

Which Fly Spray is the Best?

Determining the Efficacy of Three Equine Fly Sprays

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Abstract

Throughout history, horses have been an important part of the lives of people. To help maintain their usefulness, proper care must be taken to ensure they stay healthy, including providing them with good nutrition, keeping their feet trimmed, and keeping them disease free through the use of vaccinations, worming, and pest control. Pest control is especially important because it keeps pathogen carrying vectors at bay, and it helps keep those same pests from annoying livestock, which can affect performance and value. During late summer of 2012, three fly sprays and a control of distilled water were tested on four horses over a four week period. The objective of this project was to determine which of the three sprays was the best, and how well they all worked. This was done by recording each horse with a digital, high definition camcorder for 30 minutes following the application of fly spray that had been randomly assigned to them for that week. A different spray was used on each horse each week; a Latin square design was used. The videos were viewed, and the numbers of flies were counted along with the number of fly related leg movements, tail movements, and muscle twitches. Of the four measured variables, the number of flies was the only variable that was significantly ($P < 0.05$) affected by type of spray. For this variable, the Farnam Endure brand fly spray had the lowest mean number of flies at 30. The second most effective spray was Eqyss Marigold at 48, followed by Gordon's Permethrin at 60. Distilled water had an unsurprisingly high mean of 105 flies.

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Chapter 1. Introduction

Pests, such as flies, gnats, mosquitoes, fleas, ticks, lice, and other parasites, can harm livestock by spreading diseases and causing stress. Flies and mosquitoes are particularly aggravating as they can swarm in large numbers. Because they can breed quickly in agricultural settings, these flying pests can quickly overpopulate an area and become a huge concern. For horses, environmental stability is a necessity because their behavior directly impacts their use. A swarm of flies, or even a few persistent ones, can annoy a horse until it bucks, rears, or even bolts. This can be dangerous to a handler or a rider, who could be kicked or thrown. Some pests can inflict pain with their bite, while some use horses as a host on which to lay their eggs. According to Burgess (1993) biting flies can also cause disease, poor growth, and gastrointestinal issues. The state of Tennessee is home to many kinds of flies including common horse flies (*Tabanus* sp.), deer flies (*Chrysops* sp.), horn flies (*Haematobia irritans*) and stable flies (*Stomoxys calcitrans*), just to name a few. Flies spread many pathogens, including those that cause equine infectious anemia, and stomach worms. Mosquitoes are notorious for their ability to spread Eastern Equine Encephalitis, Western Equine Encephalitis, and West Nile Virus (Burgess 1993). There are many different options for horse owners looking for a way to control the pest population in their barns. Fly sprays, traps, drops, and feed-through fly controls are readily available at most feed and farm stores.

There are several brands of fly spray on the market, containing many chemicals in many mixtures. Prices can range anywhere from \$4.00, to more than \$20.00 per bottle. A large amount of research has been done to determine the effectiveness of fly spray chemicals on cattle, but relatively few studies on horses have been reported in the literature. It was also very

difficult to find studies done on name brand fly sprays that can be purchased in retail stores.

Searches of the product websites yielded no studies of their products to back up any claims

they make, and no studies comparing these fly sprays could be found. More work needs to be

published on the effectiveness of fly sprays used on horses.

Chapter 2. Literature Review

The Horse Industry

Horses are important in America for economical and emotional reasons. According to the American Horse Council (2010), there are approximately 9.2 million horses in the United States, owned by 2 million people. In Tennessee alone, there were over 142,000 horses as of the 2007 Census of Agriculture statistics, placing the state in sixth place for number of horses in the U.S. (Menard et al., 2010). The industry has an annual economic impact of 39 million dollars and provides 460,000 jobs. While economics has a huge impact on why the horse industry is important, people would not generally own horses if they did not have some sort of attachment to them. The University of Tennessee at Knoxville's Tennessee Equine Veterinarian webpage states "In addition, a significant economic impact of the equine industry is on suppliers of agricultural products. The greatest impact, however, is the value of horses to their owners" (TEVRO). Whether they enjoy pleasure riding, competition, or breeding, horse owners are the driving force behind the horse industry.

