach balls and placed directly into vials of 70% ethanol and the plastic wrap was immediately removed and discarded.

Because of the proximity of the balls to the northern pelican creche, a 4x4 Latin square design was used to determine and correct for any potential bias of the balls that were nearest the creche. The design consisted of four replicates of each color treatment with a longitudinal and a latitudinal gradient towards the pelican colony. Stable flies were collected on these modified beach balls every other week between 8 July and 26 Aug.

Leg Counts

To determine if a relationship exists between Coroplast® card collections and biting activity in the field, weekly point counts of adult stable flies were taken from both front legs of 7-25 cows in 2008, 2009, and 2010 while cards were deployed in the field. All observations were made by the same individual (KMH) between 11 am and 1 pm using binoculars.

Pelican Surveillance

So that inferences could be made concerning stable fly-pelican interactions, the pelican colony on Bridgerman Point was monitored 3-4 days/week between April and Oct in 2009, May and Oct in 2009, and June and August in 2010. In 2009 and 2010, pelicans began roosting on Big Island, approximately 1 km from the tip of the peninsula, and were also counted. In 2008, nests were counted 27 May and chicks were banded 7 July. In 2009 and 2010, nests were counted 28 May and 15 June, respectively, but due to resources, chicks were not banded. As WNV became apparent in the colony, juveniles were euthanized and tissues were collected for WNV processing.

Weather

Daily mean temperature, minimum temperature, maximum temperature, and precipitation were recorded by a weather station located next to refuge headquarters and submitted to the National Climatic Data Center at www.ncdc.noaa.gov. Mean temperatures were calculated as the [(maximum - minimum) / 2]. Based on lab-derived larval? developmental data, degree-day accumulations were calculated using a threshold

of 10°C (Lysyk 1993a). So that comparisons could be made with other reports, degree-days were calculated beginning 1 January.

Statistical Analysis

Adult fly data were ln(y+1)-transformed before being subjected to analysis of variance and (post hoc test?) to determine if significant differences exist in stable fly abundance among and within transects, between microhabitats, and between colors on the beach ball traps. Back transformed values are shown in text and tables. Weekly changes in abundance were calculated as $(N_{t+1} - N_t)/N_t$ where N_t is the mean abundance of the previous week and N_{t+1} is the mean abundance of the current week. After the weekly change in abundance values were normalized by ln(y+1)-transformation, stepwise multiple regression was used to determine if weekly average temperature, precipitation, or degree-day accumulations accounted for the variation in abundance. Akaike's information criterion was used for model selection. Z-scores were calculated to test the hypothesis that males and females were collected in equal proportion on cards in 2008 and 2009 and on each color type of beach ball in 2010. All statistics were performed using R© software (version 2.12).

Results

Sampling for the presence or absence of immature stable flies revealed the lack of appropriate developmental conditions on the peninsula (Site 1) and in the pasture (Site 2). Decaying, moist vegetation was never identified in the pasture or on the peninsula. Algal blooms were washed ashore typically in late August, but immature muscids were not

recovered from the subsequent accumulated aquatic vegetation. The confinement lots (Site 3), however, did support stable fly development throughout most of July in 2008. Larvae and pupae were primarily recovered near the round hay bale used to feed cattle. As precipitation decreased in August, larval abundance appeared to decrease and few immature stable flies were collected for the remainder of the season. In 2009, very few immature stable flies were collected from the confinement lots. Immature stable flies were present throughout the season in 2010 at the larger feedlot and up to 500 stable fly pupae were collected between mid-July and mid-August.

Activity of adult stable flies at the MLNWR this location in varied by year depending on weather conditions, but generally appeared to begin between mid-May and late June (Figure 1.2). Accumulation of degree-days until the first capture varied substantially between years. In 2008, stable flies were monitored with a total of 484 cards on 14 dates between 13 April and 24 October. During this year, the first stable fly was collected on the peninsula after 70 DD_{10} on 16 May. A total of 565 cards was deployed on 16 dates between 16 May and 26 September in 2009. Stable flies were first detected on both the peninsula and the pasture in 2009 on 25 June, or after 491 DD_{10}. In 2010, 237 DD_{10} accumulated before the initial fly capture on 16 June. A total of 164 cards was deployed on nine dates between 16 June and 25 August.

Stepwise multiple regression showed that for each year analyzed, the rate of weekly change was explained by weekly average temperature and precipitation (Table 1.1). In 2008 and 2010, rates of change were also explained by accumulated degree days. These regressions moderately predicted seasonal abundance (Figures 1.3-1.5). In 2008,

one peak occurred at 475 degree-days above 10°C (Figure 1.3). Two peaks in 2009 were identified 768 and 1020 degree days (Figure 1.4) and with a degree-day interval of 252. In 2010, two peaks occurred at 647 and 824 degree-days with an interval of 177 (Figure 1.5).

Temporal variation in abundance differed at each of the three microhabitats in 2008 ($F = 7.4$; df = 16, 191; $P < 0.001$) and 2009 ($F = 17.7$; df = 14, 270; $P < 0.001$). In 2008, stable flies were monitored at various distances from grazing cattle from 10 June to 24 October. Drought conditions prohibited cattle from grazing in designated treatment areas on the refuge as originally scheduled. When moisture is adequate, vegetation may be abundant enough to support grazing. But, in this area, irrigation is not widely used and decreased precipitation negatively impacts vegetative growth. As a result, the cattle spent most of the trapping season (3 July – 14 August) closest to transect #4 or at some distance from the transects (26 August – 24 October). Analysis was restricted to data obtained between 3 July and 14 August and comprised six collections. While no difference in abundance was detected within transects ($F = 0.01$; df = 1, 84; $P = 0.92$), a significant difference was detected among transects ($F = 5.2$; df = 3, 84; $P = 0.002$). In pasture, stable flies were most abundant near the grazing cattle and gradually decreased with distance away from the herd (Figure 1.6). Because of the difference among transects, data from transect #4 was used to compare stable fly abundance in the three microhabitats. A range of 3-487 flies and a mean (± SE) of 50 ± 8 were collected on cards in the pasture with a peak occurring 25 July. The population returned to a relatively lower level of adult activity by 26 August and the last fly was collected 2

October. On the peninsula, a range of 10-185 and a mean of 83 ± 9 flies was collected per card while adults were active. Although the first fly was collected 16 May, none was collected on 11 June. The population began to increase 25 June with the peak average of 185 flies per card occurring 15 July. Fluctuations in abundance were detected through 26 August, after which the population declined. The last stable fly collected on the peninsula was on 2 October. The number of adults collected on cards placed near the confinement lots remained relatively low for most of the season with the exception of the last two weeks in August when the peak averaged 103 ± 32 and 169 ± 49 flies per card. The seasonal mean collected was 41 ± 10.

Stable fly populations were markedly lower in 2009 and 2010 compared to 2008 (Table 1.2). In 2009, cattle were allowed to graze near the peninsula but were absent from transects investigated in 2008. Because no significant difference was detected either between (F = 01.2; df = 3, 17; P = 0.35) or within transects (F = 0.5; df = 3, 17; P = 0.70), all data collected from the pasture were used for location comparisons. In both the pasture and the lots, the average number of stable flies collected per card ranged between 1-4 with an overall mean of 2 ± 1. Abundance was higher on the peninsula (F = 159.6; df = 3, 270; $P < 0.001$) with a mean of 20 ± 2 and an average range between 1-73 flies per card with the peak occurring 25 August. In 2010, the average number of flies collected on the peninsula ranged from 1-75. A peak in the population was observed 11 August. Populations in the pastures investigated were also low and ranged between 1-27 in site 2 with a mean of 10 ± 3.

Stage of ovarian development was determined for 926 and 261 females collected on the white Coroplast® cards in 2008 and 2009, respectively (Figure 1.7). In 2008, ovarian development was initially advanced (older flies) with a mean of 3.1 ± 0.3 (SE) (Table 1.3). Afterwards, mean ovarian stage was 1.6 ± 0.2 (younger flies) until the end of the season. With the exception of the first week in July, the average ovarian stage of females collected near the lots was consistently lower compared to flies collected on the peninsula and in the pasture. In 2009, again, ovaries were initially advanced with a mean stage of 3.4 ± 0.3 between 26 June and 17 July (Table 1.4). Average ovarian stage decreased to a mean of 1.8 ± 0.2 at all microhabitats between 30 July and 26 September.

A total of 18,815 flies collected from cards in 2008 and 2009 was sexed. A difference in sex ratio between sites might have occurred initially in 2008. During the first two weeks, 5:1 (n = 251) and 13.2:1 (n = 142) male-female ratios were observed on the peninsula, contrasting with ratios of 1.3:1 (n = 42) and 1.3:1 (n = 254) observed on the pasture and 1.5:1 (n = 6) observed near the lots. After 3 July, this difference was no longer seen and the average sex ratio leveled to 2:1 for the rest of the season. In contrast, no discernable pattern emerged from the sex ratio of flies in 2009 (N = 3695) during which averages fluctuated between 0.3:1 and 5.7:1. In 2008 and 2009, seasonal collections of stable flies on Coroplast® cards were male-biased with an average of two males per female (2008: Z = 73.4, P < 0.001; 2009: Z = 32.6, P < 0.001).

Color of the beach balls used to trap stable flies in 2010 significantly explained variance (F = 9.3; df = 3, 41; P < 0.001) while individual trap location (F < 0.001; df = 1, 46; P = 0.98) and gradient (gradient 1: F = 0.75; df = 1, 41; P = 0.39, gradient 2: F =

0.07; df = 1, 41; $P = 0.80$) did not. Flies were more abundant on white balls than on white/black, multi colored, or black balls (Table 1.5). No difference in abundance was detected between the white/black and multi colored balls. Black balls collected the least number of flies. Stage of ovarian development was also similar on all colors investigated. The mean stage of ovarian development in 192 females dissected was 1.0 ± 0.1 (SE). Collections were male biased on the white ($Z = 7.3$, $P < 0.001$), white/black ($Z = 4.0$, $P < 0.001$), and multi-colored beach balls ($Z = 3.2$, $P = 0.001$), but not on the black beach balls ($Z = 0.7$, $P = 0.5$). A mean of 1.9 ± 0.3 males per female were collected on the white, white/black/and multi-colored balls.

Stable flies were counted on the two front legs of a total of 270 cattle in 2008-2010. Flies were first documented attacking cattle on 10 June in 2008 and on 3 July in 2009 and 9 July in 2010 (Table 1.6). In 2008, 175 cattle were observed and the highest level of stable fly attack was documented on 5 August. Close-range access to pastured cattle limited observations in 2009 and only 37 cattle were monitored. The peak attacking activity was documented on the last day of observations, 24 July. After this date, cattle moved beyond the range of the observer. A total of 58 cattle was observed in 2010. Mean attack rates of 4 ± 1 (SE) flies per front pair of legs was observed between 15 July and 5 August.

Each year between 2008 and 2010, approximately 1,500 pelican nests were counted on the peninsula. During all years, nests became active in mid-May and hatching began by the end of May or the first half of June. Pelicans were distributed among two main creches, one on the south shore and one on the north shore, with about 80% roosting

on the south shore. Pelicans were also found roosting on Big Island and in 2009 and 2010, 905 and 260 nests were counted, respectively. In 2008, three juveniles exhibiting ataxia or immobility were euthanized between 5 August and 15 August. A total of 105 juveniles succumbed between 23 July and 26 August in 2009, with 81 of these being on Big Island. In 2010, eight juveniles were euthanized between 22 July and 4 August on the peninsula. Pelicans departed their roosting site for the season by mid- to late-September.

Temperatures were close to historic average temperatures throughout 2008 (Figure 1.7). However, lower than average precipitation from January to August resulted in severe drought conditions for the area (Table 1.7). Medicine Lake experienced higher than normal precipitation from September to November. In 2009, mean monthly temperatures tended to be lower than those in 2008 with the exception of September. Precipitation was lower than average during May and June, but higher than average in July and August.

Discussion

Stable fly larvae are typically found in confined feedlots, dairies and pastures in accumulated and fermenting vegetation, soil, urine, and water (Meyer and Petersen 1983, Schmidtmann et al. 1988, Skoda et al. 1991, Lysyk 1993b, Broce et al. 2005), Similar conditions were present in the lots near MLNWR, but not on the peninsula or in the pasture. After a thorough visual examination of potential substrates at all three microhabitats, the pens with hay bales were likely to be the only location supporting stable fly development. It is possible that larval development sites on the peninsula or in

the pasture, were not identified, but environmental conditions make this unlikely. The peninsula and grazing area, composed of silty and sandy loam soils, rarely had any build up decaying vegetation or moisture.

During peak temperatures, green algal blooms in Medicine Lake were common once-a-year occurrences from elevated fertilizer use on surrounding farms. Following strong winds, algae would occasionally wash ashore and build up as deep as two feet, however larval development was never found in these substrates during these studies. Also known as the shore fly, *S. calcitrans* has been documented along the shores of lakes and marine beaches following inland weather fronts that push the flies out towards the shore (Voegtline et al. 1965, Hogsette and Ruff 1985). If the accumulation of aquatic grasses is deep enough, moisture is adequate, and fermentation is active, stable flies may use this substrate for oviposition (Simmons and Dove 1941, Hansens 1951, Pickard 1968). However during a 4-year study in coastal northwest Florida, Simmons and Dove (1941) found stable fly larvae in bay grasses on only one occasion.

In Kansas, sites where round hay bales were fed to pastured cattle during the winter were shown to support larval growth during the late winter and early spring because of the accumulation of spilled hay (Broce et al. 2005). At Medicine Lake, cattle are brought into the confinement lots during the winter and this is when hay feeding begins. The absence of developmental sites on the peninsula and grazing areas indicates that stable fly adults at those locations are dispersing from nearby developmental sites, such as those at the confinement lots. Additionally, cattle come back to the confinement

lots at least once during mid-day for water. Thus, local dispersal is constantly occurring throughout the season despite presence of oviposition sites and host and mate availability.

