Attack Intensity of Pest Flies and the Behavioural Responses of Pastured Dairy Cows

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ABSTRACT

ATTACK INTENSITY OF PEST FLIES AND THE BEHAVIOURAL RESPONSES OF PASTURED DAIRY COWS

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Pest fly species and abundance, fly avoidance behaviours, grazing behaviour, milk cortisol, and milk production were studied to understand the impact of pest flies on dairy cows at pasture. Treatment of cows with a natural fly repellent based on sunflower oil and essential oils, lemongrass and geranium, was used to compare cows with reduced fly attack intensity to untreated cows. Treatment of cows with the fly repellent resulted in a 69.5 % reduction in pest fly attack intensity. Treated cows also had reduced rates of tail flicks, skin twitches, head throws, and leg stamps by 44.8 %, 54.0 %, 78.9 %, and 45.1 % respectively. The reduction of flies on treated cows had no effect on milk cortisol and milk production. Treatment of cows with an effective, natural fly repellent may improve cow comfort with reduced levels of painful biting flies and fly avoidance behaviours.

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Chapter 1: Introduction

Dairy production in North America has changed dramatically over the last few decades, with the use of intensive management systems to maximize efficiency and production. Modern intensive systems allow the producer to have improved cow management and greater control over factors influencing the cow such as feeding, housing and health practices. Although these systems have allowed for greater control of certain environmental factors, there are environmental stressors that remain a challenge for producers. This is particularly relevant to organic producers who are required to provide cows access to pasture during the grazing season, but cannot use synthetic insecticides available to conventional dairy farms (Public Works and Government Services Canada, 2011).

Environmental stressors are elements in the animals' environment that they cannot control and can create physiological and/or psychological stress in the animal. Key environmental stressors in current dairy production systems include pathogens, heat stress, and pests (Cortinas and Jones, 2006; Baumgard and Rhoads, 2012; Paduch et al., 2013). Environmental stressors can negatively impact production and animal welfare, especially in situations where animals have no opportunities to avoid the stressors (Shütz et al., 2008; West et al., 2003).

Animal welfare in food production systems has arisen as a major concern of consumers, becoming a central point of ethical debate and research. The concept of animal welfare and quality of life was described by Fraser et al. (1997) with three main components: natural living, affective state/mental health, and biological functioning.

Natural living refers to animals having the ability to carry out natural behaviours adapted to the species. Affective state refers to the animals' feelings and emotions. Good welfare would imply that animals are free of feeling negative states of emotion such as fear and pain for prolonged and intense periods of time. Biological functioning refers to animals being in good health and performing physiological functions such as growth and reproduction. Traditionally, animal welfare was a concern of producers who believed that good welfare consisted of animals with good production and being free of illness and injury (von Keyserlingk et al., 2009). Animal welfare has recently become an important issue to consumers who are concerned about painful procedures and "unnatural living conditions" used in current production practices (Fraser et al., 1997; von Keyserlingk et al., 2009). While it is generally accepted that all three components are important in evaluating welfare, challenges can arise when improvements to one component lead to reduced welfare in another (von Keyserlingk et al., 2009). For example, placing dairy cows on pasture improves the natural living aspect of welfare in comparison to cows living in a tie-stall barn setting (von Keyserlingk et al., 2009). However, if the cows have no shade at pasture and become heat stressed, frustrated and have reduced milk production, then there is a reduction in both their affective state and biological functioning (von Keyserlingk et al., 2009).

Animal welfare research over the last couple of decades has seen tremendous growth and practical solutions for problems facing modern dairy production systems (von Keyserlingk et al., 2009). While recent research has focused on specific environmental factors such as heat stress, the influence of pest flies on dairy cattle welfare has been an area lacking study. Simply understanding how pest flies influence the behaviour and physiology of cattle can begin to help reveal the importance of this issue in cow welfare and comfort, and potentially to animal productivity.

Chapter 2: Literature Review

2.1 Pest flies in cattle production

Pest flies are an economic and welfare concern in cattle production systems as they transmit pathogens, disturb feeding time, and negatively affect weight gain and milk production (Steelman et al., 1991; Byford et al., 1992; Dougherty et al., 1993b). Fly avoidance behaviours, disrupted grazing and increased stress of cattle can lead to energy expended on pest flies that could otherwise be used for biological functions (Schwinghammer et al., 1986b; Dougherty et al., 1993a, 1994; Mullens et al., 2006). Cattle defensive behaviours can range from tail flicking, leg stamping, head throwing and skin twitching to evasive displacement and bunching behaviour in an effort to lessen parasite attack intensity (Ralley et al., 1993). It is evident that increased disease transmission and reduced biological functioning (milk production, growth rate) are an impairment of cattle welfare; however, little research has focused on affective state.

Understanding how pest flies influence production in cattle can be technically difficult and it is challenging to tease apart and understand individual factors (Skyes, 1994). Milk production is influenced by numerous factors such as genetics, days in milk, and type and quality of feed (Blott et al., 2003; Caccamo et al., 2012). Although pest flies can disrupt grazing meals, Dougherty et al. (1994) found that dry matter intake increased linearly with increased stable fly numbers. Cattle with higher numbers of face flies appeared to take fewer but larger bites of grass in a suspected effort to displace flies near the face (Dougherty et al., 1993a). The pattern of decreased bite rate and increased bite mass has also been observed in several stable fly studies, reflecting that cattle compensate grazing disruption with increased bite size to maintain herbage intake (Dougherty et al, 1993b, 1994). If observed, reductions in weight gain and milk production may therefore be a result of increased energy expenditure and blood loss rather than feed intake disruption (Jonsson and Mayer, 1999; Campbell et al., 2001). Clear evidence of the influence of pest flies on milk production is hard to find and may indicate that cattle are able to adapt to the situation and continue their top priority of milk production (Jonsson and Mayer, 1999).

The main pest flies of cattle in North America include the house fly (*Musca domestica*), the face fly (*Musca autumnalis*), the horn fly (*Haematobia irritans*), the biting stable fly (*Stomoxys calcitrans*), and deer and horse flies (Family Tabanidae) (Cortinas and Jones, 2006).

2.1.1 House flies

House flies are often a dominant fly species found on dairy farms, as the environment is favourable for high reproductive rates. House fly larvae complete their life cycle in areas that contain organic matter such as straw and manure, and with favourable conditions the life cycle can be completed within 10 days. House flies do not take blood meals from cattle but are a nuisance due to their disturbance of behaviour and ability to mechanically transfer bacteria.

Both male and female house flies feed on organic matter from cattle and the surrounding environment by using sponge-like sucking mouthparts for ingestion (Cortinas and Jones, 2006). House flies will feed on cattle saliva, eye secretions and blood from open wounds, and within the environment feed on manure, milk replacer and other organic material (Christensen, 1982; Lysyk, 1993a; Cortinas and Jones, 2006; Malik et al., 2007).

2.1.2 Face flies

Face flies are similar in size and colouring to house flies and similarly do not bite cattle, they mainly feed on organic matter such as saliva and eye secretions (Van Geem and Broce, 1985). Although named after their tendency to congregate around moist areas of the face such as eyes and nostrils, face flies can also be found on other body parts especially near open wounds (Cortinas and Jones, 2006). Due to their attraction to the facial area of cattle, ear flapping and head shaking behaviours are particularly associated with face flies (Schmidtmann, 1985a).

Face flies are known for their ability to carry and transmit *Moraxella bovis*, the causative agent for infectious bovine keratoconjunctivitis (IBK) or bovine pink-eye (Berkebile et al., 1981; Glass and Gerhardt, 1984). Berkebile et al. (1981) found that face flies can harbour viable *M. bovis* bacterium for up to 3 days. Although rarely fatal, IBK is considered the most important ocular disease of cattle worldwide due to significant financial losses and animal suffering (Postma et al., 2008). Financial losses associated with IBK include treatment costs, reduced market values due to eye disfigurement, and decreased weight gain due to impaired vision (Thrift and Overfield, 1974; Slatter et al., 1982). Little research has focused on the pain and suffering that is associated with IBK however the obvious ocular tissue damage is certainly a welfare concern (Postma et al., 2008).

Due to the large economic impact of IBK there has been extensive research focusing on face flies and cattle. Studies on face fly abundance show a positive correlation between face fly attack intensity and IBK disease incidence (Brown and Adkins, 1972). Behavioural studies of cattle have shown that face flies increase host defensive behaviours, however there have been no significant impacts observed of face flies on productivity measures such as weight gain and milk production (Schmidtmann et al., 1981, 1984; Schmidtmann and Valla, 1982; Dougherty et al., 1993a).

2.1.3 Stable flies

A major pest fly species of cattle known for its large economic losses and ability to spread disease is the biting stable fly, *Stomoxys calcitrans.* Stable flies are a worldwide nuisance and are estimated as one of the most damaging pest fly species of cattle in North America (Byford et al., 1992; Taylor et al., 2012). Losses in dairy and beef cattle industries combined in the United States from 2005 to 2009, due to stable flies alone, was $2.2 billion per year (Taylor et al., 2012). In the dairy industry alone, economic losses were estimated at $360 million per year between 2005 and 2009 (Taylor et al., 2012). Losses associated with stable flies include reduced milk production and weight gain, and costly fly control treatments (Drummond et al., 1981; Catangui et al., 1997; Taylor et al., 2012).

Although similar in size to house and face flies, stable flies feed on blood and both sexes are equipped with a mouthpart that can slash through skin and blood vessels (Cortinas and Jones, 2006). Unlike some biting insects, the saliva of stable flies does not contain an anaesthetic and is therefore believed to inflict considerable irritation

(Cortinas and Jones, 2006). Even though the irritating influence of stable flies on cattle behaviour is suspected to be a large cause for losses in performance, determining the cost associated with specific avoidance behaviours has not been attempted (Taylor et al., 2012). Schwinghammer et al. (1986b) found that cattle with 25 or 50 stable flies had increased respiration and heart rates in comparison to fly-free cattle.

Stable flies are suspected of being mechanical vectors for many significant livestock pathogens and have been shown to be mechanical carriers of pathogens that cause West Nile Virus, bovine leukosis and anaplasmosis (Weber et al., 1988; Scoles et al., 2005; Doyle et al., 2011). Research regarding bovine virus diarrhoea, an economically important disease complex in cattle production, has shown that stable flies can carry the virus for up to 96 hours after feeding on an infected animal (Tarry et al., 1991). Stable flies have also been implicated as a possible mechanical vector for Rift Valley Fever virus, primarily a livestock disease that has had recent attention for the outbreaks of infection among livestock and humans in Africa (Turrell et al., 2010).

Seasonal effects significantly influence stable fly populations, with temperature as the most dominant weather variable in determining stable fly abundance (Berry and Campbell, 1985; Taylor et al., 2007; Gilles et al., 2008). Berry and Campbell (1985) observed that stable fly feeding rates were correlated with weather conditions and that feeding rates decreased with increased wind and radiation. In a 5 year study of stable fly populations in Eastern Nebraska, temperature and precipitation were the most important weather variables that accounted for fly variations (Taylor et al., 2007). Mullens and Peterson (2005) also found precipitation to be a key weather variable for stable fly numbers; more specifically, they reported that rainfall amounts in March significantly affected stable fly populations in May and June.

Stable fly populations observed on Canadian farms during summer months have also been notably influenced by populations of stable flies that overwintered on farms (Lysyk, 1993a; Beresford and Sutcliffe, 2009). Stable fly research from dairy farms in central Ontario found that spring and summer stable fly populations are influenced by a population of overwintering flies on that particular farm and by overwintering flies from neighbouring farms (Beresford and Sutcliffe, 2009). Overwintering insect abundance depends on suitable habitat sites and is influenced by husbandry practices on the farm (Berkebile et al., 1994). For example stable fly pupae can overwinter in manure piles and silage (Berkebile et al., 1994). Broce et al. (2005) found that areas where round hay bales were provided on pasture for winter-feeding provided suitable breeding sites for stable flies and was the main source of stable flies in the subsequent spring and summer.

2.1.4 Horn flies

The horn fly, *Haematobia irritans*, is a common pest fly found in many areas of the world and is noted as one of the most economically damaging pests of cattle (Byford et al., 1992; Cortinas and Jones, 2006). In the U.S.A., cattle production losses associated with horn flies have been estimated at close to one billion dollars annually (Kunz et al., 1991; Cupp et al., 1998). Production losses associated with horn flies include reduced weight gains, reduced milk production and disruption of feeding behaviour (Steelman et al., 1991; Byford et al., 1992; Jonsson and Mayer, 1999).

Due to the obligate blood-feeding nature of adult horn flies, production losses are also assumed to be associated with the pain and stress of flies feeding on cattle (Byford et al., 1992). Horn fly infestation of cattle under laboratory conditions found that horn fly densities of 100 and 500 flies per animal led to increased physiological stress measures in comparison to animals with no horn flies (Schwinghammer et al., 1986a). In comparison to control cattle with zero horn flies, cattle with densities of 100 and 500 horn flies had significantly higher heart and respiration rates, and higher blood cortisol concentrations (Schwinghammer et al., 1986a).

The negative impact of horn flies on cattle health and production is also associated with their ability to transmit pathogens to and between cattle (Torres et al., 2012). Horn flies have been shown to be a mechanical vector for several livestock pathogens including bovine anaplasmosis (Rodriguez et al., 2009), *Corynebacterium pseudotuberculosis* (Spier et al., 2004) and a mastitis-causing agent, *Staphylococcus aureus* (Gillespie et al., 1999).