Horse Behavior

Blood feeding insects can cause itching, irritation, blood loss, annoyance, fatigue, diseases and even malnutrition. Horses and ponies have many ways of dispersing pests including stomping, swishing their tails, biting, and muscle twitching; these behaviors are symptoms of pests (Daufman and Rutz, 2010). Animals may also seek refuge from pests. Keiper and Berger (1982) found that in two geographically separated herds of wild ponies, both groups engaged in patterns of activity that corresponded with fly densities. In the herd of ponies found

in the desert of Nevada, members grazed in the morning while it was cool and the pest population was low, whereas during the heat of the day they sought refuge by resting on slopes and ridges, away from the fly populations. The ponies located on the coasts of Virginia and Maryland sought refuge in water and snow (Keiper and Berger, 1982). In another study, in Mongolia, reintroduced Prezwalski horses annoyed by flies moved to higher elevations to avoid them, even though there was less food (King and Gurnell, 2010).

The following species of biting insects are examples of common pests in the United States that horses can encounter in their environments.

Fly Species

Tabanus spp.

Tabanids are more commonly known as horse flies. Halstead (2009) describes the horse fly as a large fly, dark grey to dark brown in color, about 25mm long, with clear wings. Fly sprays appear to have little to no obvious effect on horse flies. The females are the blood feeders and have biting and cutting mouthparts. Tabanids are a diverse and widely dispersed type of biting fly, consisting of more than 4000 species that cause disease by spreading viruses, bacteria, protozoans, and helminths (Muzari et al., 2010). Examples of diseases transmitted by horse flies include Equine Infectious Anemia, Anthrax, *Pasteurella*, and Brucellosis (Krinsky, 1976).

Chrysops spp.

Chrysops spp., or deer flies, look similar to horse flies as they are about the same color, but are less than half the size, 6 to 10mm (Halstead, 2009). Males and females feed on nectar,

but the females are also blood feeders. They lay their eggs in moist, vegetative areas, and after hatching, the larvae can take years to mature. Like horse flies, their bite can be painful, but they can be somewhat controlled by commercial fly sprays (Halstead, 2009). A 1986 study determined that deer flies could be included in a list of vectors that spread Lyme disease (Magnarelli et al., 1986). Deer flies also transmit zoonotic bacteria known as tularemia, which cause rabbit fever (Peterson et al., 2008).

Haematobia irritans

Haematobia irritans, also known as the horn fly, is a small fly (about 3 to 4mm) that feeds on the midsection of livestock. Both females and males are blood feeders. The females, like stable flies, lay their eggs in fresh manure, with the life cycle being completed in as few as ten days. Because they reproduce quickly, horn fly populations can quickly overwhelm animals. Spray on fly controls can be effective, but need to be reapplied frequently (Halstead, 2009). Horn flies can become a serious problem for horses because both sexes feed many times a day, leaving only occasionally to lay eggs. This can cause fatigue and loss of blood. Nature gives a lot of help in the fight against these flies in the form of attacking predacious insects and dung beetles which disturb the eggs and larvae of the flies (Daufman and Rutz, 2010).

Stomoxys calcitrans

Stomoxys calcitrans, the stable fly, is a species of fly that is much smaller than the common horse fly: about 7mm to 8mm versus 25mm. Both males and females are blood feeders that have a very painful bite. They breed in manure, so the first step in control should be proper sanitation. Chemical control appears to be difficult, so adhesive traps should be used

(Halstead, 2009). Stable flies prefer to feed from the legs of equines (Bittencort and Borja, 2000) and they are a vector of *Bacillus anthracis* (Turell and Knudson, 1987). There are several types of parasitic insects that seek out the pupae of stable flies and use them to lay their own eggs, killing them in the process. These include *Spalangia cameroni*, *Spalangia endius*, and *Muscidifurax raptorellus* (Pitzer et al., 2011).

Mosquitoes

Mosquitoes are pests that affect all land dwelling mammals. They can be very annoying to horses because they emit a high pitched buzzing noise. However, their bite is not as painful as the bite of a blood feeding fly. Mosquitoes can transmit several diseases to horses. West Nile Virus, the most recent disease scare associated with mosquitoes, is spread to horses by mosquitoes in the *Aedes* and *Ochlerotatus* genera (Kulasakera et al., 2001). Another disease that can be spread by mosquito vectors is Encephalomyelitis, which can be fatal (Kissling et al., 1956). There are vaccines for these diseases, but reducing mosquito breeding sites will cut down on their populations. Mosquito females lay their eggs in or near water after feeding on blood, and the larvae and pupae live in water, so removing these breeding sites will reduce the mosquito population (Sutherland and Crans, 2009).