In 2006, Beresford and Sutcliffe (2006) published an extensive investigation that compared stable fly collections on Alsynite® to sticky Coroplast® cards of various colors. They found that Coroplast® cards caught more stable flies, likely a reflection of increased collections of males and nulliparous females. When the efficacy of white, grey, red, green, yellow, blue, and black colored cards were investigated, white cards collected the most stable flies. This is also true for Alsynite® (Ruff 1979). For these reasons, white Coroplast® cards were used to collect stable flies in the current investigation.

Throughout the study, distinct population trends were observed on the peninsula, in pasture, and near confinement lots, all of which were within just three km of each other. With the exception of one collection point, stable flies were more abundant in the pasture and on the peninsula than near the confinement lots. This is interesting because resting sites, hosts, mates, and oviposition substrate were plentiful in or adjacent to the lots. However, the flies need the cattle for blood meals, and stable flies tend to collect on peninsulas, even those without pelicans (Hogsette et al. 1987). This demonstrates that stable flies, unlike house flies, for example, cannot necessarily feed, mate and oviposit all at the same site. As might be expected, the importance of precipitation, temperature, and degree-day accumulation as explanatory variables in weekly stable fly populations varies by geographic location. Precipitation is thought to be the limiting factor of population change in Nebraska and Kansas, where temperatures are warm but rainfall is limited during the short summer fly season (Taylor et al. 2007). However, temperature and

degree-day accumulations are thought to explain variation Alberta where moisture levels might be right, but low temperatures limit flight activity (Lysyk 1993a). In central and southern California, population change was related to spring rainfall and degree-day accumulations (Mullens and Peterson 2005).

At MLNWR, population changes were explained by temperature, precipitation, and degree-day accumulation. A wide range of degree-day accumulations until first capture was observed during this study. In 2008, the first stable fly was collected when only 70 degree-days above 10°C had accumulated. This was in contrast to 2009 and 2010 when the first stable flies were collected after 492 and 237 cumulative degree-days, respectively. The earlier appearance and higher seasonal abundance of stable flies in 2008 when compared to 2009 and 2010, even though spring and summer temperatures between years were similar, suggests that local overwintering might have been successful in 2008 but not in 2009 and 2010. Stable flies overwinter as quiescent larvae and will resume development given appropriate exogenous conditions such as open silage storage systems or large piles of fermenting vegetation (Simmons and Dove 1941, Scholl et al. 1991). However because winter conditions were also similar between years and larvae were not sampled in the MLNWR during the winter months, factors affecting overwintering success are unknown and remain an area for future investigation.

If overwintering success does explain the reason for the disparity between cumulative degree-days on initial capture during the three study years, long-distance migration may have been responsible for this phenomenon. Synoptic weather systems passing through the MLNWR in early summer may bring flies from further south.

Additional research stable fly dispersal is needed in the Montana area to help define local stable fly behavior .

Additionally, ovarian age-grading indicated an initial population of older females that transitioned into mostly newly emerged females as the season progressed. This also indicates that stable flies are migrating into MLNWR, then establishing local populations over the summer. In Florida, at least one stable fly was found to have migrated 225 km (Hogsette and Ruff 1985). The definitions of long-distance migration and local dispersal is somewhat ambiguous and subjectively defined, but a larger confined feedlot, located approximately 30 km northwest of the refuge, brings livestock into a large, closed pen during the winter, and may serve as a potential overwintering site for stable flies.

Similar physiological status and sex ratios were documented in stable fly populations collected on both the white Coroplast® cards and the white beach balls, indicating that the color white attracts similar demographics. However, abundance on white beach balls was approximately 25% less per cm^2 than on white cards, which could suggest that shape alone did not account for stable fly attacks on the pelicans. Alternatively, the effects of wind might have caused the comparative reduction in adult collection. While the cards were stapled to posts, and generally were rigidly upright throughout the collecting period, the beach balls were hung from string and subject to freer movement during weather events. In this area, wind may often be sustained at 10-15 mph with gusts up to 60-80 mph. In fact, cards were sometimes blown off the wooden stakes despite being stapled several times. Stable flies tend to not land on swinging

objects (J Hogsette, personal communication), and the swaying movement of the balls in these kind of conditions may have deterred stable flies from landing.

Contrary to what Cilek (2002) observed in Florida, white balls collected the most stable flies when compared to white/black, multi colored, or black balls. This disparity might be a result of thermoregulation. When the balls were placed in the field, the average air temperature was 21°C. During this time, adults might preferred the cooler white surface as waiting stations or as shelter from prevailing winds (Buschman and Patterson 1981, Lysyk 1993b). Regardless, the sex ratios on all color schemes is in agreement with Cilek's (2002) observations.

Interestingly, the average sex ratio of more than 1,200 stable flies aspirated from sick juvenile pelicans was four males per female (Johnson et al. 2010a), a ratio that is more skewed than the M/F ratio found in this study. If both the cards and the beach balls on the peninsula collected approximately two males per female, it is possible that factors other than shape and color are attracting a different demographic of stable flies to the pelicans.

Leg counts revealed attack rates similar to reports from Nebraska (Campbell et al. 2001) and California (Mullens et al. 2006) with the exception of one day in early August, 2008 when counts were highest and averaged 6.5 flies per leg. Campbell et al. (1987) demonstrated that on confined cows in feedlots, point counts of two stable flies per leg was the economic threshold. In pastured yearling cattle in Nebraska, Campbell et al. (2001) observed a range of 1-6 flies per leg and found decreased weight gains of 0.2 kg per steer per day when 3 flies per leg were observed. Alternatively, when peak stable fly

attack ranged between 3.0-3.5 flies per leg on cows held in open dirt lots on a southern California dairy (Mullens et al. 2006), no significant decrease in milk production was detected. Because of the contrast in these published reports and because the effects of stable fly loads observed in this study were not measured, it is hard to draw definitive conclusions about the impact of the populations observed on the pastured cattle. During all three years, though, cattle were observed to bunch and wade in the lake during peak fly activity (personal observation). Although the economic threshold was met during all three years at this location, the threshold was recorded only once or twice throughout the season. Measurable economic impacts from stable fly attacks probably require a duration of more than one week of attack above the economic threshold. Hence, defensive behaviors in response to stable flies were probably not exhibited long enough for an economic impact to occur.

This is the first description of the composition and temporal dynamics of adult stable fly populations in Montana. Stable flies are opportunistic and vary behaviorally and physiologically at the microhabitat level. At this location, temperature, precipitation, and accumulated degree-days explained population changes. Local dispersion is constant throughout the season and may be occurring despite resource availability for survival and reproduction. Even during years of relatively lower abundance, stable fly attacks on grazing cattle approached economic thresholds. Although the duration of these attacks don't appear to be sustained, MLNWR offered an opportunity to study stable fly phenology in a unique location where cattle and stable fly development sites were less abundant compared to other studies (i.e., larger cattle herds and more consistent stable fly

development sites in confined facilities such as dairies and feedlots). Clearly, given the economic importance of the cattle industry in Montana, further research exploring seasonal abundance of stable flies is warranted for other regions of the state, especially near larger livestock facilities.

Table 1.1. Parameters for linear equations explaining weekly rate of stable fly population change with temperature as the explanatory variable. Standard errors of estimates are provided in parentheses.

Year	n	b_0	b_1	b_2	b_3	*F*	r^2
2008[a]	218	-11.67 (3.7)	0.029 (0.004)	0.07 (0.015)	0.94 (0.25)	12.2	0.13
2009[a]	72	-14.41 (0.91) (1.403)	---	0.032 (0.010)	0.76 (0.49)	164.6	0.82
2010[a]	105	-17.06 (4.35)	-0.07 (0.023)	0.015 (0.011)	1.09 (0.29)	13.8	0.27

[a] Linear regression for rate of change $= b_0 + b_1$*weekly degree-days above 10°C + b_2*weekly precipitation (mm) + b_3*weekly mean temperature (°C)

Table 1.2. The mean number of stable flies ± standard error collected on white Coroplast cards at Medicine Lake NWR, MT 2008-2010.

	Mean ± SE (n)				
Month	2008		2009		2010
May	0.06 ± 0.06	(18)	0 ± 0	(40)	
June	7.2 ± 2.2	(40)	0.12 ± 0.05	(57)	0.2 ± 0.13 (10)
July	58.5 ± 7.8	(161)	7 ± 1.9	(99)	22.4 ± 4.2 (39)
August	113.8 ± 11.4	(120)	32 ± 4.8	(62)	33.28 ± 5.41 (40)
September	9.3 ± 1.3	(41)	17.6 ± 3.0	(43)	
October	4.4 ±0.99	(80)			

Table 1.3. Stage of ovarian development and sex ratio of stable flies collected at Medicine Lake NWR, MT 2008. The score is the sum of all ovarian stages for the given date and location.

Date	Location	N	Score	Score/N	M	F	M/F
25-Jun	Peninsula	25	80	3.2	200	37	5.4:1
	Pasture	18	58	3.2	24	18	1.3:1
	Peninsula				5	2	2.5:1
	Overall	43	138	3.2	229	57	4.0:1
3-Jul	Peninsula	9	30	3.3	132	10	13.2:1
	Pasture	2	6	3	494	95	5.2:1
	Peninsula				25	13	1.9:1
	Overall	11	36	3.3	651	118	5.5:1
10-Jul	Peninsula	25	57	2.3	152	78	1.9:1
	Pasture	17	56	3.3	44	20	2.2:1
	Peninsula	7	26	3.7	14	8	1.8:1
	Overall	49	139	2.8	210	106	2.0:1
15-Jul	Peninsula	25	51	2	1123	642	1.7:1
	Pasture	25	67	2.7	154	100	1.5:1
	Peninsula	25	22	0.9	90	60	1.5:1
	Overall	75	140	1.9	1367	802	1.7:1
25-Jul	Peninsula	25	52	2.1	1689	504	3.4:1
	Pasture	25	37	1.5	1229	334	3.7:1
	Peninsula	25	21	0.8	128	59	2.2:1
	Overall	75	110	1.5	3046	897	3.4:1
5-Aug	Peninsula	25	38	1.5	1337	597	2.2:1
	Pasture	25	40	1.6	1374	640	2.0:1
	Peninsula	25	9	0.4	456	294	1.6:1
	Overall	75	87	1.2	3167	1531	2.1:1
14-Aug	Peninsula	25	48	1.9	1083	553	2.0:1
	Pasture	25	47	1.9	855	551	1.6:1
	Peninsula	25	12	0.5	229	97	2.4:1
	Overall	75	107	1.4	2167	1201	1.8:1
26-Aug	Peninsula	244	285	1.2	1067	498	2.1:1
	Pasture				50	34	1.5:1

Table 1.3. Continued.

	Peninsula				104	55	1.9:1
	Overall				1221	587	2.1:1
11-Sep	Peninsula	4	10	2.5	153	74	2.1:1
	Pasture				51	37	1.4:1
	Peninsula				23	26	0.9:1
	Overall				227	137	1.7:1
2-Oct	Peninsula	35	73	2.1	46	38	1.2:1
	Pasture	40	77	1.9	121	45	2.7:1
	Peninsula	17	18	1.1	70	28	2.5:1
	Overall	92	168	1.8	237	111	2.1:1
	TOTAL	1,238	2,145	1.7	25,044	11,094	2.3:1

Table 1.4. Stage of ovarian development and sex ratio of stable flies collected at Medicine Lake NWR, MT 2009. The score is the sum of all ovarian stages for the given date and location.

Date	Location	N	Score	Score/N	M	F	M/F
26-Jun	Peninsula	3	14	4.7	3	4	0.8:1
	Pasture	1	3	3	3	1	3.0:1
	Pen						
	Overall	4	17	4.3	6	5	1.2:1
2-Jul	Peninsula	9	25	2.8	20	9	2.2:1
	Pasture	1	3	3	2	2	1:1
	Pen				3	1	3.0:1
	Overall	10	28	2.8	25	12	2.1:1
7-Jul	Peninsula	24	73	3			
	Pasture	8	28	3.5			
	Pen						
	Overall	32	101	3.2			
17-Jul	Peninsula	23	51	2.2	23	38	0.6:1
	Pasture	11	40	3.6	21	24	0.9:1
	Pen	3	15	5	1	4	0.3:1
	Overall	37	106	2.9	45	66	0.4:1
23-Jul	Peninsula				15	6	0.7:1
	Pasture				15	8	0.7:1
	Pen				1	2	0.3:1
	Overall				31	16	0.7:1
30-Jul	Peninsula	25	57	2.3	366	173	2.1:1
	Pasture				4	0	
	Pen	12	27	2.3	29	13	2.2:1
	Overall	37	84	2.3	399	186	2.1:1
4-Aug	Peninsula	25	27	1.1	533	186	2.9:1
	Pasture	11	22	2	7	9	0.8:1
	Pen	19	17	0.9	56	19	2.9:1
	Overall	55	66	1.2	596	214	2.8:1
19-Aug	Peninsula	12	20	1.7	32	12	2.7:1
	Pasture	4	10	2.5	13	7	1.9:1
	Pen	6	6	1	19	8	2.4:1

Table 1.4. Continued.