As previously mentioned, adult horn flies are obligate blood-feeding pests of cattle and both sexes engage in numerous small blood meals on a daily basis (Cortinas and Jones, 2006). Both sexes of adult horn flies are small in size, with a dark black body usually less than 5 mm long and a head with a bayonet-like appearance (Cortinas and Jones, 2006). Adult horn flies spend the majority of their life on host animals, leaving the host only to lay eggs and find other hosts (Foil and Hogsette, 1994). The necessity for blood in their diet leads to high fly densities, averages can easily reach 200 adult horn flies on a single animal during peak fly periods (Quisenberry and Strohbehn, 1984; Guglielmone et al., 2002).

Like many other fly species, seasonality of horn fly densities is highly dependent on weather factors influencing the development of larvae (Lysyk, 1992). Development of immature stages of horn flies is highly correlated with environmental temperatures and the entire life cycle can be completed within 2 to 4 weeks (Lysyk, 1992; Lysyk and Floate, 2002). Densities of adult horn flies found on cattle also appear to be correlated with weather factors such as rainfall, temperature and relative humidity (Lima et al., 2003; Castro et al., 2008; Maldonado-Siman et al., 2009). The majority of horn flies present on cattle develop from local, on-farm populations that overwinter in manure pats, with a small population of immigrant flies (Marley et al., 1991). Immigrant adult horn flies have been shown to travel several kilometres in search of a host and can migrate from neighbouring farms despite natural boundaries such as trees (Marley et al., 1991; Sheppard, 1994).

2.1.5 Horse and deer flies

Horse and deer flies are part of a large family of biting flies, Tabanidae, comprising over 4000 fly species (Foil and Hogsette, 1994). In Canada alone there have been 144 species of horse and deer flies identified (Teskey, 1990). The distinction between horse and deer flies is based on body size with deer flies having a body length ranging from 6 to 10 mm and horse flies from 10 to 30 mm (Cortinas and Jones, 2006). They are major pests of humans and animals worldwide due to their painful bite and ability to transmit pathogens (Iranpour and Galloway, 2002). Unlike stable flies in which both sexes feed on cattle for blood meals, only female horse and deer flies ingest blood meals (Foil and Hogsette, 1994). Blood meals are required by female horse and deer flies to ensure development of their eggs (Iranpour and Galloway, 2002).

Economic losses in cattle production include disease treatment but are more notably linked to reduced weight gains and losses in milk production (Perich et al., 1986; Ralley et al., 1993). Reduction in fitness and performance of the host is related to blood loss, energy expenditure for avoidance behaviours and altered grazing behaviour (Ralley et al., 1993; Iranpour and Galloway, 2002). During the mid-1980's production losses in beef cattle due to horse and deer flies was estimated at $54 million dollars in the United States alone (Iranpour and Galloway, 2002). Perich et al. (1986) found that heifers exposed to an average of 90 horse flies per day had a 0.1 kg reduction in weight gain per day. While there is an assumption that they negatively impact milk production, there have been few studies looking specifically at tabanids and milk production.

The biting mechanism of horse and deer flies is presumed to be extremely painful as their mouthparts tear through skin and blood vessels for a meal (Cortinas and Jones, 2006). Anti-thrombosis components from horse and deer fly saliva prevent the wound from rapidly clotting and attract other flies to the site such as house and face flies (Kazimírová et al., 2001; Cortinas and Jones, 2006). Adult horse and deer flies present significant health and economic concerns due to their ability to transmit pathogens including bacteria, viruses, trypanosomes and helminths (Foil, 1989; Iranpour and Galloway, 2002). Pathogens that influence cattle production and are associated with horse and deer fly transmission include bovine leukemia virus (Foil, 1988), bovine anaplasmosis (Hawkins et al., 1982) and lyme disease (Magnarelli et al., 1986).

Population fluctuations of horse and deer flies have been linked to factors such as current environmental factors, environmental factors from the preceding year and location of pasture in relation to wetlands (Mikuška et al., 2012). Environmental factors such as temperature, precipitation and flood duration can influence seasonal fluctuations of adult horse flies during the following year (Mikuška et al., 2012). Populations of tabanids increase on pastures in close proximity to wetlands and wooded habitats (Burger and Pechuman, 1986). While adults of a single species are only present for a few weeks, a series of multiple species in one area emerging can lead to a steady fly pressure throughout the summer (Foil and Hogsette, 1994; Cortinas and Jones, 2006).

2.2 Individual fly attraction

Pest fly abundance on individual animals can vary greatly, even among cattle within the same herd that are located in close proximity to each other (Birkett et al., 2004). Pest flies of cattle are suspected to use specific visual and/or olfactory cues from the cattle for host identification and location (Birkett et al., 2004; Tangtrakulwanich et al., 2011). Birkett et al. (2004) used field and laboratory tests to show that the natural differential attractiveness of dairy heifers to pest flies was partly due to volatile semiochemicals that cattle emit. Natural volatile profiles of cattle are shaped by many physiological factors such as genetics, diet, health status of animal, and reproductive status (Vale, 1981; Birkett et al., 2004). Wind tunnel experiments have shown that stable flies are attracted to carbon dioxide, octenol and acetone, all of which are semiochemicals that cattle emit (Schofield and Brady, 1997). Torr et al. (2006) looked at several animal factors and found that stable fly attraction to individual cattle was likely due to the variation in carbon dioxide production.

Identifying the visual cues used by pest flies has become a recent research interest as the use of flytraps has become a key point of many integrated pest management systems (Horváth et al., 2008). In order to design effective flytraps it is essential to know how flies are identifying their hosts. Horse and deer flies have been found to be significantly more attracted to dark colours and dark coloured hair on horse and cattle, while animals with white hair coats have reduced horse and deer flies (Horváth et al., 2010; Blaho et al., 2012). Horváth et al. (2008, 2010) found that this difference in attraction to coat colours is due to their polarizing properties. The different coat colours polarize light at different angles, producing different images to animals and insects that can detect polarized light (Horváth et al., 2008). Flies of the tabanid species are able to detect polarized light and use it for locating important objects and places such as bodies of water necessary for egg-laying sites (Horváth et al., 2008). Field studies have also revealed that tabanids are much less attracted to coat patterns with numerous spots and stripes in comparison to solid coloured coats (Blaho et al., 2012; Egri et al., 2012). This recent research into the visual capabilities of horse and deer flies has allowed for a better understanding of why some cows attract more flies and how flies detect and choose their host animals.

2.3 Behavioural responses to pest flies

Cattle have developed a wide range of fly avoidance behaviours that use up valuable energy and may disrupt their feeding behaviour (Dougherty et al., 1993b).

Behavioural responses observed in cattle include tail flicking, skin twitching, head throwing, foot stamping, ear flicking, and group bunching (Dougherty et al., 1993a,b; Ralley et al., 1993). There have been several factors aside from fly attack intensity that have been suggested to possibly influence fly avoidance behaviour rates in individual animals such as satiation, habituation, and age of animal (Dougherty et al., 1994; Mullens et al., 2006).

2.3.1 Tail flicking

Tail flicking is one of the most common and frequent fly avoidance behaviours observed in cattle (Dougherty et al., 1993b). It is described as the movement of the tail to the animal's side or back (Ralley et al., 1993). Tail flicking is effective at removing flies located on the animal's sides, back, and rear legs (Ralley et al., 1993). Multiple studies have found tail flicking to be the most frequent biting fly avoidance response in comparison to other common behaviours such as ear flicking, skin twitching, and leg stamping (Ralley et al., 1993; Dougherty et al., 1993b,1994,1995). Cattle subjected to both outdoor environments and enclosed environments with only stable flies or face flies, increased tail flick rates with increasing fly attack intensities (Dougherty et al., 1993a,b,c; Ralley et al., 1993; Mullens et al., 2006). For example, cows subjected to releases of 125 and 250 stables flies had mean rates of tail flicking at 36.7 and 57.5 min^{-1} per cow, respectively (Dougherty et al., 1993b). Cattle in enclosures with no flies present did not perform any tail movements (Dougherty et al., 1995). Harris et al. (1987) looked at natural fly intensities on pasture and found that tail flick rates were most correlated with stable fly numbers observed on legs.

In an effort to mimic natural fly attacks, Dougherty et al. (1993b) subjected cows to waves of stable flies and found that the rate of tail flicks remained constant as the grazing meal progressed. Cows subjected to releases of 125 and 250 stable flies every 15 min for a 1 h period, showed no significant change in tail flick rates with reported behavioural means of 36.7 min^{-1} and 57.5 min^{-1}, respectively ($P > 0.05$) (Dougherty et al., 1993b). Mullens et al. (2006) also found no change in tail flick rates over the summer among individual animals, suggesting that there is no habituation to stable flies.

2.3.2 Skin twitching

Skin twitching is also a frequently observed fly avoidance behaviour and has been grouped with tail flicking as being more frequent and less energy-intensive fly (Mullens et al., 2006). Less energy is required to perform these behaviours and they may in part be more frequently used, as they do not interfere with grazing behaviour (Dougherty et al., 1993c). Skin twitching is an involuntary reaction to irritation on the animal's skin. Muscles under the skin of cattle and other quadrupeds known as cutaneous trunci muscles, automatically contract in response to stimulus commonly referred to as the panniculus reflex or cutaneous trunci reflex (Blight et al., 1990).

Skin twitching rate is observed to increase as fly attack intensity increases and is effective at alighting flies that land on an animal's sides (Dougherty et al., 1993a,c; Mullens et al., 2006). Studies with enclosed cattle allowing exposure to select species of flies showed that skin twitch rates increased as numbers of face flies and stable flies increased (Dougherty et al., 1993a,c). Exposure of cattle to releases of 0, 400, and 800 face flies were found to induce skin twitches at rates of 0.3, 3.5, and 4.1 min^{-1} cow^{-1}, respectively (Dougherty et al., 1993a). In comparison, cattle exposed to releases of 0, 125, and 250 stable flies were found to induce skin twitches at rates of 5.0, 13.1, and 22.6 min^{-1} cow^{-1}, respectively (Dougherty et al., 1993b). Cattle on pasture also showed increased rates of skin twitches as stable fly intensity increases, and no signs of habituation to flies over time (Mullens et al., 2006).

2.3.3 Head throwing

One of the less frequent and more energy-intensive acts performed by cattle for fly avoidance is head throwing behaviour (Mullens et al., 2006). Head throwing is described as the movement of the head toward the body directed towards either the sides or front legs (Ralley et al., 1993). Head throws directed towards the front legs are regarded as important for dislodging feeding stable, horse and deer flies that do not displace from the leg following leg stamps (Ralley et al., 1993). Cattle in enclosures with stable flies had a positive linear relationship between head throw rate and fly intensity, particularly due to abundant flies landing on the forelegs (Dougherty et al., 1994,1995). Cattle exposed to pulses of 0, 50, and 100 stable flies every 15 min for a 1 h period, displayed means rates of 0.8, 1.6, and 3.3 head throws min^{-1} per cow, respectively (Dougherty et al., 1995).

Head throwing is a behaviour that expends more energy in comparison to other behaviours such as skin twitching, and it highly disrupts grazing behaviour (Dougherty et al., 1994,1995). Dougherty et al. (1993b) found that prehension and biting were increasingly disrupted by violent head throws as grazing time progressed and suggested that increased satiety level may influence the frequency of head throws.

Habituation to biting stable flies may also play a role in head throwing behaviour. Mullens et al. (2006) found that cattle followed for a long time period (12 weeks) showed a significant reduction in the ratio of head throws to fly numbers, indicating that cattle may become habituated to biting flies. Head throw behaviours at the start of the summer occurred at a ratio of > 0.6 (behaviour: fly number) and dropped to a ratio of < 0.4 by the end of the season, with a significant difference found in two of four herds observed ($P < 0.01$) (Mullens et al., 2006).

2.3.4 Leg stamping

Leg stamping or foot stomping is the movement of the leg in an effort to dislodge biting flies, and is a movement not related with locomotion (Dougherty et al., 1993b,c; Ralley et al., 1993). While leg stamping is often associated with biting stable flies that prefer feeding on the legs, it is often not effective at removing flies that are in the process of feeding (Dougherty et al., 1993b,c). Cattle exposed exclusively to face flies did not display any leg stamping behaviour, even at enclosure release rates of 800 flies per animal (Dougherty et al., 1993a). Leg stamping is referred to as a less frequent and energy-intensive fly avoidance behaviour (Mullens et al., 2006). The fact that this behaviour is not effective and is energy-intensive may help in understanding why this behaviour is less frequently observed (Dougherty et al., 1993b; Mullens et al., 2006).

Several studies have reported that increasing numbers of stable flies result in increased rates of leg stamping; however the rate still remains low in comparison to other fly avoidance behaviours such as tail flicking (Harris et al., 1987; Dougherty et al., 1993b,c, 1995; Mullens et al., 2006). For example, exposure to 2500 stable flies resulted in 69 tail flicks min^{-1} in comparison to only 8 front leg stamps min^{-1} (Dougherty et al., 1993c). Dougherty et al. (1993b) found a linear increase of hind leg stamps in response to stable flies, as cows exposed to releases of 125 and 250 stable flies every 15 min had mean rates of 0.5 and 1.7 stamps min^{-1} per cow, respectively. Harris et al. (1987) found that the rate of leg stamps and kicks were correlated with stable fly numbers found on the legs. Habituation to biting stable flies has been suggested as the ratio of leg stamps to fly numbers dropped significantly in cattle followed for a 12 week period (Mullens et al., 2006).

2.3.5 Side licking

Side licking behaviour in cattle is not commonly studied in fly avoidance behavioural studies. It is recognized as a behaviour that is a direct and immediate reaction to a biting fly, but it is more often mentioned in studies of autogrooming behaviour among ungulates (Mooring et al., 2006). Autogrooming is generally considered an automatic behaviour that is performed without consciously thinking to perform the behaviour (Mooring et al., 2006). This is in comparison to a conscious behavioural response to a painful fly bite such as throwing the head to the side of the body (Mooring et al., 2006). The side lick behaviour is believed to be a preventative behaviour to remove pests and prevent ticks and other external pests from successfully attaching and feeding (Mooring et al., 2006). Ralley et al. (1993) mention that cattle were observed licking the front legs to remove feeding horse and deer flies, but to date, there are no fly avoidance studies of dairy cattle that recorded side licking rates.

