Stable Flies, Winter Bedding, and Summer Dairy Cow Comfort

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Introduction

Adequate management is essential for optimum dairy cow health and production. Producers are faced with several choices and must choose management strategies that work best for their herd. Maintaining comfortable animals is critical for optimizing production. Housing is one important element to cow comfort (Lobeck et al., 2011) due to daily resting requirements. When selecting housing, producers decide whether to keep cows indoors or outdoors and what bedding types to use. The ultimate goal of such choices is to maintain comfortable animals that are productive and profitable. In northern climates, fly management is another important aspect of cow comfort during summer months. Flies stress cattle, inducing defensive behaviors that affect normal grazing and resting behaviors (Dougherty et al., 1993). High fly populations can hinder production, impact animal health and can irritate people in addition to livestock.

This chapter reviews literature relevant to cow comfort in regards to different housing systems and important nuisance flies in dairy. Flies discussed in this chapter are horn flies, face flies and stable flies, with an emphasis on stable fly biology and management. Dairy cattle will exhibit a number of defensive behaviors in response to fly irritation and this chapter further describes such behaviors on an individual and herd basis.

Housing Dairy Cattle

There are many successful housing systems for dairy cows and all are designed with cow comfort in mind. Comfortable resting facilities are important for optimum production and designing adequate housing helps alleviate stress (Uzal and Ugurlu, 2010). Providing adequate resting time is an important aspect of dairy management, both for production and welfare (Haley et al., 2000). Dairy cows in confinement should lie down for approximately 12 hours/day (Jensen et al., 2005); however, if facilities are not sufficiently clean or comfortable, cows will often remain standing (Leonard et al., 1994, Haley et al., 2001). Insufficient lying time can negatively affect milk production. Producers can assess cow comfort using tools such as behavior indexes, which include cow comfort index and stall usage index (Seyfi, 2013b) to make any necessary adjustments. Maintaining a dairy herd with inadequate comfort leads to increased incidence of lameness. Lameness can be detrimental to a dairy herd, with significantly decreased production (Hernandez et al., 2005a), reproductive performance (Hernandez et al., 2005b) and early culling (Booth et al., 2004).

Several housing options for dairy cows are available depending on herd size, economics, and production goals. To increase milk production and modernize facilities, producers began expanding their herds (Stahl et al., 1999). Newer housing methods were necessary to effectively accommodate larger dairy herds. Free stall barns became more frequently used with decreased labor intensity and increased efficiency (Stahl et al., 1999). This type of barn provides cows with freedom to move from stalls to feed and water as desired, which is beneficial to cow welfare (Endres and Barberg, 2007). Factors such as flooring and stall partition are important in establishing comfortable free stall barns (Drissler et al., 2005).

Compost Barns

An alternative confinement housing option is the compost bedded pack, which is more commonly used in smaller dairies (Lobeck et al., 2012). Producers using compost barns have reported increased cow comfort and milk production (Janni et al., 2007). Cows may be kept in compost packs during winter months or throughout the year. These barns are free of confining stalls and other barriers, allowing unrestricted movement. A barn consists of enough space where each cow can simultaneously lie down with space remaining to allow a walking cow to pass by (Janni et al., 2007). Sawdust is the most frequently used bedding substrate, although alternate materials are acceptable provided adequate management (Shane et al., 2010). Packs should begin with 30-50cm of material (Janni et al., 2007). Properly managed compost packs may vary in depth, but should have core temperatures exceeding 40°C with moisture ranging from 50-60% (Janni et al., 2007, Black et al., 2013).

To facilitate composting, bedding must be tilled at least once daily. Convenient times to do so are when cows are removed for milking. Tilling incorporates fresh manure and urine while aerating the material. Aeration promotes microbial activity, which increases temperature (Janni et al., 2007, Black et al., 2013). Heat from composting dries the surface layer (Black et al., 2013), but new bedding should be added as needed to maintain a dry resting area. If dry bedding is not available, cows will spend significantly less time lying down (Fregonesi et al., 2007).

Outwintering

Faced with potentially high building costs and environmental concerns, many producers are investigating options of keeping cattle outdoors throughout winter (Barnes et al., 2013). In some countries, beef cattle are often kept outdoors through winter without adverse effects to welfare or growth (Redbo et al., 1996). There are few outwintering studies in the upper Midwest of the United States, an area known for harsh winters.

In northern climates, housing in winter must protect cattle from cold weather to maintain production. Cows are cold hardy and thrive in cooler temperatures. However, protection from elements should be provided (Redbo et al., 2001), especially for younger animals that are more susceptible to cold (Young, 1981). Wind and excess moisture are stressors to cows exposed to inclement weather (Young, 1983). Dairy cows exposed to winter temperatures below zero usually opted to spend time indoors (Krohn et al., 1992). According to Tucker et al. (2007), higher body condition scores helped insulate cows against winter effects and cows would position themselves to minimize surface area exposed to harsh weather.

Producers in Ireland often use woodchip outwintering pads during winter as a lower cost option compared to conventional freestall housing (O’Driscoll et al., 2008). Use of a straw yard in overwintering is currently being investigated. When bedding on a straw pack, cows select their own lying areas. Straw is routinely added to the pack to maintain a dry surface (Phillips and Schofield, 1994).

Cows kept outdoors in cold temperatures require more feed due to increased metabolic demands (Young, 1983). Increased metabolic demands result in less energy being directed toward production, and therefore, milk production and growth will be reduced (Young, 1981). Boyle et al. (2008) concluded that heifers housed outdoors on wood chips over winter grew more slowly than heifers housed indoors, but still considered outdoor winter housing to be a good alternative to indoor housing in stalls. Increased feed costs should be considered when choosing housing options for cattle in cold weather.

Pests of Dairy Cattle: Muscid Flies

Flies are present wherever there are cattle and these insects have long been a problem for livestock producers. Nuisance flies are typically most active from May to October in northern regions, but are active year round in warmer climates. These flies feed on cattle, whether housed indoors or on pasture. During summer, flies often migrate from facilities and can become a nuisance to nearby residences, potentially resulting in lawsuit (Axtell, 1986).

Cows irritated by fly feeding behavior can become very stressed under excessive fly populations. Cattle expend energy in an attempt to remove flies, which leads to reduced grazing time. Cows also become restless and spend less time lying down when under heavy fly pressure. Prolonged exposure to fly irritation can lead to decreased production. Although flies cannot realistically be eliminated from a farm, producers benefit from effective fly management with more comfortable animals and people. Proper management can keep fly populations low and minimize negative effects (Axtell, 1986).

There is extensive literature detailing effects of weather on development, fecundity, population dynamics, and activity of flies. Weather is an important factor to activity and feeding behaviors of flies, although results describing how weather affects activity are variable. Temperature is consistently an important variable for fly activity. Feeding activity of stable flies ceases at temperatures below 15˚C (Bailey and Meifert, 1973). Smith and Hansens (1975) fed stable flies at varying combinations of temperature and humidity in the lab and found the highest percentage of stable flies feeding at 32˚C with relative humidity below 43%. Additionally, the lowest percentage of flies was found feeding at 23˚C with relative humidity above 75%. Berry and Campbell (1985) noted that while stable fly feeding behavior was influenced by varying weather effects, feeding was partially dictated by time, regardless of weather.

The most important nuisance flies to the dairy industry are in the family Muscidae. The remainder of this chapter focuses on three species of muscid flies that are economically significant in dairy, including: stable flies, horn flies, and face flies. Future reference to “muscid flies” is in reference to these three species.

Stable Flies

Stable flies are economically significant biting pests of cattle and other large livestock. Adults closely resemble house flies, but are easily distinguished by piercing mouthparts that protrude from under the head. These flies are obligate blood feeders and are often found on the legs of cows. Both sexes will bite, ingesting a blood meal up to three times the average body weight (Parr, 1962). When allowed to feed without interruption, stable flies usually take 2-5 minutes to fully engorge (Bishopp, 1913). Flies typically feed once per day, but will feed twice per day in very favorable or warm conditions (Bishopp, 1913). Stable flies usually only approach their host to feed (Bailey and Meifert, 1977), then leave and perch nearby to digest the blood meal. Feeding can occur at any time during daylight hours (Mitzmain, 1913), with the most activity between 10am and 4pm (Hoffman, 1968). Adults tend to aggregate in the vicinity where hosts remain for extended periods (Gersabeck and Merritt, 1985, Hogsette et al., 1989), but are capable of flying several kilometers if necessary (Eddy et al., 1962, Bailey et al., 1973).

Stable flies have a holometabolous life cycle consisting of an egg, three larval instars, pupa and adult. Females require multiple blood meals to develop eggs prior to oviposition (Bishopp, 1913). Flies use olfactory cues when searching for an oviposition site from a distance (Jeanbourquin and Guerin, 2007). After finding a suitable media for oviposition, females lay batches of approximately 35 eggs, which hatch 12-24 hours later (Parr, 1962). Development time depends on a number of factors, such as temperature and moisture. According to Aguiar-Valgode (1992), the ideal temperature for developing maggots is 25˚C, and temperatures exceeding 35˚C are harmful for development. While heat does speed development time, moisture is another critical factor (Todd, 1964). Substrates too dry or too wet are unsuitable for developing maggots. Development is also dependent on live bacterial colonies within the substrate (Talley et al., 2009). These microbes attract ovipositing females. More eggs are laid in hay and manure mixtures with live microbial activity as opposed to a sterile substrate of the same mixture (Romero et al., 2006).

Maggots can develop in an array of decaying organic material and environmental conditions (Rasmussen and Campbell, 1981). Known breeding sites on a dairy farm include: straw bedding in calf hutches (Schmidtmann , 1988), bale hay feeding sites (Hall et al., 1980, Broce et al., 2005, Taylor and Berkebile, 2011) and accumulated silage (Williams et al., 1980, Meyer and Petersen, 1983). Silage, piled feed and grass are known overwintering sites for third instar larvae and pupae (Berkebile et al., 1994). Adults were long considered pests of cattle kept indoors due to abundance of soiled bedding, an ideal breeding site for stable flies. However, with increased use of round bale hay feeding systems in pasture, stable flies are now considered important pests of pastured cattle as well (Hall et al., 1982, Campbell et al., 2001, Broce et al., 2005, Taylor and Berkebile, 2011).

Fly size is positively correlated with fecundity (Schmidt and Blume, 1973). Moon (1980) and Easton and Lysyk (1986) found an association with head capsule width and number of ovarioles present in an adult face fly. Follicular development in stable flies begins within 24 hours after the first blood meal (Kunz, 1982). Reproductive ages of adult female stable flies can be determined through dissection and examination of the ovaries. Scholl (1980) outlines the dissection and classification process. Females are dissected in a saline solution. Abdominal terga are gently pulled apart with dissecting forceps to reveal and separate the ovaries. Ovaries are then ranked according to follicular development. Stages 0 and 1 show no obvious follicular development, referred to as previtellogenic. Reproductive stages 2 and above are considered vitellogenic. Stages 2-4 reflect further yolk development, while eggs at stage 5 are considered mature. Parous flies are distinguished by the presence of yellow bodies remaining at the base of ovariole pedicels (Anderson, 1964). Yellow bodies become more apparent as the number of ovipositions increases (Anderson, 1964).

Bites from stable flies are painful. Frequent biting stresses cattle, which can lead to decreased weight gain (Campbell et al., 2001) and milk production (Bruce and Decker, 1958). Taylor et al. (2012) estimated that when fly counts exceed 15, each additional fly decreased milk production by 0.22kg per cow per day. An economic injury threshold is necessary to determine when intervention is needed. Timing intervention is crucial to an effective integrated pest management program, but economic thresholds vary with study. The presence of only a few flies is enough to cause unrest in cattle. Campbell et al (1987) concluded that 2-5 flies per leg were enough to cause reduced weight gain and feed efficiency.

Control of adult stable flies is difficult. Only a small percentage of total population are found on cows at any given time and most chemical sprays are rubbed or washed off of cows before achieving satisfactory results (Campbell et al., 2001). Reduction or elimination of breeding sites is one of the most common methods of managing populations. Poor sanitation can create a myriad of breeding sites for stable flies, making source reduction a challenge. Any effort to manage breeding sites must often be coordinated with neighboring farms (Meyer and Hunter, 1991) to prevent dispersal.

Understanding requirements for successful development of immatures is important for maximizing control of stable flies (Meyer and Petersen, 1983). Knowledge of breeding sites will influence management strategies on a dairy farm, particularly given the range of suitable habitats. Some areas, such as drainage ditches, are more difficult to manage due to limited accessibility. However, facility modifications with fly control in mind, including the use of bedding unsuitable for fly development, can be quite useful to efforts in managing adult populations. Schmidtmann (1991) found significantly reduced densities of stable flies in calf hutches with sawdust bedding as opposed to straw bedding. Removing accumulated waste feed eliminates a prolific breeding site for stable flies. Broce et al. (2005) found a wide range of stable flies emerged from hay waste at feeding sites, some sites producing over 3,000 flies/ m^2. In addition to fly control, waste management has added benefits of providing fertilizer, preventing runoff and improving odor control (Campbell and Berry, 1989).

Carefully placed sticky traps are useful for monitoring stable flies as well as control. Several types of traps have been investigated over time. Williams (1973) found that panel traps coated with adhesive were seven times more effective in capturing stable flies than box traps. Translucent Alsynite® fiberglass was used to create 35 x 45cm panels that were slotted on a 168cm wooden stake. Alsynite® is particularly attractive to stable flies, usually capturing more males than females (Buschman and Patterson, 1981). Cylindrical Alsynite® traps were later designed to save costs, but maintain efficiency (Broce, 1986). Other types of fiberglass are not as effective in capturing stable flies, indicating a correlation between spectral sensitivities of flies and reflective properties of Alsynite® (Agee and Patterson, 1983).

Color and placement of sticky traps are important considerations for successful trapping. Stable flies are most attracted to white panels over other colors, black capturing the fewest flies (Williams, 1973, Beresford and Sutcliffe, 2006). Traps are best placed in an open area where cattle are congregating, yet far enough away to avoid trampling or other damage to the trap. Weather conditions can have an effect on catch rates. Berry et al (1986) found that more stable flies were captured on traps with increasing temperature, radiation and humidity. Sticky traps have proven effective in reducing stable fly numbers, but traps require regular maintenance and cleaning (Rugg, 1982), arguably making these traps impractical on a large scale.

Horn Flies

Haematobia irritans (Diptera: Muscidae), commonly known as horn flies, are small, biting flies that are primarily pests of cattle. Adults measure 5mm or less in length, approximately half the size of a housefly. Both male and female adults are obligate blood feeders with piercing mouthparts. These flies spend almost all their time on a host, often along the back or sides, where they feed several times per day. Cows can temporarily dislodge horn flies with head throws or tail flicks, but flies will quickly settle on the same cow once it becomes quiescent or a nearby cow. Horn flies prefer to stay on or close to a herd, but are capable of flying several kilometers if necessary (Byford et al., 1987, Sheppard, 1994). Females oviposit only on fresh dung pats and eggs hatch within 2 days (Foil and Hogsette, 1994). Depending on temperature and weather conditions, the life cycle from egg to adult is completed within 10-20 days (Campbell, 2006).

Horn fly numbers can vary greatly among cows in a single herd, with some cows attracting very large numbers of flies, while others consistently attracting very few (Steelman et al., 1993, Jensen et al., 2004). Several factors may influence horn fly attraction to a particular cow, including color (Ernst and Krafsur, 1984, Schreiber and Campbell, 1986), hair density (Steelman et al., 1997), breed of cow (Steelman et al., 1991), time of day (Schreiber and Campbell, 1986) and innate, heritable resistance mechanisms (Pruett et al., 2003).

Horn flies are among the most significant pests of dairy cattle, with production losses approaching $1 billion (Cupp et al., 2004). Heavy infestations result in decreased grazing and feed efficiency, leading to reduced production (Steelman et al., 1991, Byford et al., 1992). Cattle hides can also be damaged from extensive horn fly feeding, which reduces leather quality (Gugliemone et al., 1999, Pruett et al., 2003). Great effort is spent on controlling this pest. Chemical, mechanical and biological control methods have been developed as ways to manage horn fly populations. Intervention is usually needed when populations exceed 200 flies per animal (Hogsette et al., 1991).

Face Flies

Face flies, *Musca autumnalis* (De Geer), are pest flies of cattle introduced to North America in 1952, and have since rapidly spread (Pickens and Miller, 1980). These flies resemble house flies and feed on bodily secretions, usually around the eyes and mouth of cows. Face flies spend relatively little time on their host. Fly counts on a cow is thought to represent less than 5% of total population (Miller and Treece, 1968). These flies are most active during the day and are typically a problem for pastured cattle (Pickens and Miller, 1980), as they seldom enter barns or animal shelters (Ode and Matthysse, 1967). Males will occasionally feed on cattle, but most flies found on the face are female (Dobson and Matthew, 1960).

The life cycle consists of an egg, three larval instars, pupa and adult. Females oviposit on fresh dung pats, where eggs hatch within 24 hours (Wang, 1964). Development time from egg to adult ranges from 11-14 days depending on temperature and other conditions (Wang, 1964, Krafser and Moon, 1997). These flies overwinter as unmated adults (Teskey, 1969). After diapause, face flies begin breeding, producing several overlapping generations in a season.

Although feeding habits of face flies are annoying to cattle, there is little evidence of negatively affected growth or milk production (Krafsur and Moon, 1997). Schmidtmann et al. (1984) found that face fly numbers had little-to-no impact on quantity or quality of milk produced. Heifers infested with a mean of 13 flies per cow showed no significant difference with feed consumption, average daily gain or feed efficiency (Arends et al., 1982).

Face flies are mechanical vectors of parasites and disease, including pinkeye and *Thelazia* (Hall, 1984, Geden and Stoffolano, 1982), making control desirable if herds become infested with many flies. Management of face fly numbers focuses on animal comfort and reducing incidence of pinkeye (Krafser and Moon, 1997). Control can be difficult, as combinations of different methods seldom reduce numbers satisfactorily (Drummond et al., 1988). Use of insecticides is limited due to feeding habits.

Cattle Behavioral Responses to Flies

Cattle have a myriad of associated parasites, including several species of flies. Parasites and their hosts have a well-documented history of coevolution. Hart (1990) described how vertebrate behavior could have evolved to increase fitness against parasites. Flies induce several behaviors from cattle, some of which may serve as a deterrent, such as skin twitches and tail flicks, while others such as leg stamps and head throws may be a more direct response to pain (Dougherty et al., 1993). Predictably, cows exhibit almost no defensive behaviors when nuisance flies are absent (Dougherty et al., 1993b).

Fly presence encourages cows to move more frequently to newer areas (Distel et al., 1991) and to alter grazing bouts. Defensive behaviors not only interrupt grazing, but can increase energy costs of grazing (Dougherty et al., 1993). Cows annoyed by flies divert energy once directed toward production in an attempt to dislodge flies (Seyfi, 2013a). Intensity of attacks varies with time of day and weather conditions. Flies are particularly active when winds are low and temperatures are high (Todd, 1964). Under intense attack, cows often abandon grazing and bunch close together. Bunching is a herd response to fly activity where cows attempt to limit surface area exposed to attack. Oftentimes, cows will gather in a tight circle with heads in the center. However, cows in such close proximity have increased risk of heat stress and weight loss (Foil and Hogsette, 1994).