Fly Control

There are many things a horse owner can do to prevent an increase in the fly population around their stock. According to Burgess (1993), the first step to take would be to minimize their ability to breed. Piles of horse manure should be removed or spread out, and other fly breeding materials should be sought out and disposed of at minimum of once per week. Most

flies have a life cycle of between 7 and 21 days, so disposing of materials no later than every six days should generally be sufficient. If manure is kept to be used as fertilizer, water should be added to it to prevent breeding. If this is not possible, the manure should be used as quickly as possible. The next step in controlling the fly population is to promote dry areas through moisture control. This is especially important for the control of mosquitoes since they breed in or around water. The third step is to use insecticides, such as those that are used in this experiment. However, there are more ways than just to spray the horse itself. Horse owners can spray the walls and ceilings of their barn, use insecticide dusts, or hang up fly strips and traps to control pest populations. There are also intermittent sprayers which can be placed in a horses' stall and set to go off at certain intervals of time. Mechanical controls such as screens and fans will help keep these pests away from horses as well. Another method that is gaining popularity in the horse industry is the use of fly predators. These insects lay eggs in the pupae of certain flies; when the eggs hatch, the larvae use the pupae as a food source, thus killing the fly pupae in the process.

While there are many things that can be done to control fly populations, a popular method is applying a repellent or insecticide directly to the animal. What product and how much to use is up to the user. Cost and effectiveness can be issues. Burgess' "Horse Pest Management" contains a table with several chemicals commonly used on livestock to deter pests and application rates are listed for some products (Burgess, 1993). Several of them, however, say to see the manufacturer's recommendations. Both the Eqyss Marigold and Farnam Endure brand bottles simply say to "apply generously" (Farnam, 2008; Eqyss, nd). Gordon's brand permethrin has a pamphlet attached to the bottle with the dilution and

application instructions, categorized by use and animal (PBI/Gordon 2013). Because they are liquids, fly sprays should be reapplied after the horse has been out in the rain or after heavy sweating. The Endure brand fly spray bottle claims that it is water-resistant due to conditioners in its formula, but according to the label, one application does not last the full 14 days as the label says in those conditions, and so must be reapplied (Endure). Permethrin and Eqyss Marigold sprays should be reapplied daily as they do not make any claims to last longer.

Permethrin

Many people purchase a generic form of permethrin, which is generally cheap and sold in a concentrate, then mixed in a separate spray bottle. Permethrin is a type I pyrethroid that works by modifying the sodium channels, causing nerve damage in insects (Sadeghi-Hashjin et al., 2010). Because it has been around for so long, many studies have been done to evaluate the effectiveness of permethrin in controlling insects. Schmidtman et al. (2001) studied permethrin use on cattle and ponies. They found that permethrin reduced the number of black flies and mosquitoes on the ponies by up to 98%, but gradually wore off (Schmidtman et al., 2001). A study conducted at the air force base in the Philippines found that applying permethrin spray to horses decreased the number of flies on them by 50% (Lang et al., 1981). Insects have begun to develop a resistance to permethrin, so other chemicals have been added in other formulations to make it more effective (Matowo et al, 2010). Insects become resistant to chemicals by either producing more enzymes which metabolize or bond to the chemicals, or the target site of the chemical in the insect is mutated (Matowo et al, 2010).

Eqyss Marigold

Because of the recent “Green Movement”, many companies have opted to manufacture natural fly sprays, which have become rather popular. The Eqyss company (www.eqyss.com), claims that their Premier Marigold Spray is “marigold scented” and is “a safe, non-insecticidal botanical spray” (Eqyss, nd). Marigolds have long been used to deter pests from other plants, and have recently been found to have a nematocidal effect due to the same volatile thiopenes that make it an effective insecticide (Ratnadass et al., 2012).