2.3.6 Group bunching

Group bunching or aggregation behaviour of cattle in response to fly attack pressure has been observed in several studies (Schmidtmann and Valla, 1982; Ralley et al., 1993). Bunching behaviour is described as the grouping of several animals or the entire herd in an effort to lessen pest fly attack (Ralley et al., 1993). The idea is that animals in the middle of the bunch are subjected to fewer flies than those more exposed on the exterior of the bunch (Schmidtmann and Valla, 1982; Ralley et al., 1993). Schmidtmann and Valla (1982) found that during bunching, heifers with their face protected inside the group had a mean number of 1.7 face flies on their face in comparison to 17.1 face flies on heifers with their faces exposed. Constant agitation of the group occurs as animals on the exterior try to work their way into the middle, with dominant animals often pushing more subordinate animals to the exterior (Ralley et al., 1993). This behaviour not only expends valuable energy, but may also increase the risk of heat stress in animals (Schmidtmann and Valla, 1982).

Ralley et al. (1993) found that cattle performed the bunching behaviour in response to horse flies but did not perform the behaviour in response to stable flies and mosquitoes. The application of a permethrin fly repellent on the face of heifers reduced face fly abundance and significantly reduced the number and duration of bunching behaviour on treated animals in comparison to untreated heifers (Schmidtmann and Valla, 1982). Heifers that were treated with permethrin had bunching episodes ≥ 60 min on 4 out of 51 trial days; in comparison, untreated heifers had bunching episodes ≥ 60 min on 27 out of 51 trial days (Schmidtmann and Valla, 1982). Schmidtmann and Valla (1982) found that bunching behaviour was initiated when face flies averaged 9-12 flies per face, in comparison to Teskey (1969) who reported bunching initiation at 30 flies per head.

2.3.7 Modification of grazing behaviour

Grazing behaviour is clearly an economically important component of cattle production and remains a key issue when studying the influence of pest flies and fly avoidance behaviour. While many studies state that flies negatively impact production through their influence on grazing behaviour, conflicting literature shows that cattle can be able to counterbalance grazing disruption with increased grazing efficiency (Dougherty et al., 1994, 1995). Dougherty et al. (1994, 1995) found that increased levels of stable flies resulted in a linear decrease in bite rate and linear increases in bite mass and dry matter intake. This increase in grazing efficiency was theorized to be the result of cattle placing their face deeper in the grass in an effort to displace flies, resulting in increased herbage intake per bite (Dougherty et al., 1994). Increased rates of herbage intake may also help to compensate for reduced grazing time in cows that are subject to increased fly attack intensities (Schmidtmann et al., 1981; Schmidtmann and Valla, 1982). The satiety level of cattle can also interact with grazing behaviour disruption from flies, as cattle that were fasted showed fewer disruptions due to fly avoidance behaviours compared to animals near satiety (Dougherty et al., 1993b). Studies of grazing cattle found that animals approaching satiety were more likely to quit grazing due to the harassment by pest flies (Schmidtmann et al., 1981; Schmidtmann and Valla, 1982). Harris et al. (1987) found that while the use of fenvalerate ear tags significantly reduced fly attack intensity, untreated and treated cattle spent the same amount of time grazing throughout the day.

2.4 Control and management of pest flies in grazing animals

Over the last 50 years, management of pest flies in animal production has relied heavily on the use of synthetic insecticides, often to control the adult stage. Insecticides that target larvae populations are not as common, as it is difficult to ensure thorough application to all areas where larvae can mature. Current pest management recommendations for on-farm practices aim to reduce areas that promote larvae growth such as wet bedding under water bowls and piles of spoilt silage. Recommendations of on-farm practices also include the use of physical fly traps such as sticky tape or pheromone-baited traps. Recently, the use of biological control agents such as releasing natural predators and parasitoids of larvae and pupae within barns have been used to reduce fly populations (Kaufman et al., 2012). Using a multi-level approach of the practices mentioned above is the best method for reducing fly populations on-farm especially within buildings. These practices however may not be effective for reducing the fly populations when animals are released onto pastures, hence the necessity for insecticides such as repellents. The problem may be exacerbated in organic animal production, as the animals must have access to grazing during the pasture season (Public Works and Government Services Canada, 2011).

There are many methods of insecticide application on cattle such as sprays, back-rubbers, pour-ons and dust bags. Treatment of cattle with pour-on insecticides has had success in long-term control of horn flies, achieving fly levels below 50 flies per side for up to 8 weeks following treatment (Lysyk and Colwell, 1996; Foil et al., 1998; Andress et al., 2000). This fly density is termed successful for achieving fly levels below the economic threshold of horn flies, which is estimated at 50 to 200 flies per animal (Andress et al., 2000). Long-term horn fly control has also been achieved with ethion insecticide ear tags, reducing horn fly levels more than 85% for 16 weeks following ear tagging (Anziani et al., 2000).

2.4.1 Essential oil repellents

Concerns over growing insect resistance to conventional treatments, environmental and health impacts, and a lack of new active compounds for insecticides have also created a need for new and unique strategies for pest management (Wall, 2012). While there has been a recent movement towards organic food, organic dairy producers have no registered pesticides available to use, leaving organic herds particularly vulnerable to high pest fly levels. One approach for natural and effective pest control that has received recent attention with encouraging results is the use of essential oils (Hieu et al., 2010).

Essential oils are volatile substances found in a wide variety of plant species and their terpenoid compounds can be natural insecticides (Isman, 2006). With hundreds of essential oils to choose from, studies have mainly focused on the laboratory bioassays to evaluate repellency and toxicity (Hieu et al., 2010; Baldacchino et al., 2013). Bioassays using an exposed human hand to stable flies found that binary mixtures of essential oils (clove bud, clove leaf, patchouli, savory or thyme white) and tamanu oil (*Calophyllum inophyllum* nut oil) effectively repelled flies for over 2 h (Hieu et al., 2010).

Baldacchino et al. (2013) found that stable flies avoided a zone containing blood mixed with lemongrass oil, spending more time in a zone with untreated blood (median values 64 s vs. 218 s). Lachance and Grange (in press) found that basil, geranium, lavender, lemongrass and peppermint essential oils repelled more horn flies than sunflower oil alone used as a carrier, for a period ranging between 1.5 and 4 h following treatments applied to dairy heifers. Effective stable fly repellency was also found using catnip oil in laboratory and field studies by Zhu et al. (2012). Field trials looking at stable flies on the legs of cattle found that a 15% catnip oil-based treatment and a 30% catnip water-based treatment had >90% repellency up to 5 h following application (Zhu et al., 2012). Kumar et al. (2011) were able to achieve >95% repellency of stable flies on cattle for 3 h post-treatment using a 10% menthone oil (*Mentha piperita*) solution. Menthone oil reduced fly levels from 137 flies h^{-1} landing on control animals to 5.5 flies h^{-1} landing on treated animals (Kumar et al., 2011). Khater et al. (2009) also found promising results on field trials with water buffalo, obtaining significant fly reductions using peppermint, camomile, onion and camphor oils. Essential oils provide a unique and encouraging new approach to pest management and repellents that requires further investigation.

2.5 Research objectives

The aim of the present study was to investigate the relationships between pest flies and behavioural responses of dairy cattle on pasture. The first objective was to assess the abundance of important pest flies in Eastern Ontario. Identification of individual fly species allowed for the assessment of unique abundance patterns through the summer months and the impact of abiotic and biotic factors on their abundance on cows. In order to evaluate the impact of varying fly densities on cattle behaviour, an organic fly repellent was developed to allow for use on the trial's organic dairy herd. Therefore the second objective was to evaluate the efficacy of the essential oil fly repellent (lemongrass oil and geranium oil), against multiple fly species throughout the summer months of July and August. The third objective was to determine the impact of pest fly species on cow behaviour, stress, and productivity. Cow behavioural measures included fly avoidance behaviours, pasture distance travelled, and time spent grazing and ruminating. Stress was measured using milk cortisol obtained from milk samples. Cow productivity was measured using milk production values.

Chapter 3: Attack intensity of pest flies and the behavioural responses of pastured dairy cows

3.1 Introduction

Pest flies are an economic and welfare concern in cattle production systems as they transmit pathogens, disturb feeding time, and negatively affect weight gain and milk production (Steelman et al., 1991; Byford et al., 1992; Dougherty et al., 1993b). Fly avoidance behaviours, disrupted grazing and increased stress of cattle can lead to energy expended on pest flies that could otherwise be used for biological functions (Schwinghammer et al., 1986b; Dougherty et al., 1993a, 1994; Mullens et al., 2006). Cattle defensive behaviours can range from tail flicking, leg stamping, head throwing and skin twitching to evasive displacement and bunching behaviour in an effort to lessen parasite attack intensity (Ralley et al., 1993).

Organic dairy herds are particularly vulnerable to pest fly implications as organic producers are required to provide cows and heifers access to pasture during the grazing season, but cannot use synthetic insecticides available to conventional dairy farms (Public Works and Government Services Canada, 2011). Fly control options for organic producers are limited, and present conflict between providing cows with the improved comfort of “natural living conditions” and reduced comfort of increased fly attack intensity. There is also a need for new and unique strategies for pest fly control, as there are growing concerns over insect resistance to synthetic insecticides, along with environmental and health impacts.

The aim of this study was to investigate the relationships between pest flies and behavioural responses of dairy cattle on pasture. The first objective was to assess the abundance of important pest flies in Eastern Ontario. The second objective was to evaluate the efficacy of an essential oil fly repellent (lemongrass oil and geranium oil), against multiple fly species throughout the summer months of July and August. The third objective was to determine the impact of pest fly species on cow behaviour, stress, and productivity. The impact of pest fly abundance on cows was evaluated by examining fly avoidance behaviours, grazing behaviour, pasture travel distance, milk cortisol and milk production.

3.2 Materials and methods

3.2.1 Animals and diet

Twenty-one lactating Holstein dairy cows were used in this experiment (9 primiparous, 12 multiparous, DIM mean ± SD = 122 ± 72) between June and September 2011. All animals were housed at the University of Guelph Organic Dairy Research Centre (Alfred, ON, Canada) and were cared for according to the Canadian Council on Animal Care guidelines (CCAC, 2009). Animals were on pasture for the entire duration of the experiment except for milking from approximately 0600-0730 h and 1700-1830 h. Animals were provided with a grain (oats and rolled corn) and vitamin-mineral supplement meal in the barn following milking, and water was available ad libitum. Animals were rotated between pastures at the Organic Dairy Research Centre to ensure continuous feed availability, and water was also available ad libitum on pasture. Pastures were composed of approximately 50% legumes and 50% grasses, with legume species being red clover, white clover, alfalfa, trefoil, and grass species being brome grass, fescue, timothy and orchard grass.

3.2.2 Experimental design

Animals were randomly divided into 2 groups of 10 animals, but were only physically separated during observation days. The trial length was 9 weeks with each group alternating between treated (essential oil repellent) and untreated each week. Group A was untreated on week one and therefore was treated on week 2. Group B was treated on week one and was untreated on week 2, creating a crossover experimental design with the following two sequences of treatment (U = Untreated, T = Treated):

Group A: U T U T U T U T U

Group B: T U T U T U T U T

Animals were treated on the observation day, usually on the Monday of the week, following morning milking. The essential oil mixture was applied < 30 min before the animals were released to pasture in the morning (usually at about 0800 h). The repellent mixture was manually applied, wearing nitrile laboratory gloves, to the cow's entire body except for the udder, hooves, and sensitive facial areas (i.e. close to the eyes, nose and mouth). The two groups were walked to pasture separately and were kept separated in the same pasture by a single fence wire, allowing for visual contact between the two groups. Cows were washed with dishwashing liquid soap (Green Works scent free, Clorox, Brampton, Ontario, Canada) and water on observation days following PM milking. One animal was removed after week 4 due to illness and another cow was added in week 5 of the trial.

3.2.3 Essential oil repellent

The fly repellent applied to all treated cows contained 95% sunflower oil, 2.5% geranium oil (*Pelargonium asperum*), and 2.5% lemongrass oil (*Cymbopogon citratus*). Both essential oils were produced by Aliksir Inc. (Quebec, Canada) and were certified organic by Ecocert Canada. Essential oils were stored in a refrigerator. The sunflower oil was produced by La Maison Orphée Inc. (Quebec, Canada) and was certified organic by Quality Assurance International (QAI). The volume of repellent applied to each animal was adjusted according to metabolic body weight (MBW=kg of $BW^{0.75}$), with a mean (± SD) of 115 ± 11 mL fly repellent mixture applied to animals. Individual mixtures were prepared the evening before the morning application and were stored in a refrigerator overnight.

3.2.4 Fly counts and fly avoidance behaviours

Direct observation in the field occurred at three time periods throughout each observation day; Period 1: 0900-1030 h, Period 2: 1200-1330 h, Period 3: 1500-1630 h. All cows were observed once during each time period, therefore every cow was observed three times on the observation day. Direct observations included a fly count by visual observation from a distance of approximately 1 m from cows, followed by 6 min of continuous recording of fly avoidance behaviours (see Table 1). Fly counts were recorded according to fly species and location on the cows' body. Locations on the cows' body used for recording fly location were: head, neck, back, left side, right side, belly, udder, and legs. Fly species that were identified were categorized as follows: house and face flies, horn flies, stable flies, deer and horse flies. House and face flies were grouped together because of the difficulty to differentiate between them in the field. Stable flies were recorded when identified on the legs of cattle and during weeks 3 to 9 as they were not present on cows at the onset of the trial. A single observation of bunching behaviour was recorded after each 6 min direct observation period. Bunching was recorded as the number of standing cows whose head was within 30-46 cm of the observation cow's head was recorded (adapted from Schmidtmann and Valla, 1982).