Some quantitative studies examine the relationship between defensive behaviors and nuisance flies. Quantifying defensive behaviors in response to flies is useful to producers planning the best management strategies for increasing production. However, most studies focus on a single pest fly species. Oftentimes, cattle are infested with multiple species, making it difficult to attribute effects to a specific fly (Mullens et al., 2006).

Cattle Behavior in Response to Stable Flies

Dougherty et al. (1993a, 1993c, 1994, 1995) manipulated stable fly numbers by using cages in a field setting to examine herd mean responses to stable fly attack and concluded that defensive behaviors increased with numbers of flies. In these studies, 12 beef cows were tethered to an individually enclosed area with released stable flies and allowed to graze for one hour. During that time, various behaviors were observed and recorded for one minute in 12 minute intervals. Behaviors observed included head movements, leg stamps, skin twitches and tail flicks. Leg stamps apparently caused by stable flies were recorded separately for front and back legs. A skin twitch event, measured on one side of a cow, could be counted as a single twitch in one area, or continuous rippling through the flank for several seconds. Tail flicks were marked when the tail moved from resting position to one side of the cow.

Mullens et al. (2006) monitored four groups of 25 cows twice a day, five times per week over 12 weeks to study behavior responses to stable flies on cows housed outdoors in a dirt lot. Stable flies were counted, and then responses (head throws, leg stamps, skin twitches and tail flicks) were recorded for two minutes. After the observation period, stable flies were recounted. This procedure was repeated until all cows were observed, using behavior definitions outlined by Dougherty.

Stable fly numbers vary among individual cows and cows differ in response to attack (Mullens et al., 2006). Cows subjected to harassment through the fly season showed decreases in leg stamping and head throws, indicating habituation to bites (Mullens et al., 2006). Habituation is a form of learning where an animal no longer responds to a particular stimulus (Alcock, 1989). Warnes and Finlayson (1987) found that cows earnestly exhibiting defensive behaviors were attacked by fewer flies than calmer animals. Front leg stamps are a good indicator of stable fly presence, though tail flicks may be easier for producers to observe as part of an integrated pest management strategy (Mullens et al., 2006).

Cattle Response to Horn Flies

Cows infested with horn flies differ in grazing behavior from uninfested cows. Cows not disturbed by horn flies were spread more widely in pasture (Duren, 1975).

Harvey and Launchbaugh (1982) noted that steers infested with 300 or more horn flies tended to walk more and differed in grazing/rumination behavior than steers without horn flies. In this study, two pastures were stocked with 9 yearling Hereford steers, one herd controlled for horn flies and one herd without control. Over nine days, activity from all steers was recorded from morning rise to bedding down at night. Tail flicks were recorded for 2 minutes at 15 min. intervals. Behaviors such as leg stamps or head throws were recorded intermittently. Infested steers had significantly more tail flicks than steers treated for horn flies.

Other physiological responses to horn fly infestations include increased heart rate, respiration, temperature and cortisol levels (Schwinghammer et al., 1986). Harvey and Launchbaugh (1982) concluded that infested steers likely have an increased energy requirement, which would need to be offset by increased feed or feed efficiency.

Cattle Behavior in Response to Face Flies

Feeding habits of face flies annoy cattle, evidenced by observed defensive behaviors. Irritation from face flies alters grazing behavior, reducing energy intake (Dougherty et al., 1993b). Ear flaps in particular are a good indicator of face fly presence. According to Schmidtmann (1985), ear flaps are adaptive behaviors that interrupt face fly feeding in that fly numbers are greater before flaps than after. In this study, face flies were counted every 20 seconds along with numbers of ear flaps during that interval.

Behavior was recorded on both the right and left side by two observers positioned 3-5m away from cattle.

When face fly numbers are high, cattle may be seen bunching, which is defined by Schmidtmann and Valla (1982) as bouts exceeding 15 minutes where at least 8 heifers positioned in a circle with heads pointed medially. To better understand the relationship between face fly numbers and herd density, Schmidtmann and Berkebile (1985) observed seven herds of 14-16 cows 3 times per day over 10 consecutive day periods. During that time, face flies were counted and proximity to another cow was recorded on a scale from 0 (0-0.5m distance) to 3 (>3m distance). Cows protecting their face tended to have fewer face flies (Schmidtmann and Valla, 1982, Schmidtmann and Berkebile, 1985).

Conclusions

Many elements must come together for a successful dairy herd. Maintaining comfortable cows not only addresses public concern for animal welfare, but optimizes production. Adequate resting facilities with clean, dry bedding are critical throughout the year. Cows must be provided shelter from harsh conditions during winter. Some bedding types are better for immature stable flies than other bedding types. Soiled bedding left over from winter may provide suitable habitats for maggots, which can lead to very high fly numbers in summer. Harassment from nuisance flies may need to be addressed during summer, because fly activity negatively affects cow behavior. Uncomfortable cows can have a significant decrease in production, whether the cause is a lack of dry bedding or attack from flies.

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Production of stable flies (Stomoxys calcitrans) from sawdust compost barns and straw bedding packs, two alternative cold winter housing systems for dairy cows

Summary

Stable flies, *Stomoxys calcitrans* (L.), are important biting pests of dairy cattle and other livestock. Immature flies develop in decaying organic matter, such as soiled animal bedding. As part of a larger study of management options in organic dairy, we asked how leftover debris from two winter housing systems, outdoor straw packs and indoor sawdust compost barns differ in numbers and size of stable flies produced the following summer. This study was conducted at the University of Minnesota's West Central Research and Outreach Center in Morris. In winter 2013 and 2014, independently managed groups of ~20 cows were housed from November to May in replicated housing systems. After transfer to summer pasture, we assembled fly traps at leftover piles (n=4): emergence traps to quantify stable fly emergence, and Olson traps to study ambient adults. Beginning 2014, we measured size of emerged flies and 30 ambient adult females. Sampled females were also dissected to determine gonotrophic age. During peak emergence in both years, straw piles produced significantly more stable flies than compost bedding, but adults were equal in size. Olson traps showed adults were equally abundant at both sources, indicating that either eggs were not laid in compost or maggots did not survive. Over 60% of females dissected were previtellogenic, indicating local emergence. These results show that compost is useful in managing stable fly numbers, while straw presents a serious stable fly production liability if not disposed of properly.

Key words: stable fly, winter housing, compost, straw, stable fly management

Introduction

Cattle welfare and comfort are important considerations when selecting housing and bedding type, particularly in Minnesota, where cold and snowy winters are the norm. Deep bedded compost barns are a housing system that has been increasing in popularity. Dairy producers reported using these barns for increased cow comfort and longevity (Barberg et al., 2007). However, high building costs and environmental concerns have resulted in many farmers investigating options to keep cattle outdoors throughout winter (Barnes et al., 2013). In Ireland, cows housed outdoors on outwintering pads through winter showed no ill effect on production (O'Driscoll et al., 2009) or udder health (O'Driscoll et al., 2008). However, outwintering research is lacking in the Upper Midwest, where winters are much colder.

Stable flies are economically significant pests of dairy cattle and other livestock during summer, with national losses to the cattle industry exceeding $2 billion annually (Taylor et al., 2012). Adult flies are obligate blood feeders, whose painful bites annoy and stress cattle. Stress from biting flies negatively affects weight gain and milk production (Campbell et al., 2001; Bruce and Decker, 1958). Immature flies develop in decaying organic matter, including soiled animal bedding. Previous studies have shown that soiled straw calf bedding yielded significantly higher numbers of stable flies and house flies than wood chips or sawdust bedding (Schmidtmann et al., 1989). Leftover hay from bale feeders is also a suitable substrate for developing maggots (Taylor and Berkebile, 2011, Broce et. al, 2005). With several types of potential breeding sites, control of stable flies through source reduction can be difficult.

This study is part of a larger study to develop sustainable and profitable management strategies for dairy cattle, including winter housing and fly management. We compared stable fly risk from leftover debris of two winter bedding systems for organic dairy cows: outdoor straw packs and covered sawdust compost barns. Our objectives were to characterize ambient stable fly populations in summer around remaining debris from straw bedding piles and compost bedding piles, and to characterize stable fly populations that emerge from bedding debris piles

If piles are equally attractive, we would expect to observe similar numbers of ambient adults surrounding piles. Comparing numbers and sizes of flies emerging from piles provides an assessment of substrate quality. Based on results from previous studies, we hypothesized that when compared with compost piles, straw piles would produce higher numbers of larger flies.

Materials and Methods

Study Site and Weather

We worked with the certified organic dairy herd at West Central Research and Outreach Center (WCROC) in Morris, MN, where research in swine and dairy management, horticulture and renewable energy is conducted (Fig.1). Surrounding the WCROC are organic pastures for grazing and field crops. This site also includes a conventional dairy herd independently managed from the organic herd. Conventional cows were housed in a compost barn adjacent to the study barn during most of the year, and transferred to a dry lot for part of each summer. The research herd comprised of crossbred and Holstein dairy cows. Daily temperatures and precipitation were recorded at the onsite weather station from November, 2012 through October, 2014 and used to characterize weather from both winters and the following summer grazing seasons.

Experimental Design

Winter housing systems compared were indoor compost bedding packs and outdoor straw bedding packs. In November of 2012 and 2013, sites for both treatments were cleaned out and prepared to house cattle for the upcoming winter. Each treatment had two replicates in both years, which are referred to as “Straw 1, Straw 2” and “Compost 1, Compost 2”. A group of 21-22 cows, balanced by breed, parity and calving date, was randomly assigned to one of the four replicates in December of both years. Each group remained in their assigned housing except for twice daily milking, and was fed a TMR consisting of organic corn silage, alfalfa silage, organic dry alfalfa hay, organic expelled soybean meal, organic corn, and vitamins and minerals throughout winter.

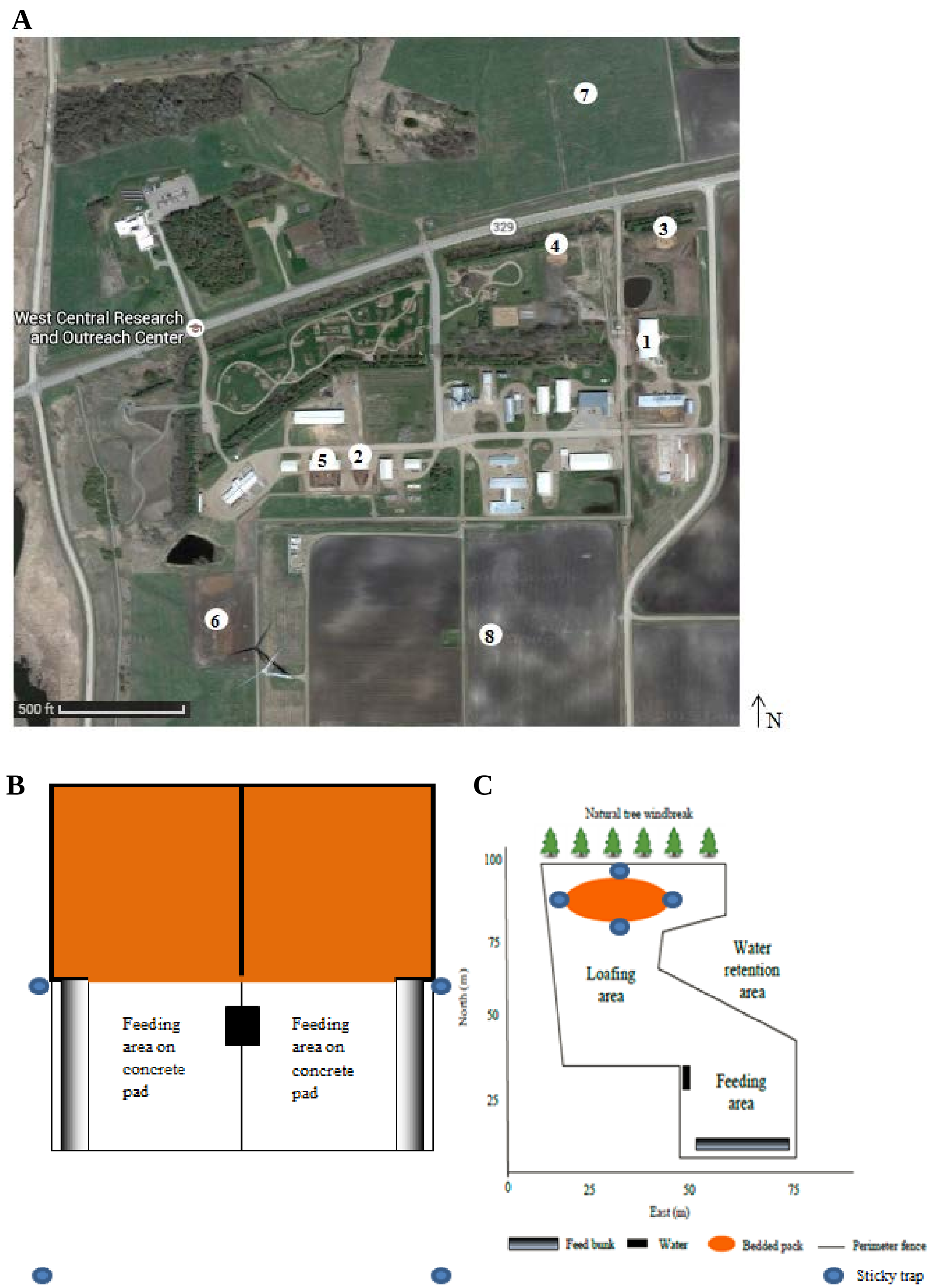


Figure 1- (A) Google satellite image of WCROC showing location and distance of : 1-milking parlor, 2- Compost barn, 3- Straw replicate 1, 4- Straw replicate 2, 5- Conventional dairy herd barn, 6- Conventional dairy herd dry lot, 7- organic pasture, 8- field crops. Diagram of (B) compost barn and (C) straw lot including locations of Olson traps.

Replicate straw piles were located ~100m apart, with each pile >100m away from the milking parlor (Fig. 1A). Roughly 1500kg of organic wheat straw was laid to create a pile measuring ~12 x 21m. New straw was added on top of old straw as needed to maintain a dry lying surface. Cows also had a loafing area to access feed and water. To the north of each straw lot was a natural tree windbreak (Fig.1C).

One existing open front compost barn ~385m from the milking parlor was divided in half to create two replicated barns with independently managed groups on each side (Fig.1B). Each barn measured ~9 x 12m. Immediately outside was a concrete floored loafing area where cows had access to feed and water. In December of each year, ~1600kg of new organic sawdust was laid to create a deep bedding pack. Compost was tilled twice each day during milking using a skid loader, and new sawdust was added as needed to maintain a dry lying surface. In February of each year, compost piles became too high to effectively till using the skid loader, necessitating removal of ~12,700kg of compost from barns. Removed material from each compost replicate was piled separately nearby and probed for maggots throughout summer.

After cows were moved to pasture May 28, 2013 and June 4, 2014, remaining straw piles were flagged and measured to calculate area and create a scaled map of each pile. Barn dimensions were used to create scaled maps of compost piles. These maps were used to indicate weekly trap assignments.

Ambient Adult Fly Population

After cows vacated their assigned housing, we assembled Olson biting fly traps (Fig. 2A) adjacent to each pile to measure ambient adult density and test the hypothesis that the two bedding pile types attracted different numbers of adult stable flies. A sheet of Alsynite fiberglass (66 x 30.5cm) was bent into a cylinder with a surface area of ~3000cm^2 and secured ~0.5m above ground with a wooden stake. Each cylinder was wrapped with a replaceable sticky sleeve, secured in place with a binder clip. Olson traps were placed at straw pile edges in all cardinal directions and diagonally from the compost barn. Locations of Olson traps in relation to debris piles are shown in Figure 1B and 1C.

In 2013, traps at one straw pile and both compost piles were assembled May 22 after cows were removed. Olson traps were assembled at the second straw pile on June 6, after cows were transferred to pasture. Each pile had four traps placed on compass points for a total of 12 traps. In summer 2014, traps were assembled April 24 to document when flies first became active. An additional four traps were placed 500-1000 away from leftover piles, to evaluate fly density at distant locations away from potential breeding sites. Two of the field traps were placed to the south of piles, and the remaining two were placed to the north. All sleeves were inspected on a regular basis and replaced as needed, once or twice weekly. Full sleeves were frozen until counted. In both years, sticky traps were maintained until the second week in October.

Figure 2- Types of traps used in this study: (A) Olson traps and (B) emergence traps with (C) collection container. Emergence traps had weekly random locations on each (D) replicate compost pile and (E) replicate straw pile.

To test the hypothesis that ambient adult females around debris piles differ in size and gonotrophic age, we began sampling female flies from Olson traps in summer 2014. Olson traps at all locations were inspected daily through the week until 30 adult females were sampled from all locations. Weeks when 30 females could not be sampled due to low catches, males were collected until head widths of 30 flies were measured. Sampled flies were transferred to the lab for measurement and dissection. Heads were removed, lined up occiput down on a petri dish and measured at the widest point using a dissecting microscope at 12X. Bodies of flies were saved for dissection. Females were dissected the day of collection in a 0.7% saline solution using methods outlined by Scholl (1980). Abdominal terga were gently pried apart using forceps to expose the ovaries. Ovaries were then inspected and scored as being previtellogenic (scored as 0 or 1) or vitellogenic (scored as 2 and above) based on presence of visible yolk in developing follicles.

Stable Flies Emerging from Piles

We placed funnel emergence traps (Fig. 2B) on piles to quantify numbers of stable flies emerging from remaining debris piles and test the hypothesis that the two bedding types would differ in numbers and sizes of flies emerged. Thirty traps were assembled on each pile after cows vacated their assigned housing. In both years, random sections of piles were probed to search for developing maggots with a trowel. Hauled compost debris was also probed for maggots through summer. In 2014, head widths of emerged flies were measured to examine substrate quality.

Each emergence trap was a 7.57L (2 gallon) plastic bucket (Leaktite, Leominster, MA) with bottom removed. A screen sided 1.89L (2 quart) funnel (Midwest Can, Melrose Park, IL) was inserted midway into the plastic bucket and capped with a 32oz (~0.95L) plastic deli container (Delitainer, Lake Forest, IL) to collect trapped flies (Fig. 2C). A plastic 1.25oz (~37ml) portion cup (Solo, Lake Forest, IL) with its bottom removed was glued inside a hole on the bottom of each deli container to help secure the container to the funnel and to facilitate quick removal and replacement. Each container included a laminated note card coated in a 50% Stikem Special (Seabright Laboratories, Emeryville, CA) spread to aid in trapping flies. Wooden stakes anchored bucket traps to straw piles in case of high winds (Fig. 2E). Traps in compost barns were sheltered by walls, as such wooden stakes were not needed (Fig. 2D). Total possible trap locations within each pile (Table 1) were used to extrapolate cumulative fly emergence from the entire pile in a given summer.

Emergence traps were randomly assigned locations on all piles in both years. All traps were inspected, emptied and assigned a new random location each week. Containers with flies were retrieved and frozen until flies could be counted. Emergence traps were initially assembled June 14, 2013 using stratified random sampling. Each pile contained 10 traps at the edge and 20 in the center to determine if more flies emerged from the pile's edge or center.

In summer 2014, all emergence traps were assembled using unrestricted random sampling (n=90). Thirty emergence traps were assembled in each straw pile May 13 shortly after the first observation of stable flies on sticky traps. Each compost barn contained 15 emergence traps in 2014, which were set May 28 after cows were moved to pasture. Traps could not be assembled until after cows were removed from their assigned housing. Emergence traps from compost barns were dismantled in the second week of August in both years after consecutive weeks of not trapping any stable flies. Straw pile traps were maintained until the second week of September of both years as stable flies continued emerging.