	Overall	22	36	1.6	64	27	2.4:1
25-Aug	Peninsula	25	30	1.2	785	377	2.1:1
	Pasture	11	24	2.2	13	11	1.2:1
	Pen	2	2	1	8	2	4.0:1
	Overall	38	56	1.5	806	390	2.1:1
12-Sep	Peninsula	25	50	2	390	158	2.5:1
	Pasture	4	11	2.8	13	4	3.3:1
	Pen	3	6	2	17	3	5.7:1
	Overall	32	67	2.1	420	165	2.5:1
26-Sep	Peninsula	25	45	1.8	133	72	1.8:1
	Pasture	2	2	1	6	3	2.0:1
	Pen	2	5	2.5	2	2	1.0:1
	Overall	29	52	1.8	141	77	1.8:1
	TOTAL	592	1,226	2.1	5,021	2,250	2.2:1

Table 1.5. Mean ± standard error stable flies collected on white, white/black, multi colored, and black beach balls as well as the male to female ratio (M/F) and stage of ovarian development

Color	N	Mean ± se	M/F (N)	Stage of ovarian development (N)
White	723	59.75 ± 28.58	1.7:1 (723)	1.07 (81)
White/Black	161	13.42 ± 7.44	2.41:1 (106)	1.13 (31)
Multi colored	113	9.42 ± 4.61	1.2:1 (53)	0.91 (58)
Black	180	4.42 ± 4.65	1.59:1 (180)	1.04 (23)

Table 1.6. Mean ± standard error stable flies counted on both front legs of cows, 2008-2010. Flies were counted between 11 am and 1pm.

Date	2008			2009			2010		
	No. cattle	Range of stable flies	Mean no. stable flies ± SE	No. cattle	Range of stable flies	Mean no. stable flies ± SE	No. cattle	Range of stable flies	Mean no. stable flies ± SE
Jun 10	17	0 - 0	0 ± 0						
Jun 25	17	0 - 68	4.4 ± 4.0						
Jul 3	22	0 - 2	0.1 ± 0.1	12	0 - 5	1.3 ± 0.5			
Jul 9	19	0 - 7	1.9 ± 0.5	7	0 - 1	0.3 ± 0.2	13	0 - 6	1.5 ± 0.5
Jul 15	17	0 - 6	0.9 ± 3.6	8	0 - 1	0.3 ± 0.2	7	1 - 7	3.6 ± 0.9
Jul 24	24	0 - 14	3.3 ± 0.8	10	0 - 22	8.4 ± 2.4	13	0 - 17	4.3 ± 1.6
Aug 5	19	1 - 35	13.3 ± 2.2				25	0 - 22	4.4 ± 1.0
Aug 14	20	0 - 3	0.9 ± 0.2						
Aug 26	20	0 - 20	1.5 ± 1.0						

Table 1.7. Average temperature (°C) and precipitation (mm) at Medicine Lake NWR, MT 2007-2010.

	2007		2008		2009		2010	
Month	Temperature	Precipitation	Temperature	Precipitation	Temperature	Precipitation	Temperature	Precipitation
January			-14.2	0.508	-13.7	7.620	-11.5	0.760
February			-10.0	0.762	-11.9	2.290	-12.6	2.030
March			-1.3	10.160	-6.1	2.290	-0.1	2.540
April			5.3	7.112	5.2	75.690	7.8	23.110
May			12.2	28.450	11.8	12.700	10.6	107.950
June			16.3	54.100	16.6	31.500	17.9	65.790
July			21.7	48.000	18.9	133.100	20.1	71.370
August			21.2	30.000	18.6	48.500	19.5	83.570
September	13.8	15.49	13.1	46.000	17.6	23.370	12.7	20.320
October	7.1	21.34	6.4	25.400	3.1	35.810	8.0	28.700
November	-1.6	1.52	-0.4	38.600	1.2	0.510	-3.8	10.920
December	-9.9	0	-14.8	0.250	-14.7	5.330	-12.0	3.560

Figure 1.1. Map of Medicine Lake National Wildlife Refuge 2008 grazing areas with the peninsula (red circle), pasture (transects #1-4 identified as T #1-4 and green lines), and confinement lots (purple box). Adult stable flies were monitored in the same locations during 2008-2009. In 2010, adults were monitored on the peninsula and in pasture along the north and south shores (areas identified in boxes).

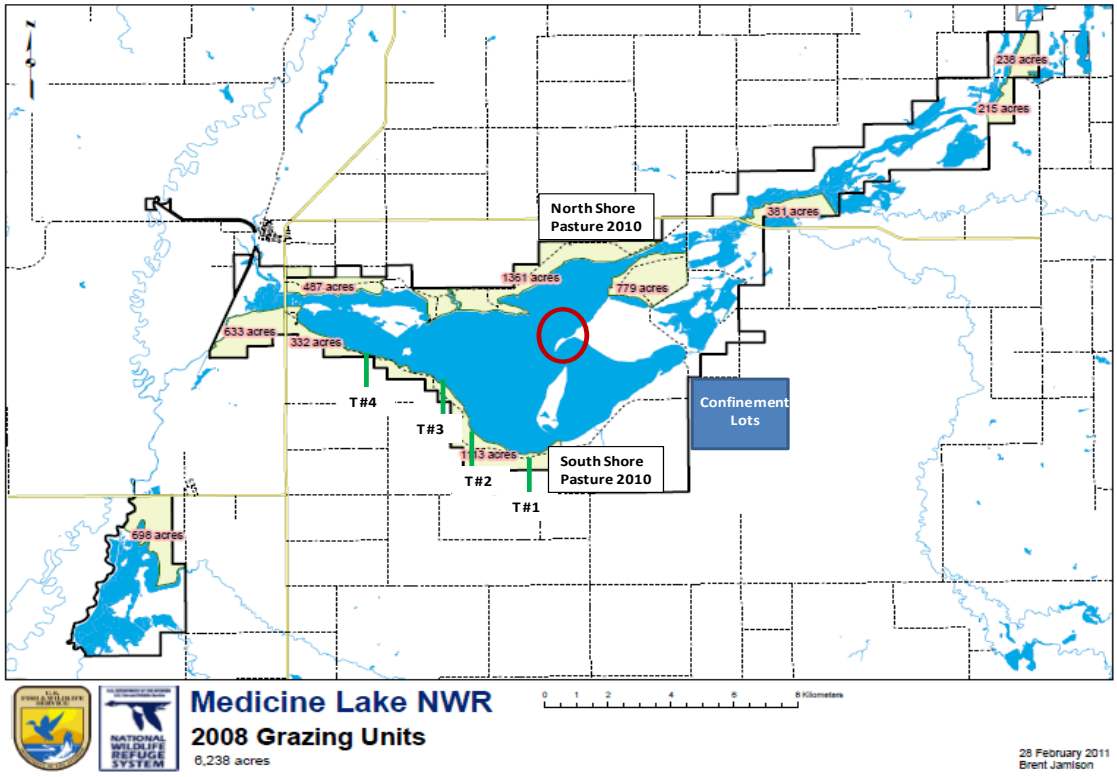


Figure 1.2. Temporal and spatial abundance of stable flies at and around Medicine Lake NWR, MT 2008-2010.

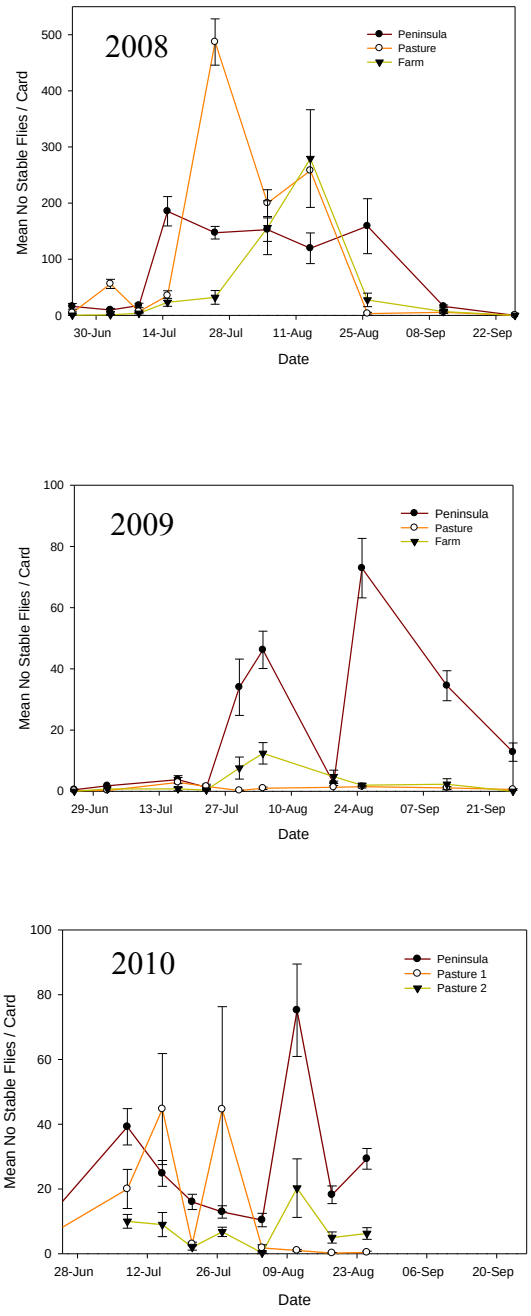


Figure 1.3. Mean weekly rate of change as degree-days above 10°C accumulated in 2008.

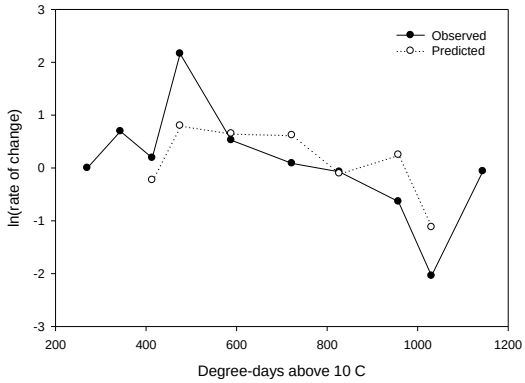


Figure 1.4. Mean weekly rate of change as degree-days above 10°C accumulated in 2009.

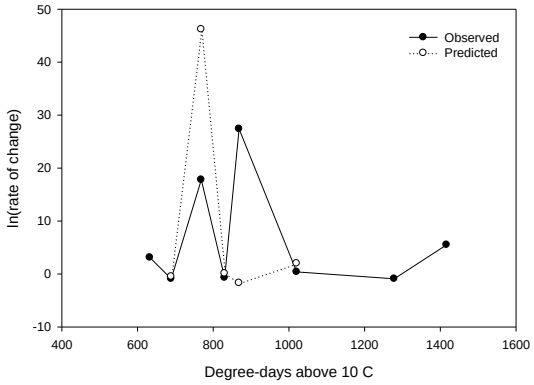


Figure 1.5. Mean weekly rate of change as degree-days above 10°C accumulated in 2010.

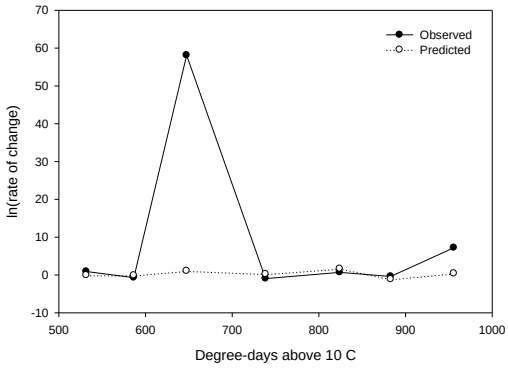


Figure 1.6. Mean number of stable flies ± SE collected from Coroplast® cards placed along transects in pasture during 2008.

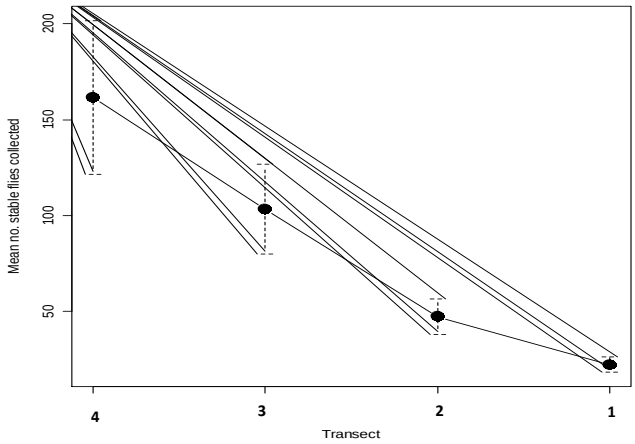


Figure 1.7. Average ovarian stage of stable flies collected at Medicine Lake NWR, MT 2008-2009.