Farnham Endure

Endure fly spray, manufactured by Farnham, claims to provide up to 14 days of fly control. The formula consists of pyrethrin, cypermethrin, piperonyl butoxide, and butoxy polypropylene glycol (Farnam, 2008). The first chemical, pyrethrin, is a commonly used pesticide that is the basis of pyrethroids. However, many flies and mosquitoes have developed some genetic immunity to pyrethrin (Foil et al., 2010 and Sarkar et al., 2010). Pyrethrins, as they are used now, are synthetic copies of pyrethrum, which is a natural compound, found in the chrysanthemum (Isman, 2006). The second chemical in Endure, cypermethrin, is a pyrethroid like permethrin, but it is what is known as a type II alpha-cyano pyrethroid. It also affects the sodium channels in insects, but instead of modifying them, it prevents them from closing, causing “depression of the nerve impulse” (Sadeghi-Hashjin et al., 2010). Piperonyl butoxide, another component of Endure, is what is known as a synergist for pyrethrins, meaning that it helps in the effectiveness of pyrethrin (Amweg et al., 2006). Butoxy polypropylene glycol was first registered by the Environmental Protection Agency in the 1960s,

and the report states that it repels deer flies, face flies, gnats, horse flies, etc. (EPA, 2007). Also found in Endure is permethrin, which is found in many fly sprays (see previous section on permethrin). All of these chemicals work together to ensure that, even if a pest is immune to one of them, there is another that it is likely not immune to. These three fly sprays are examples of the many products available to horse owners to control flies.

An overpopulation of biting flies and mosquitoes can cause many problems with horses including diseases, annoyance, blood loss, and weight loss. There are many different types of flies and several types of mosquitoes, all of which have different control methods. There are hundreds of chemical means that have been developed to repel these insects, but there is limited evidence to back up many of the claims companies make about their products.

Objectives

The objective of this study was to determine which of three selected, commercially available fly sprays, Farnam's Endure, Eqyss Marigold, or Gordon's Permethrin, were the most effective against flies commonly found around horses in Tennessee.

Chapter 3. Materials and Methods

This project was conducted at The Equine Training Center, a horse boarding and training facility located in Mount Pleasant, Tennessee in late summer (August/September) of 2012. The horses were pasture kept with a run-in shed, and included: a 28 year old, 15.3 hand grey mare (Figure 1), a 22 year old, 14.3 hand palomino mare (Figure 2), a 15 year old, 15 hand grey mare (Figure 3), and a ten year old, 15.1 hand sorrel mare (Figure 4). Each horse was fed a diet of 12% Producer's Pride All Stock Feed according to their weight, as well as free choice grass hay, salt block, and fresh water. Their separate pastures were approximately two acres of short-cropped grass, which were dragged to spread manure every two weeks.



Figure 1. Horse A, grey mare, 15.3 hands, 28 years old.



Figure 2. Horse B, palomino mare, 14.3 hands, 22 years old



Figure 3. Horse C, grey mare, 15.0 hands, 15 years old.



Figure 4. Horse D, sorrel mare, 15.1 hands, 10 years old.

Experimental Design

A 4x4 latin square design was used to assign weekly a fly spray treatment to each horse over a 4 week period (Table 1). Each horse received Farnam Endure, Eqyss Marigold, Gordon's Permethrin, and the water control, in a random order over the 4 week trial period. Each spray treatment was applied in the same manner and amount daily for 5 days in each week of the trial (this experiment took place during August and September, 2012).

To determine how much fly spray each horse should receive in one treatment, a small survey was taken (July 21-27, 2012) of 17 horse owners and riders at the same boarding facility the experiment took place. Unfortunately, no one seemed to know how much they used. Each person seemed to use enough to layer the coat with a light mist. With that in mind, for a week prior to the beginning of the trial, water was sprayed on each horse several times using the same spray bottle that would be used in the trial. It was determined that between 1.5 and 2 ounces, less for the smaller horses and more for the larger horses, would provide the light mist

Table 1. Latin square design used to assign fly sprays to each horse for each week.

Week of Study	Horse			
	A	B	C	D
1	Marigold	Endure	Permethrin	Water
2	Endure	Marigold	Water	Permethrin
3	Water	Permethrin	Endure	Marigold
4	Permethrin	Water	Marigold	Endure

needed. During the trial, horse A received approximately 2 ounces of spray, horse B received approximately 1.5 ounces, and horses C and D received approximately 1.75 ounces each time they were sprayed. Each spray was poured into a separate spray bottle (32 ounces) with ounce designations (Tractor Supply brand bottles were used for all treatments).