A vial of approximately 30 lab reared house fly pupae was also planted inside emergence traps in both summers to measure trap efficiency. These marker houseflies were easily distinguished from wild type flies. Sand was added to vials with a hole punched in the bottom in 2014 to protect pupae from weather and predators. Each week when emergence traps were reassembled, adult marker flies captured in deli containers and empty puparia inside vials were counted. These counts were used to calculate percent of emerged flies that were captured in overhead emergence traps.

To estimate egg to adult development time for stable flies developing in the debris pile, we created degree day models based on pile temperature. Temperatures within one of each kind of debris piles were recorded every 30 minutes in summer 2014 using 4 probe HOBO temperature loggers installed on May 29. Each probe was designated different locations within the pile. In straw, all four probes were assembled in the pile's center at varying depths from 2.5cm to ~100cm below the surface. In compost, we placed 2 probes at the pile's edge and the other 2 probes at the center to assess possible temperature differences. At both edge and center, probes were positioned ~75cm and 2.5cm below the surface. HOBOs were maintained on the pile until emergence traps were dismantled. Pack temperatures were not recorded during winter when cows inhabited the packs.

Statistical Analysis

All analyses began with a full repeated measures model with fixed effects of date and treatment and random effects of pile and trap within pile. Random effects accounting for less than 15% of total variation were removed and analyses proceeded only fixed effects. Log transformations were used as needed to satisfy analytical assumptions of normal distribution and equal variance. Residual plots were graphed and inspected to confirm these assumptions. Insignificant effects were removed to create a minimally sufficient model.

Ambient Adult Fly Populations

To analyze differences in ambient adult population of flies surrounding piles, we conducted a repeated measures nonlinear mixed effects analysis on log transformed data and tested for an interaction of treatment and date. Similarly, proportion vitellogenic and mean head widths of flies sampled in 2014 were compared by date and location to assess any differences between bedding types and if flies captured away from piles were significantly different than flies captured next to piles.

Stable Flies Emerging from Piles

In 2013, emergence traps were set using stratified random sampling. Raw catch rates reflected weekly catches, from which we calculated log transformed daily catch rates by taking the log of weekly catch rates (plus one) divided by the number of days that traps were assembled. Summer emergence was calculated using pooled means of catch rates from edge traps and center traps. After determining emergence was not significantly different between edge and center traps, emergence traps were set using unrestricted random sampling in summer 2014.

In both years, we used a repeated measures nonlinear mixed effects analysis on log transformed daily catch rate to assess differences in numbers of stable flies emerging by date and pile type. Size of emerged flies was compared by date and location using the same methods previously described for size of ambient adult flies.

Marker flies were analyzed with a repeated measures model with fixed effects of date and treatment and random effects of pile and trap within pile. To calculate trap efficiency, we divided the number of marker flies captured in the container by total emerged. After determining trap efficiency, we adjusted stable fly catches accordingly by dividing raw daily catch rates by matching estimates of trap efficiency. We extrapolated total numbers of stable flies emerging from each pile by multiplying the mean daily catch rate by number of possible trap locations within a pile. These numbers were used to estimate cumulative emergence over the summer and proportion of the total that had emerged at any given time.

We used degree day models to compare timing of emergence and capture of ambient adults on Olson traps by pile type in 2014. Degree days were calculated from temperatures recorded every 30 minutes in bedding piles. In both types of piles, one probe was selected for these calculations, based on where stable fly maggots were predicted to develop. In compost, the center probe 2.5cm below surface was chosen. In straw, temperatures from the probe 2.5cm below surface were used until mid-July, when we switched to the second shallowest probe, ~40cm below surface. This switch was made due to observations that the top layer of straw was drying out, which would have forced maggots to develop deeper within the pile. One generation was defined as 327.1 degree days with 7˚C as the lower developmental threshold and 34˚C as the upper developmental threshold (Moon, unpub). When HOBO loggers were not in place, pile temperatures were estimated based on records obtained from the weather station.

All statistical analyses were conducted using R version 3.0.1, using package "nlme" and "lme4".

Results

Study Site and Weather

Removed compost debris was piled within 50m of the compost barn and regularly probed for maggots in spring and summer of both years. Maggots were never found in removed debris in either year.

Cows were removed from straw bedding packs prior to pasture turnout due to muddy conditions from rainy weather. In 2013, cows from one replicate straw pile were removed on April 22, approximately one month prior to scheduled pasture transfer. In 2014, all cows in straw piles were removed April 23, approximately one month before cows were moved to pasture. Due to limited housing in both years, cows remained in compost until turn out to pasture. Total days that cows inhabited their respective housing systems are listed in Table 1.

Throughout both summers, the conventional dairy herd was independently housed in a compost barn less than 15m from the study barn. In August 2014, young cows were housed in the compost barns after emergence traps were dismantled due to limited housing space.

Average monthly temperatures during winter 2012-2013 were close to normal averages. However, with snow remaining until the second week of May and colder than average temperatures, the growing season was delayed. After spring, weather was warm through September. Two strong storms passed through, including strong winds and tennis-ball sized hail. Several emergence traps at both pile types were displaced or damaged from these storms, but were repaired and replaced in less than 24 hours.

In early November 2013, winter conditions returned with permanent snow cover on the 4th of December. Most snowfall during winter 2013-2014 occurred in December 2013. Mean winter temperature onsite ranged from -14.4˚C to 5˚C. Summer temperatures in 2014 were cooler than average. In both years, precipitation levels were comparable to long term averages.

Table 1- Summary of mean characteristics from replicate winter bedding systems in 2013 and 2014.

	2013		2014	
	Straw	**Compost**	**Straw**	**Compost**
Size (m)	12 x 21	9 x 12	12 x 22	9 x 12
Depth (m)	~1	~1	~1.2	~1
Moisture (%)	NA	NA	71.86	30.14
Temperature	NA	NA	27	34.5
Housing Start Date	12/11/2012	12/11/2012	12/3/2013	12/3/2013
Housing End Date	4/23/2013 (straw 2) 6/4/2013 (straw 1)	6/4/2013	4/23/2013	5/28/2014
Days in Housing	126 (straw 2) 168 (straw 1)	168	141	176
Total Possible Trap Locations	2,659 (straw 2) 2,267 (straw 1)	1198	2,976 (straw 2) 3,391 (straw 1)	1198

Pile Characteristics

In both years, both straw piles produced flies into September when emergence traps were dismantled. In contrast, compost piles were relatively dry. Few maggots were found during troweling and emerging stable fly adults were infrequently captured in emergence traps. In straw, pile surface temperature fluctuated with ambient air temperature, ranging from 15-37˚C. At lower depths, temperatures were more consistent at ~20^{o}C, but steadily increased above 30^{o}C as summer progressed (Fig. 3B). Compost temperatures remained hot throughout summer, ranging from 29^{o}C on the surface of the pile's edge, up to 53^{o}C deeper in the pile's center (Fig. 3A). Compost at the surface had more weather exposure, with greater variance in temperature than probes buried ~30cm below the surface. Further descriptions of pile characteristics in both years are shown in Table 1.

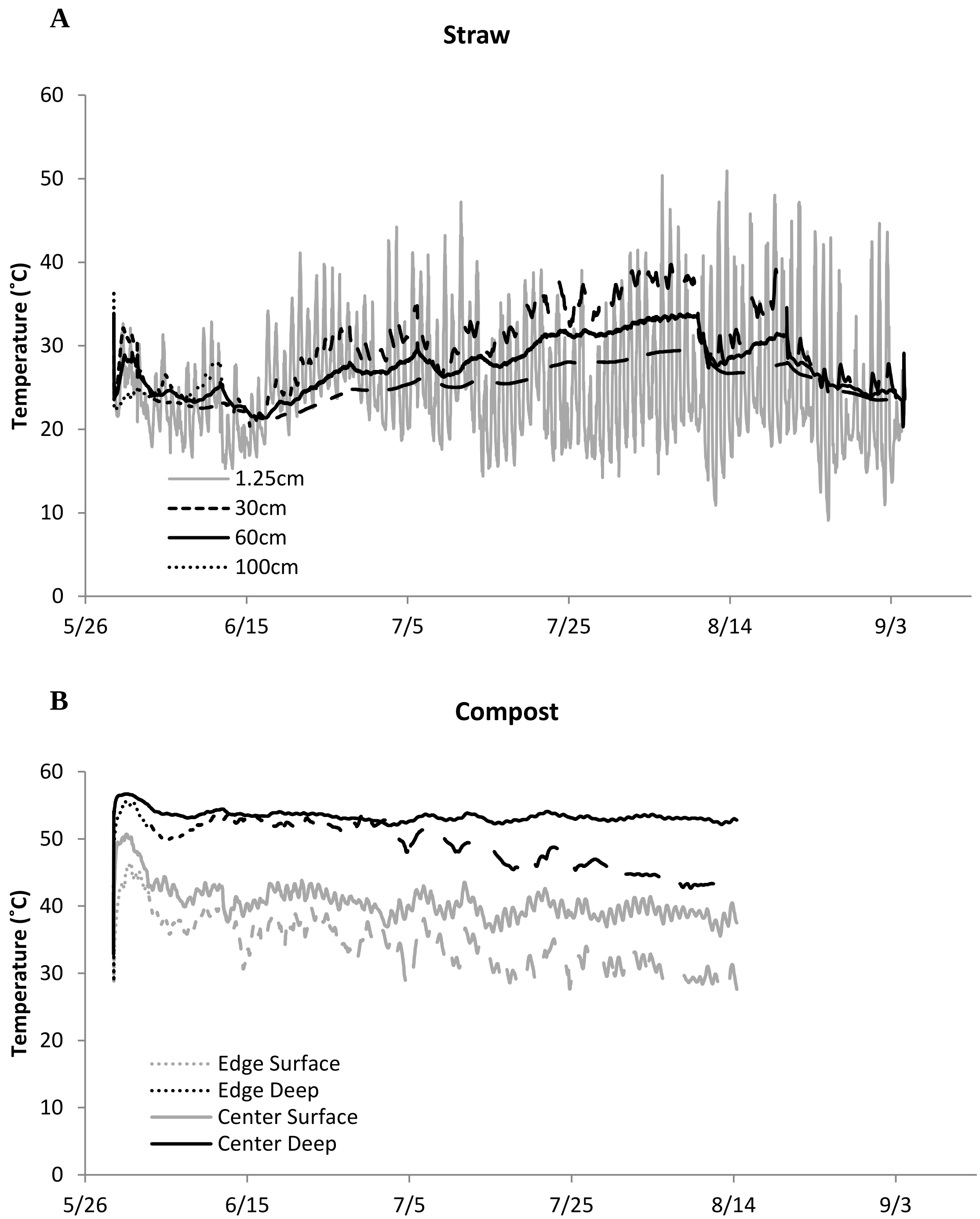


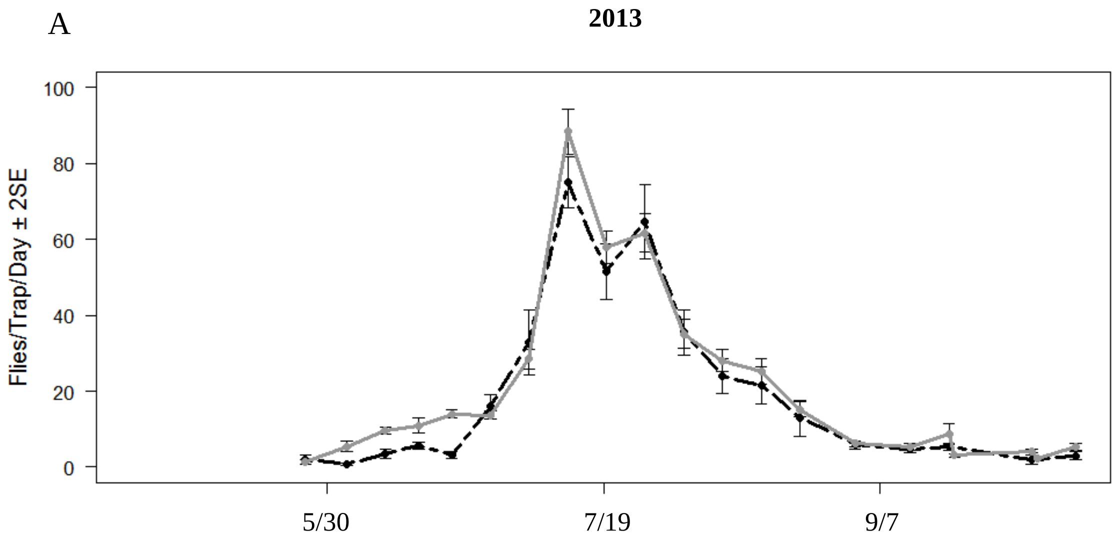
Figure 3- Pile temperatures recorded using 4-probe HOBOs with probes at varying depths in the (A) center of a straw pile and (B) edge vs. center temperatures in a compost barn in summer 2014.

Ambient Adult Fly Population

Stable flies became abundant at all locations where Olson traps were set. Flies were already active when traps were set May 22, 2013, with an average of 13 flies per trap per day (Fig. 4A). Catch rates were lower in compost than straw for the first five weeks of the study, and then compost became equal to straw after June 25. Numbers between compost and straw were similar, although we did detect significant differences over time (Table 2). Catch rates peaked in mid-July, with ~500 stable flies per trap before catches tapered off in August.

Traps in 2014 were set before stable flies became active. The first stable flies were captured in the third week, between 5/8 and 5/13. No flies were caught in field traps until mid-June, between 6/18 and 6/25. Numbers on field traps remained low until the first week of July (Fig. 4B). Catch rates peaked the week of 7/15, with ~ 450 flies per straw trap and ~300 flies per field trap. Captured flies at compost traps peaked the week before with ~ 300 flies per trap. All numbers tapered off in July and August. In the week of 8/25, catch rates at compost piles increased to ~200 flies per trap, while traps at straw and in the field captured ~100 flies (Fig. 4B). Field trap numbers were consistently lower than traps near breeding sources, except for late July through early August when numbers at all piles were more equal. Sticky sleeves were not changed as frequently in October 2014 as in 2013.

A



B

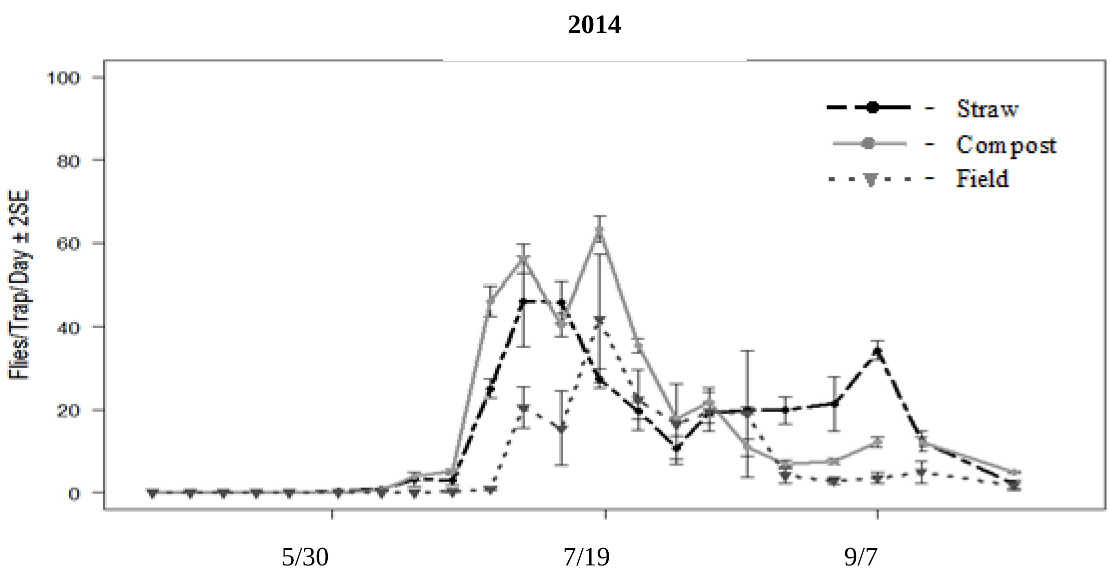


Figure 4- Mean number of stable flies captured per Olson trap per day by treatment in summer (A) 2013 and (B) 2014.

Numbers of immature flies in straw were higher than compost in the beginning of both years. More flies were captured throughout 2013, but catch rates in September and October 2014 were higher than those months in 2013. All Olson traps were dismantled mid-October in both years when fly numbers were declining, but still active.

In both years, random effects of pile and trap within pile accounted for less than 15% of total variation and were consequently removed from the model. We found a significant interaction between treatment and time in both years (Table 2), indicating that different numbers of flies were captured at pile types with differing patterns during the summer (Fig. 4). These differences were most apparent at the beginning and end of the season as incoming flies dispersed within the site.

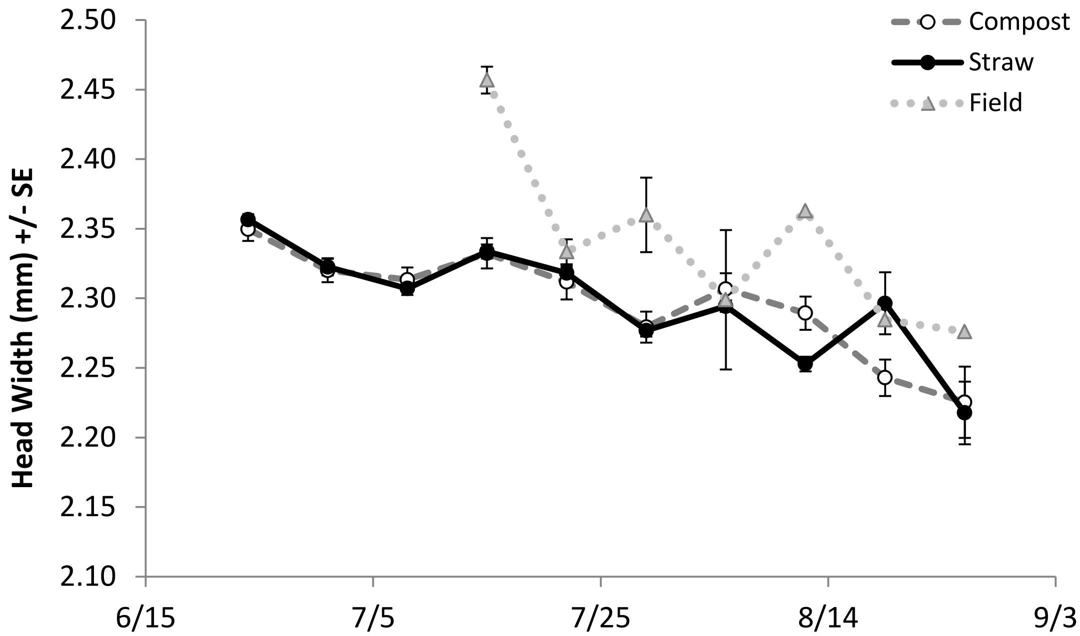
Table 2- Results from ANOVA examining variation in catch rates from Olson traps. In 2014, field traps were analyzed in addition to straw and compost piles.