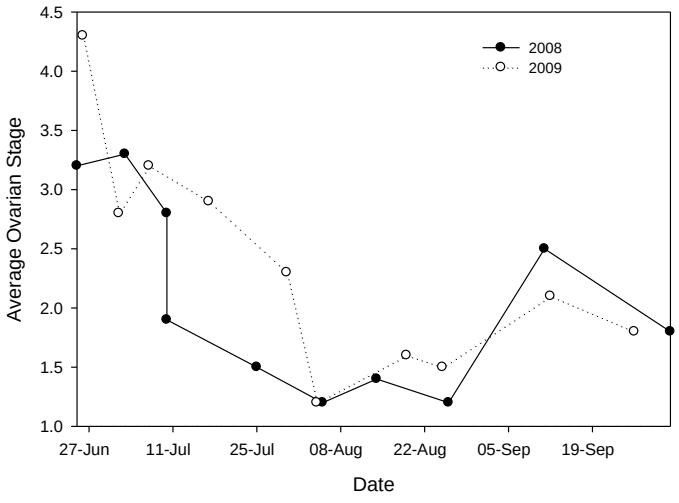
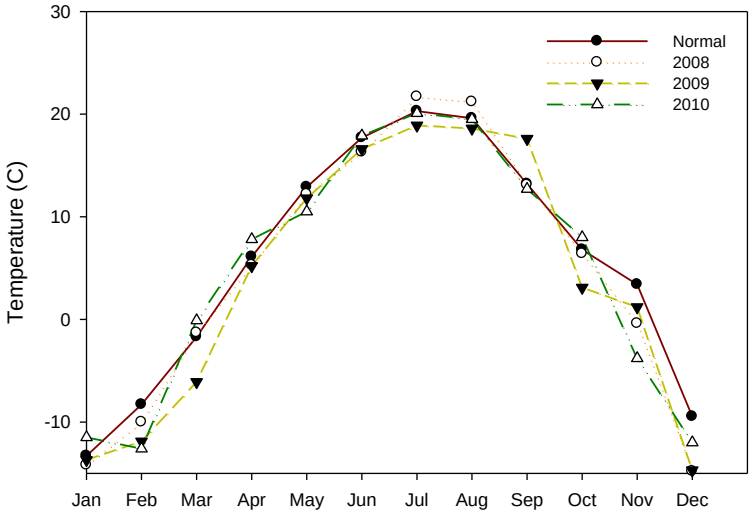
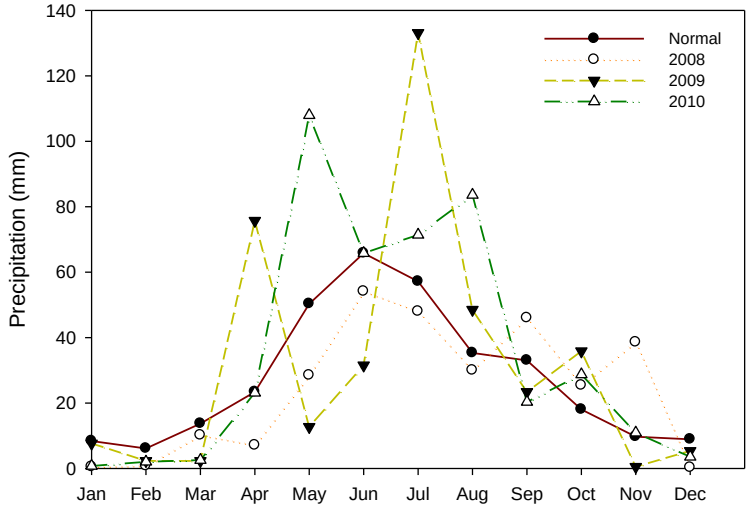


Figure 1.8. Temperature and precipitation at Medicine National Wildlife Refuge, 2008-2010.





COMPARATIVE EFFECTS OF CATTLE, HORSE, AND CHICKEN BLOOD ON STABLE FLY (*STOMOXYS CALCITRANS* (L.)) FECUNDITY

Abstract

Stomoxys calcitrans (L.) (Muscidae: Diptera) is a cosmopolitan pest of livestock, domestic animals, and humans. While stable flies occasionally feed on birds such as chickens, their preferred host are mammals, including cattle. During a West Nile virus outbreak in a colony of American white pelicans ((*Pelecanus erythrorhynchos* Gmelin) in northeast Montana, stable flies fed on moribund pelicans at a level that has not been previously documented for any avian species. Furthermore, previous work has indicated that stable flies are not capable of successfully reproducing when fed chicken blood. The objective of this investigation was to determine if stable fly daily and lifetime fecundity rates would significantly differ when fed cattle, horse, or chicken blood. Each treatment was replicated three times and followed through F_2 adult emergence. Lifetime fecundity rates between treatments were similar ($\chi^2 = 3.4$; df = 2; $P = 0.2$). Daily fecundity rates were highest for flies fed chicken blood (vs. cattle: $P = 0.008$; vs. horse: $P = 0.05$), but oviposition periods were shorter. Eggs deposited by females fed chicken blood had a smaller volume than those produced by the other cohorts ($F = 16.6$; df = 2, 333; $P < 0.001$). Females tended to live longer than males except when cohorts were fed chicken blood. In all treatments, females also had longer wings than males ($F = 46.8$; df = 1, 140; $P < 0.001$). These results suggest that the compositional differences in blood do not explain host preferences. The potential of host defenses to explain the feeding behavior of stable flies is discussed.

Keywords: Diptera, reproduction, host preference, defensive behavior

Introduction

The hematophagous stable fly (*Stomoxys calcitrans* (L.)) is a cosmopolitan pest (Moon 2002) that can cause decreased weight gains in confined and pastured cattle (Campbell et al. 1987, Campbell et al. 2001). And, while it readily feeds on domestic livestock, most companion animals, and humans, it rarely feeds on birds (Bishopp 1913). When the blood meals of 158 engorged stable flies collected on poultry ranches in California were identified, 155 were from cattle, two from horse, and one from a dog (Anderson and Tempelis 1970). When flies were fed blood from eight different animals (Sutherland 1978), all the females that had been fed chicken blood died before oviposition. This was presumed to be due to a protein deficiency in chicken blood which may have caused the follicles to resorb the yolk, preventing further maturation. Given both the relative rarity of reports of *S. calcitrans* feeding on birds and fecundity studies that have illustrated possible reasons for this behavior, much research has focused on the interaction between stable flies and cattle.

In northeast Montana, however, stable flies have been recently documented feeding en masse on American white pelicans (*Pelecanus erythrorhynchos* Gmelin) infected with West Nile Virus (WNV) (Johnson et al. 2010b). Following the introduction of WNV into the area in 2003, mortality of approximately 1,200 pre-fledged pelicans increased from a 10-yr average of 4% to more than 30% (Madden and Restani 2003, B. Madden, personal communication). Symptoms of WNV in moribund chicks include

ataxia and immobility (Johnson et al. 2010a). Stable flies were seen feeding so vigorously on these weakened birds that wounds were openly weeping blood. Flies tended to congregate around the crown of the head and the eyes, where, occasionally, scabbing was so severe as to seal the eyes shut. While stable flies have occasionally been observed to attack birds (Golding 1946, Greenberg 1971, Hogsette and Farkas 2000), this level of attack on any avian species has not been documented. Furthermore, the location of the roosting pelicans was along the sandy shoreline of a peninsula that is protected from the intrusion of possible predators with an electric fence separating it from the rest of the refuge. The exclusion of other hosts that would be more suitable for protein required for mating and/or oogenesis prompted us to investigate the possible benefit of the stable flies from feeding on pelicans.

A preliminary fecundity trial was conducted for the purpose of feeding colony-maintained stable flies diets of cattle, pelican, or chicken blood and then measuring daily and lifetime fecundity rates. Results from this trial showed that stable flies will lay eggs when given either pelican or chicken blood, but indicated that there might be differences in both daily and lifetime fecundity rates (Figure 2.1). The objectives of this study, therefore, were to 1) further investigate the effects of host source on the fecundity of stable flies using blood from cattle, horse, and chicken and 2) test the null hypothesis that chicken blood causes a reduction in fecundity in the F_0 generation that can be restored in the F_1 generation by feeding cattle blood.

Materials and Methods

Stable Flies

Stable flies were obtained through Dr. J. Hogsette from colonies maintained at the United States Department of Agriculture Center for Medical, Agricultural, and Veterinary Entomology colony in Gainesville, Florida. Flies were received as pupae and allowed to eclose in cages of various sizes (see Appendix for rearing procedures).

Blood

Blood was obtained 2 or 3 November, 2010 and treated with 3.7 g sodium citrate per liter (J Hogsette, personal communication). Bovine blood (4 liters) obtained by the author at the Ranchland Packing Co. Butte, M. was a homogenate of blood from two bulls. Animal Technologies, Inc. (Tyler, TX) was the source of chicken blood and Quad Five (Ryegate, MT) provided the horse blood. Chicken and horse blood (4 liters each) was shipped overnight on blue ice to Montana State University in Bozeman, MT. One liter of blood of each type was refrigerated at 1.6 °C for immediate use and the other three were frozen at -17°C until needed.

Trial 1

Fecundity trials began with three replications of three F_0 cohorts fed cattle, horse, or chicken blood. To control for possible variation due to sex ratio, adults up to two days old and maintained on 10% sucrose solution were anesthetized with CO_2, sexed, and up to 50 males and 50 females were placed in each of nine round, plastic GladWare®

containers (The Glad Products Company, Oakland, CA, 1.89 L, 82.6 mm diameter x 50.8 mm height) which served as cages for the trial. Two rectangular holes approximately 114.3 mm wide x 63.5 mm tall were cut on opposite sides of the containers and fitted with white cloth mesh to allow for ventilation. Lids of the containers were modified so that a square measuring 102 mm x 102 mm was cut out and replaced with a long stockinet sleeve to allow entry into the containers. Containers were kept in ambient room conditions where temperature and relative humidity were recorded every hour for the duration of the experiment by a HOBO Pro series data recorder (Onset Corporation, Pocasset, MA). The average temperature was 23.6 ±0.04 °C and average relative humidity was 13% ± 0.12.

One super jumbo cotton ball (Walmart Stores, Inc, Bentonville AR) was treated with 5 ml of a 10% sucrose solution and 10 ml of one blood type and wrapped in black Sheermist Batiste cloth (65 polyester/ 35 cotton) to serve as an oviposition substrate. Wrapped cotton balls were then placed in Glad® plastic cups (The Glad Products Company, Oakland, CA, 118 ml, 82.6 mm diameter x 50.8 mm high) which were then placed in the containers for 24 h. Eggs were removed from fresh cotton balls daily and counted. Dead flies were removed from cages daily, counted and sexed.

Up to 250 eggs from each container were harvested 12 d after emergence of the parental adults, which coincided with 5-6 d into the oviposition period, by cutting sections of the black oviposition cloth containing a known number of eggs that were counted. Eggs and cloth were placed in stable fly larval medium and covered with approximately 0.6 cm of the medium [2 parts wheat bran (223 g): 1 part pine shavings

(36.5 g): 1 part Purina® fly chow (200.2 g, Nestle S.A., Vevey, Switzerland) , 50 mg Munton's Active Brewing Yeast® (Munton's Plc, Suffolk England), and 1 L of water (T Lysyk, personal communication)] to obtain an F_1 generation. Diets for each container were mixed individually and placed into Gladware® containers (1.89 L, 203 mm wide x 142 mm long x 102 mm tall) that were secured over the top with white cloth.

Pupae were separated from medium by spooning them into a container of water and skimming floating pupae with a strainer. Because of high levels of adult mortality on the day following CO_2 anesthetization, adults for the F_1 generation were obtained by placing up to 150 pupae into clean containers, and allowing emergence for 2 days before removing any remaining uneclosed pupae and empty puparia. Treatments and processing were repeated as previously described for the F_1 adults to produce an F_2 generation.

Wing length and egg length x width for F_1 adults were measured with a 2 megapixel MiScope (Zarbeco, LLC, Randolph, NJ) that was calibrated with a 2-mm stage micrometer (Leica, Wetzlar, Germany) divided into 0.01-mm units. Volume for the eggs, which are prolate spheroids, was calculated with the following formula: 0.523 x (egg length^2) x egg width.

Egg viability was determined by cutting sections of the black oviposition cloth described above containing eggs, counting the number of eggs, moistening the cloth and placing each of them with the eggs right-side up in a plastic Petri dish. Dishes were placed into large plastic containers of varying sizes with a moist sponge and closed with plastic lids to maintain humidity levels adequate for preventing desiccation of the eggs. Eggs were allowed to incubate at ambient conditions (previously described) for three to

four days. Viability was determined by microscopically examining eggs for indications of emergence (i.e., empty eggs). Eggs that were not readily identified as empty were prodded with a dissecting pin.

Trial 2

To determine if cattle blood can restore a reduction in fecundity caused by feeding chicken blood, six containers similar to those described in trial 1 each housed up to 50 males and 50 females obtained from the lab colony and sexed after CO_2 anesthetization. All cohorts of the F_0 generation were fed chicken blood. The subsequent F_1 generation was divided into two groups of three replications for which one group was fed cattle blood and the other group was fed chicken blood. Processing was the same as described above. All flies used to start this experiment were obtained separately from the ones used in trial 1. Therefore, all data obtained from trial 2 were separated from trial 1 for analysis.

Statistical Analysis

The non-parametric two sample Wilcoxon rank sum test was used to detect significant differences ($P \leq 0.05$) in daily fecundity rates between cohorts within each treatment, as well as between generations and treatments. Differences in average lifetime fecundity for each cohort, calculated as $\text{fecundity}_{\text{mean}}$ x oviposition period, were analyzed with the Kruskal-Wallis test. Variance in F_1 egg volume due to host type was analyzed with a one-way analysis of variance. Relative investment in egg development per treatment was measured as the lifetime $\text{fecundity}_{\text{mean}}$ x $\text{volume}_{\text{mean}}$. Differences in wing

lengths were tested with a multiple analysis of variance that included sex, blood type, and an interaction term as explanatory variables. Wing lengths of F_1 and F_2 were combined due to low F_2 adult emergence rates. All statistical analysis was performed using R© software (version 2.12).

Results

Significantly more eggs were laid daily when flies were fed chicken blood than when they were fed cattle ($P = 0.008$) or horse blood ($P = 0.05$) (Table 2.1). No significant difference in daily fecundity was detected between cohorts fed cattle and horse blood ($P = 0.36$). However, there were no significant differences between lifetime fecundity rates ($\chi^2 = 3.4$, df = 2, $P = 0.20$). Oviposition periods for all flies typically began 4-6 days after emergence (Figure 2.2).

Eggs produced by chicken blood-fed flies fed on chicken blood were significantly smaller than those produced by cattle blood-fed and horse blood-fed flies fed on cattle or horse blood ($F = 16.6$; df = 2, 333; $P < 0.001$). No significant difference in egg volume was detected between cattle- and horse blood-fed flies fed cattle and horse blood. Mean egg length was 1.1 ± 0.003 (SE) mm and 0.3 ± 0.001 mm wide. Average volume of eggs from flies fed on cattle and horse blood was 0.18 ± 0.003 and 0.19 ± 0.006, respectively, and 0.16 ± 0.002 for flies fed on chicken blood.