Observations were divided between 3 trained observers with each observer switching between treatments for each observation. Cows were observed in the same order at each of the three time periods within each observation day. Climate conditions were recorded at each observation time period using a hand held thermometer/anemometer (Mini Thermo-Anemometer, model 45158, Extech Instruments Corp., New Hampshire, USA). These were temperature, relative humidity, and mean and maximum wind speed. Observation days were not performed on rainy days, to avoid potential washing away/dilution of fly repellent treatment.

3.2.5 Grazing Behaviour

To monitor distance travelled on pasture, GPS devices (Foretrex 301 Personal Navigator, Garmin Ltd., Kansas, USA) were attached to the collar of two untreated and two treated cows each weekday. GPS devices were placed on the cows prior to leaving the barn after AM milking. They were removed immediately following entry into the barn at PM milking or during the day if cows were moved to a forested pasture for shade.

Every day data was downloaded from each GPS and converted into a Google Earth .kml file using GPS visualizer software (www.gpsvisualizer.com). Time and distance points recorded allowed for analysis of the first 3 h on pasture.

To monitor grazing behaviour on pasture, graze halters (IGER Behaviour recorder, version 3.6, model GREC 3, Ultra Sound Advice, London, UK) were placed on two untreated and two treated cows each weekday. Graze halters were placed on the cows prior to leaving the barn after AM milking. They were removed immediately following entry into the barn at PM milking or during the day if cows were moved to a forested pasture for shade. Data from each graze recording box was downloaded and saved onto a computer. Recordings were analyzed using the Graze program (version 0.80) developed for use with the graze halters. Raw data recordings were analyzed by Graze to identify jaw movements (grazing or rumination) and converted into an Excel (version 14.2.3) results file showing the duration of jaw movement bouts. For the final analysis only the first 3 h of data recording were used, as data for afternoons was insufficient due to mechanical issues occurring with the long-term use of the graze halters.

3.2.6 Milk yield and milk cortisol

Portable milk meters (WB Ezi-Test meter, Tru-Test Ltd., Auckland, New Zealand) were obtained from CanWest DHI and installed in the milking parlour to manually record individual milk yields on observation days and the evening milking the day prior. Milk samples (60 mL) were collected at each Sunday PM milking and at the PM milking following test day for analysis of milk cortisol. Milk samples were of foremilk, where the farm technician hand-stripped the first milk into a disposable cup before attachment of the milking unit. The observer recording milk yields would obtain the sample and place it into a labeled sample vial. Milk samples were placed in a freezer (-24°C) directly after milking time and remained frozen until sample analysis. Samples were centrifuged (15 min, 4 °C, 5000 rpm) and skim milk cortisol was analyzed using a commercial EIA kit (Product #EA65, Oxford Biomedical Research, Inc., Oxford, MI, USA). Details of this kit are available at (http://www.oxfordbiomed.com/cortisol-eia-kit) (last accessed on March 25th, 2013).

3.2.7 Statistical analysis

Residual plots were created using PROC PLOT to test for variance heterogeneity and ensure normality of data. Count data of flies and behaviours was square root transformed using the statement: SQRT(count + 0.375). Grazing time, rumination time and pasture travel distance data were subjected to an arcsine transformation using: ARSIN[SQRT(X/10800sec)]. The differences between the post-treatment values and the pre-treatment values for milk yield and milk cortisol were calculated in preparation for statistical analysis.

Data was analyzed using the PROC MIXED procedure in SAS (2011, version 9.3) with a crossover design. Sequence of treatment, week and treatment were tested as a fixed effect for all variables. Sequence of treatment was not significant for any variables. Cow within treatment group was tested as a random effect for all analyses. PROC MIXED including carry-over was run to test for possible carry-over effects of treatment, but none was found.

Estimated partial correlation coefficient values were generated using the ESTIMATE slope statement. For the analysis of factors associated with fly species, covariates that were tested for significance included temperature, wind, relative humidity, % black hair, lactation number, cow weight, and DIM. For the analysis of factors associated with avoidance behaviours, grazing, rumination, and pasture travel distance, the covariates tested for significance included house/face flies, horn flies, stable flies, and deer/horse flies. Estimates of least square means and standard errors were generated using the LSMEANS statement. For comparison between weeks, Tukey's method (Kramer, 1956) for multiple comparison adjustment of LS means was used.

Due to stable flies only being recorded on weeks 3 to 9, analysis for significant covariates associated with behaviours only included data for weeks 3 to 9. Only weeks 3 to 9 were used for analyzing treatment and week effects on stable fly data.

3.3 Results

3.3.1 Fly counts over the summer

The number of horn flies counted on untreated cows showed a linear increase over the summer months (α, β, R^2 = 7.46, 12.11, 0.96 and 34.96, 12.21, 0.86 for group 1 and group 2, respectively) (Figure 1). In contrast, the number of house and face flies and horse and deer flies remained fairly consistent over time (house flies: α, β, R^2 = 28.23, 1.30, 0.20, and 40.98, 2.03, 0.23, for group 1 and group 2, respectively; horse and deer flies: α, β, R^2 = 0.58, -0.02, 0.07, and 0.77, -0.04, 0.15 for group 1 and group 2, respectively) (Figure 1).

Table 2 shows the significant week effect observed on the mean number of total flies and on all fly species observed on treated and untreated cows (Table 2). For fly counts on cows combining all fly species, August 23 had the highest mean for total flies and July 4 had the lowest mean for flies. The lowest mean of horn flies was also observed on July 4, with the highest mean for horn flies observed on July 25. House and face flies also peaked on July 25, showed another peak on August 23rd, and then had their lowest mean observed on August 29. Deer and horse flies peaked the first week of the trial on July 4 and were not observed on the last three weeks of the trial on August 16, 23, and 29. Stable fly numbers fluctuated between weeks with their highest mean on August 8 and their lowest mean the following week on August 16.

3.3.2 Location and abundance of fly species on cows

The recording of fly species abundance according to location on the cows' bodies showed that fly species were not evenly distributed among the different body parts of untreated cows (Table 3). The body location with the highest abundance of all fly species totaled together was the belly where 37.0 % of flies were observed. The back, sides, and belly accounted for 21.2 %, 18.7 %, and 13.2 % of flies on the body, respectively. House and face flies were the most abundant fly species observed on the head of cows, accounting for 96.7 % of all flies. Horn flies and house and face flies were the most abundant fly species observed on the neck area of cows, accounting for 70.2 % and 29.7 % of all flies, respectively. Horn flies were also the most abundant fly species observed on the back, side, belly and leg areas of cows, accounting for 79.8 %, 88.1 %, 82.2 %, and 52.5 % of all flies, respectively.

3.3.3 Treatment effect on fly counts and fly avoidance behaviours

Treatment of cows with the essential oil fly repellent reduced the total number of flies on cows by 69.5 % and that of all individual fly species (Table 4). Horn flies were the most abundant species observed on cows, and were reduced by 82.5 % on treated cows. House and face flies were the second most abundant species observed on cows and were reduced by 36.5 % on treated cows. Deer and horse flies were the least abundant fly species observed on cows and were reduced by 54.3 % on treated cows. During weeks 3 to 9, stable fly counts on the legs of cattle showed that the repellent treatment reduced mean fly counts on treated cows by 26.6 %, relative to untreated cows.

Fly avoidance behaviours of cows treated with the essential oil fly repellent were also reduced in comparison to untreated cows (Table 5). Total tail flicks, representing partial and full tail flicks combined, were the most abundant fly avoidance behaviours observed in cows. Total tail flicks, partial tail flicks and full tail flicks were reduced by 44.8 %, 38.7 % and 51.4 % respectively, in cows treated with the fly repellent. Skin twitches were the second most abundant fly avoidance behaviours observed. Treated cows had a 54.0 % reduction in skin twitches, relative to untreated cows. Head throws, leg stamps, and side licks were all reduced on treated cows by 78.9 %, 45.1 %, and 33.3 % respectively, in comparison to untreated cows. A treatment effect was also found for cattle bunching behaviour, with treated cows having a 70.6 % reduction in bunching.

3.3.4 Week effect on fly avoidance behaviours

Table 6 shows a week effect observed for all fly avoidance behaviours except side licks. Total tail flicks, partial tail flicks, and full tail flicks had the highest mean rate of behaviour on the last week of the trial on August 29, and the lowest rate for total and full flicks were observed on July 19, and for partial flicks, on August 2. Mean rates of skin twitches, head throws, and leg stamps peaked on August 8, with the lowest mean rates of skin twitches and leg stamps on August 29 and July 19 for head throws. The number of cows involved in bunching behaviour was observed to be the highest on August 8 and the least on August 29.

3.3.5 Effect of abiotic and biotic factors on fly counts and fly avoidance behaviours

Several abiotic and biotic factors were found to be associated with total fly counts and counts for individual fly species (Table 7). Temperature was found to have the strongest positive correlation with total number of flies, and also showed a positive association with house and face flies and stable flies. Wind speed was the factor most positively associated with house and face flies along with deer and horse flies, and also showed a positive association with total flies and stable flies. Negative associations were found between temperature and deer and horse flies, as well as between relative humidity and stable flies.

Analysis of associations between fly avoidance behaviours and fly species revealed that two groups of biting flies, the stable flies and horse and deer flies, were positively associated with the majority of the behaviours (Table 8). Stable flies were found to have a positive partial correlation with total tail flicks, partial tail flicks, skin twitches, head throws, leg stamps, bunching, and to be negatively correlated with full tail flicks. Horse and deer flies were found to have a positive partial correlation with partial tail flicks, skin twitches, head throws, leg stamps, bunching and a negative partial correlation with full tail flicks. Horn flies were partially correlated with head throws, with a positive association between the two variables. House and face flies were positively associated with partial tail flicks and leg stamps. There were no associations found between the side lick behaviour and fly species.

3.3.6 Treatment effect on pasture behaviours, milk yield and milk cortisol

Treatment of cows with the essential oil fly repellent had an effect on grazing, rumination, and travel distance on pasture (Table 9). Cows treated with the fly repellent had a 6.3 % increase in the median amount of time spent grazing in comparison to untreated cows. Conversely, untreated cows had an 86.4 % increase in the median amount of time spent ruminating, in comparison to treated cows. Untreated cows had a 3.8 % increase in the median pasture travel distance, relative to cows treated with the fly repellent.

Production data of the trial cows showed that the average milk yield at PM milking was 14.1 ± 3.0 kg (mean ± SD) and average milk cortisol level was 1.36 ± 1.18 μmol ml^{-1}. No treatment effects were found for differences in milk yield (post treatment/observation day – pre-treatment/previous Sunday, which were 0.10 ± 0.15 and 0.22 ± 0.15 kg per animal (LS mean ± SE), respectively for treated and untreated cows (P = 0.43). Milk cortisol differences (post treatment/observation day – pre-treatment/previous Sunday) were not affected by treatment either, which were -0.10 ±

0.07 and -0.05 ± 0.07 μmol ml^{-1} (LS mean ± SE), respectively for treated and untreated cows (P = 0.31).

3.3.7 Week effect on pasture behaviours, milk yield and milk cortisol

Table 10 shows a week effect observed in grazing time, travel distance, milk yield, and milk cortisol among both treated and untreated cows. Cows had the highest median time spent grazing, within the first 3 h on pasture, on August 2 and the lowest median on August 29. The highest median for pasture distance travelled by cows was on August 8, with July 4 having the lowest median for distance travelled. Milk yield and milk cortisol differences showed fluctuating weekly LS means, with LS means among weeks having both positive and negative differences. Milk yield difference had the highest positive increase in milk on August 8, with the highest reduction in milk on August 2. Milk cortisol difference showed the highest increase in milk cortisol to be on July 11, with the highest reduction in milk cortisol on August 2.

3.4 Discussion

3.4.1 Fly counts

Many researchers who have focused on house and face fly levels observed only on the face of cattle, therefore our weekly means that included house and face flies on other body parts were higher than weekly means reported in previous studies (Schmidtmann and Valla, 1982; Schmidtmann, 1985; Skoda et al., 1987). Schmidtmann and Valla (1982) observed a mean of 17.1 face flies per face on heifers that were on the outside of bunching groups.

The pattern in horn fly abundance during summer observed in the present study was similar to patterns observed in other studies from both temperate and tropical environments. Unimodal patterns with low fly densities observed at the beginning of summer with a single peak in late summer have been observed in other horn fly studies (Castro et al., 2008; Maldonado-Siman et al., 2009). The peak in mean horn flies observed on untreated cows observed in our study (191 flies per animal) is similar to peak means reported in other studies of temperate and subtropical environments (165 flies per animal: Lima et al., 2003; 200 flies per animal: Jensen et al., 2004; 200 flies per animal: Castro et al., 2008; 193 flies per animal: Maldonado-Siman et al., 2009).

The seasonal pattern observed in horse and deer fly abundance during July and August was similar to patterns observed in Canadian studies by Lewis and Leprince (1981) and Ralley et al. (1993). Both studies also found that horse and deer fly abundance was lower in August in comparison to July. Lewis and Leprince (1981) studied horse and deer flies in Southwestern Quebec and found that the number of deer and horse fly species observed on cows were lower in August (1 species), in comparison to July (10 species). Attack intensity of horse and deer flies on cattle in Oklahoma varied between months with mean fly attack intensities of 117 and 9 flies per animal in July and August, respectively (Perich et al., 1986). Weekly means observed in horse and deer fly abundance were lower than means observed in several studies from the southern United States, but similarly showed fluctuations between weeks (Wright et al., 1984; Perich et al., 1986; Hribar et al., 1992).