	F	df	P value
2013			
Treatment	33.58	1, 144	<0.001
Week	40.79	18, 144	<0.001
Treatment:Week	2.5	18, 144	0.001
2014			
Treatment	122.54	2, 219	<0.001
Week	245.08	21, 219	<0.001
Treatment:Week	6.89	42, 219	<0.001

We were able to sample 30 females from each pile type the week of June 23 for head width measurement and dissection and continued sampling until September. Prior to this date, males were also measured to obtain a sample of 30 flies. On average, approximately one in six flies on Olson traps was female. Once 30 females were procured from each treatment, male flies were discarded.

Random effects of pile and trap within pile accounted for less than 15% of total variation in head widths, and so these effects were removed and analyses proceeded with fixed effects only. There was no significant interaction between treatment and week ($F= 1.03$; $df= 2, 888$; $P = 0.42$), and so the interaction was also removed from the model. Flies significantly differed in size over time ($F= 5.27$; $df = 9, 888$; $P<0.001$). Head width of flies sampled at the end of June from both straw and compost piles averaged 2.35mm in late June, and decreased to 2.22mm in August (Fig. 5). Mean head widths ranged between 2.46mm and 2.22mm (Fig. 5), with significant differences between treatments ($F= 7.23$; $df = 2, 888$; $P=0.001$). However, when analyzing a subset of data with head widths of flies sampled from only compost and straw piles, we found no significant difference in size ($P=0.62$). Flies sampled from field traps were larger than flies sampled from straw or compost piles (Fig. 5).

A



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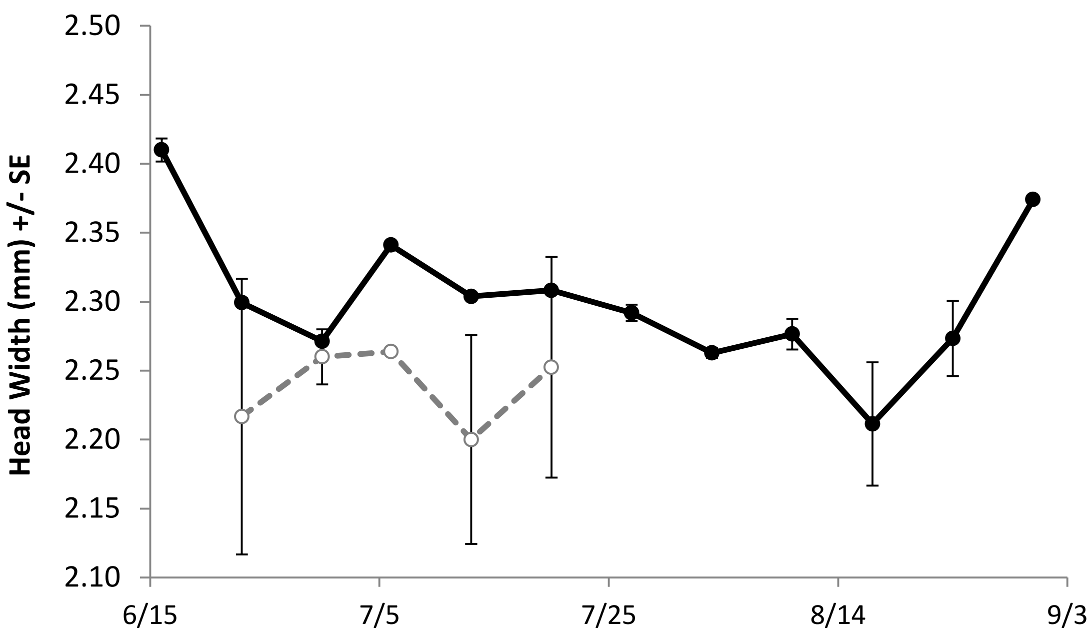

Figure 5- Mean head widths (mm) of adult female stable flies sampled in 2014 from: (A) Olson traps set at straw, compost, and at more distant field locations and (B) emergence traps set at straw and compost debris piles.

Of females dissected through summer, over 60% were previtellogenic, indicating recent emergence (Fig. 6). More vitellogenic females were captured at field traps than straw or compost piles.

In summary, flies became abundant at all trap locations as summer progressed. Catch rates at field traps in 2014 were lower than compost or straw locations until the first week of July. Fly numbers were decreasing when Olson traps were dismantled in both years, however flies were still active. Catch rates differed significantly over time between locations in both years, despite similar numbers in mid-summer. Flies were less abundant at compost piles than straw piles in the beginning and end of trapping. Flies trapped at straw and compost piles were not different in size, whereas flies captured at field traps were significantly larger. Mean head widths decreased from 2.35mm to 2.22mm as summer progressed. Of females sampled from straw and compost piles, ~80% scored as previtellogenic, in contrast to ~60% previtellogenic flies sampled from field traps. These numbers indicate that these females had only recently emerged.

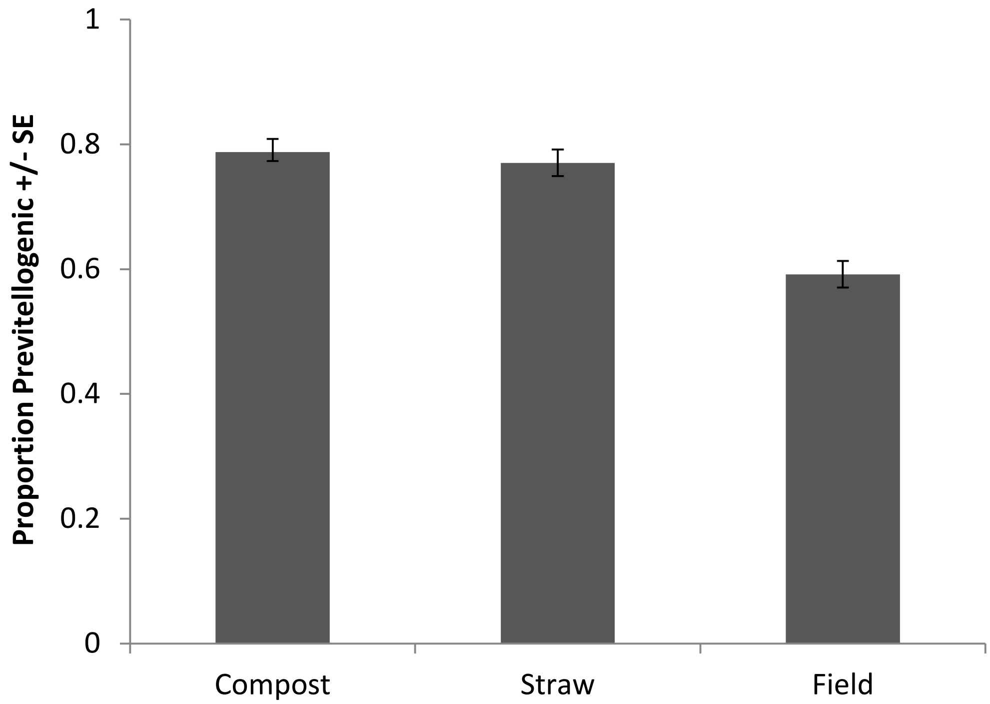

Figure 6- Proportion previtellogenic females (scored as 0 or 1 during dissection) sampled from Olson traps at three location types in summer 2014.

Stable Flies Emerging from Piles

In 2013, 120 emergence traps were assembled on leftover debris piles for 12 weeks on straw (6/14-9/6) and 10 weeks on compost (6/14-8/22). In 2014, 90 traps were set for 16 weeks on straw (5/13-9/4) and 11 weeks on compost (5/29-8/14). Emergence had not ceased at straw piles in early September, but only ~2 flies per pile were being captured when traps were dismantled. Stable flies were last captured in compost traps between 8/2 and 8/9 in 2013 and between 7/10 and 7/17 in 2014.

Marker flies were not available the last week of June in 2013 and so were not set in emergence traps (Fig. 7). Trap efficiency was highly variable at both types of piles, with proportion captured ranging from 0.39 – 0.56 at straw piles, and 0.30 - 0.66 at compost piles. In summer 2013, many marker flies either died inside vials, or failed to emerge due to water accumulation in vials. To eliminate these problems, each vial was filled with sand in 2014. After incorporating sand, virtually no marker flies died inside vials. Mean proportion of marker flies captured from compost piles had a notable increase from 0.48 in 2013 to 0.69 in 2014. In 2014, proportion captured ranged from 0.37 - 0.64 at straw piles, and 0.56 - 0.77 at compost piles.

Analyses of marker flies were conducted using a subset of data when traps were assembled at both straw and compost piles in order to compare catch rates between the two treatments. Random effects accounted for <15% of total variation in both years, so these effects were removed from the model and analyses proceeded with fixed effects only. Analyses began with a full model including treatment, time and interaction. In 2013, there was no interaction between treatment and time ($F= 0.29$; $df= 8, 18$; $P=0.96$)

and so the interaction was removed from the model. We found no significant difference in proportion marker flies captured at straw and compost piles ($F= 0.65$; $df= 1, 18$; $P=0.8$), nor any differences in proportion captured over time ($F= 0.74$; $df=8, 18$; $P=0.65$). On average, trap efficiency was 48%.

As in 2013, we found no significant interaction between treatment and time in 2014 ($F= 1.42$; $df =10, 22$; $P=0.24$). Consequently, this interaction was removed from the model. Proportion marker flies captured differed significantly between straw and compost piles ($F= 49.49$; $df= 1, 32$; $P<0.001$). 52% of marker flies were captured in the collection container of emergence traps set at straw piles, and 69% were captured in collection containers from compost. We also detected a significant change in proportion captured over time ($F= 2.39$; $df=10, 32$; $P=0.03$). More marker flies were captured at both pile types at the end of the season than the beginning (Fig. 7).

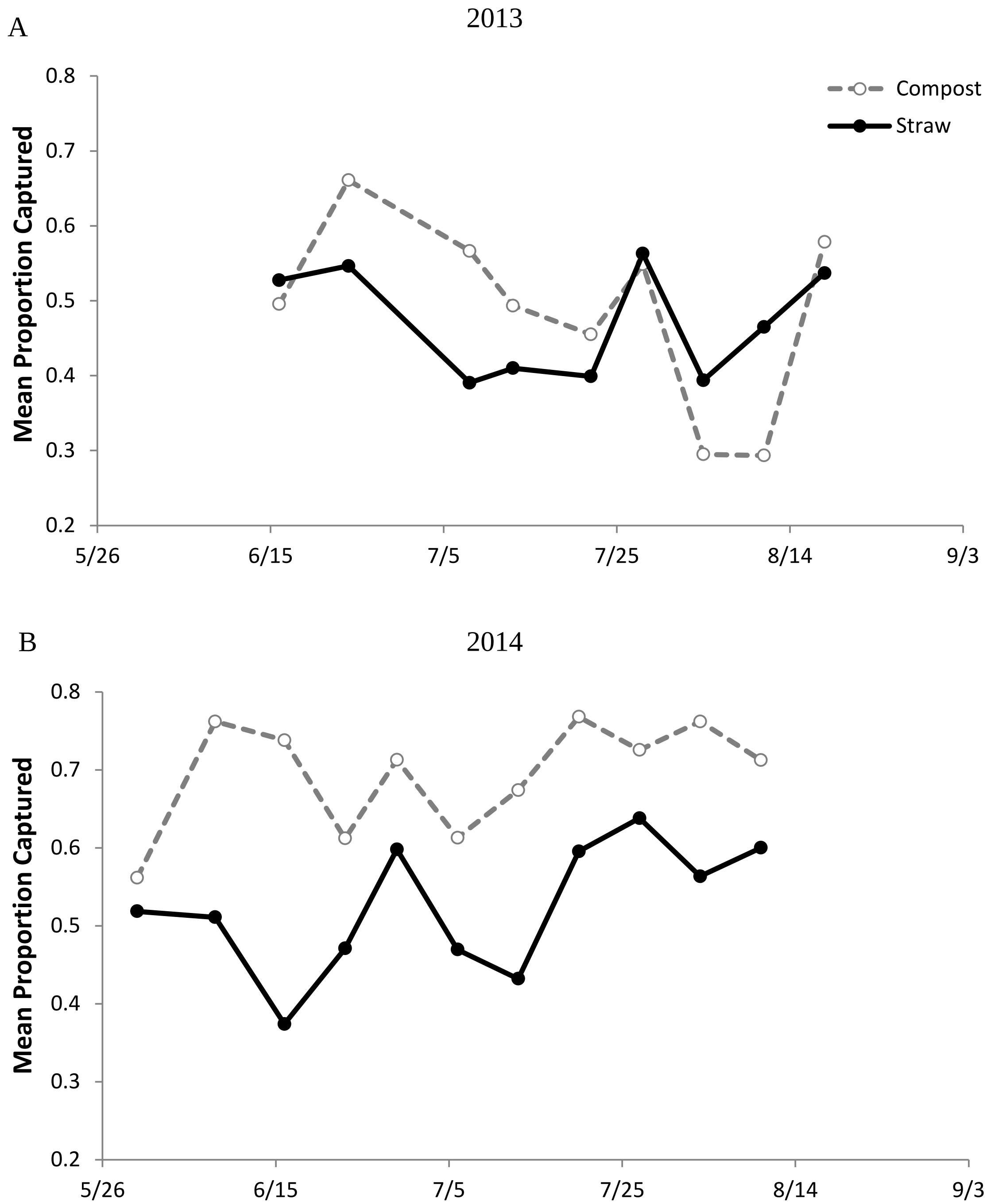

Figure 7– Weekly mean proportion of marker flies captured in straw and compost piles in (A) 2013 and (B) 2014.

We adjusted stable fly counts accordingly based on results from marker fly catch rates. Counts were adjusted upwards by dividing stable flies captured by proportion of marker flies captured. In 2013, there was no significant interaction between treatment and time in proportion marker flies captured, and so we used the same proportion, 0.48, to adjust stable fly counts. However, in 2014, we did detect a significant interaction between treatment and time and so the proportion used to adjust stable fly numbers was calculated separately for each week. All results regarding stable fly emergence from piles are adjusted catch rates from this analysis.

Stable flies were already emerging when emergence traps were placed in 2013, with peak emergence at Straw 1 in the week of June 22 (Fig. 8A). Weekly catches were used to calculate mean daily catch rate per emergence trap. Both straw piles produced higher numbers of stable flies than compost barns from late June to early August. Straw 1 was highly productive from mid-June to mid-July, with mean daily catch rates of 20-70 flies per trap (Fig. 8A). Straw 2 did not peak until the week of 7/21, with approximately 11 flies per day. Compost piles were much lower in production, averaging approximately 1-2 flies per day from 6/26-7/4. In the week of 8/4, mean daily catch rate at compost peaked again, averaging 2.5 flies. Stable flies that were captured from compost piles were typically from traps set at the front of the barn. In this area, the substrate was cooler and wetter due to accumulated rainwater.

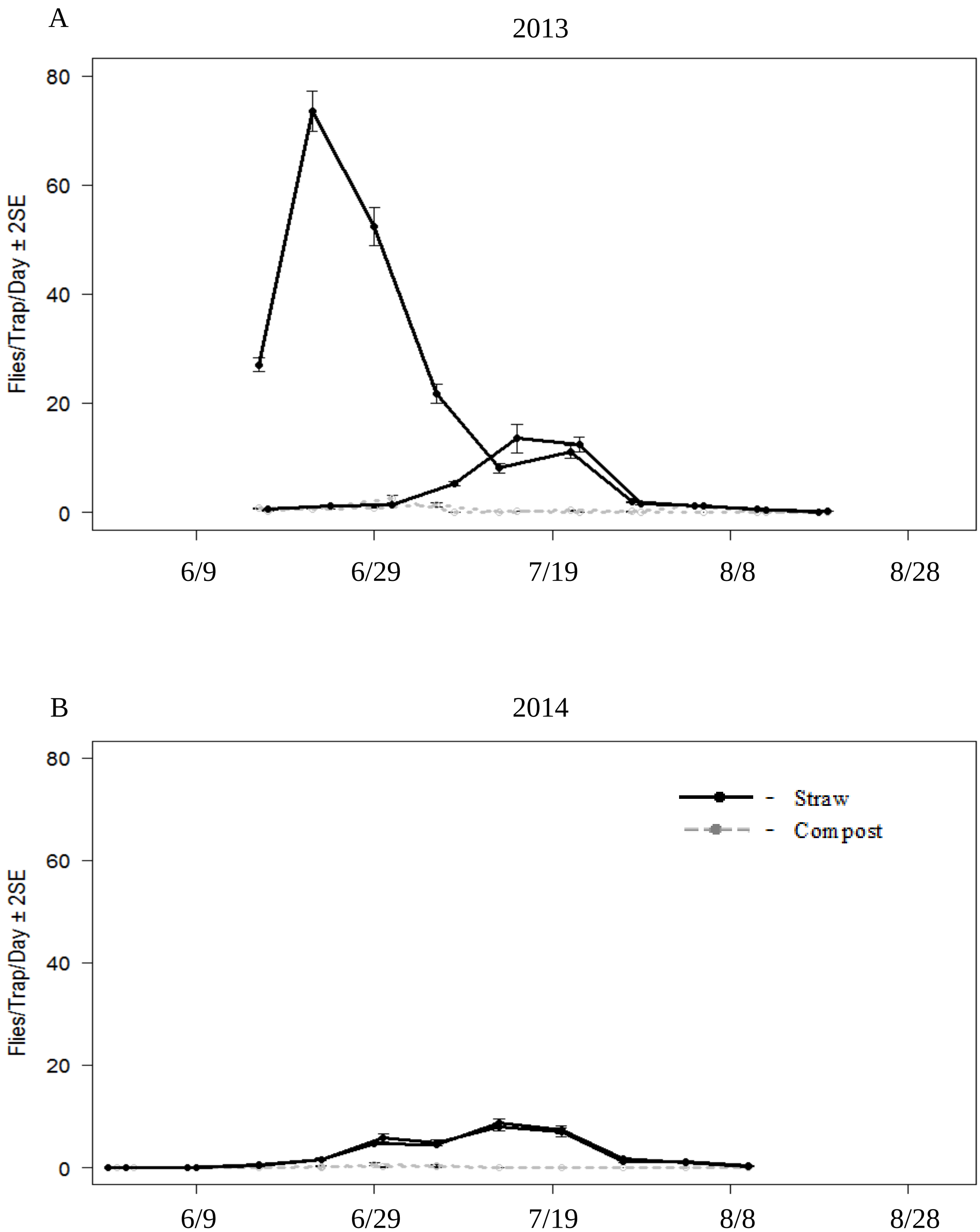

Figure 8– Mean numbers of stable fly adults captured per emergence trap per day in (A) 2013 and (B) 2014

In 2014, no flies were captured in emergence traps until the week of 6/13 (Fig. 8B). Both straw piles were very comparable in emergence rates, peaking the week of 7/10, with approximately 10 flies captured per trap per day and decreasing through August (Fig. 8B). Daily catch rate at compost piles were slightly lower than in 2013, with fewer than 1 fly captured per day consistently through summer.

We did not detect any significant differences in emergence between edge and center traps in either pile type when sampling was stratified in 2013 ($F = 3.49$; $df = 1, 88$; $P = 0.07$), and so we simplified our methods to unrestricted random sampling in 2014. When comparing stable fly emergence between compost and straw piles in 2013, we found that the random effect of pile accounted for 22% of total variation, so this effect was retained in the model. We detected a significant interaction between treatment and time (Table 3), indicating that emergence patterns over time differed between the two treatments. In 2014, random effects of pile and trap within pile were removed from the model after accounting for <1% of total variation. Again in this year, we detected a significant interaction between treatment and time (Table 3).