Average viability of the eggs also differed numerically between treatment types with flies fed on horse, cattle, and chicken blood producing eggs that hatched 59% (*n* =

179), 83% (n = 704), and 94% (n = 507) of the time, respectively. The 95% confidence intervals suggest that these differences are not significant.

Wing lengths did not differ significantly between treatments (F = 0.2; df = 2, 140; P = 0.82), and sex by treatment interaction was not significant (F = 1.1; df = 2, 140; P = 0.34). Females have significantly longer wings than males by about 2 mm (F = 46.8; df = 1, 140; $P < 0.001$). Mean female wing length was 5.4 ± 0.03 (SE) mm and mean male wing length was 5.1 ± 0.07 mm. Flies fed on horse blood (both sexes) had the largest wings. The females with the smallest wings had been fed on cattle blood and the males with the smallest wings had been fed on chicken blood (Table 2.1).

In all treatments, males tended to die before the females (Table 2.2). Although the period before all flies died was identical for males and females in most of the treatments, this usually represented only one or two flies and most males had died before the females.

Total maternal investment calculated here as the average lifetime fecundity x volume of the egg, revealed maternal investment of resources to egg production in females fed cattle blood was 70.1 mm^3. Females fed horse (55.2 mm^3) and chicken (54.0 mm^3) blood had markedly lower investments.

Discussion

In this study, stable flies fed cattle, horse, and chicken blood had similar lifetime fecundity rates. Although survival and oviposition periods were longer for flies fed cattle and horse blood than for flies fed chicken blood, stable flies fed chicken-blood had higher

daily fecundity rates. So that oviposition period may be accounted for, lifetime fecundity was calculated as the average number of eggs laid per female per day and multiplied by the oviposition period. The resulting lifetime fecundity should be interpreted with caution because this assumes that the average number of eggs laid daily remains constant, which may not be an accurate description of stable fly oviposition. To address this, fecundity studies investigating variance in individual females should be conducted. Keeping this in mind, many other endogenous and exogenous factors may also affect oviposition and survival.

Blood meals are necessary to the production of sex pheromones and successful mating. Meola et al. (1977) demonstrated that production of cuticular hydrocarbon-based female sex pheromones is dependent on the digestion of a blood meal. Effects of different types of blood meals on the qualitative and quantitative production of these pheromones have not been addressed. A blood meal is also required for the successful transfer of spermatazoa from the male to the female (Anderson 1978). Because females mate only once (Harris et al. 1966) and lifetime fecundity was similar between the three blood-source treatments, pheromone production and sperm transfer are also likely to be similar and independent of host type.

In our stable flies, the amount of blood digested may have had an effect on the number of eggs produced. A study involving the sandfly *Lutzomyia longipalpis* (Lutz and Neiva) showed a positive correlation between egg production and the size of the blood meal (Ready 1979). *Aedes aegypti* (Linnaeus) produce more eggs as blood meal size increases from 0.4 mg to 2.0 mg, or about double the female's body weight (Woke et

al. 1956). Blood meals greater than 2.0 mg only slightly increased egg production until the upper threshold of 4.9 mg of blood was ingested. In the current investigation, stable flies were allowed to feed *ad lib*, and provided with enough blood so that at no time during these trials were cotton balls completely dried out. Cotton balls treated with blood only would have dried within 24 hrs, but the addition of a 10% sucrose solution eliminated this problem. It should be noted that this addition of sucrose to the blood meal could have also affected fecundity rates by stimulating engorgement (Lang and Wallis 1956). Although it has not been formally measured, it is likely that stable flies exhibit prediuresis (Mitzmain 1913) and will feed until nutritional requirements have been met. Prediuresis is the active concentration of nutrients from a meal while feeding (compared to diuresis where concentration occurs after feeding). Preiduresis has been documented in several *Anophelese* mosquito species and *Phlebotomus* sandflies (Sadlova et al. 1998). The rate of prediuresis and concentration of erythrocytes in the rectum varies according to species and the type of blood being fed upon, possibly due to the size of the erythrocytes (which vary according to host source) and the size of the pyloric armature, or spicules in the rectum (whose function is to trap the erythrocytes) (Vaugan et al. 1991, Briegel and Rezzonico 1985, Nijhout and Carrow 1978). If this is the case, then blood meals from the different blood types need to be measured to determine if differences exist because of nutritional requirements of the stable fly. However, while it is possible that the amount of blood ingested may have varied across treatments and may have had a slight effect on fecundity rates, it likely had no major impact.

Varying concentrations of protein and lipid in the blood source could also have had an effect on oocyte development. Sutherland (1978) conducted an in-depth investigation of stable fly fecundity rates on various host sources and reported mean reproductive potentials as low as 0 when flies were given chicken blood and as high as 3,600 when given cattle blood. These differences were attributed to nutritional deficiency. Amino acid and lipid concentrations may vary greatly on an individual level due to a variety of reasons including genetics, sex, age, physiological status, and diet. Generally, blood lipid concentrations are similar in cattle, horses, and chickens (Chen et al. 2005, Wehrman et al. 1991, Djik and Wensing 1989) but blood cell amino acid concentrations are higher in birds than in mammals (Pons et al. 1986). This is partially due to protein synthesis in the nucleated cells of birds (mammalian red blood cells are not nucleated).

If amino acid concentration is the regulating factor for stable fly fecundity, specific amino acids, then, rather than total protein, would be involved. If this were the case, detailed research in adult stable fly nutritional requirements should be explored. However, ovipositional and survival periods in Sutherland's(1978) study were short with maximum values of 10 days of oviposition and 16 days until 100% mortality was recorded. Other studies, including the current one, typically record survival periods of six weeks and, occasionally, over three months and oviposition periods spanning a month (Berry and Kunz 1977, 1978, Gilles et al. 2005). Temperature may explain the shortened periods. But, according to the temperature reported (27°C) and Lysyk's (1998) regression derived from maintained-colony developmental data on a defibrinated cattle

blood diet, Sutherland's (1978) oviposition and survival periods are still significantly lower than expected.

Although stable flies prefer cattle, they will feed on most hosts available to them (Pitzer et al. 2011). If host source had such a profound impact on survival and fecundity, selection pressure should be high enough to result in an almost exclusive relationship between cattle and stable flies. The data obtained in the current study suggest that because no difference in lifetime fecundity was detected, stable flies opportunistically rather than preferentially feed on cattle. Further evidence for this comes from the identification and characterization of a vast and complex mixture of enzymes in stable fly salivary glands (Wang et al. 2009). However, it is possible that two generations were not enough to detect cumulative effects of the various diets (Nation 2002). It should also be stressed that the stable flies used in the current investigation were obtained from a Florida-based laboratory colony and that caution should be used when extrapolating these results to describe feeding behavior documented at MLNWR or other field sites.

Host blood composition has been shown to alter life history parameters for other hematophagous insects. For example, because isoleucine enhances egg production and survival in *Ae. aegypti*, females will take smaller but more frequent meals when feeding on low-isoleucine hosts (i.e., humans) when compared to high-isoleucine hosts (Harrington et al. 2001). Interestingly, Harrington et al. (2001) showed that survival and fecundity were greater and population replacement rates were faster when females fed on human blood compared to the high-isoleucine host (mouse) regardless of the amount ingested, indicating that the physiological processes relating host source to *Ae. aegypti*

life history has not yet been fully described. Results from sand fly fecundity trials vary with species. *Phlebotomus papatsi* Scopoli showed no difference in fecundity rates given eight different mammalian blood types while *Lutzomyia ovallesi* Ortiz did show differences given chicken, cow, goat, pig, dog, human, or horse blood (Harre et al. 2001, Noguera et al. 2006). Noguera et al. (2006) postulated that the differences they detected were related to the species-specific synthesis and rupture of the peritrophic matrix and the synthesis of digestive enzymes.

While the possible differences in gut activity given different host sources have not been studied in stable flies, there has been some research exploring the synthesis and activity of proteases and lipases. The midgut metabolism of blood lipids such as triacylglycerols, phospholipids, and sphingomyelin may have a profound effect on stable fly longevity and fecundity (Spates et al. 1990). Deloach and Spates (1980) postulated that this reduction in fecundity was due to a reduction in either *de novo* synthesis or activation of digestive enzymes. Spates (1979) showed that female stable flies that were given blood meals treated with a phospholipase inhibitor exhibited longer digestion periods, less than normal fat body, and little or no yolk deposition. Additionally, in some of the groups exposed to the phospholipase inhibitor, the spermathecae were relatively fragile and filled with microorganisms rather than spermatazoa.

In many insects, gut microflora may influence the release of nutrients from the meal required for survival and/or fecundity (i.e., termites). Alternatively, like all other animals, insects may be detrimentally affected by any pathogens they ingest due to toxins or costs of immunity (Kraaijeveld and Wertheim 2009). And although microorganisms

were not quantitatively or qualitatively described, blood that was used during these trials was refrigerated for up to three weeks before end of use. Additionally, a strong, pungent odor came from the chicken blood that was absent in the bovine or horse blood meals. Blood that has been refrigerated or frozen for extended periods of time and is used to feed stable flies will lead to a decrease in colony vigor that is restored as soon as fresh blood is administered (personal observation). The anterior midgut tissue of stable flies, where the peritrophic matrix is produced and blood is stored, does constitutively produce antibacterial peptides that are regulated by three broad classes of defense mechanisms, each with functional redundancies (Lehane et al. 1997, Munks et al. 2001). Therefore, that microbial contamination may have influenced survival rates is only speculative until further investigation.

If stable flies are able to digest avian blood and produce as many eggs as when fed mammalian blood, why are there not more field observations of *S. calcitrans* feeding on birds? Even when more than 150 engorged stable flies were collected on the outside walls on poultry ranches and their hosts identified, none had fed on birds (Anderson and Tempelis 1970). The answer for this anomaly may be because of the opportunistic behavior of stable flies.

Smaller animals are attacked less by flying insects because of the effectiveness of their defensive behaviors compared to that of larger animals (Lehane 1991). In an open-field environment, defensive behaviors of dairy cattle in response to stable fly attack were defined as head throws, tail flicks, skin twitches, and leg stomps (Mullens et al. 2006). At peak stable fly attack, which was considered moderate with four flies per leg, the

maximum number of defensive behaviors by an individual could be up to 32 responses in a two-minute interval. Translating into 960 defenses per hour, this hourly estimate is likely to be higher than what actually occurs. Mullens et al. (2006) also found that individuals will become habituated. In a separate study, five eight-week old chickens were exposed to 300 female *Culex nigripalpus* Theobald mosquitoes and their defensive behaviors, defined as head shake, foot shift, foot peck, wing flip, body peck, body shake, and bill snap, were quantified per hour (Edman et al. 1974) and reached a maximum of 985 behaviors per bird. Lehane (1991) remarked that some species of birds will perform up to 3,000 defensive movements per hour when at roost. When various hosts were caged and restrained or unrestrained, *Cx. nigripalpus* tended to feed on the animal that was restrained regardless of host size or species (Edman et al. 1974).

In northeast Montana, stable fly populations were monitored near a colony of American white pelicans due to the high level of attack and feeding on local populations on WNV-infected juveniles in 2007. Subsequent adult stable fly surveillance during 2008-2010 showed that although *S. calcitrans* was present near the roosting site and large potential hosts other than pelicans were rare, the level of attack documented in 2007 has not reoccurred. During outbreaks of WNV within the colony, it is not unusual to find 100 carcasses per week (personal observation). Juveniles with advanced symptoms were easily approached and unresponsive. In addition to stable fly attacks, feather and pouch lice appeared to be much more abundant on sick juveniles than on the healthy ones. An ectoparasite recovery study from 14 sick juveniles revealed average loads of 4,7011 pouch lice (range of 160 - 13,200) and 758 feather lice (range of 55 - 2,161) (G Johnson,

unpublished data). Masses of blowfly eggs covering the undersides of the wings were also often seen before the juvenile succumbed. Healthy juveniles spent a large amount of time preening, flipping pouches inside out, and, when stable fly attacks increased, were seen dispersing into the lake and settling along the shores of an island approximately 0.8 km from the main roosting site.

In summary, stable flies will successfully mate and produce eggs when fed at least two types of avian blood. Feeding behavior in the field is likely to be dictated by host defensive behaviors rather than any physiological difference that might occur in stable flies in response to blood quality or meal quantity. Field observations of stable flies feeding on birds may therefore be limited to immunocompromised hosts.

Table 2.1. Physiology and fecundity rates from F_0 and F_1 cohorts fed cattle, horse, or chicken blood.
Columns with means followed by the same letter were not significantly different.

	Mean ± SE (n)						
Host Source	Daily fecundity[a]	Oviposition period (d)	Lifetime fecundity[b]	F_1 Egg volume (mm^3)	F_1 Egg viability	Female wing length (mm)[c]	Male wing length (mm)[c]
Trial 1							
Cattle	19 ± 2A		389 ± 47A	0.18 ± 0.003A	0.83 ± 0.05	5.328 ± 0.042A	5.026 ± 0.052A
	(97)	21	(6)	(130)	(704)	(31)	(13)
Horse	18 ± 3A		291 ± 52A	0.19 ± 0.006A	0.59 ± 0.13	5.485 ± 0.038A	5.327 ± 0.063A
	(61)	17	(5)	(31)	(179)	(29)	(29)
Chicken	24 ± 2B		337 ± 10A	0.16 ± 0.002B	0.94 ± 0.02	5.387 ± 0.059A	4.974 ± 0.068A
	(52)	14	(5)	(175)	(507)	(30)	(14)
Trial 2							
$F_{0\ (Chicken)}$	15 ± 2	14	238 ± 58	---	---	---	---
	(66)		(6)				
$F_{1(Bovine)}$	17 ± 3	27	461 ± 61	0.172 ± 0.003A	0.89 ± 0.03	5.422 ± 0.022	5.194 ± 0.023
	(46)		(2)	(117)	(308)	(75)	(59)
$F_{1\ (Chicken)}$	33 ± 7	7	236 ± 91	0.162 ± 0.004B	0.91 ± 0.06	5.011 ± 0.163	4.946 ± 0.045
	(18)		(3)	(116)	(285)	(2)	(7)

[a] Numbers in parentheses are the total number of days in which fecundity was calculated.
[b] Numbers in parentheses are the total number of cohorts during F_0 and F_1.
[c] Numbers in parenthesis are the total number of flies measured.