Once a day at around 6:00 pm, for five consecutive days, each horse was sprayed with a measured amount of the assigned spray. A Sanyo VPC-FH1A digital, high definition camcorder was used to record one side of each horse for 30 minutes to capture leg movement, tail movement, muscle twitches, and the number of flies. The horses were recorded from the knees up, and from the middle of the neck back to produce a video image clear enough to count flies (see Figures 1 through 4). At the end of the five days, each horse was bathed to remove residual fly spray and allowed to rest for two days before the next spray was applied according to the Latin square design (Table 1). Each horse was assigned a new fly spray each week. This process was repeated for four weeks. After each week, the videos of the horses were viewed using a Toshiba Satellite laptop, a Sony Playstation 3 game system, and a 73" Mitsubishi DLP television. The laptop was sufficient to count leg movements, tail movements, and muscle twitches; however, to count flies a larger screen was needed. The Playstation 3 and the laptop were networked together over a wireless broadband connection, and the videos were viewed on the television in 1080p high definition. The number of flies that landed on each horse during the 30 minute time period was counted, as well as obvious signs of annoyance including leg movement, tail movement, and muscle twitches. The means for the four variables were then calculated using Excel 2010. Descriptive statistics and correlation coefficients among the four variables were calculated using Proc Corr in SAS 9.3. Analysis of variance for the Latin square

design was run using Proc GLM in SAS 9.3 and Duncan's Multiple Range Test was used for mean separation.

Chapter 4. Results and Discussion

The ANOVA for the number of flies (Table A.1), indicated that there were significant ($P=0.0003$) differences among fly sprays. Of the three chemical sprays, Farnam's Endure brand fly spray was the most effective at repelling flies, with a mean number of 30 flies per 30 minute interval averaged over all horses over the 4 weeks (Table 2, Figure 5). Eqyss Marigold had the next lowest mean (48 flies per 30 minute interval), and was not significantly different from Endure (Table 2). The Gordon's Permethrin had a mean of 60 flies per 30 minute interval. All sprays had significantly lower fly numbers than the control (105 flies per 30 minute interval). The number of ingredients in Endure spray more than likely contributed to its effectiveness. The combination of the permethrin and pyrethrin (and its synergist, piperonyl butoxide), attack the insect's nervous system by interfering with sodium channels. The conditioners Farnam adds also may act to make the spray last longer by preventing their evaporation.

Table 2. Effect of fly spray on mean numbers of leg movements, tail movements, muscle twitches, and fly numbers over a 30 minute interval.

Spray	Leg Movement	Tail Movement	Muscle Twitches	Fly numbers
Water	117a*	552a	680a	105a
Permethrin	110a	335a	512a	60b
Marigold	83a	451a	728a	48bc
Endure	62a	259a	358a	30c

*Within a column means with the same letter are not significantly different ($P < 0.05$) according to Duncan's Multiple Range Test.