Table 3- Results from ANOVA examining variation in log transformed daily catch rate of stable flies captured in emergence traps set at straw and compost piles during summers 2013 and 2014.

	F	df	P value
2013			
Treatment	4.86	1, 1178	0.03
Week	26.46	9, 1178	<0.001
Treatment:Week	15.76	9, 1178	<0.001
2014			
Treatment	207.38	1, 968	<0.001
Week	40.98	10, 968	<0.001
Treatment:Week	14.07	10, 968	<0.001

Cumulative emergence from total straw and compost piles in both years was predicted using extrapolated numbers calculated from daily catch rates in emergence traps (Fig. 9). Based on daily catch rates, straw 1 alone produced over 120,000 stable flies in 2013 (Fig. 9A). Seasonal totals averaged approximately 69,000 flies per straw pile, about 46 times more than the average compost pile for the same number of cow-months the previous winter. Although emergence was overall lower in 2014 (Fig. 8), we still observed a large difference in cumulative emergence between compost and straw piles. Seasonal totals averaged approximately 45,000 flies per straw pile, over 70 times more than the average compost pile (Fig. 9B).

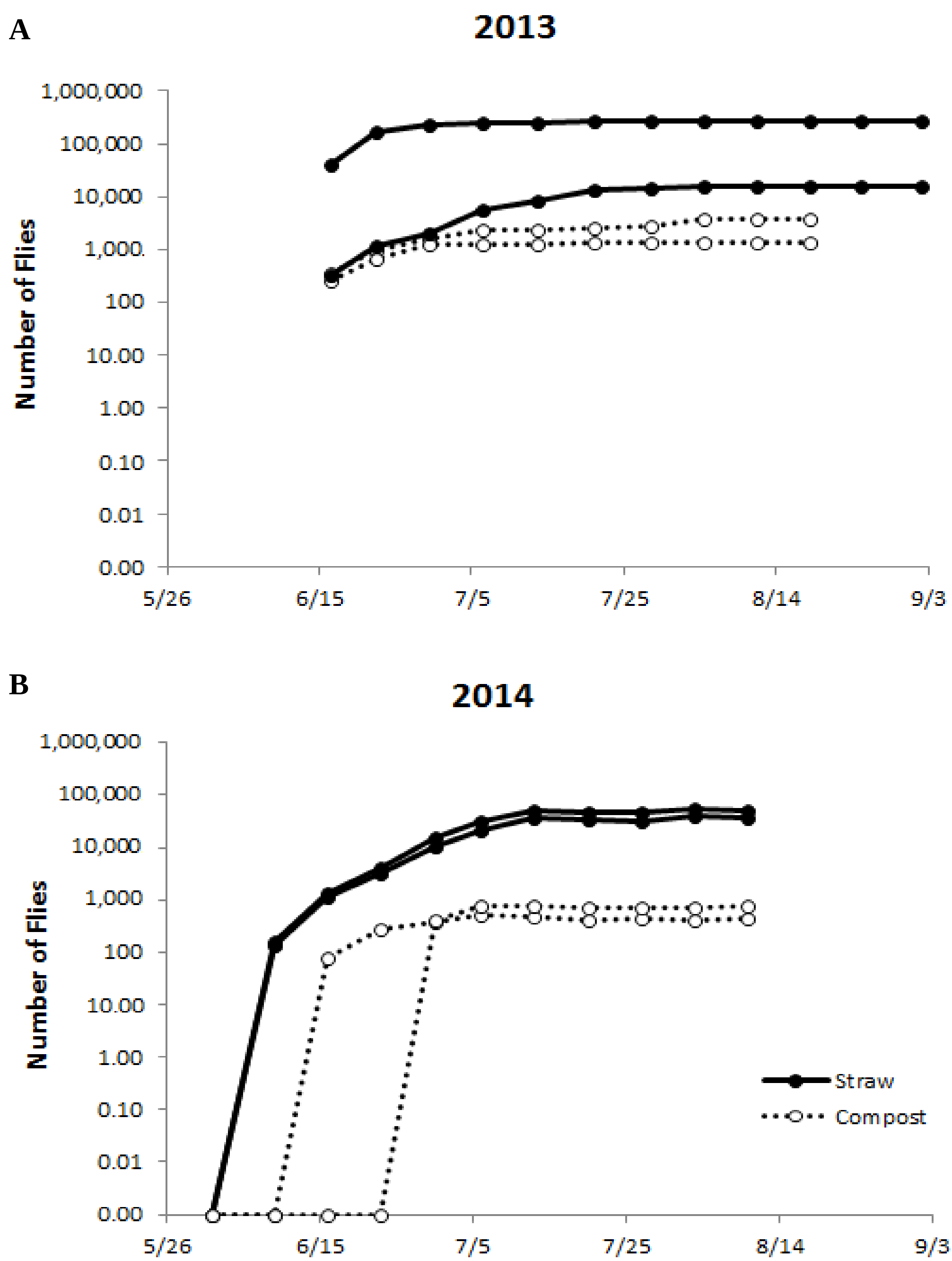

Figure 9– Extrapolated total emergence of stable flies from debris piles vs. end date of weekly trapping intervals in summer 2013 and 2014. Traps in 2013 were not assembled until after flies began emerging.

Head Width

Of the 16 weeks that emergence traps were assembled in 2014, stable flies were captured in compost piles only in weeks 6-10 (6/23-7/20). Less than 30 total stable flies were recovered from emergence traps in compost barns for measuring in summer 2014. Head widths of flies emerged from straw piles averaged 2.3mm (Fig. 5B). Mean head width of flies emerged from compost piles was 2.25mm. Random effects of pile and trap within pile were removed from the model after accounting for <15% of total variation. We detected no significant interaction between time and treatment (F= 0.09; df= 3, 403; P=0.96), and so this effect was subsequently removed. Treatment was also not significant (F= 2.07; df= 1, 403; P=0.15), indicating flies were equal in size, however, fly size did change significantly with time (F= 2.45; df=10, 403; P=0.008).

When analyzing a subset of data from weeks 6-10, we again detected no significant interaction between treatment and time (F= 0.12; df= 4, 236; P=0.97), or treatment (F= 3.24; df=1, 240; P= 0.07). Although not statistically significant, flies captured from straw piles tended to be larger than flies captured from compost piles (Fig. 5B). In analyzing this subset, we also did not detect a significant difference through time (F= 1.58; df=4, 240; P= 0.18).

To compare seasonal pattern of abundance in ambient populations of adults and local emergence from the debris piles, catch rates from Olson traps in summer 2014 were superposed on catch rates from emergence traps on a degree day scale (Fig. 10). The first stable flies were captured on Olson traps around straw piles in the second week of May, but no flies were captured in emergence traps on debris piles until the second week of

June, approximately one month after initial stable fly activity. Approximately 600 degree days passed between the first flies captured on Olson traps and the first flies captured in emergence traps set on straw piles. On compost, the first flies were captured in emergence traps 885 degree days after the first flies captured on Olson traps.

In summary, emergence rates were significantly higher at straw packs than compost packs between June and August of both years. In straw piles, emergence peaked in late June to early July and decreased through August into September. Stable fly catches per trap in compost piles were consistently close to zero through all of both summers. Head widths of flies emerging from straw piles were larger than flies emerging from compost piles, but these differences were not significant. When catches from Olson traps were superposed onto catches from emergence traps, we saw a difference of approximately 1 month, or degree days between first activity on Olson traps and catches in emergence traps, consistent with stable fly development time.

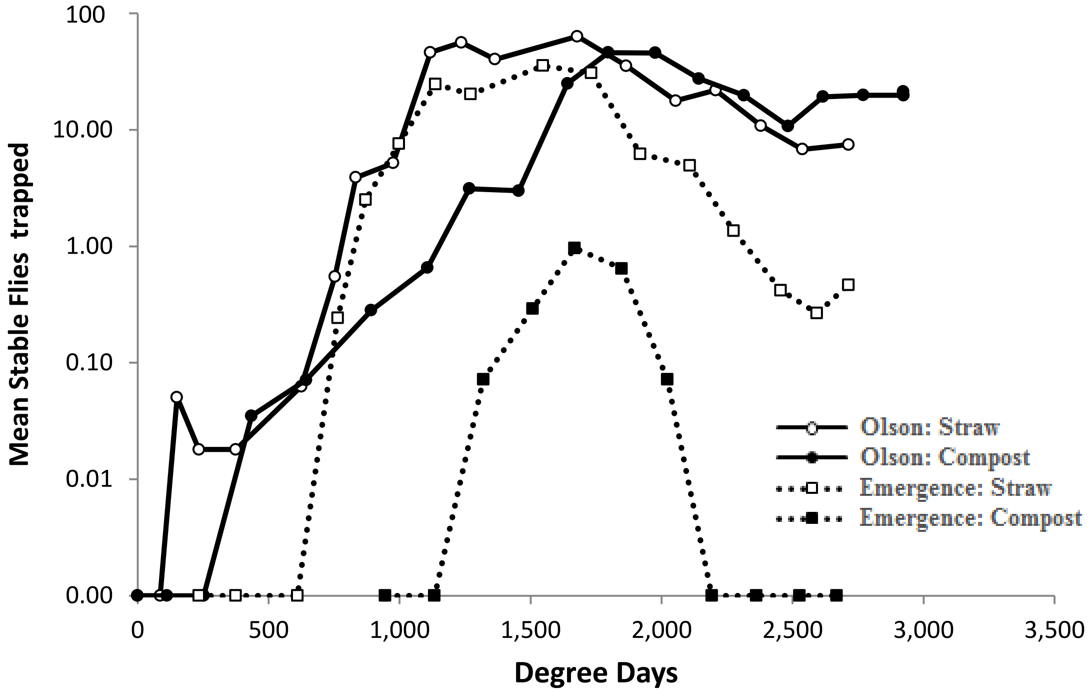

Figure 10– Number of stable flies captured daily on sticky and emergence traps at debris piles in summer 2014 as seen on a degree day scale. Degree days were calculated based on temperatures within debris piles.

Discussion

Ambient populations of adult stable flies were higher in 2013 than in 2014, though at different times of the season. Olson trap catch rates in October 2014 were higher than catch rates in October 2013. When left unattended, sleeves can become clogged with insects, dust and other debris and lose effectiveness at trapping stable flies. It is possible that flies were declining at a similar rate in both years, but sleeves were not changed frequently enough to observe the decrease. The decrease in head widths of flies sampled from Olson traps could indicate that substrate quality of developing maggots degraded at the end of summer compared with the beginning of summer.

Stable flies prefer to remain in the vicinity of hosts (Gersabeck and Merritt, 1985), but are strong fliers capable of flying several kilometers (Eddy et al., 1962, Bailey et al., 1973). Predictably, when cows are moved, stable flies will follow. In 2014, the numbers of flies captured on Olson traps near compost had decreased through July and increased again in August, a pattern not observed on Olson traps set near straw or away in the field, nor in catch rates from 2013. In early August, just prior to the observed increase, the conventional dairy herd was transferred from their summer dry lot back to a deep bedded compost barn adjacent to the study barn. With a herd of cows now <10m away from set Olson traps, an increased catch of flies is sensible. Therefore, it is possible that flies were not equally attracted to all piles and Olson trap catches appeared similar between piles because cows were housed close to compost debris. In future studies, ensuring that no cows are housed close to any leftover debris would help clarify if flies are equally attracted to both pile types for oviposition. Had no cows been present at compost, it is possible that Olson trap counts would have been lower.

It is possible for developing stable flies to overwinter in piled silage or manure (Berkebile et al., 1994) as a pile's internal temperature gradient could protect immatures from freezing (Berry et al., 1978). To ensure that previous piles were not an overwintering source for stable flies, all winter bedding packs were hauled away in fall and new packs were laid. As such, any flies emerging from piles had to have developed from eggs laid that spring by ambient females. We observed significantly higher numbers of flies emerging from straw piles compared to compost piles, yet ambient adult density was similar between types of piles. These results indicate that all piles were equally attractive to adult flies. In 2014, flies captured on Olson traps in the beginning and end of the season likely were flies from elsewhere that were drawn to piles. The first stable flies in 2014 occurred on Olson traps approximately one month before any flies were captured in emergence traps on debris piles, which is consistent with stable fly development time. However, the number of degree days that passed after flies were first captured on Olson traps was approximately two times longer than the generation definition of 327.1 degree days. These results imply that if the first flies on site oviposited on debris piles, maggots took longer than expected to develop. However, females were either not ovipositing in compost piles or maggots did not survive to adulthood. As summer progressed, catch rates on Olson traps remained higher as emergence traps decreased. Differences in seasonal patterns of catch rates between sticky and emergence traps imply that stable flies have other breeding sites. Decreasing emergence from straw toward the end of summer indicated that straw piles became less suitable for developing maggots as summer progressed.

Overall differences in emergence rates between straw and compost are likely due to a number of factors. Our results are comparable to results from Schmidtmann (1989), who demonstrated that straw bedding in calf hutches produced significantly more stable flies than wood chip bedding, and this difference could be attributed to microbial content, temperature and moisture content. Little research has been done on stable fly oviposition behavior, including microbial composition in larval development substrates (Romero et al., 2006) and maggot development is dependent on live bacterial colonies within the substrate (Talley et al., 2009). Compost piles in Morris were consistently above 40˚C below the surface, temperatures too hot to support developing maggots.

Differences in emergence between straw piles were especially apparent in the beginning of summer 2013. Heavy rain and muddy conditions necessitated the removal of cows from Straw 2 one month before cows from Straw 1. As a result, Straw 1 had an additional months' worth of accumulated soiled debris, which could have increased pile attractiveness to ovipositing females. Additionally, cows were still being housed in Straw 1 when stable flies first became active. Flies could have initially been drawn to the pile in search of hosts and aggregated due to the close proximity of hosts, mates and a suitable oviposition site. To ensure equal treatment of piles the following year, all cows were removed from both straw piles at the same time. Emergence rates between straw piles were comparable in summer 2014 and similar to emergence from Straw 2 in 2013, supporting the hypotheses described.

Beginning June 25, 2013, a herd of heifers were housed in the concrete loafing area outside the compost barn while emergence traps were set with a fence blocking entrance into the barn. However, on July 1, the herd broke into the compost barn during the night and was not discovered until the next morning. All emergence traps were destroyed and significant amounts of fresh manure was added to both piles. Afterwards, in the first week of August, more stable flies were captured than what was trapped in the previous month. Average development time from egg to adult is approximately one month, indicating that females oviposited shortly after the break in and maggots were able to survive to adulthood. No cows breached compost barns when emergence traps were assembled in 2014 and we did not observe an increase in catch rate during August 2014 as seen in 2013. As such, we can conclude that the addition of fresh urine and manure on compost at that time either supported maggots to adulthood, or attracted additional females to oviposit.

In both years, 70% of the seasonal total of stable flies had emerged from debris piles by the end of June and 90% of captured flies had emerged by the end of July (Table 4). Seasonal totals overall were lower in 2014 possibly from having a longer and colder winter.

Table 4- Stable fly production from leftover debris piles in summers 2013 and 2014, with hypothetical cleanout dates, which would have prevented further fly production.

Cleanout date	**No. flies. produced to date**	**Percent flies eliminated by cleanout**
<u>2013</u>		
6/16	9,816	86
6/22	41,788	40
6/29	55,191	21
7/13	64,127	8
7/21	67,292	3
8/04	69,287	1
<u>2014</u>		
6/16	507	98
6/23	1856	93
6/30	8124	70
7/13	19,213	30
7/20	23,828	13
8/04	26,708	3

The present findings are significant to dairy herd management for producers selecting housing for cows during winter months. Cows performed equally well in terms of milk production, dry matter intake and body condition scores when housed in both systems. Initial costs for housing cows in compost barns could be greater than overwintering on a straw bedding pack if a new barn must first be constructed. However, cows housed outdoors during winter consume up to 30% more feed and so additional feed cost must be taken into consideration when selecting winter housing. Producers having difficulty with stable flies during summer should consider sawdust compost bedding over winter, as compost piles produced 98% fewer flies than straw. Dairy herds housed on sawdust compost bedding during winter are likely to experience fewer stable flies the following summer, depending on how much additional fly breeding substrates are available on site and proximity of neighboring dairies. In contrast, straw bedding presents a much more serious liability for stable flies if not disposed of properly in summer, particularly if cows are still present on straw when flies become active. If straw bedding is used during winter, we recommend hauling and spreading debris by June 1st to minimize maggot development.

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Defensive behaviors of pastured dairy cattle in response to three species of muscid flies, stable flies, horn flies and face flies

Summary

Two groups of 17 organic crossbred dairy cows were observed on pasture six times per week from June to August 2014 in Morris, MN for defensive behaviors in response to three species of muscid flies. Counts of stable flies (*Stomoxys calcitrans* (L.)), horn flies (*Haematobia irritans* (L.)), and face flies (*Musca autumnalis* DeGeer) were recorded before and after observation. Individual cows were monitored for 5 minute intervals to observe frequencies of 5 different defensive behaviors: front and back leg stomps, head tosses, skin twitches and tail swishes. Fly numbers averaged 5 stable flies per leg, 37 horn flies per side and 1 face fly per face during the study. Fly counts and behavior frequencies both increased with ambient temperature. Results showed a very strong relationship between numbers of flies and numbers of defensive behaviors ($p<0.0001$), though correlations between specific flies and behaviors were low ($r^2 <0.3$). We found younger cows usually hosted fewer stable flies and horn flies than older cows. Absence of relations between fly numbers and daily milk yields indicate injury thresholds for pastured organic dairy cows were greater than 5 for stable flies, 37 for horn flies and 1 for face flies.

Key words: stable fly, horn fly, face fly, behavior, pasture, milk production

Introduction

Cattle have a myriad of associated parasites, including several species of flies. Hart (1990) described how vertebrate behavior could have evolved to increase fitness against parasites such as flies. Defensive behaviors in response to flies not only interrupt grazing, but can increase energy costs of grazing (Dougherty et al., 1993). Stress from flies can lead to serious production losses in feed efficiency, weight gain and milk production. The most important pest flies in dairy are muscid flies, including stable flies, horn flies, and face flies. Future references to muscid flies refer to these three species.

Stable flies (*Stomoxys calcitrans* (L.)) are blood feeding flies typically found on the legs of cattle. These flies were long considered pests of confined cattle, but the introduction of round bale hay feeders has resulted in increased populations on pastured cattle (Broce et al., 2005). Horn flies, (*Haematobia irritans* (L.)) are another blood feeding species, which are most often found on backs, sides or bellies of cattle. Horn flies feed multiple times per day and spend almost all of the adult stage on their host. Heavily infested animals can host several thousand horn flies at any given moment. The face flies (*Musca autumnalis* DeGeer) is a nonbiting fly that feeds on liquid secretions, typically around the eyes and muzzle of cows. These flies cause irritation and they can vector eye inhabiting parasites and pathogens.

Nuisance flies and defensive behaviors exhibited by cows in response to flies are easily observed interactions. Previous studies have focused on how cow behavior is affected by a single species of fly. Dougherty et al. (1993a, 1993b, 1994, 1995) released starved laboratory raised stable flies on grazing beef cattle to observe behavioral responses to fly feeding behavior. Mullens et al. (2006) documented stable flies on dairy cows in a feedlot throughout the fly season to assess relationships between stable flies, defensive behaviors and milk production. Also examined were the effects of face flies on cattle behavior, individually and as a herd (Schmidtmann, 1985a, Schmidtmann, 1985b, Schmidtmann and Valla, 1982, Dougherty et al., 1993). All of these studies showed that pest flies can increase frequencies of defensive behaviors, and many concluded that production was negatively affected by fly infestations. However, results showing the extent to which production is reduced is variable. For example, Bruce and Decker (1958) found that stable flies suppressed milk production well past the end of the fly season, while Mullens et al. (2006) was unable to detect effects on milk production.