Weekly fluctuations in stable fly abundance were also observed by Mullens et al. (2006), who reported that stable fly abundance varied between weeks during the spring on a California dairy. Stable fly means observed on cattle in the last two weeks of July during our trial were comparable to counts observed by Dougherty et al. (1993c). Stable fly means observed on the legs of cattle on July 19 and 25 were 4.0 and 12.1 per animal, respectively, while Dougherty et al. (1993c) had a mean number of 6.5 stable flies per animal on the legs only between July 17 and 21.

The fact that two groups were used in an alternating pattern must be taken into account when looking at the week effect and weekly means observed for fly counts. If one group had several cows that were naturally more attractive to flies, than some of the differences in weekly means could be related to fly attractiveness of the group rather than changing fly abundance or weather variables.

3.4.2 Factors associated with fly counts

While several studies have found that face fly abundance increases during spring and summer months in temperate environments, none has associated weather variables with face fly abundance (Schmidtmann and Valla, 1982; Schmidtmann, 1985a; Skoda et al., 1987). This is also the case for house fly abundance on cattle and weather variables.

The positive associations between wind speed and total flies, house and face flies, and deer and horse flies, indicate that this weather variable may be an important factor that has been overlooked in previous studies. The positive correlation found between wind speed and stable fly abundance is in disagreement with Berry and Campbell (1985), who found that feeding rates of flies on cattle decreased with increasing wind.

The positive association between horn flies and percentage black hair observed in the present study is in disagreement with other studies that have focused on different coat colours of cattle (Ernst and Krasfur, 1984; Guglielmone et al., 2002). Guglielmone et al. (2002) found no difference in horn fly abundance between Holstein (black and white coat) and Holstein x Jersey (black coat) cattle, however the effect of coat colour in that study may have been confounded by the breed differences between the cattle.

In the present study, no associations were found between horn fly abundance and the weather factors that were recorded (temperature, relative humidity, and wind speed). In contrast, a two-year study by Maldonado-Siman et al. (2009) found horn fly abundance to be positively correlated with rainfall ($r = 0.67$, $P < 0.05$), and abundance in the second year alone was also positively correlated with relative humidity ($r = 0.75$, $P < 0.05$). Several studies have also found a positive correlation between horn fly abundance and preceding rainfall, suggesting that environmental precipitation may play a critical role in their larvae development (Barros, 2001; Lima et al., 2003; Urech et al., 2012). While several studies have found temperature to be an important factor in horn fly abundance, (Guglielmone et al., 1997; Lima et al., 2003; Castro et al., 2008), the absence in this study may in part be due to the narrow range of temperatures observed (24.0 – 32.2 °C) in comparison to the other studies.

While a negative association was found between temperature and horse and deer fly abundance, some studies have found positive associations (Guglielmone et al., 1997; Castro et al., 2008). Disagreement may be due to the narrow range of temperatures during the present trials, in comparison to the other studies that followed fly levels through an entire year.

The positive correlation that was observed between black hair coat and horse and deer flies is in agreement with several other studies that have found dark hair coats to be more attractive than white coat colour (Horváth et al., 2008, 2010; Blaho et al., 2012). The differential attraction to coat colours is due to their different polarizing properties and the polarotactic behaviour of horse and deer flies (Horváth et al., 2010). Dark hair coats such as black and brown have a higher degree of polarization and are thus more attractive than white coats with a low degree of polarization (Horváth et al., 2010).

The positive association between stable flies and temperature was in agreement with findings by Gilles et al. (2008), who reported positive partial regression coefficients between temperature and stable flies ($\beta = 0.53\text{-}0.95$). The negative association between stable flies and humidity observed was also in agreement with findings by Gilles et al. (2008), who found negative partial regression coefficients between humidity and flies ($\beta = -0.15\text{-}(-0.57)$). Berry and Kunz (1977) also found that adult stable fly survival decreases with increasing relative humidity.

Most studies, including the present one, correlating weather factors and fly populations tend to overlook a couple of fundamental elements of population dynamics (Castro et al., 2008). The first is the fact that a population observed at a given time is related to the population of the previous generation; therefore weather factors influencing the previous year's fly population will most likely play a large role in the current year's population (Castro et al., 2008). The second key point is that weather variables such as temperature and rainfall can play a large role in the developmental time of larvae, thus weather conditions during the weeks prior to observations may play a larger role than current weather conditions (Maldonado-Siman et al., 2009).

3.4.3 Treatment effect on fly counts

The reduction in fly abundance that was observed with the essential oil fly repellent was in agreement with other studies that have also found essential oils to have effective fly repelling properties (Hieu et al., 2010; Kumar et al., 2011; Zhu et al., 2012). While few studies have field-tested essential oils on livestock species, the reduction in fly attack intensities shows a promising use for these oils as fly repellents (Kumar et al., 2011; Zhu et al., 2012). The choice of essential oils for the present study was based on previous results found by Lachance and Grange (in press), who noted that a 5 % lemongrass and sunflower oil mixture and a 5 % geranium oil and sunflower oil mixture both had over 90 % repellency up to 8 h following application on cows. The lower repellency that was observed in the present study may be due to the application of repellent on the entire body, in comparison to Lachance and Grange who only tested repellents on a 4675 cm^2 square area on the flanks of cows. Khater et al. (2009) found that several essential oils (7.5 % each of camphor, onion, peppermint, chamomile, rosemary) were effective at reducing house flies, stable flies and horn flies on water buffaloes for up to 6 days following application. Although repellency length was not tested in the present study, studies such as Khater et al. (2009) show that further work is needed in determining effective concentrations of essential oils to ensure successful efficacy over time.

3.4.4 Fly avoidance behavioural counts and associations with fly species

The finding that tail flicks were the most abundantly performed fly avoidance behaviour in cattle is in agreement with several other studies (Ralley et al., 1993; Dougherty et al., 1993b,1994,1995). Weekly changes in tail flick rates are most likely related to changes in fly species abundance as observed in the associations between fly species and the behaviour. The mean rate of total tail flicks in untreated cattle that was observed (5.1 min^{-1}) was similar to the peak mean rate of tail flicks (4.5 min^{-1}) observed by Mullens et al. (2006). The mean rate was higher due to the recording of tail flicks on both sides of the animal, as opposed to flicks directed to only one side as reported in Mullens et al. (2006), and also due to the higher numbers of horse and deer flies observed in the present trial.

There was a positive association between partial tail flicks and the number of stable flies and horse and deer flies, and conversely, there was a negative association between full tail flicks and stable flies and horse and deer flies. This finding may suggest that, at low stable fly and low horse and deer fly intensities, cattle use full tail flicks to remove flies, but when fly intensities increase, cattle use only partial tail flicks and increase other avoidance behaviours such as head throws and leg stamps. Dougherty et al. (1993b) found that although there was a positive linear association between tail flick rates and stable fly abundance, as meal time progressed there was an increase in the rate of aggressive head throwing behaviour. It is believed that as grazing progresses and cows approach satiety, they are more likely to perform energy intensive behaviours that disrupt grazing such as head throwing (Dougherty et al., 1993b). Habituation to stable flies during a 1 h grazing meal was reported by Dougherty et al.

(1993c), who observed a reduction in tail flick rate from 69 to 17 flicks min^{-1} per animal following the release of 2500 stable flies.

The finding that skin twitches were the second most abundantly performed fly avoidance behaviour in cattle is in agreement with several other studies (Dougherty et al., 1993b,c; Mullens et al., 2006). The positive association found between skin twitch rate and stable flies is in agreement with several studies (Dougherty et al., 1993b,c; Mullens et al., 2006). The mean skin twitch rate observed in untreated cattle (3.7 min^{-1}) was similar to mean rates obtained by Dougherty et al. (1993c) on grazing cattle exposed to natural pest populations (2.8 min^{-1}).

The mean rate of head throws observed in our untreated cows (0.57 min^{-1}) was similar to rates observed by Dougherty et al. (1993c) (0.64 min^{-1}) in grazing cattle exposed to natural pest populations. The low frequency of head throwing behaviour in comparison to frequent behaviours such as tail flicking was made evident by Dougherty et al. (1993c). These authors observed that releases of 2500 stable flies resulted in peak tail flicks rate of 69 min^{-1} and peak head throws rate of 8 min^{-1}, during the first 0.2 h of grazing. These rates of tail flicks and head throws declined over the grazing period, indicating that habituation may play a factor in behavioural responses over time (Dougherty et al., 1993c). The positive association between stable flies and head throws in the present study is in agreement with positive correlations observed by Dougherty et al. (1993b,c,1994). The positive association between horse flies and head throws observed in the present study was also observed by Ralley et al. (1993).

The mean leg stamp rate observed in untreated cows (0.51 min^{-1}) was similar to rates observed by Dougherty et al. (1993c) (0.57 min^{-1}) and Ralley et al. (1993) (0.42

min^{-1}). While an association was observed between house and face flies and leg stamps, Dougherty et al. (1993a) found that house flies had no effect on leg stamp rate. On the other hand, a positive association found between stable flies and leg stamps has also been observed in several studies (Dougherty et al., 1993b,c,1995; Mullens et al., 2006). The frequency of leg stamps has been suggested as an ideal way to determining stable fly abundance levels for use in pest management strategies, due to the feeding behaviour of stable flies on the legs (Mullens et al., 2006). Although not correlated in other studies, the present results suggest that other species such as deer and horse flies appear to also have an influence on leg stamp behaviour.

The increased bunching behaviour observed in untreated cattle is in agreement with observations made by Ralley et al. (1993). These authors reported that bunching behaviour was a response to biting flies, especially horse flies. While Schmidtmann and Valla (1982) found a positive association between face flies and bunching behaviour, numbers of other fly species were not counted. More research on the flies associated with this behaviour is required to better understand the role it plays in cattle pasture behaviour. It has also been noted that bunching behaviour may cause animals to increase in body temperature, which can be further detrimental in hot temperatures experienced during summer months (Ralley et al., 1993).

3.4.5 Grazing time, rumination time, and travel distance

The increased grazing time observed in treated cows in the present study is in agreement with the findings of Schmidtmann and Valla (1982). They found that during the daytime hours (0500-2200 h), treated cattle grazed for an average of 31 min/d more than untreated cattle. Dougherty et al. (1994,1995) found that increased levels of stable flies resulted in a linear increase in bite mass and DMI, thus cattle may be able to compensate for reduced grazing time due to flies. Rumination time has not been studied in previous studies comparing cattle treated with fly repellents and untreated cattle, as many of these studies focused on food ingestion or grazing behaviour (Dougherty et al., 1993b,c,1994,1995). The low values of rumination time observed may be due to cows focusing their energy on grazing versus rumination during the first 3 h on pasture following milking time. For example, Schirmann et al. (2012) found that peak rumination does not occur until approximately 4 h following feeding.

The increased pasture travel distance that was observed in untreated cattle was in agreement with Harvey and Launchbaugh (1982), who found that untreated cattle travelled an extra 0.5 km day^{-1} in an effort to avoid fly attack. The increased travel distance observed in our untreated cattle may be more related to the increased bunching, as it is an active behaviour in which animals move around in an effort to be in the centre of the group (Ralley et al., 1993).

3.4.6 Milk yield and milk cortisol

The absence of a treatment effect on milk production has been noted in several other studies (Burton et al., 1984; Harris et al., 1987). However, there have been some earlier studies that did find significant differences in milk yields between treated and untreated cattle (Bruce and Decker 1947,1958). The absence of a treatment effect may be due to several factors such as below-threshold fly levels, treatment duration, and the large variability in trial cow milk yields. Schwinghammer et al. (1986a,b) previously found that cattle subjected to 500 horn flies or 50 stable flies on their bodies had increased blood cortisol, as well as increased respiration and heart rate. The absence of a treatment effect is similar to milk yield in that fly levels may have been below a "stressful threshold", the treatment duration could have been insufficient to reduce cortisol levels, and variability in the individual cortisol levels between cows and days may have been too high.

3.5 Conclusion

This study provided a unique overview of the pest fly species and their abundance during summer months on pastured dairy cows in Eastern Ontario. A better understanding of the relationship between pest fly abundance and fly avoidance behaviours can aid in making effective pest management decisions. Even though the essential oil fly repellent did not influence milk production or milk cortisol, its reduction in pest flies and fly avoidance behaviours can offer an effective alternative to synthetic chemical fly repellents. Reduced fly attack intensity can provide cows with increased comfort, to focus energy on grazing behaviour and avoid the pathogens that pest flies can transmit.

Table 1. Ethogram of fly avoidance behaviours recorded, adapted from Ralley et al., 1993; Dougherty et al., 1995.

Fly avoidance behaviour	Definition
Tail flick (Full and partial tail flicks)	The movement of the tail from its resting position to either side of the cow and back towards its resting position. If the tail is brought back across the resting position to the opposite side of the body it is counted as a second tail flick. *Full tail flick*. When the tail flick reaches to the top of the back (reaching to or beyond the vertebral column). *Partial tail flick*. When the tail flick does not reach the top of the back (tail hits the side, belly, or back below the vertebral column).
Skin twitch	Skin twitching (also known as the panniculus reflex) is the contraction of the cutaneous trunci muscle located under the skin. Skin twitches in a localized area or for a continuous duration over the entire side are both counted as a single event.
Head throw	The movement of the head around to one side of the body (judged to be in response to flies).
Leg stamp	The movement of a front or rear leg off the ground judged to have been induced by flies; this includes both vertical motion of the leg or a horizontal leg kick towards the belly. It does not include leg movements related to locomotion.
Lick side	A single event involves the movement of the head around to the side of the body and licking of side (self-grooming). Both a single lick of the side or licking for a continuous duration is recorded as a single event.