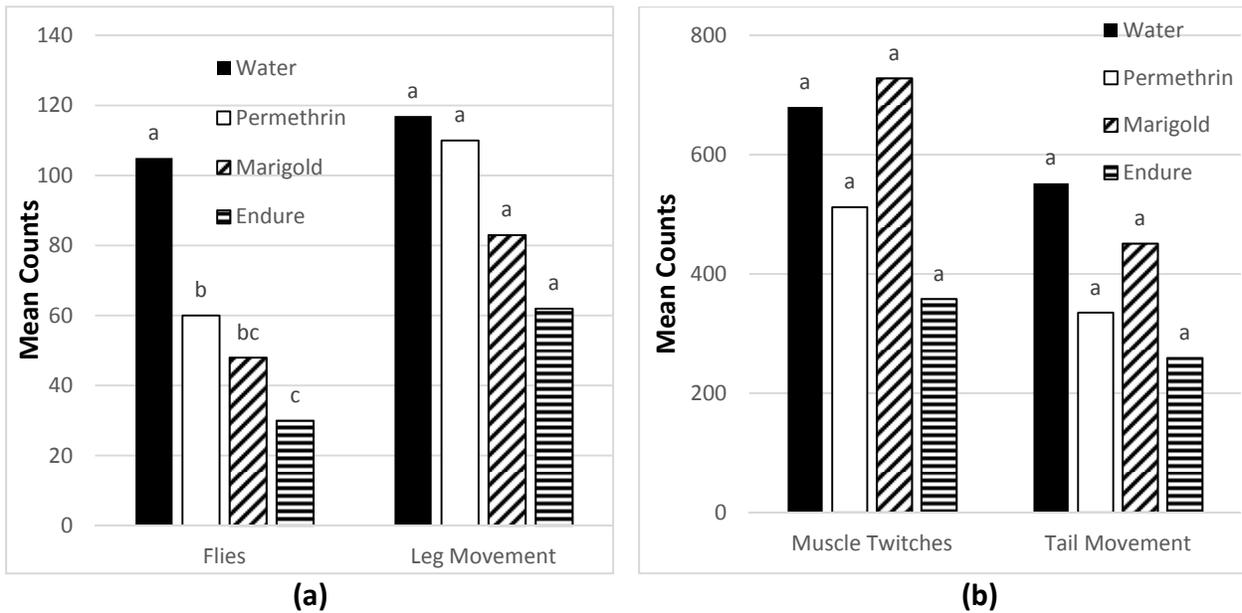


Figure 5. Effect of fly spray on mean counts of (a) flies and leg movements and (b) tail movement and muscle twitches. Within measurement types, means with the same letter are not significantly different ($P < 0.05$) according to Duncan's multiple range test.

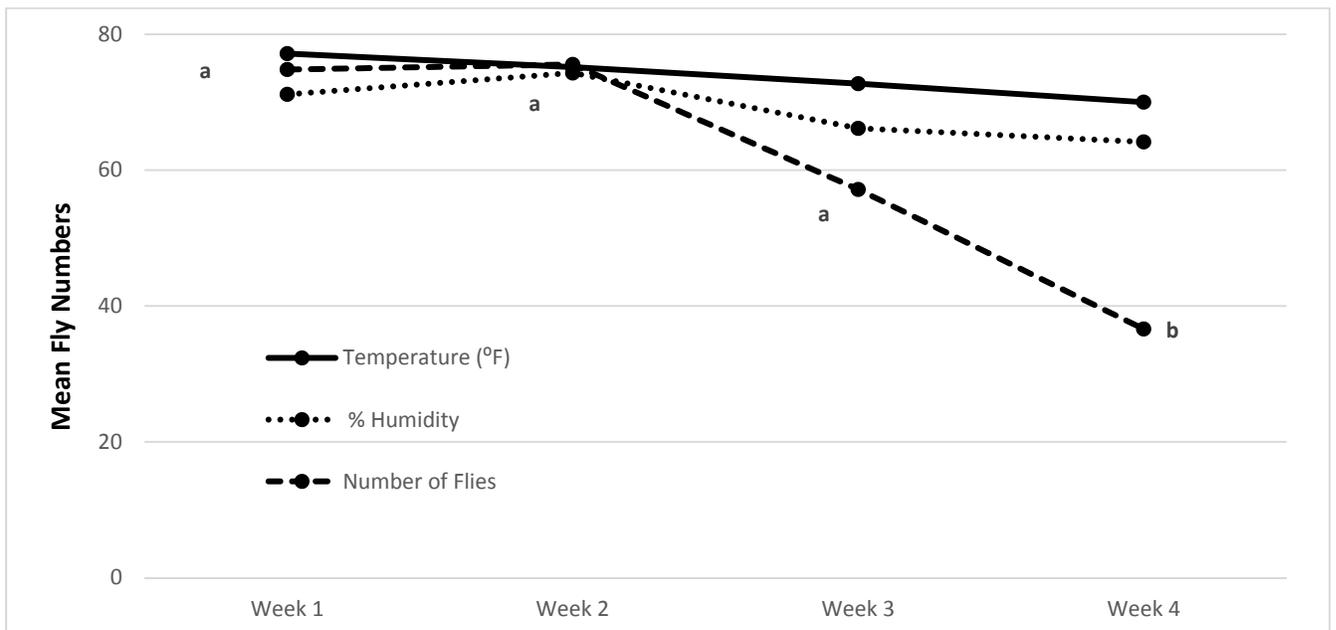


Figure 6. Mean Fly numbers compared to weekly temperature and humidity. Fly means with the same letter are not significantly different ($P < 0.05$) according to Duncan's multiple range test.

The ANOVA for fly numbers (table A1) also showed that there were significant differences in fly numbers with regards to the week and the horse. The significance with regard to fly numbers and what horse was used could simply be because some horses are more attractive to flies than others. Over all four weeks, horse A had the lowest significant mean number of flies, while horse D had the highest (89); horses B (63) and C (65) were significantly different from A and D, but not from each other. Horse A had a significantly lower mean number of flies than the other three (27) (Table3). It is worth noting that horse A is a very light grey, and horse D is a dark sorrel; her dark color may make her more attractive to flies. As far as the significance with what week the trial was in, the weather could have contributed. As the temperature and humidity dropped over the course of the four weeks, so did the number of flies (Figure 6). Flies, like most other insects, are most active on warm days. The drop in humidity levels probably reduced their breeding sites, lowering the population.