With many fly species associated with cattle, attributing a species with a specific behavior can be difficult when observing free roaming cattle in pasture. Behaviors are easier to observe from a distance than flies, and so knowing the relationship between defensive behaviors and fly numbers can be a useful tool to producers. Rather than counting flies, a producer could potentially observe cows for a brief period to estimate if fly management is necessary to maintain production. There are several objectives in this study. The first objective was to examine muscid fly populations on two groups of pastured cattle. Fly counts were then compared with observed behavior frequencies to understand how cows respond when attacked by multiple fly species simultaneously. Fly counts and defensive behaviors were then compared with electronically recorded milk weights, to assess associations among fly counts, frequencies of behavior and milk production.

Materials and Methods

Study Site

This study was conducted with the certified organic dairy herd at the University of Minnesota's West Central Research and Outreach Center (WCROC) in Morris during summer 2014. The site housed independently managed herds of organic and conventional crossbred and Holstein cattle. Pastures suitable for grazing surrounded the milking parlor. We worked with 2 independently managed groups of certified organic crossbred dairy cows. Each group consisted of 17 cows, balanced by breed, parity and production.

Cows were turned out to pasture on May 28 and remained on pasture throughout summer except while being milked. Groups grazed primarily cool season grasses, including smooth bromegrass (*Bromus inermis)*, red clover (*Trifolium pretense)*, white clover (*Trifolium repens),* meadow fescue (*Festuca pratensis)*, perennial ryegrass (*Lolium perenne)* and alfalfa (*Medicago sativa)*. Observation days occurred three times each week with cows being observed twice within that day, except during extreme weather conditions. The study began June 5 and concluded on August 15. Cows were milked in a swing-9 para-bone milking parlor at 06:00 and 17:00. Milk weights were electronically recorded for each cow at every milking.

Weather records were obtained from the WCROC weather station to assess associations of temperature, wind speed, relative humidity and/or precipitation during observation periods with fly counts and behaviors. Temperatures were recorded during all observation periods, as well as the observation day's minimum, maximum and mean temperature.

Fly Counts and Defensive Behaviors

Both groups of cows were observed during summer to measure fly abundance and concurrent frequencies of defensive behaviors. Cows were observed between 9:00 and 11:00, and again between 13:00 and 15:00. Two observers were used during each period so both groups could be observed simultaneously. Individual cows were identified with numbered ear tags. Cows were observed from a distance of 1-2m to allow for accurate fly counts without disturbing the cow's natural behaviors.

An observer would approach an individual focal cow as available, then count and record the number of muscid flies present on the animal. Stable flies were counted separately on front and back legs. Leg counts were defined as number of flies visible from brisket to hoof when viewed from a single angle where both legs are visible (Taylor et al., 2012, Berry et al., 1983). Horn flies were counted along one side, from back and withers to the belly. Face flies were counted on faces, viewed head on.

After counting flies, the focal cow was observed for five minutes to tally defensive behaviors. A stopwatch was used to keep track of time, and behaviors were tally marked on a data sheet. Behaviors recorded were head throws, front leg stamps, back leg stamps, skin twitches and tail flicks using definitions found in Mullens et al. (2006) and Dougherty et al. (1993):

- Head throw: nose crosses transverse plane at front of chest on the observer's side
- Front or back leg stamp: either front or back leg lifts enough to clear ground while animal is not walking
- Skin twitch: skin ripple ~2 seconds or more (duration not recorded)
- Tail flick: tail tip moves forward enough to cross imaginary plane across rear of animal

After five minutes, flies were counted again, and then pre- and post-observation counts were averaged to characterize abundance during the observation period. These processes were repeated until all cows were observed. Observations were compared with the next day's recorded milk production, presuming that any stress effects would be observed the following day.

Statistical Analysis

We used analysis of covariance to examine variation in numbers of counted flies, observed behavior frequencies, and milk production. For each response variable, summary statistics were examined, followed by an examination of variation in relation to fixed and random effects. We began with a full repeated measures model, using fixed effects of observer, date, fly species, parity and interactions, and random effects of group and cow within group. Random effects that accounted for less than 15% of total variation were removed to conduct a simple analysis of covariance with just fixed effects.

Appropriate 4- or 3-way interaction terms were used in denominations to conduct conservative F-tests. Insignificant fixed effects and interaction terms were removed to create a minimally sufficient model. Interaction plots were constructed to evaluate the nature and magnitude of interactions. If an interaction was small in magnitude, then it was considered non-significant and removed to further simplify the model.

Added variable plots were used to graphically examine the relationships between response variables of interest and continuous predictors after adjusting for other factors in the chosen model. Diagnostic plots were created to check analytical assumptions of the final model. Log transformations of fly counts and behavior frequencies were used as needed to satisfy analytical assumptions of equal variance and normal distribution in errors. Graphical inspection of residual plots confirmed these assumptions.

All statistical analyses were conducted using R 3.0.2, with packages “lme4”, “nlme” and “car”.

Variation in Fly Counts

We hypothesized that more flies would be observed in the afternoon than in the morning, and that counts would increase with temperature and humidity. We began with a linear mixed effects model with observer, date, parity, fly species, time of day (morning vs. afternoon) and time (start vs. end count) as fixed effects. Temperature, relative humidity, wind speed and precipitation were included as covariates in the initial model and group and cow within group were included as random effects.

Analyses using the same methods were also performed for individual fly species. In the case of stable flies, location (forelegs vs. hindlegs) was added as a fixed effect to determine if more stable flies were found on front or back legs.

Variation in Behavior Frequencies

To test the hypothesis that behavior frequencies increase with fly counts, we compared counts of all five behaviors combined with counts of all three fly species combined. After detecting a significant interaction between time and observer, fly counts were adjusted to account for observer differences when analyzing behavior frequencies. We began with a linear mixed effects model with observer, date, parity and fly species as fixed effects. Temperature, relative humidity, wind speed and precipitation were also included as fixed covariates with group and cow within group as random effects. A polynomial term for fly count was also included in the model to test for curvilinearity, or saturation. We hypothesized that we would observe a positive curvilinear relationship, in that behavior frequencies would increase with fly count to an extent, but not indefinitely.

Observation days were grouped into three time periods at the beginning, middle and end of the study to test the hypothesis that cows became habituated to fly activity. The previously described analysis was repeated, replacing date with time period. We hypothesized that if cows were becoming habituated to flies, there would be more defensive behaviors in the first time period at the beginning of the study, and fewer behaviors in the third time period.

These analyses were also performed for individual fly species to determine if any given species was a cause of greater irritation. In the case of stable flies, counts from front and back legs were added together to remove the effect of location, our rationalization being that stable fly presence causes irritation regardless of location. Analyses were then repeated for individual behaviors, beginning with a full linear mixed effects model and simplifying when possible.

Milk Production

To test the hypothesis that milk production decreased with increasing fly counts and behavior frequencies, we compared electronically recorded milk weights from the day after each observation day with fly counts and behaviors observed the day before. Milk weights from the morning and afternoon milking were added together to calculate daily milk production. We examined associations between parity, days in milk (DIM), fly counts, fly species, behaviors and temperature on the next day's recorded milk production, presuming that stress effects would be observed the following day.

Results

The study occurred between June 5 and August 15, 2014 and included 27 observation days, with over 3,000 fly counts and 1,500 behavior tallies. The first observation day on June 5 included observer training for primary observers and alternates, and so was removed from analyses. Through the first week of July, both groups were transferred to different pasture due to limited forage. Observations did not take place during that time and resumed July 10 when cows were moved back to their original pastures. One afternoon observation period in July was postponed due to severe lightning. A subset of data including observation days with only primary observers was analyzed after detecting a significant difference between observers.

Temperatures ranged from 11˚C to 29˚C, with a summer average of 19.7˚C (Fig. 1A). Mean temperature was 19.7˚C during morning observations and 23˚C during afternoon observations. Humidity ranged between 59 and 85% and average daily wind speed ranged from 3 - 34 km/hr (Fig.1B). Daily precipitation ranged from 0-5mm, with the exception of 4 days, where precipitation ranged from 13-24mm in a day (Fig. 1C). Weather trends were overall consistent with 10 year averages at the research site.

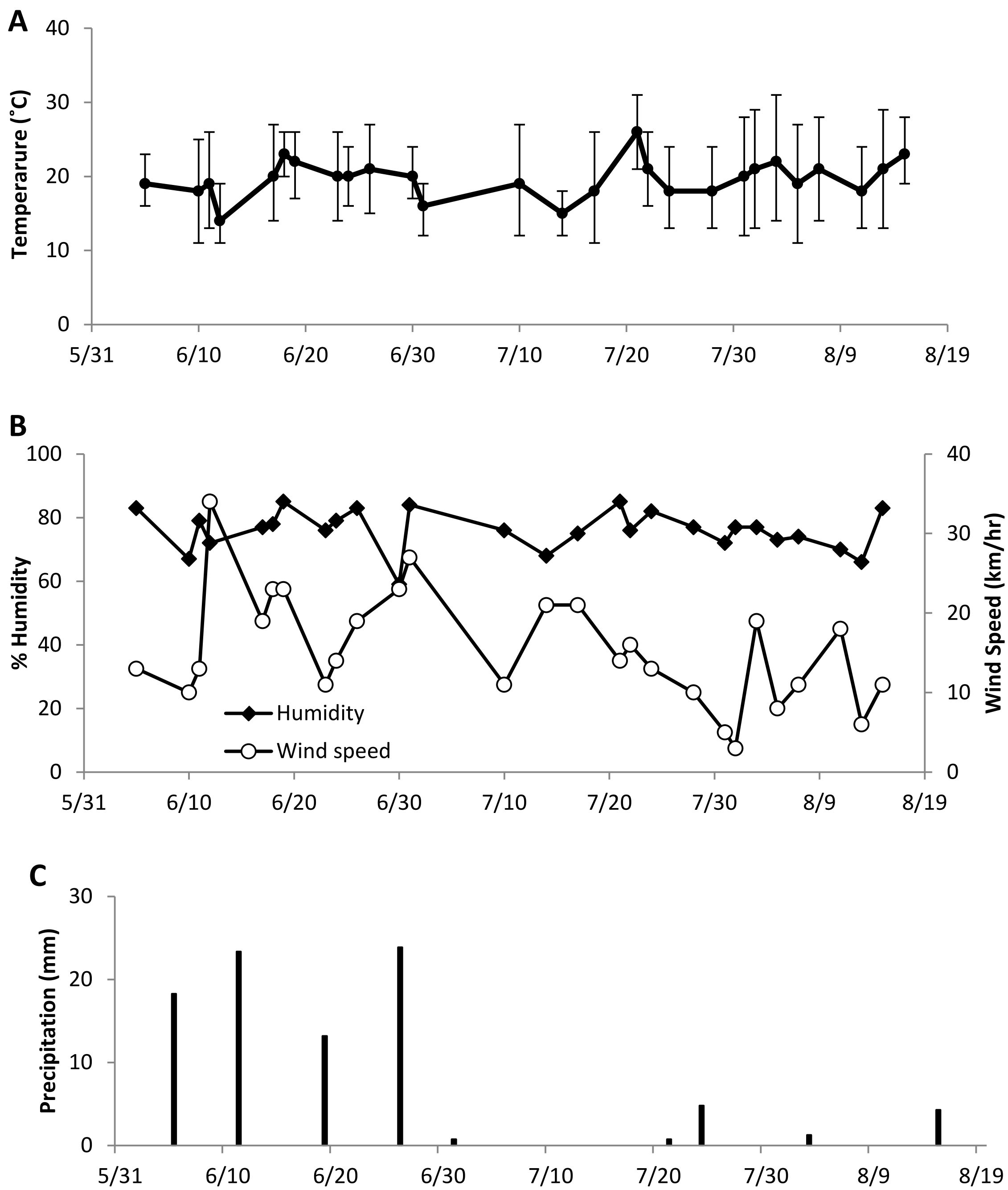

Figure 1- Weather conditions in Morris, MN during summer 2014 on 27 group observation days.

(A) Mean temperature with daily minimum and maximum temperature shown in error bars.

(B) Daily average relative humidity and wind speed. (C) Daily total precipitation.

Variation in Fly Counts

We began with a comparison of total fly counts of all species combined with various weather and animal variables to examine any associations. Formal analyses of total combined fly counts indicated that random effects of group and cow within group accounted for less than 15% of total variation. Consequently, these effects were removed, and analysis proceeded with fixed effects only. We noted a significant interaction between observer and date, which indicated that observers counted different numbers of flies as the study progressed (Fig. 2A). Interaction plots showed that Observer 1 typically recorded more flies than Observer 2 except for the last six observation periods (Fig. 2A). Differences in daily means between observers were usually between 0 and 6 flies.

Fly counts were lower on July 14 (Fig. 3A). Temperatures were cooler at 12˚C in the morning and 16˚C in the afternoon, with average winds of 21 km/hr and 68% humidity. Similarly, stable fly counts were noticeably low on June 12 (Fig. 2B, 2C), with temperature at 10.5˚C and 16˚C in the morning and afternoon respectively, winds averaging 34 km/hr and 72% humidity.

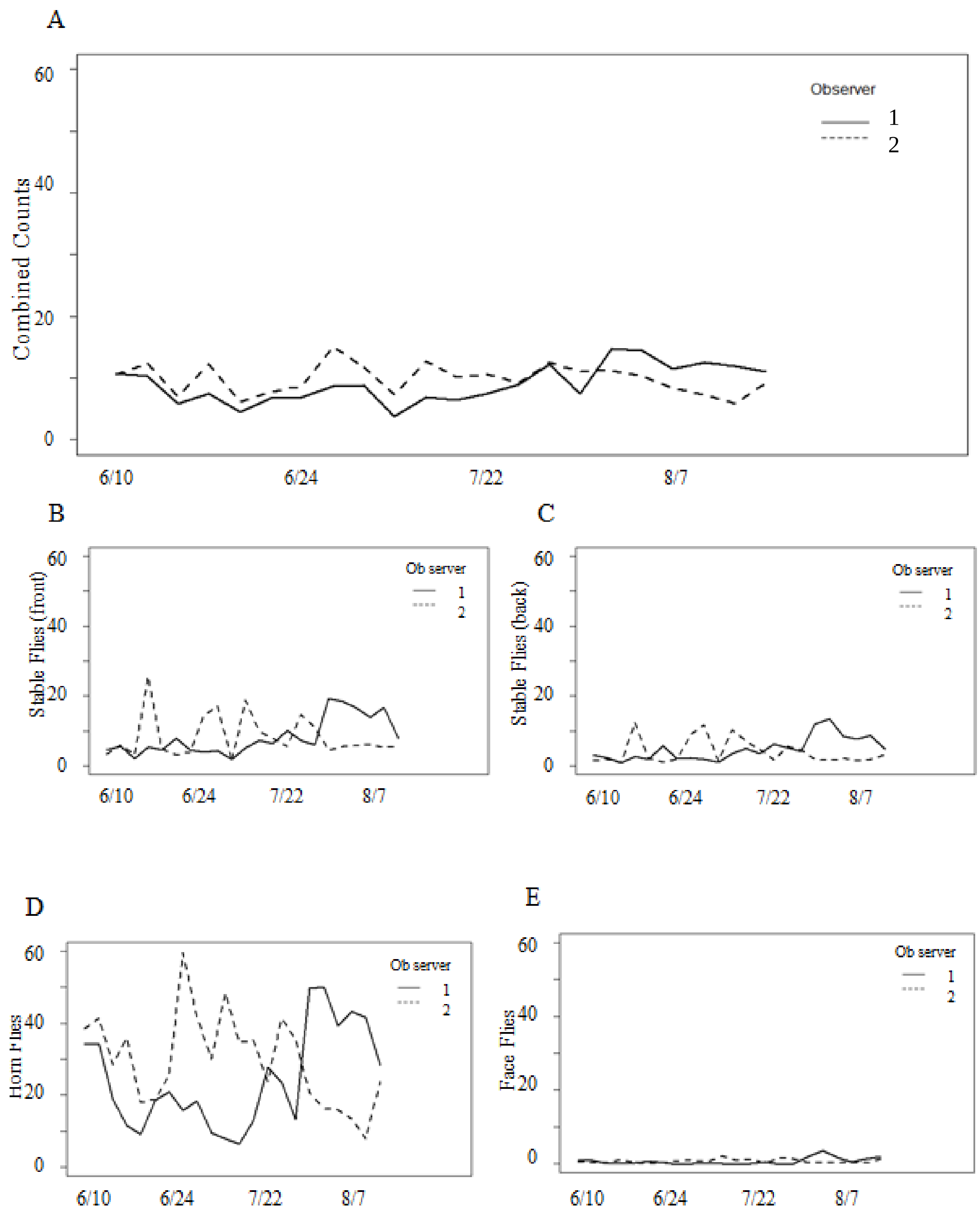

Figure 2- Daily average fly counts by date and observer: (A) combined counts of all three species, (B) stable flies on front legs, and (C) back legs, (D) horn flies per side and (E) face flies per face. Each mean represents 8 observations on 17 cows from the morning and afternoon.

Total combined fly counts covaried significantly with temperature, precipitation, and wind speed (Table 1), but were independent of relative humidity (F=1.4; df= 1, 84; P=0.24). Humidity was subsequently removed from the model. Fly counts were slightly higher in the afternoons, though this difference was not statistically significant (F= 0.97; df= 1, 84; P=0.33), and so fly counts were calculated as daily average for use in further analysis. There were no significant differences in fly counts before or after the observation period (F= 0.014; df= 1, 84; P=0.91). Consequently, the effect of time was removed from the model and counts adjusted for observer were used in subsequent analyses.