Table 2.2. Mortality of stable flies fed either cattle, horse, or chicken blood.

Host Source	No. Males	No. Females	No. Cohorts	Male $Mortality_{50}$	Female $Mortality_{50}$	Male $Mortality_{100}$	Female $Mortality_{100}$
Trial 1							
Cattle	141	131	6	14 ± 1	16 ± 2	37	37
Horse	100	89	5	10 ± 3	13 ± 2	23	23
Chicken	55	130	5	10 ± 1	9 ± 1	22	22
Trial 2							
$F_{0\ (Chicken)}$	117	124	6	11 ± 1	12 ± 1	25	25
$F_{1\ (Cattle)}$	27	26	2	13 ± 3	17 ± 1	27	37
$F_{1\ (Chicken)}$	15	11	3	11 ± 1	13 ± 3	21	18

2.1. Average number of eggs laid per female per day in preliminary fecundity trial. Cohorts were fed cattle, pelican, or chicken blood.

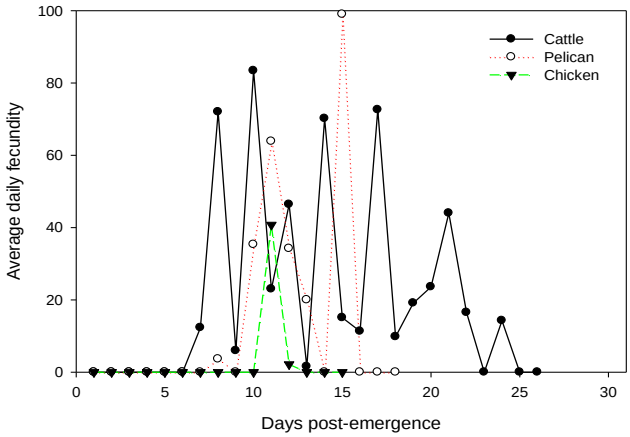
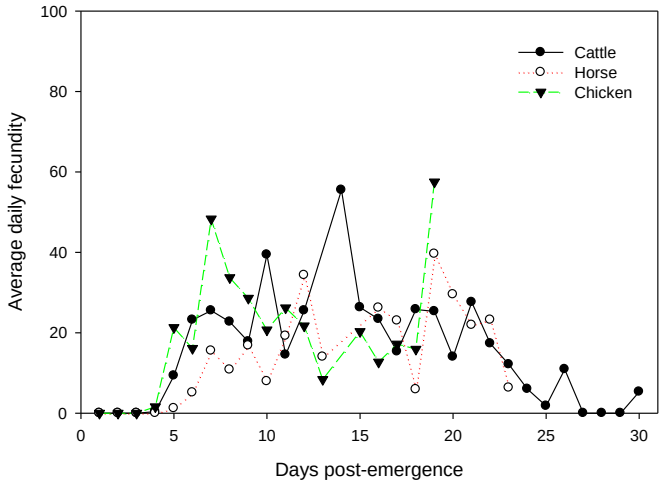


Figure 2.2. Average number of eggs laid per female per day in cohorts fed cattle, horse, or chicken blood.



References Cited

Agee H and R Patterson. 1983. Spectral sensitivity of stable, face, and horn flies and behavioral responses of stable flies to visual traps (Diptera: Muscidae). Environ Entomol. 12: 1823-1828.

Alzogaray R and D Carlson. 2000. Evaluation of *Stomoxys calcitrans* (Diptera: Muscidae) behavioral response to human and related odors in a triple cage olfactometer with insect traps. J Med Entomol. 37: 308-315.

Anderson J. 1978. Mating behavior of *Stomoxys calcitrans*: Effects of blood meal on the mating drive of males and its necesity as a prerequisite for proper insemination of females. J Econ Entomol. 71:379-386.

Anderson J and C Tempelis. 1970. Precipitin test identification of blood meals of *Stomoxys calcitrans* (L.) caught on California poultry ranches, and observations of digestion rates of bovine and citrated human blood. J Med Entomol. 7: 223-229.

Bailey D, T Whitfield, and B Smittle. 1973. Flight and dispersal of the stable fly. J Econ Entomol. 66: 401-411.

Beresford D and J Sutcliffe. 2006. Studies on the effectiveness of Coroplast sticky traps for sampling stable flies (Diptera: Muscidae), including a comparison to Alsynite. J Econ Entomol. 99: 1025-1035.

Beresford D and J Sutcliffe. 2008. Stable fly (*Stomoxys calcitrans*: Diptera, Muscidae) trap response to changes in effective trap height caused by growing vegetation. J Vector Ecol. 33: 40-45.

Beresford D and J Sutcliffe. 2009. Local infestation or long-distance migration? The seasonal recolonization of dairy farms by *Stomoxys calcitrans* (Diptera: Muscidae) in south central Ontario, Canada. J Econ Entomol. 102: 788-798.

Berry I and J Campbell. 1985. Time and weather effects on daily feeding patterns of stable flies (Diptera:Muscidae). Environ Entomol. 14: 336-342.

Berry I and S Kunz. 1977. Mortality of adult stable flies Environ Entomol. 6: 569-574.

Berry I and S Kunz. 1978. Oviposition of stable flies in response to temperature and humidity. Environ Entomol. 7: 213-216.

Bishopp F. 1913. The stable fly (*Stomoxys calcitrans* L.), an important livestock pest. J Econ Entomol. 6: 112-126.

Briegel H and L Rezzonico. 1985. Concentration of host blood protein during feeding by anopheline mosquitoes (Diptera: Culicidae). J Med Entomol. 22: 612-618.

Broce A. 1988. An improved Alsynite trap for stable flies, *Stomoxys calcitrans* (Diptera: Muscidae). J Med Entomol. 25: 406-409.

Broce A, J Schwenke, and K Hampton. 1991. Landing pattern of stable flies (Diptera: Muscidae) on the Alsynite cylinder trap: effect of wind speed and direction. J Med Entomol. 28: 730-733.

Broce A, J Hogsette, and S Paisley. 2005. Winter feeding sites of hay in round bales as major developmental sites of *Stomoxys calcitrans* (Diptera: Muscidae) in pastures in spring and summer. J Econ Entomol. 98: 230-2312.

Buschman and Patterson. 1981. Assembly, mating, and thermoregulating behavior of stable flies under field conditions. 1981. Environ Entomol. 10: 16-21.

Burg J, D Powell, and F Knapp. 1991. Arthropod faunal composition on Kentucky equine premises. J Med Entomol. 28: 658-662.

Burg J, D Neely, N Williams, and F Knapp. 1994. Retention and attempted mechanical transmission of *Ehrlichia risticii* by *Stomoxys calcitrans*. Med Vet Entomol. 8: 43-46.

Buxton B. 1985. Role of insects in the transmission of bovine leukosis virus: potential for transmission by stable flies, horn flies, and tabanids. Am J Vet Res. 46: 123-126.

Campbell J and C McNeal, Jr. 1979. A guide to integrated pest management at feedlots and dairies. Univ Nebr Coop Ext Serv. EC 80-1536. 21 pp.

Campbell J, I Berry, D Boxler, R Davis, D Clanton, and G Deutscher. 1987. Effects of stable flies (Diptera: Muscidae) on weight gain and feed efficiency of feedlot cattle. J Econ Entomol. 80: 117-119.

Campbell J, S Skoda, D Berkebile, D Boxler, G Thomas, D Adams, and R Davis. 2001. Effects of stable flies (Diptera: Muscidae) on weight gains of grazing yearling cattle. J Econ Entomol. 94: 780-783.

Chamberlain W and J Matter. 1986. Control of stable flies (Diptera: Muscidae) with a unique nitrogen fertlizer, calcium cyanamide. J Econ Entomol. 79: 1573-1576.

Chen K, W Chi, and P Chiou. 2005. Caponization and testosterone implantation effects on blood lipid and lipoprotein profile in male chickens. Poul Sci. 84: 547-552.

Chia L, J Baxter, and P Morrison. 1982. Quantitative relationship between ingested blood and follicular growth in the stable fly, *Stomoxys calcitrans*. Can J Zool. 60: 1917-1921.

Chihota C, L Rennie, R Kitching, and P Mellor. 2003. Attempted mechanical transmission by lumpy skin disease virus by biting insects. Med Vet Entomol. 17: 294-300.

Christophers S. 1911. The development of the egg follicle in anopholines. Paludism. 2: 73-88.

Cilek J. 1999. Evaluation of various substances to increase adult *Stomoxys calcitrans* (Diptera: Muscidae) collections on Alsynite cylinder traps in north Florida. J Med Entomol. 36: 605-609.

Cilek J. 2002. Attractiveness of beach ball decoys to adults *Stomoxys calcitrans* (Diptera: Muscidae). J Med Entomol. 39: 127-129.

Deloach J and G Spates. 1980. Effect of soybean tripsin inhibitor-loaded erythrocytes on fecundity and midgut protease and hemolysis activity on stable flies. J Econ Entomol. 73: 590-594.

Detinova T. 1962. Age-grouping methods in Diptera of medical importance. WHO Monogr Ser. 47: 1-216.

van Dijk S and T Wensing. 1989. Comparison of the lipoprotein pattern of the horse, the pony and the lactating and non-lactating cow obtained by a combination of an ultracentrifugation and a precipitation technique. Comp Biochem Phys. 94B: 735-738.

Eddy G, A Roth, and F Plapp. 1962. Studies on the flight habits of some marked insects. J Econ Entomol. 55: 603-607.

Edman J, L Webber, and A Schmid. 1974. Effect of host defenses on the feeding pattern of *Culex nigripalpus* when offered a choice of blood sources. J Parasitol. 60: 874-883.

Ferris D, R Hanson, R Dicke, and R Roberts. 1955. Experimental transmission of vesicular stomatitis by Diptera. J Infec Dis. 96: 184-192.

Foil L and C Younger. 2006. Development of treated targets for controlling stable flies (Diptera: Muscidae). Vet Parasitol. 137: 311-315.

Geden C and R Moon. 2009. Host ranges of gregarious muscoid fly paraitoids: *Muscidifurax raptorellus* (Hymenopter: Pteromalidae), *Tachinaephagus zealandicus* (Hymenoptera: Encryrtidae), and *Trichopria nigra* (Hymenoptera: Diapriidae). Environ Entomol. 38: 700-707.

Gingrich R. 1960. Development of a synthetic medium for aseptic rearing of larvae of *Stomoxys calcitrans* (L.). J Econ Entomol. 53: 408-411.

Gilles J, J David, and G Duvallet. 2005. Effects of temperature on the rate of increase of *Stomoxys calcitrans* and *Stomoxys niger niger* (Diptera: Muscidae) from La Reunion Island. J Med Entomol. 42: 959-965.

Golding F. 1946. A new method of trapping flies. Bull Ent Res. 37: 143-154.

Greenberg B. 1971. Flies and Disease. Vol I: Ecology, classification and biotic associations. Princeton University Press.

Greene G, J Hogsette, and R Patterson. 1989. Parasites that attack stable fly and house fly (Diptera: Muscidae) puparia during the winter on dairies in northwestern Florida. J Econ Entomol. 82: 412-415.

Guo Y, G Greene, and M Butine. 1998. Population profile of stable flies (Diptera: Muscidae) caught on Alsynite traps in various feedlot habitats. J Econ Entomol. 91: 159-164.

Hafez M and F Gamal-Eddin. 1966. The feeding habits of the horn fly, *Siphona irritans* (L.) , with special reference to its biting cycle in nature in Egypt. Bull Soc Ent Egypte.70:

Haines T. 1955. Breeding media of common flies II: in rural areas. Am J Trop Med Hyg. 2: 933-940.

Hansens. 1951. The stable fly and its effect on seashore recreational areas in New Jersey. J Econ Entomol. 44: 482-487.

Harre J, K Dorsey, K Armstrong, J Burge, and K Kinnamon. 2001. Comparative fecundity and survival rates of *Phlebotomus papatsi* sandflies membrane fed on blood from eight mammal species. Med Vet Entomol. 15: 189-196.

Harrington L, J Edman, and T Scott. 2001. Why do Aedes aegypti (Diptera: Culicidae) feed preferentially and frequently on human blood? J Med Entomol. 38: 411-422.

Harris R, E Frazar, P Grossman, and O Graham. 1966. Mating habits of the stable fly. J Econ Entomol. 59: 634-636.

Hennig W. 1964. Muscidae. In Lindner E. (editor): Die fliegen der palaearktischen Region. Schweizerbart, Stuttgart.

Hogsette J. 1983. An attractant self-marking device for marking field populations of stable flies with fluorescent dusts. J Econ Entomol. 76: 510-514.

Hogsette J and J Ruff. 1985. Stable fly (Diptera: Muscidae) migration in northwest Florida. Environ Entomol. 14: 170-175.

Hogsette J and J Ruff. 1986. Evaluation of flucythrinate- and fenvalerate-impregnated ear tags and permethrin ear tapes for fly (Diptera: Muscidae) control on beef and dairy cattle in northwest Flordia. J Econ Entomol. 152-157.

Hogsette J and J Ruff. 1990. Comparative attraction of four different fiberglass traps to various age and sex classes of stable fly (Diptera: Muscidae) adults. J Econ Entomol. 83: 883-886.