Table 3. Effect of horse used on variable numbers. Means with the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$).

Variable	Horse			
	A	B	C	D
Flies	27c	63b	65b	89a
Muscle Twitches	1330a	216b	225b	507b
Leg Movements	88ab	121a	37b	126a
Tail Movements	578a	159b	230b	630a

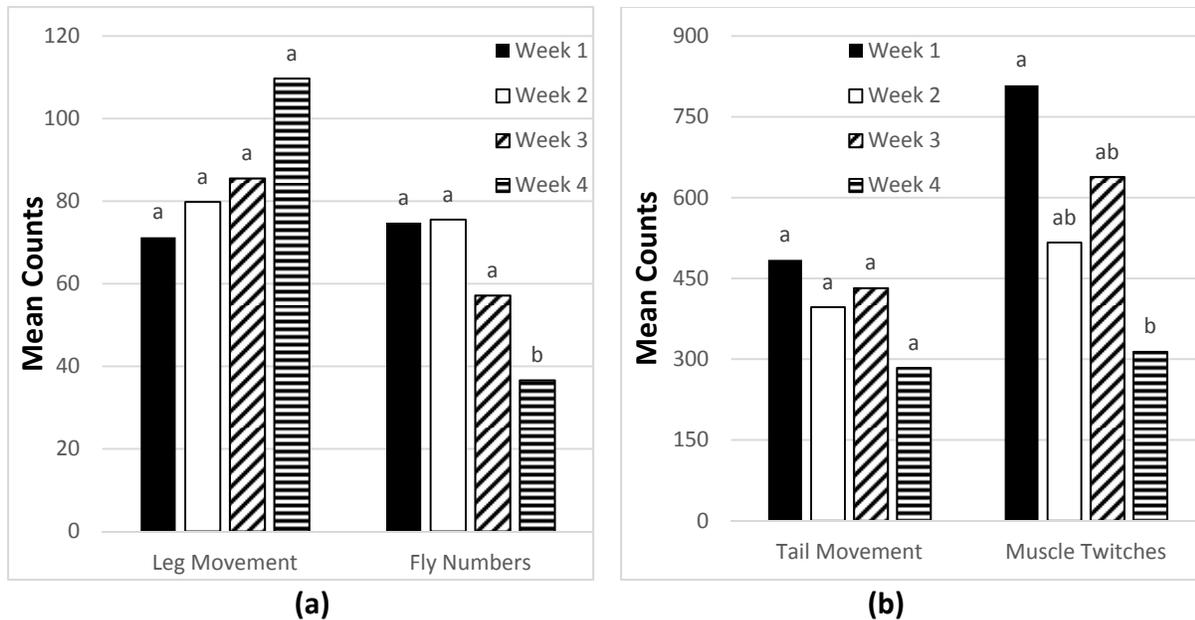


Figure 7. Effect of horse used on (a) leg movements and fly numbers, and (b) tail movements and muscle twitches. Means with the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$).

For the number of leg movements, tail movements, and muscle twitches, ANOVA indicated that there were no significant differences among fly spray treatments (Tables A3, A4, and A5). It is likely that increased variability for these measurements prevented significant differences. In Table 3 and Figure 6, the variables can be compared by horse, and at first glance the variables that stand out the most are the muscle twitches for horse A; this particular horse had a significantly high number of muscle twitches when compared to the other three horses, which may have to do with the sensitivity of the skin for this particular horse. This is likely the cause of the increased variability. There may be other variables affecting these outcomes including flying insects that cannot be seen in the recording (such as mosquitoes), and flies landing on the side of the horse that was not videotaped.

When Pearson correlation coefficients were calculated for each pair of variables, the only significant correlations observed were the correlation ($r=0.721$, $P=0.002$) between tail movements and muscle twitches, and the correlation ($r=0.666$, $P=0.005$) between tail movements and fly numbers (Table 4). To a horse person, this may seem odd, as fly numbers should have a direct effect on all of these physical signs of annoyance. This may partially be related to the difficulty in observing muscle twitches when compared to tail movements.

Table 4. Pearson correlation coefficients among variables measured in the experiment.