Combined fly counts were variable with parity, with no distinct pattern, despite statistically significant differences in fly loads for cows of different parity (Fig. 3A). Between mid to late June and early to mid-August, cows in their second lactation hosted on average, an additional 1-5 flies than other cows. By fly species, differences were approximately 5-15 additional horn flies for cows in their second lactation through most of the study (Fig. 3C), and approximately 1-4 additional stable flies for cows in their second lactation during mid-August (Fig. 3B). Significant interactions between species and parity, as well as species and date, indicated a need to analyze variation in counts for individual fly species (Table 1)

Table 1- Results from ANOVA examining variation in combined total counts of three muscid fly species

	F	df	P-value
Temperature	1218.8	1, 84	<0.001
Wind Speed	6.9	1, 84	0.01
Precipitation	5.0	1, 84	0.03
Species	16509	2, 84	<0.001
Parity	23.4	2, 84	<0.001
Observer	3.9	1, 84	0.05
Date	479.6	21, 84	<0.001
Observer: Date	283.7	21, 84	<0.001
Species: Parity	17.0	4, 84	<0.001
Species: Observer	177.38	2, 84	<0.001
Species: Date	936.4	42, 84	<0.001

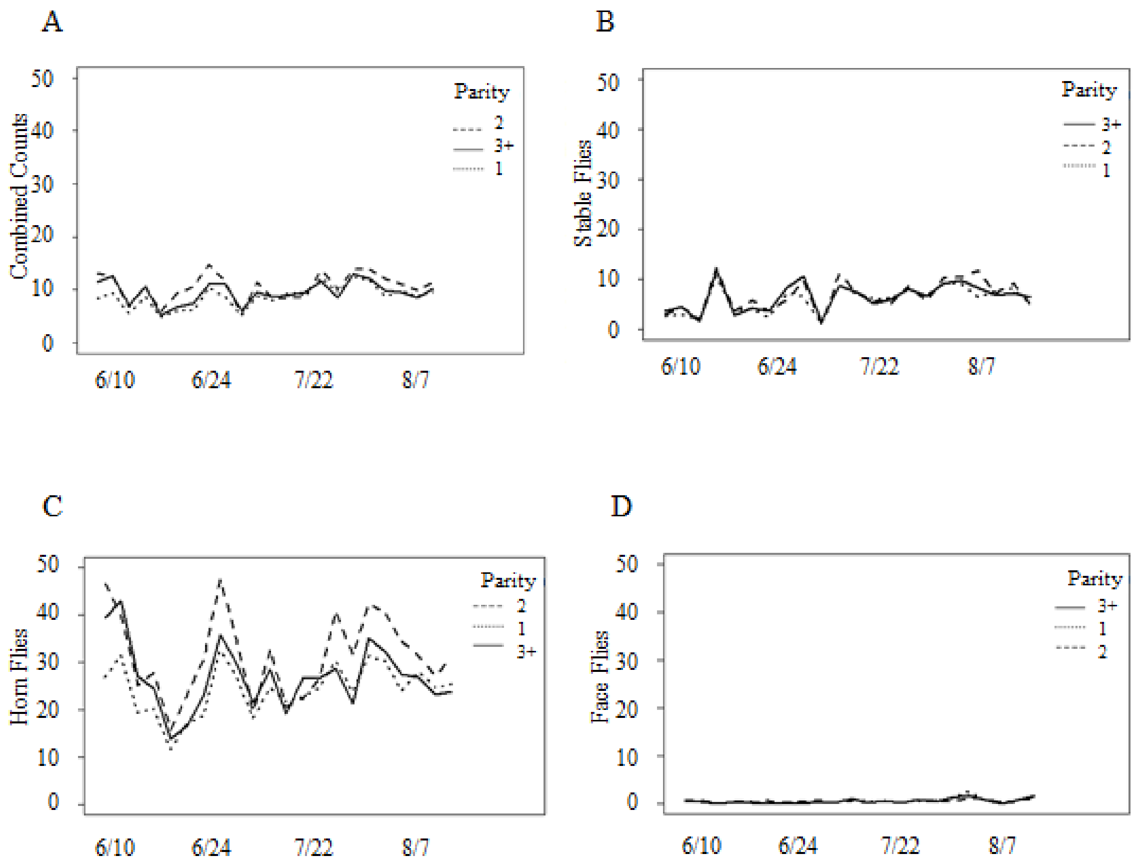

Figure 3- (A) Combined counts of three muscid fly species by cow parity (1st, 2nd, and 3+ lactations), (B) stable fly, (C) horn fly, and (D) face fly.

Stable fly activity began in the first full week of May, approximately one month before the study began. Stable flies averaged 3 flies per leg and gradually increased in numbers over the summer (Fig. 2A, 2B). Analyses of stable fly counts indicated that daily average on front legs ranged from ~1-15 flies and ~1-7 flies on back legs throughout the study (Fig. 4). We consistently observed a significant difference of approximately twice as many stable flies on front legs than back legs throughout the study (Table 2). Counts differed significantly with parity (Table 2), with cows in their first lactation hosting fewer flies throughout most of the study (Fig. 3B). We noted that counts significantly increased with temperature and precipitation, but decreased with humidity (Table 2). Wind was not significant to stable fly count ($F = 0.29$; $df = 1, 42$; $P = 0.59$). Examination of added variable plots indicated that these relationships were weak (not shown). There was no significant difference in counts from the morning and afternoon ($F = 0.62$; $df = 1, 42$; $P = 0.43$), nor in start vs. end count ($F = 0.51$; $df = 1, 42$; $P = 0.47$), so those effects were removed.

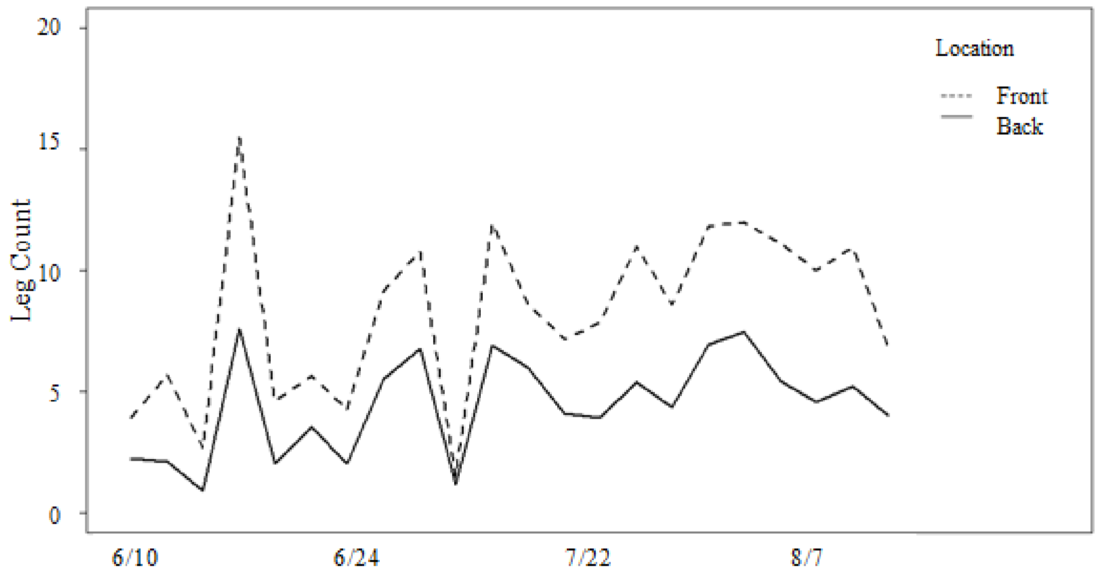

Figure 4 – Mean number of stable flies counted on front and back legs in summer 2014.

Daily average counts of horn flies ranged from 0 to 150 per side. Through the duration of the study, cows hosted a mean of approximately 35 horn flies (Fig. 2C), with an observed increase in fly counts as temperature increased. In the range of temperatures recorded during this study, we observed an increase of 1.2 horn flies with every 2˚ increase in temperature. Parity was a statistically significant factor for horn fly counts (F= 38.91; df=2, 42; P <0.001), with fewer flies on first lactation cows for most of the study (Fig. 3C). Counts decreased with increasing wind speeds and precipitation, but increased with humidity (Table 2). However, examination of added variable plots indicated that these relationships are weak. We observed more horn flies in the afternoon than in the morning, but these differences were not significant (F= 2.14; df=1, 42; P =0.08). Horn fly counts were the same before and after behavior observation (F= 0.36; df=1, 42; P = 0.55). Consequently, effects of morning vs. afternoon and start vs. end were removed from the model.

Analyses of face fly counts showed that very few face flies were observed throughout the study. At any given observation period, only one or two flies were counted on all animals in the group, resulting in a mean count of <1 face fly per cow (Fig. 2D). Toward the end of the study, there was a slight increase in face fly counts, averaging 1 fly per cow. Counts were significantly related to all weather variables (Table 2). Face fly counts also increased with temperature, though not as much as stable flies or horn flies. Added variable plots showed a slight decrease in face fly counts with increasing humidity and increase in counts with increasing wind speeds and precipitation. However, when adjusted for other factors, the relationships between weather components and face fly counts were weak. We detected no significant differences in start vs. end count ($F= 0.18$; $df=1, 42$; $P = 0.67$), nor in morning vs. afternoon counts ($F= 3.3$; $df= 1, 42$; $P= 0.08$), so these effects were removed from the model. Cows harbored the same number of face flies regardless of parity ($F= 0.37$; $df=2, 42$; $P= 0.69$).

In summary, fly counts varied throughout the study, with significant differences between observer and date. Horn flies were the most frequently observed species, with more flies on second lactation cows than other parity states. Stable flies had the second highest counts, with more flies on front legs than back legs. The least frequently observed species were face flies, with fewer than one fly per cow on average. Cows in their first lactation hosted fewer horn flies and stable flies through most of the study. Relationships between weather components and fly counts were weak when adjusted for other factors, but we observed an increase in counts of all species with increasing temperature.

Table 2– Results from ANOVA examining variation in log transformed counts of three species of muscid flies in summer 2014.

	F*	DF	Coefficient	SE
Stable Flies				
Temperature (˚C)	3035.0	1, 42	0.15	0.004
Precipitation (mm)	71.1	1, 42	2.92	0.60
Humidity (%RH)	64.6	1, 42	-0.75	0.16
Location (front legs)	984.5	1, 42	0.75	0.02
Observer (GP)	177.7	1, 42	0.44	0.12
Date	57.5	21, 42	NA	NA
Parity (2)	9.8	2, 42	0.23	0.14
Parity (3+)		2, 42	0.02	0.12
Observer*Date	23.4	21, 42	NA	NA
Parity*Date	1.9	42, 42	NA	NA
Horn Flies				
Temperature	81.2	1, 42	0.04	0.01
Precipitation	106.0	1, 42	10.20	1.84
Humidity	21.7	1, 42	-2.83	0.51
Wind	83.7	1, 42	1.00	0.18
Observer (2)	182.3	1, 42	-0.08	0.13
Date	30.5	21, 42	NA	NA
Parity (2)	38.7	2, 42	0.70	0.16
Parity (3+)			0.43	0.15
Observer*Date	34.0	21, 42	NA	NA
Parity*Date	2.2	42, 42	NA	NA
Face Flies				
Temperature	128.6	1, 42	0.02	0.00
Precipitation	13.2	1, 42	-1.90	0.99
Humidity	16.0	1, 42	0.56	0.28
Wind	46.1	1, 42	-0.21	0.09
Observer (2)	18.1	1, 42	0.09	0.1
Date	22.2	21, 42	NA	NA
Observer*Date	10.6	21, 42	NA	NA

* P-values for all F-tests were <0.001

Variation in Behavior Frequencies

Analyses began with all behaviors added together to create a count for total behaviors per observation period and compared with observer adjusted fly counts of a given species. Because random effects of group and cow within group accounted for less than 15% of total variation, these effects were removed, and analysis proceeded with fixed effects only. Frequencies of behaviors observed during the study were highly variable; some observation periods passed without observing any defensive behaviors, whereas tallies were 4-5 times greater than the seasonal means in other periods (Table 3). Skin twitches were the most frequently observed defensive behavior, followed by tail flicks, front leg stamps, back leg stamps and head throws (Table 3). A significant interaction between observer and date indicated that observers counted different numbers of behaviors as the study progressed (Fig. 5).

Table 3- Summary statistics of tallied defensive behaviors

Behavior	Mean	Median	Maximum	SD
Skin Twitch	16	10.5	84	9.0
Tail Flick	9.3	7.0	46	7.2
Front Leg Stamp	4.7	3.0	50	5.9
Head Throw	4.0	2.8	17	2.6
Back Leg Stamp	2.0	2.0	30	3.6

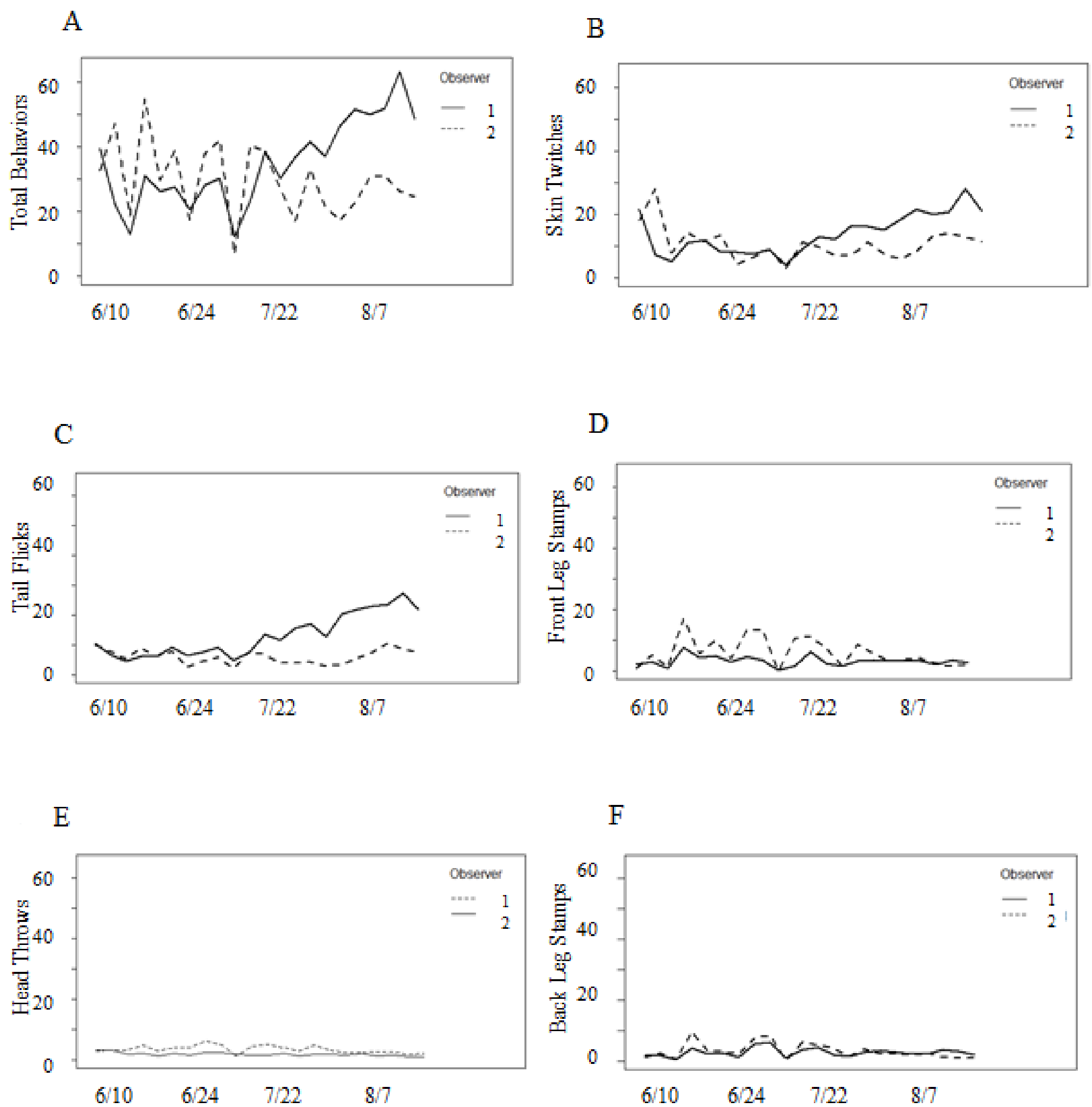

Figure 5- Mean behavior tallies by observer: (A) all behaviors combined, (B) head throws, (C) skin twitches, (D) tail flicks, (E) front leg stamps and (F) back leg stamps

There was a very strong positive correlation between frequencies of defensive behaviors and adjusted fly counts, in that behaviors increased with fly count (Fig. 6). Of temperature, humidity, wind and precipitation, only temperature was significant to total behavior tallies (Table 4). Stable flies and horn flies were both highly associated with total behavior responses (Table 3), while face flies were not ($F = 0.39$; $df = 1, 99$; $P = 0.53$). Behavior frequencies were independent of parity ($F = 0.95$; $df = 2, 99$; $P = 0.45$) and so parity was removed from the model. This model was tested using a polynomial regression and we found no significant evidence of curvature, or saturation, for any fly species ($P > 0.05$). In the observed range of fly counts, frequencies of defensive behaviors increased with flies without any obvious curvature. We also found no evidence of habituation from our analysis using time periods ($F = 0.49$; $df = 2, 1386$; $P = 0.61$). Examination of plots showed no clear pattern between number of defensive behaviors and time period (not shown). Front and back leg stamps and head throws were consistent through the study, while skin twitches and tail flicks appeared to increase as the study progressed (Fig. 5).

Table 4- Results from ANOVA examining variation in log transformed total behavior frequencies.

	F	DF	P value
Temperature	1205.2	1, 42	<0.001
Date	18.2	21, 42	<0.001
Observer	91.0	1, 42	<0.001
Stable Flies (front legs)	308.8	1, 42	<0.001
Stable Flies (back legs)	34.0	1, 42	<0.001
Horn Flies	39.8	1, 42	<0.001
Observer: Date	9.1	21, 42	<0.001
Date: SF (front legs)	7.1	21, 42	<0.001

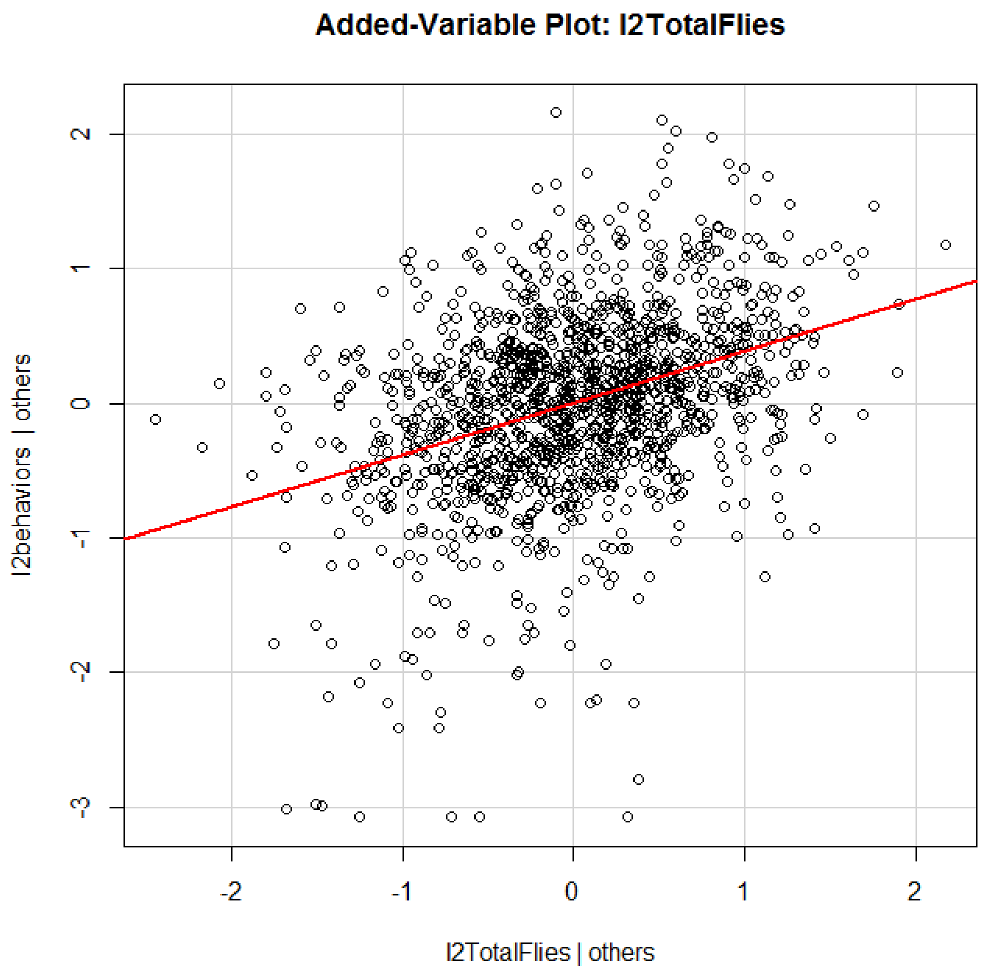

Figure 6- Added variable plot showing increase in behavior frequencies with increasing fly count, adjusting for other factors within the model.