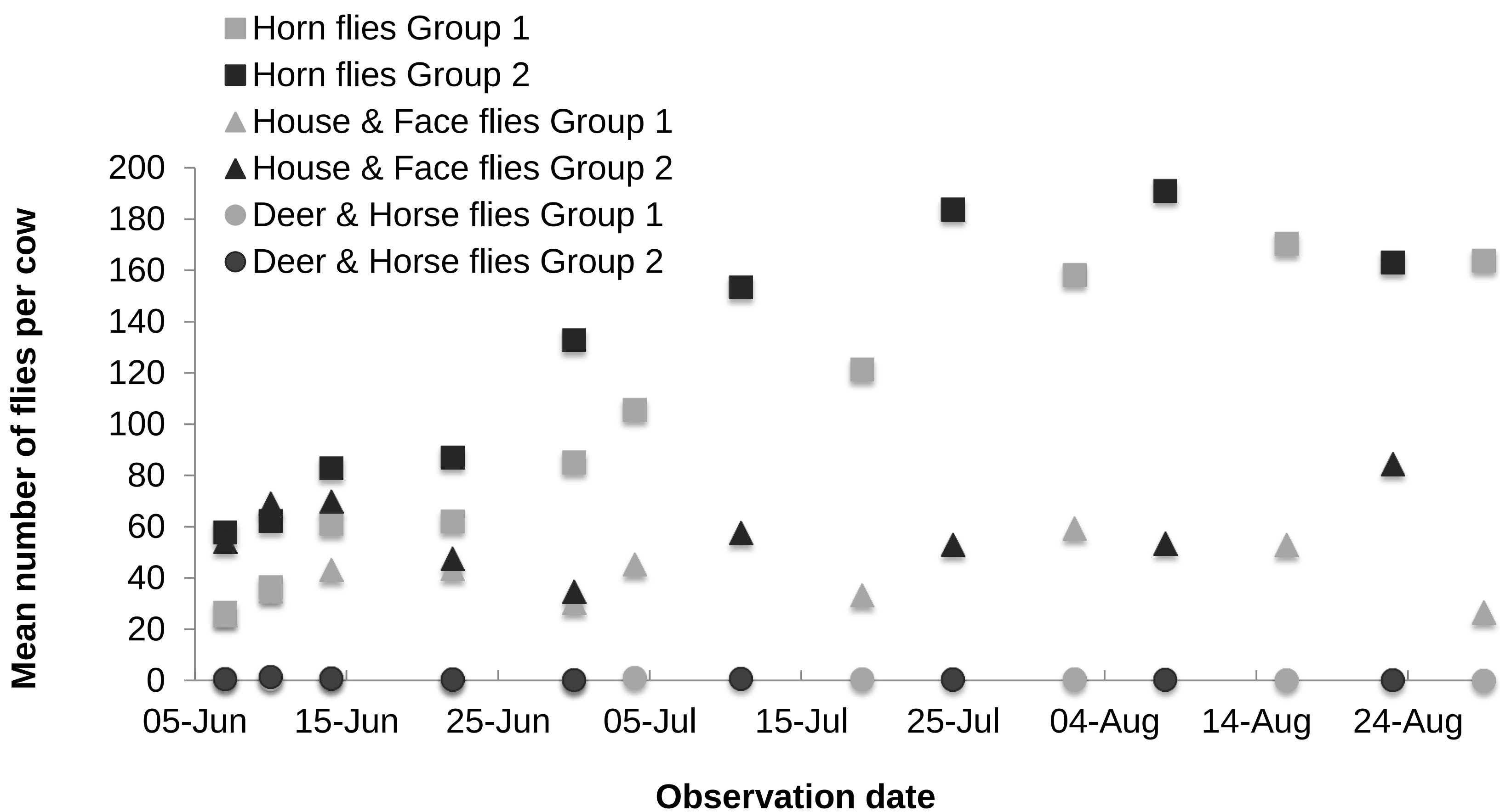

Figure 1: Mean number of flies per cow on untreated cows[1] only, according to fly species and cow group.

[1] The 9 week trial ran from July 4 to August 29.

Table 2: Week effect[1] on total fly counts per cow and fly counts according to fly species per cow.

	Week 1 July 4	Week 2 July 11	Week 3 July 19	Week 4 July 25	Week 5 Aug. 2	Week 6 Aug. 8	Week 7 Aug. 16	Week 8 Aug. 23	Week 9 Aug. 29	*P*
Total flies[2]	85.96	107.35	106.87	152.81	130.86	128.71	111.18	154.31	115.39	<0.001
Horn flies	52.52	65.94	64.15	88.16	78.51	69.42	82.02	72.85	84.56	<0.001
House & face flies	36.87	40.91	43.62	60.63	35.49	38.90	17.24	63.15	15.45	<0.001
Deer & horse flies	1.09	0.51	0.80	0.51	0.03	0.10	0	0	0	<0.001
Stable flies[2]	---	---	4.01	12.13	1.92	13.14	0	2.78	0.46	<0.001

[1] LS means presented are back-transformed following statistical analysis as data was transformed using sqrt (count + 0.375) and represent both treated and untreated cow data.
[2] Stable fly counts were done during trial weeks 3 to 9 only; other counts were done over 9 weeks.

Table 3: Location and abundance of fly species on untreated cows.

	Head	Neck	Back	Sides	Belly	Legs	Udder
Total flies	12.65	7.58	45.05	39.72	78.77	28.11	1.00
House and face flies	12.23	2.25	9.04	4.66	13.91	9.28	0.42
Horn flies	0.40	5.32	35.94	35.00	64.76	14.75	0.57
Deer and horse flies	0.02	0.01	0.07	0.06	0.10	0.14	0.01
Stable flies[1]	---	---	---	---	---	3.94	---

[1] Stable fly counts were done during trial weeks 3 to 9 only; other counts were done over 9 weeks.

Table 4: Effect of an essential oil fly repellent on total fly counts and fly counts according to fly species.

	Treatment[1]	LS mean[3]	Lower CL[3]	Upper CL[3]	*P*
Total flies					
	Untreated	200.22	180.44	221.02	< 0.001
	Treated	60.97	50.26	72.71	
Horn flies					
	Untreated	144.41	129.20	160.47	< 0.001
	Treated	25.29	19.13	32.30	
House & face flies					
	Untreated	46.41	40.26	52.98	< 0.001
	Treated	29.49	24.63	34.80	
Deer & horse flies					
	Untreated	0.35	0.29	0.42	< 0.001
	Treated	0.16	0.10	0.21	
Stable flies[2]					
	Untreated	4.02	3.21	4.91	0.012
	Treated	2.95	2.25	3.74	

[1] Treated cows received application of the essential oil repellent mixture of sunflower, lemongrass and geranium oil; an average of 115 ± 11 mL fly repellent mixture was applied to animals.
[2] Stable fly counts were done during trial weeks 3 to 9 only; other counts were done for a 9 week period.
[3] Count data was transformed using sqrt (count + 0.375), thus LS mean, lower confidence limit (CL) and upper CL presented are back-transformed following statistical analysis.

Table 5: Effect of a reduced fly intensity induced by an essential oil fly repellent on cattle defensive behaviours.

	Treatment[1]	LS mean[4]	Lower CL[4]	Upper CL[4]	*P*
Total tail flicks[2]					
	Untreated	5.13	4.49	5.82	< 0.001
	Treated	2.83	2.34	3.36	
Partial tail flicks[2]					
	Untreated	3.00	2.67	3.34	< 0.001
	Treated	1.84	1.57	2.11	
Full tail flicks[2]					
	Untreated	1.77	1.41	2.17	< 0.001
	Treated	0.86	0.59	1.17	
Skin twitches[2]					
	Untreated	3.72	3.33	4.13	< 0.001
	Treated	1.71	1.43	2.00	
Head throws[2]					
	Untreated	0.57	0.49	0.65	< 0.001
	Treated	0.12	0.07	0.18	
Leg stamps[2]					
	Untreated	0.51	0.41	0.63	< 0.001
	Treated	0.28	0.19	0.38	
Side lick[2]					
	Untreated	0.09	0.07	0.12	0.034
	Treated	0.06	0.04	0.09	
Bunching[3]					
	Untreated	0.51	0.44	0.59	< 0.001
	Treated	0.15	0.10	0.21	

[1] Treated cows received application of the essential oil repellent mixture, lemongrass and geranium oil in carrier sunflower oil; an average of 115 ± 11 mL fly repellent mixture was applied to animals.
[2] Behavioural frequencies are averaged from a 6 min observation period on each animal.
[3] Bunching behaviour was recorded as a single observation of cows in close proximity to animals following the 6 min observation period.
[4] Count data was transformed using sqrt (count + 0.375), thus LS mean, lower CL and upper CL presented are back-transformed following statistical analysis.

Table 6: Week effect on fly defensive behaviours[1].

	Week 1 July 4	Week 2 July 11	Week 3 July 19	Week 4 July 25	Week 5 Aug. 2	Week 6 Aug. 8	Week 7 Aug. 16	Week 8 Aug. 23	Week 9 Aug. 29	*P*
Total tail flicks[2]	3.76	3.53	2.95	3.34	3.18	4.03	4.66	4.08	5.95	< 0.001
Partial tail flicks[2]	2.43	2.06	1.84	1.96	1.82	2.61	2.75	2.38	3.90	< 0.001
Full tail flicks[2]	1.15	1.28	0.99	1.15	1.15	1.15	1.54	1.43	1.82	0.028
Skin twitches[2]	2.77	3.02	1.69	3.00	2.82	3.69	3.02	2.52	1.47	< 0.001
Head throws[2]	0.24	0.33	0.17	0.42	0.36	0.45	0.40	0.30	0.33	<0.001
Leg stamps[2]	0.30	0.44	0.27	0.39	0.47	1.06	0.33	0.33	0.10	< 0.001
Side lick[2]	0.07	0.08	0.05	0.11	0.08	0.06	0.11	0.07	0.07	0.412
Bunching[3]	0.07	0.18	0.29	0.33	0.44	0.62	0.54	0.50	0.06	< 0.001

[1] LS means presented are back-transformed following statistical analysis as data was transformed using sqrt (count + 0.375) and represent both treated and untreated cow data.
[2] Behavioural frequencies are averaged from a 6 min observation period on each animal.
[3] Bunching behaviour was the number of cows in close proximity to the observation animal following the 6 min observation period.

Table 7: Associations between fly counts and environmental variables (β ± SE).

Variable	Covariate	β[1]	SE	*P*
Total flies				
	Temperature	0.161	0.060	0.007
	Wind speed	0.140	0.070	0.045
	Black hair	0.049	0.013	0.002
House and face flies				
	Temperature	0.136	0.046	0.004
	Wind speed	0.372	0.054	<0.001
Horn flies				
	Black hair	0.051	0.012	0.001
Deer and horse flies				
	Temperature	-0.017	0.008	0.033
	Wind speed	0.043	0.009	<0.001
	Black hair	0.002	0.001	0.001
Stable flies				
	Temperature	0.095	0.041	0.021
	Humidity	-0.058	0.036	0.013
	Wind speed	0.091	0.017	0.001

[1] β represents the partial correlation coefficients. Only significant factors are presented.

Table 8: Associations between fly avoidance behaviours and fly counts (β ± SE).

Variable	Covariate	β[1]	SE	*P*
Total tail flicks				
	Stable flies	0.060	0.022	0.007
Partial tail flicks				
	House and face flies	0.028	0.012	0.022
	Stable flies	0.113	0.023	<0.001
	Horse and deer flies	0.167	0.080	0.036
Full tail flicks				
	Stable flies	-0.066	0.022	0.004
	Horse and deer flies	-0.235	0.082	0.005
Skin twitches				
	Stable flies	0.130	0.019	<0.001
	Horse and deer flies	0.214	0.069	0.002
Head throws				
	Horn flies	0.012	0.004	0.003
	Stable flies	0.029	0.009	0.002
	Horse and deer flies	0.187	0.033	<0.001
Leg stamps				
	House and face flies	0.032	0.007	<0.001
	Stable flies	0.055	0.014	<0.001
	Horse and deer flies	0.102	0.047	0.033
Bunching				
	Stable flies	0.050	0.016	0.002
	Horse and deer flies	0.145	0.061	0.019

[1] β represents the partial correlation coefficients. Only significant factors are presented.

Table 9. Treatment effect on grazing time, rumination time and pasture travel distance between cows treated with essential oil fly repellent and untreated cows.

	Treatment[1]	n[2]	Median	Min.	Max.	*P*
Grazing duration (min/180min)[3]						
	Untreated	42	106.6	68.0	143.4	0.002
	Treated	32	113.8	43.8	173.2	
Rumination duration (min/180min)[3]						
	Untreated	42	15.4	0	54.1	
	Treated	32	2.1	0	45.9	0.005
Pasture travel distance (km/180min)[3]						
	Untreated	71	0.26	0.14	0.54	0.0003
	Treated	63	0.25	0.09	0.58	

[1] Treated cows received application of the essential oil repellent mixture, lemongrass and geranium oil in carrier sunflower oil; an average of 115 ± 11 mL fly repellent mixture was applied to animals.
[2] Total number of data points used in analysis.
[3] Data averaged from 9 week trial period, with 180 min data collection period immediately upon entrance to pasture following morning milking.

Table 10. Week effect on grazing time, rumination time, pasture travel distance, milk yield and milk cortisol[1,2].