Hogsette J and R Farkas. 2000. Secretophagous and haematophagous higher Diptera. In L Papp and B Darvas, editors. Contributions to a manual of palearactic Diptera, Vol. 1 General and applied Dipterology. Science Herald, Budapest.

Hogsette J, J Ruff, and C Jones. 1987. Stable fly biology and control in northwest Florida. J Agric Entomol. 4: 1-11.

Hogsette J, A Nalli, and L Foil. 2008. Evaluation of different insecticides and fabric types for development of treated targets for stable fly (Diptera: Muscidae) control. J Econ Entomol. 101: 1034-1038.

Hollis J, F Knapp, and K Dawson. 1985. Influence of bacteria within bovine feces on the development of the face fly (Diptera: Muscidae). Environ Entomol. 14: 568-571.

Jeanbourquin P and P Guerin. 2007. Chemostimuli implicated in selection of oviposition substrates by the stable fly *Stomoxys calcitrans*. Med Vet Entomol. 21: 209-216.

Johnson C. 1966. A functional system of adaptive dispersal by flight. Ann Rev Entomol. 11: 233-260.

Johnson J, Panella N, Hale K, and Komar N. 2010a. Detection of West Nile virus in stable flies (*Stomoxys calcitrans*) parasitizing juvenile American white pelicans (*Pelecanus erythrorhynchus*). J Med Ent. 47: 1205-1211.

Johnson J, N Nemeth, K Hale, N Lindsey, N Panella, and N Komar. 2010b. Surveillance for West Nile virus in American white pelicans, Montana, USA, 2006-2007. Emerg Infect Dis. 16: 406-411.

Jones C and R Weinzierl. 1997. Geographical and temporal variation in pteromalid (Hymenoptera: Pteromalidae) parasitism of stable fly and house fly (Diptera: Muscidae) pupae collected from Illinois cattle feedlots. J Environ Entomol. 26: 421-432.

Jones C, J Hogsette, R Patterson, and D Milne. 1985. Effects of natural saccharide and pollen extract feeding on stable fly (Diptera: Muscidae) longevity. Environ Entmol. 14: 223-227.

Kano R. 1953. Notes on the flies of medical importance in Japan. Part VII. Eggs and larvae of
Stomoxydinae in Japan. Japan J Exp Med. 23:187-95.

Kraaijeveld A and B Wertheim. 2009. Costs and genomic aspects of *Drosophila* immunity to parasites and pathogens. In: Rolff J and Reynolds S, editors. Insect infection and immunity: Evolution, ecology, and mechanisms. Oxford University Press, USA.

Lang C, and R Wallis. 1956. The utilization of sucrose in an artificial feeding procedure for *Aedes aegypti* (Linnaeus). Am J Trop Med Hyg. 5: 915-920.

Lee R and D Davies. 1979. Feeding in the stable fly, *Stomoxys calcitrans* (Diptera: Muscidae) I. destination of blood, sucrose solution and water in the alimentary canal, the effects of age on feeding, and blood digestion. J Med Entomol. 15: 541-554.

Lehane M. 1991. Biology of blood-sucking insects. HarperCollins Academic, London.

Lehane M, D Wu and S Lehane. 1997. Midgut-specific immune molecules are produced by the blood-sucking insect *Stomoxys calcitrans*. Proc Natl Acad Sci. 94: 11502-11507.

Lysyk T. 1993a. Seasonal abundance of stable flies and house flies (Diptera: Muscidae) in dairies in Alberta, Canada. J Med Entomol. 30: 888-895.

Lysyk T. 1993b. Adult resting and larval developmental sites of stable flies and house flies (Diptera: Muscidae) on dairies in Alberta. J Econ Entomol. 86: 1746-1753.

Lysyk T. 1998. Relationships between temperature and life-history parameters of *Stomoxys calcitrans* (Diptera: Muscidae). J Med Entomol. 35: 107-119.

Lysyk T and R Axtell. 1987. A simulation model of house fly (Diptera: Muscidae) development in poultry manure. Can Entomol. 119: 427-437.

Lysyk T and E Krafsur. 1993. Relationship between pterin accumulation and ovarian development in the stable fly *Stomoxys calcitrans* (L.) (Diptera: Muscidae). Can Entomol. 125: 869-879.

Lysyk T, L Kalischuk-Tymensen, L Selinger, R Lancaster, L Wever, and K Cheng. 1999Rearing stable fly larvae (Diptera: Muscidae) on an egg yolk medium. J Med Entomol. 38: 382-388.

Madden E. and Restani M. 2005. History and breeding ecology of the American White Pelican at Medicine Lake National Wildlife Refuge, Montana. Waterbirds 28 (Special Publication I): 23-26.

Masmeatathip R, C Ketavan, and G Duvallet. 2006. Morphological studies of *Stomoxys* spp. (Diptera: Muscidae) in central Thailand. Kasetsart J. (Nat. Sci.). 40: 872-881.

Mellor P, R Kitching, and P Wilkinson. 1987. Mechanical transmission of capripox virus and African swine fever virus by *Stomoxys calcitrans*. Res Vet Sci. 43: 109-112.

Meola R, R Harris, S Meola, and D Oehler. 1977. Dietary-induced secretion of sex pheromone and development of sexual behavior in the stable fly Environ Entomol. 6: 895-897.

Meyer J and J Petersen. 1983. Characterization and seasonal distribution of breeding sites of stable flies and house flies (Diptera: Muscidae) on eastern Nebraska feedlots and dairies. J Econ Entomol. 76: 403-108.

Mihok S. 2002. The development of a multipurpose trap (the Nzi) for tsetse and other biting flies. Bull Ent Res. 92: 385-403.

Mihok S, E Kang'ethe, and G Kamau. 1995. Trials of traps and attractants for *Stomoxys* spp. (Diptera: Muscidae). J Med Entomol. 32: 283-289.

Mitzmain M. 1913. The bionomics of *Stomoxys calcitrans* Linnaeus; a preliminary account. Philip J Sci. 8: 29-48.

Moon R. 2002. Muscid flies (Muscidae). In: Mullen G and L Durden, editors. Medical and Veterinary Entomology. Academic Press, San Francisco.

Moorhouse C. 1972. Cutaneous lesions on cattle caused by stable fly. Aus Vet J. 48: 643-644.

Moorison P, K Venkatesh, and B Thompson. 1981. The role of male accessory-gland substance on female reproduction with some observations of spermatogenesis in the stable fly. J Insect Phys. 28: 611-614.

Moraes A, I Angelo, E Fernandes, V Bittencourt, and A Bittencourt. 2008. Virulence of *Metarhizium anisopliae* to eggs and immature stages of Stomoxys calcitrans. Anim Biodiv and Emerg Dis: Ann NY Acad Sci. 1149: 384-387.

Mramba F, A Broce, and L Zurek. 2007. Vector competence of stable flies, *Stomoxys calcitrans* L. (Diptera: Muscidae), for *Enterobacter sakazakii*. J Vector Ecol. 32:134-139.

Mullens B and J Meyer. 1987. Seasonal abundance of stable flies (Diptera: Muscidae) on California dairies. J Econ Entomol. 80: 1039-1043.

Mullens B and N Peterson. 2005. Relationship between rainfall and stable fly (Diptera: Muscidae) abundance on California Dairies. J Med Entomol. 42: 705-708.

Mullens B, N Peterson, C Dada, and R Velten. 1995. Octenol fails to lure stable fly to insecticide. Cal Agric. 49: 16-18.

Mullens B, K Lii, Y Mao, J Meyer, N Peterson, and C Szijj. 2006. Behavioural responses of dairy cattle to the stable fly, *Stomoxys calcitrans*, in an open field environment. Med Vet Entomol. 20: 122-137.

Munks R, J Hamilton, S Lehane and M Lehane. 2001. Regulation of midgut defensin production in the blood-sucking insect *Stomoxys calcitrans*. Insect Mol Biol. 10: 561-571.

Nation J. 2002. Insect physiology and biochemistry. CRC Press, New York.

Nijhout H and G Carrow. 1978. Diuresis after a bloodmeal in female *Anopheles freeborni*. J Insect Phys. 24: 293-298.

Noguera P, M Rondon, and E Nieves. 2006. Effect of blood source on the survival and fecundity of the sandfly *Lutzomyia ovallesi* Ortiz (Diptera: Psychodidae), vector of *Leishmania*. Biomedica 26(Suppl. 1): 57-63.

Olafson P, J Pitzer, and P Kaufman. 2011. Identification of a mutation association with permethrin resistance in the *para*-type sodium channel of the stable fly (Diptera: Muscidae). J Econ Entomol. 104: 250-257.

Parr H. 1962. Studies on *Stomoxys calcitrans* (L.) in Uganda, East Africa. II. Notes on the life-history and behaviour. Bull Entomol Res. 53: 437-443.

Perotti M, T Lysyk, L Kalischuk-Tymensen, L Yanke, and L Selinger. 2001. Growth and survival in mature Haematobia irritans (Diptera: Muscidae) is influenced by bacteria isolated from cattle manure and conspecific larvae. J Med Ent. 38: 180-187.

Petersen J. 1989. Biotic agents to control house flies and stable flies. In: G Thomas and S Skoda, editors. Rural flies in the urban environment: proceedings of a symposium

presented at the annual meeting of the Entomological Society of America. NC-154 Publication. San Antonio, TX.

Petersen J and J Meyer. 1983. Host preference and seasonal distribution of pteromalid parasites (Hymenoptera: Pteromalidae) of stable flies and house flies (Diptera: Muscidae) associated with confined livestock in eastern Nebraska. Environ Entomol. 12: 567-571.

Petersen J and B Pawson. 1988. Late-season activity of pteromalid (Hymenoptera) parasites of filth flies (Diptera: Muscidae) in eastern Nebraska. J Med Entomol. 25: 272-275.

Pickard E. 1968. *Stomoxys calcitrans* (L.) breeding along TVA reservoir shorelines. Mosq News. 28: 644-645.

Pitzer J, P Kaufman, and S Tenbroeck. 2010. Assessing permethrin resistance in the stable fly (Diptera: Muscidae) in Florida by using laboratory selections and field evaluations. J Econ Entomol. 103: 2258-2263.

Pitzer J, P Kaufman, S Tenbroeck, and J Maruniak. 2011. Host blood meal identification by multiplex polymerase chain reaction for dispersal evidence of stable flies (Diptera: Muscidae) between livestock facilities. J Med Entomol. 48: 53-60.

Pons A, F Garcia, A Palou, and M Alemany. 1986. Amino acid compartmentation in chick blood during the pre-hatching period. Comp Biochem Physiol. 85A: 237-242.

Potgieter F, B Sutherland, and H Biggs. 1981. Attempts to transmit *Anaplasma marginale* with *Hippobosca rufipes* and *Stomoxys calcitrans*. Onderstepoort J Vet Res. 48: 119-122.

Rasmussen R and J Campbell. 1981. Investigation of environmental factors and their relationship to populations of the stable fly, Stomoxys calcitrans (L.). Environ Entomol. 10: 798-800.

Ready P. 1979. Factors affecting egg production of laboratory-bred *Lutzomyia longipalpis* (Diptera: Psychodidae). J Med Entomol. 16: 413-423.

Romero A, A Broce, and L Zurek. 2006. Role of bacteria in the oviposition behaviour and larval development of stable flies. Med Vet Entomol. 20: 115-121.

Ross R and H Hunter. 1976. Climax vegetation of Montana: Based on soils and climate. Bozeman, MT: U.S. Department of Agriculture, Soil Conservation Service.

Sadlova J, J Reishig, and P Volf. 1998. Prediuresis in female *Phelobotomus* sandflies (Diptera: Psychodidae). Eur J Entomol. 95: 643-647.

Schmidtmann E. 1988. Exploitation of bedding in dairy outdoor Calf hutches by immature house and stable flies (Diptera: Muscidae) **.** J Med Entomol. 25: 484-488.

Schmidtmann E and Martin. 1992. Relationship between selected bacteria and the growth of immature house flies, *Musca domestica*, in an axenic test system. J Med Entomol. 29: 232-235.

Schofield S and S Torr. 2002. A comparison of the feeding behaviour of tsetse and stable flies. Med Vet Entomol. 16: 177-185.

Scholl P. 1980. A technique for physiologically age-grading female stable flies, *Stomoxys calcitrans* (L.). Univ Nebr Agric Exp Stn Res Bull. 298.

Scholl P, J Petersen, D Stage, and J Meyer. 1981. Open silage as an overwintering site for immature stable flies in eastern Nebraska. The Southwestern Entomol. 6:253-258.

Simmons S. 1944. Observations on the biology of the stable fly in Florida. J Econ Entomol. 37: 680-686.

Simmons S and W Dove. 1941. Breeding places of the stable fly or "dog fly" *Stomoxys calcitrans* (L.) in northwestern Florida. J Econ Entomol. 34: 457-462.

Skidmore P. 1985. The biology of the Muscidae of the world. Klower Academic Publishers. Hingham, MA.

Skoda S and G Thomas. 1993. Breeding of stable flies and house flies. In: G Thomas and S Skoda, editors. Rural flies in the urban environment. Nebr Agric Exp Sta Res Bull 317, N Cent Reg Res Bull 335.

Skoda S, G Thomas, and J Campbell. 1991. Development sites and relative abundance of immature stages of the stable fly (Diptera: Muscidae) in beef cattle feedlot pens in eastern Nebraska. J Econ Entomol. 84: 191-197.

Smith C and E Hansens. 1975. The effect of temperature and humidity on the amount of blood ingested by the stable fly, *Stomoxys calcitrans* L. (Diptera: Muscidae). J N Y Ent Soc. 83: 235-240.

Spates G. 1979. Fecundity of the stable fly: Effect of soybean trypsin inhibitor and phospholipase A inhibitor on the fecundity. Ann Entomol Soc Am. 72: 845-849.