We found that temperature, humidity, date, observer, horn flies and stable flies on both front and back legs were associated with variation in all defensive behaviors when examined individually (Table 5). Skin twitches were most strongly related with horn flies and front leg stamps were most strongly related with stable flies (Table 5). We found that horn flies and stable flies were similarly related to tail flicks, back leg stamps and head throws (Table 5).

However, examination of added variable plots indicated that the association between weather variables and defensive behaviors were weak (not shown). Face flies were associated with front leg stamps, but independent of all other behaviors (Table 5). Parity was associated with front leg stamps, tail flicks and head throws, but independent of skin twitches and back leg stamps (Table 5).

In summary, we detected a strong association between fly counts and defensive behaviors, with higher frequencies of defensive behaviors with increasing numbers of flies. On some observation periods where very few flies were observed, we observed no defensive behaviors. Of weather variables, only temperature was associated with defensive behaviors when all combined. Other weather variables were statistically significant when analyzing individual defensive behaviors, though these relationships were weak. There was no consistent pattern throughout the study with behavior frequencies and parity despite younger cows hosting fewer horn flies and stable flies on average (Fig. 7).

Table 5- Results from ANOVA examining variation in log transformed individual defensive behaviors.

	F	DF	P	Coefficient	SE
Skin Twitches					
Temperature	730.7	1, 42	<0.001	0.09	0.01
Humidity	15.1	1, 42	0.000	-0.83	0.92
Precipitation	62.8	1, 42	<0.001	2.74	3.2
Wind	49.7	1, 42	<0.001	0.35	0.31
Date	18.6	21, 42	<0.001	NA	NA
Observer 2	120.0	1, 42	<0.001	0.24	0.16
SF (front legs)	126.1	1, 42	<0.001	0.03	0.08
SF (back legs)	10.12	1, 42	0.003	0.05	0.03
Horn Flies	18.4	1, 42	0.000	0.09	0.03
Front Leg Stamp					
Temperature	897.2	1, 42	<0.001	0.14	0.01
Humidity	5.6	1, 42	0.023	-0.002	0.03
Wind	84.1	1, 42	<0.001	-0.16	0.12
Date	20.9	21, 42	<0.001	NA	NA
Observer 2	83.3	1, 42	<0.001	0.43	0.22
Parity (2)	2.6	2, 42	0.037	-.20	0.06
Parity (3+)	3.6	2, 42	0.037	-0.09	0.05
SF (front legs)	224.0	1, 42	<0.001	0.15	0.11
SF (back legs)	8.1	1, 42	0.007	0.02	0.03
Horn Flies	20.9	1, 42	<0.001	0.12	0.04
Face Flies	5.3	1, 42	0.026	0.07	0.04
Tail Flicks					
Temperature	447.0	1, 42	<0.001	0.07	0.01
Humidity	34.7	1, 42	<0.001	-0.01	0.93
Precipitation	54.9	1, 42	<0.001	-0.06	0.31
Wind	33.9	1, 42	<0.001	0.07	3.33
Date	25.9	21, 42	<0.001	NA	NA
Observer 2	670.9	1, 42	<0.001	0.08	0.16
Parity (2)	4.1	2, 42	0.025	0.08	0.05
Parity (3+)	4.1	2, 42	0.0245	0.08	0.04
SF (front legs)	114.2	1, 42	<0.001	0.13	0.08
SF (back legs)	22.6	1, 42	<0.001	-.01	0.03
Horn Flies	33.8	1, 42	<0.001	0.12	0.03
Back Leg Stamps					
Temperature	557.2	1, 42	<0.001	0.11	0.01
Humidity	5.7	1, 42	0.022	-0.02	0.03
Date	17.9	21, 42	<0.001	NA	NA
Observer 2	0.1	1, 42	0.807	0.09	0.21
SF (front legs)	89.9	1, 42	<0.001	0.13	0.11
SF (back legs)	19.9	1, 42	<0.001	0.11	0.34
Horn Flies	7.9	1, 42	0.007	0.1	0.34
Head Throw					
Temperature	117.4	1, 42	<0.001	0.04	0.01
Humidity	10.2	1, 42	0.003	-0.04	0.04
Wind	28.6	1, 42	<0.001	0.05	0.11
Date	8.1	21, 42	<0.001	NA	NA
Observer 2	165.7	1, 42	<0.001	0.23	0.21
Parity (2)	7.8	2, 42	0.001	0.07	0.06
Parity (3+)	7.8	2, 42	0.001	0.15	0.05
SF (front legs)	118.0	1, 42	<0.001	0.1	0.11
SF (back legs)	5.1	1, 42	0.03	-0.15	0.11
Horn Flies	12.4	1, 42	0.001	0.13	0.03

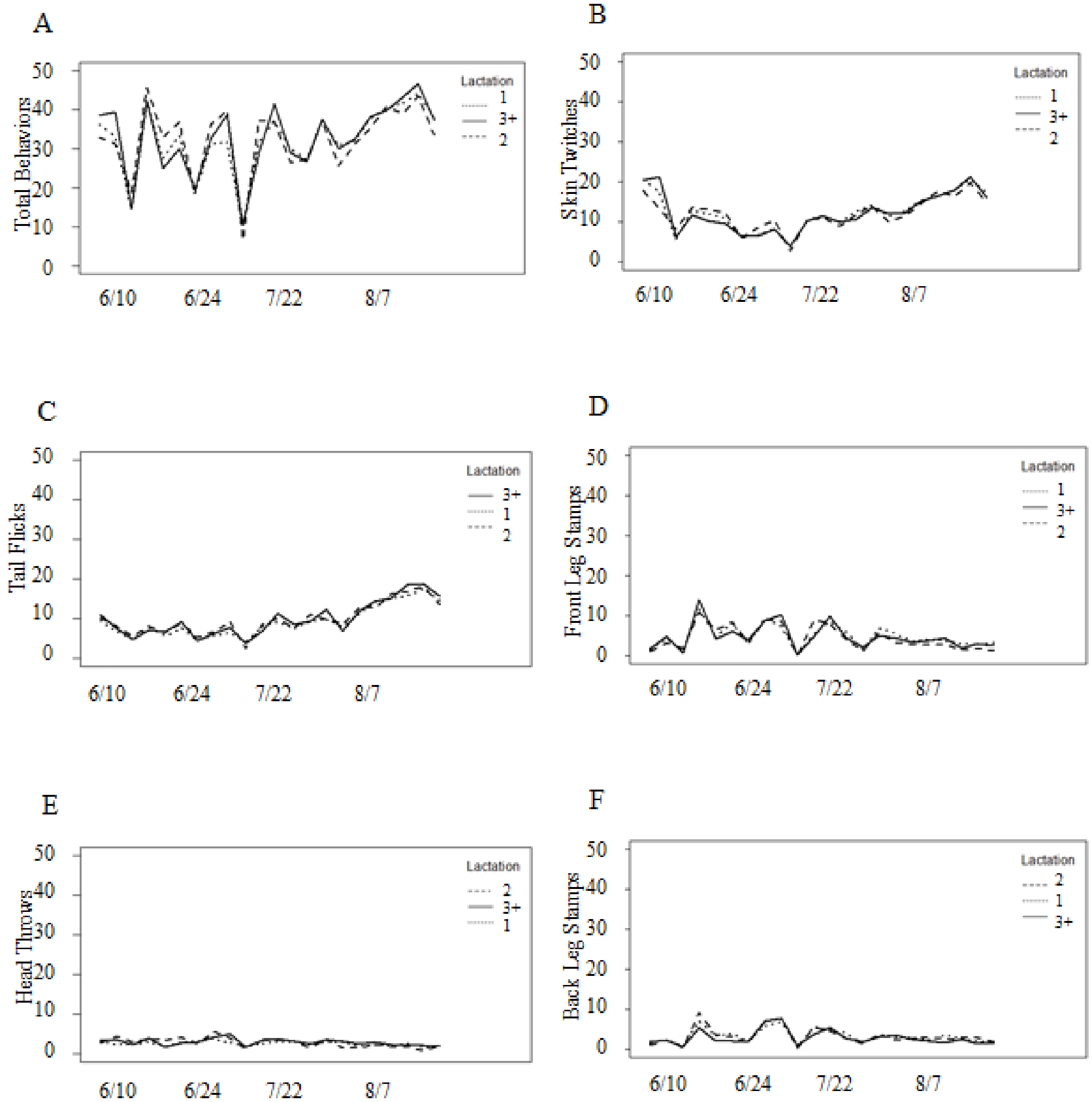

Figure 7- Behavior frequencies by parity. Parity was associated with (C) front leg stamps, (D) tail flicks and (F) head throws, but not with (B) skin twitches, nor (E) back leg stamps. There was no interaction between date and parity.

Milk Production

Milk production steadily decreased as summer progressed and leveled off as the study concluded in August (Fig. 8). Cow within group as a random effect accounted for over 60% of overall variation and so was retained in the model. There were strong associations between milk production, parity, and days in milk (DIM). Older cows produced significantly more milk than cows in their first or second lactation. For each day in milk, we observed a decrease of 0.07+/- 0.012lbs of milk produced per day.

In the observed range of fly counts, milk production was independent of fly counts of all three fly species combined ($F=1.97$; $df= 1, 1278$; $P =0.16$). Analyses were repeated for individual species. Horn flies were initially significant to milk production, but milk production was independent of behaviors (Table 6). However, when insignificant factors were removed to simplify the model, horn flies were no longer significant ($F= 1.77$; $df=1, 1300$; $P=0.18$), nor was the interaction between horn flies and date ($F=1.28$; $df= 21, 1277$; $P= 0.172$). According to our minimally sufficient model, milk production was independent of any fly species and defensive behaviors. We did observe a slight decrease in milk production with increasing behaviors, but when accounting for other factors, primarily lactation and DIM, this relationship was very weak (not shown).

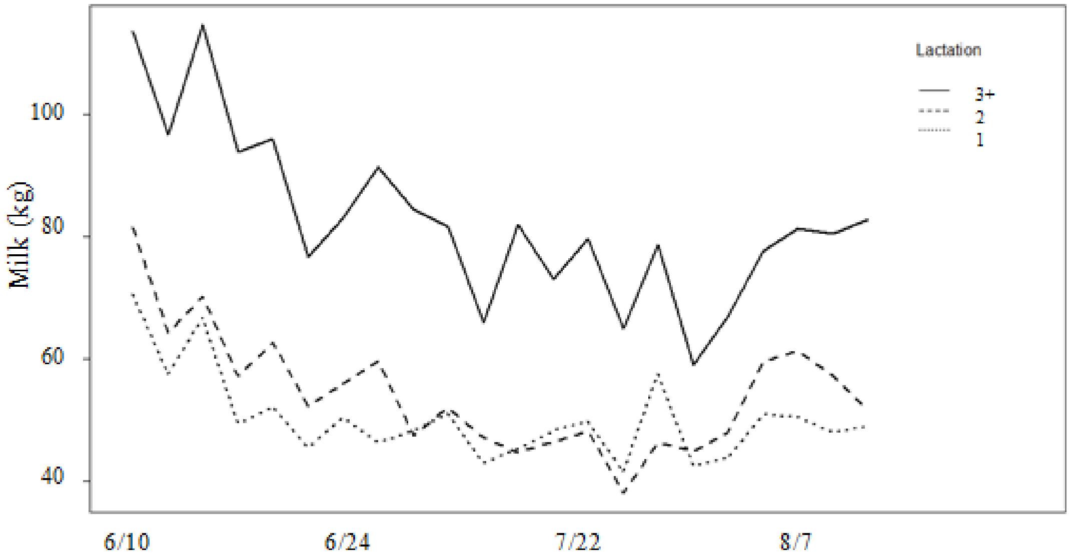

Figure 8 – Daily milk production (kg) by cows in (A) 1st lactation, (B) 2nd lactation and (C) 3+ lactations in summer 2014.

Table 6- Results of ANOVA examining associations with milk production.

	F	DF	P
Date	31.2	21, 1234	<0.001
Parity	32.1	2, 1234	<0.001
DIM	9.8	1,1234	0.002
SF	1.0	1,1234	0.313
HF	3.9	1,1234	0.050
FF	0.0	1,1234	0.922
Behaviors	0.0	1,1234	0.930
Date: Behaviors	0.4	21, 1234	0.995
Date: SF	1.4	21, 1234	0.111
Date: HF	2.3	21, 1234	0.001

Discussion

During our study, we observed varying numbers of all three species of flies with horn flies the most frequently observed and face flies the least frequently observed. We detected a strong association between fly counts and defensive behaviors, with behaviors increasing with count. There was a weak association between fly counts, defensive behaviors and weather variables, most notably an increase in counts and behaviors with increasing temperature. In the observed range of fly counts, we were unable to detect an association between milk production and fly counts or defensive behaviors. Previous research has focused on cattle behavioral responses to a single fly species. This study is the first to count three species of flies on free roaming pastured cows to detect associations with defensive behaviors and production.

In northern regions, muscid flies are most active from May through October, with peak activity mid-summer, depending on weather conditions. This study was conducted in the middle of the fly season, and we did not observe population fluctuations indicative of seasonal changes. Habituated animals are animals that stopped responding to a repeated stimulus, though this effect is not necessarily permanent (Alcock, 1989). Mullens et al. (2006) tested for and found evidence of habituation, in that intensive behaviors (head throws and leg stamps), decreased as the season progressed. These intensive behaviors are immediate responses to stable fly feeding activity (Dougherty et al., (1993a, 1994). In contrast to Mullens et al., (2006), intensive behaviors were consistent throughout the present study. Our study was conducted in the middle of the fly season, and so cows may have already habituated to fly presence by the beginning of the study. Flies were first observed on cows approximately two months before the study's start. During that time, cows could have already adjusted to hosting flies before the study began. Although counts occasionally reached over 150 flies on one animal, these numbers were not repeatedly observed. This lack of long term exposure to high numbers of flies could be an explanation for not detecting any association between fly counts and milk production.

Todd (1964) noted that under typical summer conditions, stable flies were most active between 11:00 and 15:00. He found that weather, especially temperature, was an important predictor of fly count. Similarly, we found only temperature as a consistently significant weather variable to both fly counts and behavior frequencies. Temperatures in this study ranged from 11˚C to 29˚C, well within the active range of muscid flies. During observation periods when temperature fell below ~13˚C, few flies were counted and cows exhibited little to no defensive behaviors. We observed a decrease in horn fly and face fly counts with increasing wind speed, which is consistent with previous studies. We also noticed a strong relationship between defensive behaviors and weather conditions, notably increasing behavior with temperature. There was a slight increase in fly count as temperature increased, so observing more defensive behaviors during those times is expected. Another possible explanation is the effect of warmer weather on cow behavior. Cows are prone to heat stress, and when exposed to warmer weather conditions, were possibly more irritable and sensitive to fly activity. We saw a decrease in fly numbers and defensive behaviors during rainy periods. It is possible that horn flies, although present and counted, were not biting during rain. Cows may also be somewhat desensitized to fly activity due to rain.

Economic injury levels of stable flies on beef cattle are highly variable, with daily counts ranging from 25 (Steelman, 1976) to 50 flies per cow (Campbell et al., 1977). Todd (1964) found an index of irritability for stable fly numbers up to ~15 flies per animal. Fly numbers exceeding 15 did not result in an increase in irritation shown by behaviors (Todd, 1964). Unrest in cattle can be caused by feeding activity from even a few stable flies, as 2-5 flies per leg have been shown to cause reduced weight gain and feed efficiency (Campbell et al., 1987). According to Taylor et al., (2012), when stable fly numbers range from 0-15 flies per leg, each additional fly caused daily milk losses of 0.22kg per day. Cows were obviously irritated by fly activity, but we were unable to detect such effects on milk production in the present study.

Economic thresholds and injury levels are useful for producers to determine when intervention is needed, though these levels vary with fly species. Schwinghammer et al., (1986) found that beef steers exposed to 100-500 horn flies showed increased physiological stress indicators, such as increased heart rate, respiration and rectal temperature. Irritation from horn flies can lead to decreased feed efficiency, weight gain and milk production (Steelman et al., 1991, Byford et al., 1992). Treatment is generally recommended when populations exceed 200 flies per head, or 100 flies per side (Hogsette, 1991). The average horn fly count in this study was approximately 35 flies per side, ranging from less than 10 flies to 150 flies, well below this estimate.

Virtually no face flies were observed in this study, with average counts of less than one fly per animal during the summer and counts never exceeding 10 flies per animal. There is little evidence that face fly infestations have significant impact on milk production or quality (Schmidtmann et al., 1984, Krafsur and Moon, 1997). Arends et al. (1982) found no evidence of reduced feed efficiency or average daily gain on heifers infested with 13 or more face flies and Schmidtmann et al., (1984) did not detect effects in milk yield due to face fly numbers. Therefore, it is not surprising to observe little effect on production from face flies in the present study.

Despite bearing similar fly loads, cows reacted differently based on their parity. However, there was no consistently distinct pattern as to how younger or older cows reacted to varying levels of fly activity. In contrast, Mullens (2006) observed fewer flies on younger cows, as well as more leg stamps when compared to older cows. In the observed range of fly counts, we found no clear evidence that younger cows were more sensitive to fly activity than younger cows.

Some defensive behaviors may serve as a deterrent, such as skin twitches and tail flicks, while others such as leg stamps and head throws are more of a direct response to pain (Dougherty et al., 1993). Such behaviors are seldom observed when nuisance flies are absent (Dougherty et al., 1993b). Intensity of attacks varies with time of day and weather conditions. Flies are particularly active when winds are low and temperatures are high (Todd, 1964). Hafez and Gamal-Eddin (1959) reported that stable flies fed on the sunny side of host at temperatures at or below 30˚C. At temperatures exceeding 30˚C, flies fed on the shaded side of the host or sought other sheltered locations as a form of thermoregulation. We did not have temperatures exceeding 30˚C in our study and the side of observation was random.

Skin twitches and tail flicks were observed more frequently than head throws or leg stamps. These findings are consistent with previous behavioral studies (Okumura, 1977, Dougherty et al., (1993a,b; 1994; 1995), Torr and Hargrove, 1998, Eicher et al., (2001), Mullens et al., (2006)). Dougherty et al. (1994) also found that skin twitch responses were saturated at very low populations of stable flies. There was a strong relationship between fly counts and all observed defensive behaviors. During observation periods with very few flies, cows exhibited few to no defensive behaviors. Such observation periods typically occurred in the morning, with temperatures around 13˚C.

Daily milk yields were independent of fly numbers on the same cows the day before during this study. Significant factors to milk yield were days in milk and the cow's parity. In our observed range of counts, fly load did not significantly impact milk yield, despite obvious irritation exhibited by the cows. It is also important to note that this study was done with organic grazing, crossbred dairy cows that naturally produce less milk. These results indicate that despite irritation, dairy cows can tolerate light to moderate fly loads without negative effects on milk production. However, further research is needed to investigate how the presence of additional species of nuisance flies can affect current economic thresholds currently determined for a single species. Cows may not be able to tolerate higher fly loads when multiple species are present. Further research is needed to better understand the impact of infestations from multiple species on cow comfort and productivity, especially in open field settings and high producing herds.

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