	Week 1 July 4	Week 2 July 11	Week 3 July 19	Week 4 July 25	Week 5 Aug. 2	Week 6 Aug. 8	Week 7 Aug. 16	Week 8 Aug. 23	Week 9 Aug. 29	*P*
Grazing time (min/180min)	101.13	101.93	105.81	119.13	133.50	123.61	103.58	126.25	84.82	<0.001
Rumination time (min/180min)	28.72	6.33	22.12	15.58	0	16.16	21.18	0	38.67	<0.001
Pasture travel distance (km/180min)	0.21	0.27	0.30	0.25	0.30	0.41	0.22	0.23	0.23	<0.001
Milk yield difference (kg)[3]	-0.45[de]	0.16[cd]	-0.35[de]	0.75[bc]	-1.22[f]	2.08[a]	-0.53[e]	1.01[b]	0.01[de]	<0.001
Milk cortisol difference (μmol/mL)[3]	-0.11[cd]	0.27[a]	-0.04[bcd]	0.02[bc]	-0.42[e]	0.12[ab]	-0.25[de]	-0.09[bcd]	-0.18[cd]	<0.001

[1] Medians for grazing, rumination and pasture travel distance are presented as data was transformed using arcsine(sqrt). LS means for milk yield difference and milk cortisol difference are presented as data was untransformed. Both medians and LS means represent data from treated and untreated cows combined

[2] a,b,c, $P < 0.05$

[3] Differences were calculated as: Milk yield difference = Observation day PM milk yield - Sunday PM milk yield
Milk cortisol difference = Observation day PM milk cortisol – Sunday PM milk cortisol

REFERENCES

Andress, E. R., S. M. DeRouen and L. D. Foil. 2000. Efficacy of doramectin 0.5% w/v pour-on control of the horn fly, *Haematobia irritans*. Vet. Parasitol. 90:327-331.

Anziani, O. S., G. Zimmermann, A. A. Guglielmone, M. Forchieri and M. M. Volpogni. 2000. Evaluation of insecticide ear tags containing ethion for control of pyrethroid resistant *Haematobia irritans* (L.) on dairy cattle. Vet. Parasitol. 91:147-151.

Baldacchino, F., C. Tramut, A. Salem, E. Liénard, E. Delétré, M. Franc, T. Martin, G. Duvallet and P. Jay-Robert. 2013. Repellency of lemongrass oil against stable flies, tested using video tracking. Parasite. 20,21.

Barros, A. T. M. 2001. Dynamics of *Haematobia irritans irritans* (Diptera: Muscidae) on Nelore cattle in the Pantanal, Brazil. Mem. I. Oswaldo Cruz. 96:445-450.

Baumgard, L. H. and R. P. Rhoads. 2012. Ruminant nutrition symposium: Ruminant production and metabolic responses to heat stress. J. Anim. Sci. 90:1855-1865.

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

Berkebile, D. R., R. D. Hall and J. J. Webber. 1981. Moraxella-bovis survival on female face flies *Musca-Autumnalis* laboratory studies. Trans. Mo. Acad. Sci. 15:83-88.

Berkebile, D. R., G. D. Thomas and J. B. Campbell. 1994. Overwintering of the stable fly (Diptera: Muscidae) in southeastern Nebraska. J. Econ. Entomol. 87:1555-1563.

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

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

Birkett, M. A., N. Agelopoulos, K. –M. V. Jensen, J. B. Jespersen, J. A. Pickett, H. J. Prijs, G. Thomas, J. J. Trapman, L. J. Wadhams and C. M. Woodcock. 2004. The role of volatile semiochemicals in mediating host location and selection by nuisance and disease-transmitting cattle flies. Med. Vet. Entomol. 18:313-322.

Blaho, M., A. Egri, L. Bahidszki, G. Kriska, R. Hegedus, S. Åkesson and G. Horvath. 2012. Spottier targets are less attractive to Tabanid flies: On the Tabanid-repellency of spotty fur patterns. PLoS ONE. 7:e41138.

Blight, A. R., M. E. McGinnis and R. B. Borgens. 1990. Cutaneous trunci muscle reflex of the guinea pig. J. Comp. Neurol. 296:614-633.

Blott, S., J. Kim, S. Moisio, A. Schmidt-Küntzel, A. Cornet, P. Berzi, N. Cambisano, C. Ford, B. Grisart, D. Johnson, L. Karim, P. Simon, R. Snell, R. Spelman, J. Wong, J. Vilkki, M. Georges, F. Farnir and W. Coppieters. 2003. Molecular dissection of a quantitative trait locus: A phenylalanine-to-tyrosine substitution in the transmembrane domain of the bovine growth hormone receptor is associated with a major effect on milk yield and composition. Genetics. 163:253-266.

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

Brown, J. F. and T. R. Adkins. 1972. Relationship of feeding activity of face fly (*Musca autumnalis* DeGeer) to production of keratoconjunctivitis in calves. Am. J. Vet. Res. 33:2551-2555.

Bruce, W. N. and G. C. Decker. 1947. Fly control and milk flow. J. Econ. Entomol. 40:530-536.

Bruce, W. N. and G. C. Decker. 1958. The relationship of stable fly abundance to milk production in dairy cattle. J. Econ. Entomol. 51:269-274.

Burger, J. F. and L. L. Pechuman. 1986. A review of the genus hematopota (Dipera, Tabanidae) in North-America. J. Med. Entomol. 23:345-352.

Burton, J. H., I. McMillan and G. Surgeoner. 1984. An evaluation of fenvalerate (Bovaid) eartags as an improvement in fly control methods for increased milk-production in dairy herds. Can. J. Anim. Sci. 64:113-117.

Byford, R. L., M. E. Craig and B. L. Crosby. 1992. A review of ectoparasites and their effect on cattle production. J. Anim. Sci. 70:597-602.

Caccamo, M., R. F. Veerkamp, G. Licitra, R. Petriglieri, F. La Terra, A. Pozzebon and J. D. Ferguson. 2012. Association of total-mixed-ration chemical composition with milk, fat, and protein yield lactation curves at the individual level. J. Dairy Sci. 95:6171-6183.

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

Canadian Council on Animal Care (CCAC). 2009. CCAC guidelines on: the care and use of farm animals in research, teaching and testing. Ottawa, Ontario.

Castro, E., A. Gil, J. Piaggio, L. Chifflet, N. A. Farias, M. A. Solari and R. D. Moon. 2008. Population dynamics of horn fly, *Haematobia irritans irritans* (L.) (Diptera: Muscidae), on Hereford cattle in Uruguay. Vet. Parasitol. 151:286-299.

Catangui, M. A., J. B. Campbell, G. D. Thomas and D. J. Boxler. 1997. Calculating economic injury levels for stable flies (Diptera: Muscidae) on feeder heifers. J. Econ. Entomol. 90:6-10.

Christensen, C. M. 1982. External parasites of dairy cattle. J. Dairy Sci. 65:2189-2193.

Cortinas, R. and C. J. Jones. 2006. Ectoparasites of cattle and small ruminants. Vet. Clin. Food Anim. 22:673-693.

Cupp, E. W., M. S. Cupp, J. M. C. Ribeiro and S. E. Kunz. 1998. Blood-feeding strategy of *Haematobia irritans* (Diptera: Muscidae). J. Med. Entomol. 35:591-595.

Dougherty, C. T., F. W. Knapp, P. B. Burris, D. C. Willis and N. W. Bradley. 1993a. Face flies (*Musca autumnalis* De Geer) and the behavior of grazing beef cattle (*Bos taurus*). Appl. Anim. Behav. Sci. 35:313-36.

Dougherty, C. T., F. W. Knapp, P. B. Burrus, D. C. Willis, P. L. Cornelius and N. W. Bradley. 1993b. Multiple releases of stable flies (*Stomoxys calcitrans* L.) and behaviour of grazing beef cattle. Appl. Anim. Behav. Sci. 38:191-212.

Dougherty, C. T., F. W. Knapp, P. B. Burrus, D. C. Willis, J. G. Burg, P. L. Cornelius and N. W. Bradley. 1993c. Stable flies (*Stomoxys calcitrans* L.) and the behaviour of grazing beef cattle. Appl. Anim. Behav. Sci. 35:215-233.

Dougherty, C. T., F. W. Knapp, P. B. Burrus, D. C. Willis and P. L. Cornelius. 1994. Moderation of grazing behaviour of beef cattle by stable flies (*Stomoxys calcitrans* L.). Appl. Anim. Behav. Sci. 40:113-127.

Dougherty, C. T., F. W. Knapp, P. B. Burrus, D. C. Willis and P. L. Cornelius. 1995. Behavior of grazing cattle exposed to small populations of stable flies (*Stomoxys calcitrans* L.) Appl. Anim. Behav. Sci. 42:231-248.

Doyle, M. S., B. N. Swope, J. A. Hogsette, K. L. Burkhalter, H. M. Savage and R. S. Nasci. 2011. Vector competence of the stable fly (Diptera: Muscidae) for west nile virus. J. Med. Entomol. 48:656-668.

Drummond, R. O., G. Lambert, H. E. Smalley and S. E. Terrill. 1981. Estimated losses of livestock to pests. In: CRC Handbook of Pest Management in Agriculture. CRC Press, Boca Raton, FL.

Egri, A., M. Blahó, G. Kriska, R. Farkas, M. Gyurkovsky, S. Åkesson and G. Horváth. 2012. Polarotactic tabanids find striped patterns with brightness and/or polarization modulation least attractive: an advantage of zebra stripes. J. Exp. Biol. 215:736-745.

Ernst, C. M. and E. S. Krasfur. 1984. Horn fly (Diptera: Muscidae): sampling considerations of host breed and colour. Environ. Entomol. 57:371-372.

Foil, L. D. 1989. Tabanids as vectors of disease agents. Parasitol. Today. 5:88-96.

Foil, L. D., C. L. Seger, D. D. French, C. J. Issel, J. M. McManus, C. L. Ohrberg and R. T. Ramsey. 1988. Mechanical transmission of bovine leukemia-virus by horse flies (Diptera, Tabanidae). J. Med. Entomol. 25:374-376.

Foil, L. D. and J. A. Hogsette. 1994. Biology and control of tabanids, stable flies and horn flies. Rev. sci. tech. Off. Int. Epiz. 13:1125-1158.

Foil, L. D., G. R. Strother, J. A. Hawkins, S. J. Gross, D. F. Coombs, S. M. Derouen, W. E. Wyatt, L. K. Kuykendall and B. G. Spears Jr. 1998. The use of Ivomec® (ivermectin) pour-on and permethrin ear tags for horn fly control. Southwest Entomol. 23:317-323.

Fraser, D., D. M. Weary, E. A. Pajor and B. N. Milligan. 1997. A scientific conception of animal welfare that reflects ethical concerns. Anim. Welfare. 6:187-205.

Gilles, J., J. –F. David, G. Duvallet and E. Tillard. 2008. Potential impacts of climate change on stable flies, investigated along an altitudinal gradient. Med. Vet. Entomol. 22:74-81.

Gillespie, B. E., W. E. Owens, S. C. Nickerson and S. P. Oliver. 1999. Deoxyribonucleic acid fingerprinting of *Staphylococcus aureus* from heifer mammary secretions from horn flies. J. Dairy Sci. 82:1581-1585.

Glass, H. W. and R. R. Gerhardt. 1984. Transmission of *Moraxella bovis* by regurgitation from the crop of the face fly (Diptera: Muscidae). J. Econ. Entomol. 77:399-401.

Guglielmone, A. A., O. S. Anziani, A. J. Mangold, R. E. Giorgi, M. M. Volpogni and S. G. Flores. 1997. Seasonal variation of *Haematobia irritans* (Diptera: Muscidae) in a recently infested region of central Argentina. Bull. Entomol. Res. 87:55-59.

Guglielmone, A. A., M. M. Volpogni, H. Castro, A. J. Mangold and O. S. Anziani. 2002. A study of relative horn fly, *Haematobia irritans* (Diptera: Muscidae), abundance on Holstein steers and steers of two Holstein crosses. Vet. Parasitol. 109:141-145.

Harris, J. A., J. E. Hillerton and S. V. Morant. 1987. Effect on milk production of controlling muscid flies, and reducing fly-avoidance behaviour, by the use of Fenvalerate ear tags during the dry period. J. Dairy Res. 54:165-171.

Harvey, T. L. and J. L. Launchbaugh. 1982. Effect of horn flies on behavior of cattle. J. Econ. Entomol. 75:25-27.

Hawkins, J. A., J. N. Love and R. J. Hidalgo. 1982. Mechanical transmission of anaplasmosis by tabanids (Diptera, Tabanidae). Am. J. Vet. Res. 43:732-734.

Hieu, T. T., S. Kim, S. Lee and Y. Ahn. 2010. Repellency to *Stomoxys calcitrans* (Diptera: Muscidae) of plant essential oils alone or in combination with *Calophyllum inophyllum* nut oil. J. Med. Entomol. 47:575-580.

Horváth, G., J. Majer, L. Horváth, I. Szivá and G. Kriska. 2008. Ventral polarization vision in tabanids: horseflies and deerflies (Diptera: Tabanidae) are attracted to horizontally polarized light. Naturwissenschaften. 95:1093-1100.

Horváth, G., M., Blahó, G. Kriska, R. Hegedüs, B. Gerics, R. Farkas and S. Åkesson. 2010. An unexpected advantage of whiteness in horses: the most horsefly-proof horse has a depolarizing white coat. Proc. R. Soc. B. 277:1643-1650.

Hribar, L. J., D. J. Leprince and L. D. Foil. 1992. Feeding sites of some Louisiana Tabanidae (Diptera) on fenvalerate-treated and control cattle. Vet. Entomol. 85:2279-2285.

Iranpour, M. and T. D. Galloway. 2002. *Chrysops, Hybomitra* and *Tabanus* spp., horse and deer flies (Diptera: Tabanidae). Biological Control Programmes in Canada (ed. By P. G. Mason and J. T. Huber). Pp. 132-135. CABI Publishing, Lethbridge, AB.

Isman, M. 2006. Botanical insecticides, deterrents and repellents in modern agriculture and an increasingly regulated world. Annu. Rev. Entomol. 51:45-66.