Spates G and J DeLoach. 1985. Reproductive performance of adult stable flies (Diptera: Muscidae) when fed fresh or reconstituted , freeze-dried bovine or porcine blood. J Econ Entomol. 78: 856-859.

Spates G, D Bull, and A Chen. 1990. Hydrolysis of sphingomyelin and phosphatidylcholine by midgut homogenates of the stable fly. Archives of Insect Biochemistry and Physiology. 14: 1-12.

Sutcliffe S, C d'Cambre, and A Downe. 1993. Effects of two blood-feeding regimes on mortality and female reproduction in a laboratory colony of stable flies, *Stomoxys calcitrans*. Med Vet Entomol 7: 111-116.

Sutherland B. 1978. Nutritional values of different blood diets expressed as reproductive potentials in adult *Stomoxys calcitrans* L. (Diptera: Muscidae). Onderstepoort J Vet Res. 45: 209-212.

Taylor D and D Berkebile. 2008. Sugar feeding in adult stable flies. Environ Entomol. 37: 625-629.

Taylor D, D Berkebile, and P Scholl. 2007. Stable fly population dynamics in eastern Nebraska in relation to climatic variables. J Med Entomol. 44: 765-771.

Taylor D, R Moon, J Campbell, D Berkebile, P Scholl, A Broce, and J Hogsette. 2010. Dispersal of stable flies (Diptera: Muscidae) from larval development sites in a Nebraska landscape. Environ Entomol. 39: 1101-1110.

Taylor J and Lacey J. 1994. Range plants of Montana. Montana State University Extension. EB 122.

Thomas G, I Berry, D Berkebile, and S Skoda. 1989. Comparison of three sampling methods for estimating adult stable fly (Diptera: Muscidae) populations. Environ Entomol. 18: 759-763.

Torr S, T Mangwiro, and D Hall. 2006. The effects of host physiology on the attraction of tsetse (Diptera: Glossinidae) and *Stomoxys* (Diptera: Muscidae) to cattle. Bull Ent Res. 96: 71-84.

Torr S, M Holloway, and G Vale. 1992. Improved persistence of insecticide deposits on targets for controlling *Glossina pallidipes* (Diptera: Glossinidae). Bull Ent Res. 82: 525-533.

Turrell M and G Knudson. 1987. Mechanical transmission of Bacillus *anthracis* by stable flies (*Stomoxys calcitrans*) and mosquitoes (*Aedes aegypti* and *Aedes taeniorhynchus*). Infect Immun. 55: 1859-1861.

Turrell M, D Dohm, C Geden, J Hogsette, and K Linthicum. 2010. Potential for stable flies and house flies (Diptera: Muscidae) to transmit Rift Valley Fever virus. J Am Mosq Con. 26: 445-448.

USDA National Agriculture Statistic Service) http://www.nass.usda.gov/Statistics_by_State/Montana/Publications/livestock/cattle_c.htm, updated January 8, 2011)

Uebel E, P Sonnet, B Bierl, and R Miller. 1975. Sex pheromone of the stable fly: isolation and preliminary identification of compounds that induce mating strike behavior. J Chem Ecol. 1: 377-385.

Vale G. 1993. Development of baits for tsetse flies (Diptera: Glossinidae) in Zimbabwe. J Med Entomol. 30: 831-842.

Vaughan J, B Noden, and J Beier. 1991. Concentration of human erythrocytes by anopheline mosquitoes (Diptera: Culicidae) during feeding. J Med Entomol. 28: 780-786.

Venkatesh K and P Morrison. 1980. Some aspects of oögenesis in the stable fly *Stomoxys calcitrans* (Diptera: Muscidae). J Insect Phys. 26: 711-715.

Voegtline A, G Ozburn, and G Gill. 1965. The relation of weather to biting activity of *Stomoxys calcitrans* (Linnaeus) along Lake Superior. Pap Mich Acad Sci Arts Lett. 50: 107-114.

Wang X, J Ribeiro, A Broce, M Wilkerson, and M Kanost. 2009. An insight into the transcriptome and proteome of the salivary gland of the stable fly, *Stomoxys calcitrans*. Insect Biochemistry and Molecular Biology. doi:10.1016/j.ibmb.2009.06.004.

Warnes M and L Finlayson. 1985a. Responses of the stable fly, *Stomoxys calcitrans* (L.) (Diptera: Muscidae), to carbon dioxide and host odours. I. Activation. Bull Ent Res. 75: 519-527.

Warnes M and L Finlayson. 1985b. Responses of the stable fly, *Stomoxys calcitrans* (L.) (Diptera: Muscidae), to carbon dioxide and host odours. II. Orientation. Bull Ent Res. 75: 717-727.

Watson D and J Petersen. 1991. Infectivity of *Serratia marcescens* (Eubacteriales: Enterobacteriaceae) in *Stomoxys calcitrans* (Diptera: Muscidae). J Med Entomol. 28: 190-192.

Weber A, R Moon, D Sorensen, D Bates, J Meiske, C Brown, N Rohland, E Hooker, and W Strand. 1988. Evaluation of the stable fly (*Stomoxys calcitrans*) as a vector of enzootic bovine leukosis. Am J Vet Res. 49: 1543-1549.

Wehrman M, T Welsh, G Williams. 1991. Diet-induced hyperlipidemia in cattle modifies the intrafollicular cholesterol environment, modulates ovarian follicular dynamics, and hastens the onset of postpartum luteal activity. Bio Reprod. 45: 514-522.

Williams D. 1973. Sticky traps for sampling populations of *Stomoxys calcitrans*. J Econ Entomol. 66: 1279-1280.

Williams D and A Rogers. 1976. Vertical and lateral distribution of stable flies in northwestern Florida. J Med Entomol. 13: 95-98.

Williams D, A Rogers, P Hester, J Ruff, and R Levy. 1980. Preferred breeding media of the stable fly, *Stomoxys calcitrans*, in northwestern Florida. Mosq News. 40: 276-279.

Woke P, M Ally, and C Rosenberger. 1956. The numbers of eggs developed related to the quantities of human blood ingested in *Aedes aegypti* (L.) (Diptera: Culicdae). Annals Ent Soc Am. 49: 435-441.

Zachs D and E Loew. 1989. Why is alsynite fiber glass sheet attractive to stable flies? Exp Biol. 48: 215-222.

Zhu J, X Zeng, D Berkebile, H Du, Y Tong, and K Qian. 2009. Efficacy and safety of catnip (*Nepeta cataria*) as a novel filth fly repellent. Med Vet Entomol. 23: 209-216.

Zumpt F. 1973. The Stomoxine bitine flies of the world. Fischer, Stuttgart.

APPENDICES

APPENDIX A:

PROCEDURES FOR THE MAINTENANCE OF A STABLE FLY COLONY

Every Morning

1. **Monday-Thursday** feed fresh blood. Blood is treated with sodium citrate to prevent clotting and is frozen. Place 4-5 cotton balls in small plastic cups. Add enough blood so that the cotton balls are thoroughly soaked and only a little liquid is pooled on bottom of plastic cup. Add 10% sucrose solution to the blood-soaked cotton bals until just wetted. Cut a strip of black cloth so that you have enough to wrap the cotton balls. Try to smooth the cloth as much as possible and to push it against the sides of the plastic cup. Flies will try to crawl into the sides of the cup and into the folds of the black cloth. This makes their removal more difficult. Once cloth-covered cotton balls are in plastic cups, gently push down on the cloth so that it is also soaked in blood-sugar solution. Place one fresh blood cup in each cage every morning Monday-Thursday.

2. **On Fridays**, place sugar water in cages for weekend feeding. To do this, measure 900 ml of deionized water (obtained in lab across the hall and located in designated gallon containers) in Erlenmeyer flask. To this, add pre-measured amount of sugar (located on shelf by bag of sugar). Shake. In large plastic Dixie cups, fill approximately 2/3 full with sugar water. Place wick in the solution. To prevent flies from drowning in the water, a Ziploc bag is used to cover the opening of the cup. Make a small incision with scissors in the middle of the bottom of the Ziploc bag. Gently pull over the cup and gently push the wick through the incision so that little space is available for the flies to crawl through. To fix the bag over the cup, use a rubber band.

3. When removing old blood cups **Tuesday through Friday**, harvest eggs.

a. To remove eggs, unwrap the black cloth from the cotton balls. Discard cotton balls. Use plastic squirt bottle with water to remove eggs into medium-sized square plastic container. You may gather all eggs from both cages in the same container. To concentrate the eggs in one spot, carefully tilt the container so that it is at an angle. While the eggs are settling, make larval media.

b. To make larval media add one scoop fly chow, one scoop sawdust, and two scoops wheat bran to bucket labeled "Fly Media Only" and one spoonful of brewer's yeast. Add approximately one gallon of DI water and thoroughly mix. You want the media to be moist, but not too wet. It should be just moist enough so that when you squeeze a handful of media, a little bit of water comes out. Place media in large plastic bin.

c. To place eggs in media, cut end of pipette tips. Draw 1 ml or 1000 ul of eggs at a time and dispense into pre-formed canals in media. Canals should be shallow and should be the length of the bin. This part of the procedure is similar to planting seeds. Place eggs in media until most have been harvested.

d. Once eggs have been harvested, place a piece of duct tape on the outside of the bin and write the date. Place bins in chronological order from left to right under the hood located next to the large refrigerator in the main part of the laboratory. Larvae will take between 10-15 days to pupate.

3. Check larval media for formation of pupae. When getting close to 10-15 days post-harvest, place pillow cases around media to prevent escape of adults. Secure ends of pillow cases with large black clips.

When Larvae Have Pupated

4. Larvae will migrate to the crust-moist media interface to pupate. Pupae may be found by peeling back the crust. Spoon larvae into large bin filled with water. Theoretically, most of the media should sink to the bottom while the pupae float. Some media will not sink. Skim the top of the water surface with metal sieve and place into larval bin lined with paper towels to dry. Pupae should be dry before placing into plastic cups. If still moist, fungus may form. If only a few hundred pupae are recovered, remove pupae from remaining media and place on fresh paper towel. The pupae will dry quicker this way. If more pupae are recovered, don't worry about removing from media. Once dry pupae have been placed in plastic cups, place cups into cages.

Blood Collection

Blood may be collected two ways. The easiest and primary route of blood collection will be to collect bovine blood from a slaughter house. However, this blood requires the addition of sodium citrate to prevent clotting. Sodium citrate may have reduce the vitality of the colony. If the colony appears to respond negatively from bovine blood, fresh sheep blood may be collected and given to the colony.

1. Slaughter house. Plastic gallon-sized containers are used to collect bovine blood. Before blood is collected, 100 ml of DI water and 14 grams of sodium citrate mixed in each container. Blood is obtained from Ranchland Packing Co. in Butte. The phone number is 782-6371 and the address is 1201 Centennial Avenue. Directions: Take I-90

West to Butte. Take exit 126 (Montana Street) and take a right onto Montana St. Take a left onto Centennial Avenue. You'll drive along this road for a short distance and will pass a number of businesses. Right before the road begins to slope upwards and becomes an overpass, take the small road to the left..this is the slaughter house. They begin slaughter at 5:30 am. So, try to be there by 7 am at the latest. Bring four or five containers with you. Once they're filled, place in cooler (no ice) and transport back to laboratory. To freeze blood in smaller amounts, place blood in quart-sized plastic containers with the blue screw on tops. Place blood in freezer until needed.

2. If fresh blood is needed, sheep blood may be obtained by contacting Reid for help at 994-7465. Sheep at Ft. Ellis may be used. Blood may be collected and stored long term if sodium citrate is added. However, the colony is likely to be more vigorous when fed fresh defibrinated blood. For this reason, sheep will be bled once a week between April 17, 2007 and January 31, 2008. Different sheep will be used at least once a month. Dr. Pat Hatfield will provide the sheep and skilled technicians who are skilled at bleeding sheep. The vein will be located by holding the head at a 30 degree angle and by applying pressure below the halfway point of the sheared area. Once the vein has been located, a 14 gauge needle and five ml syringe will be used to access blood flow. Between 200 and 250 ml of blood will then be collected in an Erlenmeyer flask containing glass beads. Once blood has been removed from the sheep, an alcohol swab will be used to disinfect the area of needle insertion to prevent subsequent infection. The collector will immediately begin defibrinating blood by vigorously swirling the blood for approximately ten minutes. Glass beads and any clots that form will be strained by

pouring blood into a separate screw-top glass jar through a screen sieve. Glass beads and the Erlenmeyer flask will be soaked in a 10% bleach solution for ten minutes and then washed. All needles and syringes will be disposed in a Sharps container. Blood may then be stored for up to ten days at 35°F (1.7°C).

Cleaning

It's important to keep the rearing room as clean as possible. The floors should be swept after making larval media and at least once a week (preferably on Friday). Wipe all blood spills down as they occur. Make sure the shelves and counters are clean after they've been used.

The cages are going to get dirty fast. At any given time, at least two large cages should be in use. Once one cage looks like it needs to be cleaned, begin putting pupae into new clean cage. Let adults die out in old cage. Once no more adults are alive, remove plastic cups and dead flies and hose down with water. DO NOT use bleach or ethanol or detergent to clean ANYTHING. USE WATER ONLY.

Supplies

I have a supply book containing details and pricing on items. This is only a brief list. If details are needed, check the supply book.

Black cloth- JoAnne Fabrics

Blood- Ranchland Packing Co. or Ft. Ellis

Cotton balls- WalMart

Plastic feeding cups- WalMart

Wicks- Richmond Dental

More stable fly pupae- Jerry Hogsette (Florida)

Purina Fly chow- Dallas Vaughn

Wheat bran- Murdochs

Brewer's yeast- Belgrade Brewery

Sawdust- Murdochs (***Do NOT use cedar. Cedar contains juvenile hormone that will prevent larvae from developing into adults****)