Jensen, K. –M. V., J. B. Jespersen, M. A. Birkett, J. A. Pickett, G. Thomas, L. J. Wadhams and C. M. Woodcock. 2004. Variation in the load of the horn fly, *Haematobia irritans*, in cattle herds is determined by the presence or absence of individual heifers. Med. Vet. Entomol. 18:275-280.

Jonsson, N. N. and D. G. Mayer. 1999. Estimation of the effects of buffalo fly (*Haematobia irritans exigua*) on the milk production of dairy cattle based on a meta-analysis of literature data. Vet. Entomol. 13:372-376.

Kaufman, P. E., C. Strong, J. K. Waldron and D. A. Rutz. 2012. Individual and combined releases of *Muscidifurax raptor* and *M. raptorellus* (Hymenoptera: Pteromalidae) as a biological control tactic targeting house flies in dairy calf facilities. J. Med. Entomol. 49:1059-1066.

Kazimírová, M., M. Šulanová, M. Kozánek, P. Takáč, M. Labuda and P. A. Nuttall. 2001. Identification of anticoagulant activities in salivary gland extracts of four horsefly species (Diptera, Tabanidae). Haemostasis. 31:294-305.

Khater, H. F., M. Y. Ramadan and R. S. El-Madawy. 2009. Lousicidal, ovicidal and repellent efficacy of some essential oils against lice and flies infesting water buffaloes in Egypt. Vet. Parasitol. 164:257-266.

Kramer, C. Y. 1956. Extension of multiple range tests to group means with unequal numbers of replications. Biometrics. 12:309-310.

Kumar, P., S. Mishra, A. Malik and S. Satya. 2011. Repellent, larvicidal and pupicidal properties of essential oils and their formulations against the housefly, *Musca domestica*. Med. Vet. Entomol. 25:302-310.

Kunz, S. E., K. D. Murrell, G. Lambert, L. F. James and C. E. Terrill. 1991. Estimated losses of livestock to pests. CRC Handbook of Pest Management in Agriculture (ed. By D. Pimental). Pp. 68-69. CRC Press, Boca Raton, FL.

Lachance, S. and G. Grange. 2013. Repellent effectiveness of seven plant essential oils, sunflower oil and natural insecticides against horn flies on pastured dairy cows and heifers. Med. Vet. Entomol.

Lewis, D. J. and D. J. Leprince. 1981. Horse flies and deer flies (Diptera: Tabanidae) feeding on cattle in Southwestern Quebec. Can. Entomol. 113:883-886.

Lima, L. G. F., S. H. V. Perri and A. P. Prado. 2003. Variation in population density of horn flies (*Haematobia irritans irritans*) (L.) (Diptera: Muscidae) in Nellore cattle (*Bos indicus*). Vet. Parasitol. 117:309-314.

Lysyk, T. J. 1992. Effect of larval rearing temperature and maternal photoperiod on diapause in the horn fly (Diptera, Muscidae). Environ. Entomol. 21:1134-1138.

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

Lysyk, T. J. and D. D. Colwell. 1996. Duration of efficacy of diazinon ear tags and ivermectin pour-on for control of horn fly (Diptera: Muscidae). J. Econ. Entomol. 89:1513-1520.

Lysyk, T. J. and K. D. Floate. 2002. *Haematobia irritans* (L.), horn fly (Diptera: Muscidae). Biological Control Programmes in Canada (ed. By P. G. Mason and J. T. Huber). Pp. 132-135. CABI Publishing, Lethbridge, AB.

Magnarelli, L. A., J. F. Anderson and A. G. Barbour. 1986. The etiologic agent of lyme-disease in deer flies, horse flies, and mosquitos. J. Infect. Dis. 154:355-357.

Maldonado-Siman, E., P. A. Martinez-Hernandez, H. Sumano-Lopez, C. Cruz-Vazquez, R. R. de Lara and M. A. Alonso-Diaz. 2009. Population fluctuation of horn fly (*Haematobia irritans*) in an organic dairy farm. J. Anim. Vet. Adv. 8:1292-1297.

Malik, A., N. Singh and S. Satya. 2007. House fly (*Musca domestica*): A review of control strategies for a challenging pest. J. Environ. Sci. Heal. B. 42:453-469.

Marley, S. E., J. A. Lockwood, R. L. Byford and D. G. Luther. 1991. Temporal, climatic, and physiological mediation of dispersal in the horn fly, *Haematobia irritans* (L) (Diptera, Muscidae). Environ. Entomol. 20:1612-1618.

Mikuška, A., S. Krčmar, A. Radović and T. Mikuška. 2012. The influence of temperature, precipitation and floods on the development of horse fly populations (Tabanidae) in the alluvial habitats of the Danube river in Croatia. Pol. J. Ecol. 60:395-406.

Mooring, M. S., B. L. Hart, T. A. Fitzpatrick, D. D. Reisig, T. T. Nishihira, I. C. Fraser and J. E. Benjamin. 2006. Grooming in desert bighorn sheep (*Ovis canadensis mexicana*) and the ghost of parasites past. Behav. Ecol. 17:364-371.

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

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

Paduch, J., E. Mohr and V. Krömker. 2013. The association between bedding material and the bacterial counts of *Staphylococcus aureus, Streptococcus uberis* and coliform bacteria on teat skin and in teat canals in lactating dairy cattle. J. Dairy Res. 80:159-164.

Perich, M. J., R. E. Wright and K. S. Lusby. 1986. Impact of horse flies (Diptera: Tabanidae) on beef cattle. J. Econ. Entomol. 79:128-131.

Postma, G. C., J. C. Carfagnini and L. Minatel. 2008. *Moraxella bovis* pathogenicity: An update. Comp. Immun. Microbiol. Infect. Dis. 31:449-458.

Public Works and Government Services Canada. 2011. Organic production systems: General principles and management standards. http://www.tpsgc-pwgsc.gc.ca/ongc-cgsb/programme-program/normes-standards/internet/bio-org/principes-principles-eng.html.

Quisenberry, S. S. and D. R. Strohbehn. 1984. Horn fly (Diptera: Muscidae) control on beef cows with permethrin-impregnated ear tags and effect on subsequent calf weight gains. J. Econ. Entomol. 77:422-424.

Ralley, W. E., T. D. Galloway and G. H. Crow. 1993. Individual and group behaviour of pastured cattle in response to attack by biting flies. Can. J. Zool. 71:725-734.

Rodríguez, S. D., M. A. García Ortiz, R. Jiménez Ocampo and C. Vega y Murguía. 2009. Molecular epidemiology of bovine anaplasmosis with a particular focus in Mexico. Infect. Genet. Evol. 9:1092-1101.

SAS Institute. 2011. User's Guide: Statistics. Version 9.3. SAS Institute, Inc., Cary, NC.

Schirmann, K., N. Chapinal, D. M. Weary, W. Heuwieser and M. A. G. von Keyserlingk. 2012. Rumination and its relationship to feeding and lying behaviour in Holstein dairy cows. J. Dairy Sci. 95:3212-3217.

Schmidtmann, E. T., M. E. Valla and L. E. Chase. 1981. Effect of face flies on grazing time and weight gain in dairy heifers. J. Econ. Entomol. 74:33-39.

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

Schmidtmann, E. T., D. R. Berkebile, R. W. Miller and L. W. Douglass. 1984. The face fly (Diptera: Muscidae): effect on Holstein milk secretion. J. Econ. Entomol. 77:1200-1205.

Schmidtmann, E. T. 1985a. Face flies, *Musca autumnalis*, and ear flap behavior in Holstein cows. J. Agric. Entomol. 2:69-72.

Schmidtmann, E. T. 1985b. The face fly, *Musca autumnalis* de geer, and aggregation behavior in Holstein cows. Vet. Parasitol. 18:203-208.

Schofield, S. and J. Brady. 1997. Effects of carbon dioxide, acetone and 1-octen-3-ol on the flight responses of the stable fly, *Stomoxys calcitrans*, in a wind tunnel. Physiol. Entomol. 22:380-386.

Schwinghammer, K. A., F. W. Knapp, J. A. Boling and K. K. Schillo. 1986a. Physiological and nutritional response of beef steers to infestations of the horn fly (Diptera: Muscidae). J. Econ. Entomol. 79:1010-1015.

Schwinghammer, K. A., F. W. Knapp, J. A. Boling and K. K. Schillo. 1986b. Physiological and nutritional response of beef steers to infestations of the stable fly (Diptera, Muscidae). J. Econ. Entomol. 79:1294-1298.

Scoles, G. A., A. B. Broce, T. J. Lysyk and G. H. Palmer. 2005. Relative efficiency of biological transmission of *Anaplasma marginale* (Rickettsiales: Anaplasmataceae) by *Dermacentor andersoni* (Acari: Ixodidae) compared with mechanical transmission by *Stomoxys calcitrans* (Diptera: Muscidae). J. Med. Entomol. 42:668-675.

Sheppard, D. C. 1994. Dispersal of wild-captured, marked horn flies (Diptera: Muscidae). Environ. Entomol. 23:29-34.

Shütz, K. E., N. R. Cox and L. R. Matthews. 2008. How important is shade to dairy cattle? Choice between shade or lying following different levels of lying deprivation. Appl. Anim. Behav. Sci. 114:307-318.

Skoda, S. R., J. B. Campbell and S. E. Kunz. 1987. Wide-area treatment of cattle for horn flies and face flies (Diptera: Muscidae) in south-central Nebraska. J. Econ. Entomol. 80:811-816.

Skyes, A. R. 1994. Parasitism and production in farm-animals. Anim. Prod. 59:155-172.

Slatter, D. H., M. E. Edwards, C. D. Hawkins and G. E. Wilcox. 1982. A national survey of the clinical features, treatment and importance of infectious bovine keratoconjunctivitis. Aus. Vet. J. 59:69-72.

Spier, S. J., C. M. Leutenegger, S. P. Carroll, J. E. Loyce, J. B. Pusteria, T. E. Carpenter, J. E. Mihalyi and J. E. Madigan. 2004. Use of real-time polymerase chain reaction-based fluorogenic 5' nuclease assay to evaluate insect vectors of *Corynebacterium pseudotuberculosis* infections in horses. Amer. J. Vet. Res. 65:829-834.

Steelman, C. D., A. H. Brown, E. E. Gbur and G. Tolley. 1991. Interactive response of the horn fly (Diptera, Muscidae) and selected breeds of beef cattle. J. Econ. Entomol. 84:1275-1282.

Tangtrakulwanich, K., H. Chen, F. Baxendale, G. Brewer and J. J. Zhu. 2011. Characterization of olfactory sensilla of *Stomoxys calcitrans* and electrophysiological responses to odorant compounds associated with hosts and oviposition media. Med. Vet. Entomol. 25:327-336.

Tarry, D. W., L. Bernal and S. Edwards. 1991. Transmission of bovine virus diarrhea virus by flood feeding flies. Vet. Rec. 128:82-83.

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

Taylor, D. B., R. D. Moon and D. R. Mark. 2012. Economic impact of stable flies (Diptera: Muscidae) on dairy and beef cattle production. J. Med. Entomol. 49:198-209.

Teskey, H. J. 1969. On behaviour and ecology of face fly, *Musca autumnalis* (Diptera-Muscidae). Can. Entomol. 101:561-576.

Teskey, H. J. 1990. The horse flies and deer flies of Canada and Alaska (Diptera: Tabanidae). Part 16. The Insects and Arachnids of Canada. Agr. Can. Res. Br. Rep. 1838.

Thrift, F. A. and J. R. Overfield. 1974. Impact of pinkeye (infectious bovine kerato-conjunctivitis) on weaning and postweaning performance of Hereford calves. J. Anim. Sci. 38:1179-1184.

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

Torres, L., C. Almazán, N. Ayllón, R. C. Galindo, R. Rosario-Cruz, H. Quiroz-Romero, C. Gortazar and J. de la Fuente. 2012. Identification of microorganisms in partially fed female horn flies, *Haematobia irritans*. Parasitol. Res. 111:1391-1395.

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

Urech, R., R. L. Bright, P. E. Green, G. W. Brown, J. A. Hogsette, A. G. Skerman, M. M. Elson-Harris and D. G. Mayer. 2012. Temporal and spatial trends in adult nuisance fly populations at Australian cattle feedlots. Aus. J. Entomol. 51:88-96.

Vale, G. A. 1981. An effect of host diet on the attraction of tsetse flies (Diptera: Glossinidae) to host odour. B. Entomol. Res. 71:259-265.

Van Geem, T. and A. B. Broce. 1985. Significance of cattle discharges and secretions as protein sources for ovarian development in the face fly (Diptera: Muscidae). Environ. Entomol. 14:60-64.

von Keyserlingk, M. A. G., J. Rushen, A. M. de Passille and D. M. Weary. 2009. Invited review: The welfare of dairy cattle—key concepts and the role of science. J. Dairy Sci. 92:4101-4111.

Wall, R. 2012. Ectoparasites: Future challenges in a changing world. Vet. Parasitol. 148:62-74.

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

West, J. W., B. G. Mullinix and J. K. Bernard. 2003. Effects of hot, humid weather on milk temperature, dry matter intake, and milk yield of lactating dairy cows. Am. Dairy Sci. Assoc. 86:232-242.

Wright, R. E., R. K. Whittle, M. J. Perich and A. L. Hollander. 1984. Seasonal occurrence of horse flies (Diptera: Tabanidae) in north central Oklahoma. J. Ka. Entomol. Sci. 57:209-215.

Zhu, J. J., D. R. Berkebile, C. A. Dunlap, A. Zhang, D. Boxler, K. Tangtrakulwanich, R. W. Behle, F. Baxendale and G. Brewer. 2012. Nepetalactones from essential oil of *Nepeta cataria* represent a stable fly feeding and oviposition repellent. Med. Vet. Entomol. 26:131-138.