Variable	Leg Movements	Tail Movements	Muscle Twitches
Tail Movements	0.314		
Muscle Twitches	0.099	0.721**	
Flies	0.107	0.666**	0.311

** Significant at $\alpha=0.01$ (n= 16)

Chapter 5. Conclusions

An overpopulation of biting flies and mosquitoes can cause many problems with horses including diseases, annoyance, blood loss, and weight loss. There are many species of flies and mosquitoes, all of which have different control methods. There are several chemicals that have been developed to repel these insects, but there is not a lot of evidence to back up many of the claims companies make about their products. In this experiment, three commercially available fly sprays, and a control of distilled water were used on four horses to see how they affected the number of flies that land on a horse, as well as three other variables related to pest annoyance: leg movements, tail movements, and muscle twitches. The only variable that had significant differences was fly numbers. Based on this variable, Farnam's Endure fly spray was the most effective product tested in this trial. A longer study, or a study where several horses were used for each spray at a time, might increase the reliability of this experiment. To reduce variability, horses of the same size and color could be used in the same study.

Literature Cited

- American Horse Council. 2010. National Economic Impact of the U.S. Horse Industry. <http://www.horsecouncil.org/national-economic-impact-us-horse-industry>. Accessed March 3, 2013.
- Amweg, E.L., D.P. Weston, C.S. Johnson, J. You, and M.J. Lydy. 2006. Effect of piperonyl butoxide on permethrin toxicity in the Amphipod *Hyalella Azteca*. Environ. Toxic. Chem. 25: 1817-1825.
- Bittencort, A.J. and B.E.M Borja. 2000. *Stomoxys calcitrans* (L.): preferred feeding sites on the equine body. Parasolitia al dia. 24(3/4): 119-122.
- Burgess, G. 1993. PB1478-Horse Pest Management. The University of Tennessee Agricultural Extension Service, PB1478-2M-5/93, R12-1040-18-004-93. http://trace.tennessee.edu/utk_agexani/13. Accessed August 6, 2012.
- Daufman, P.E. and D.A. Rutz. 2010. Pest management recommendations for horses. Cornell and Penn State Extension. <http://pubs.cas.psu.edu/FreePubs/PDFs/XF0276.pdf>. Accessed January 14, 2013.
- United States Environmental Protection Agency. 2007. Reregistration Eligibility Decision for Polypropylene Glycol. US Environmental Protection Agency Office of Pesticide Programs. http://www.epa.gov/oppsrrd1/REDs/poly_glycol_red.pdf. Accessed February 4, 2013.
- Farnam. 2008. Endure Sweat-Resistant Fly Spray for Horses. Label Information. http://www.farnamhorse.com/pdf_label/Endure_all.pdf. Accessed April 30, 2013.
- Eqyss. No date. Marigold Spray. http://www.eqyss.com/marigold_spray.asp. Accessed February 4, 2013.
- Foil, L.D., F. D. Guerrero, and K. G. Bendele. 2010. Detection of target site resistance to pyrethroids and organophosphates in the horn fly using multiplex polymerase chain reaction. J. Med. Entom. 47(5): 855-861.
- Halstead, J. 2009. Common Flies of Cattle. Kansas State Ag Practice updates. http://www.vet.k-state.edu/depts/vhc/agpract/articles/Common_Flies.pdf. Accessed January 14, 2013.
- Isman, M.B., 2006. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annu. Rev. Entomol. 51:45-66.

- Keiper, R.R. and J. Berger. 1982. Refuge-seeking and pest avoidance by feral horses in desert and island environments. *Applied Animal Ethology*. 9(2):111-120.
- King, S.R.B. and J. Gurnell. 2010. Effects of fly disturbance on the behaviour of a population of reintroduced Przewalski horses (*Equus ferus przewalskii*) in Mongolia. *Appl. An. Beh. Sci.* 125(1): 22-29.
- Kissling, R.E., R.W Chamberlain, D.B Nelson, and D.D Stamm. 1956. Venezuelan equine encephalomyelitis in horses. *Am. J. of Epidem.* 63(3): 274-287.
- Krinsky, W.L. 1976. Animal disease agents transmitted by horse flies and deer flies (*Diptera: Tabanidae*). *Journ. Med. Entom.* 13(3): 225-275 (51).
- Kulasakera, V.L., L. Kramer, R.S. Nasci, F. Mostashari, B. Cherry, S.C. Trock, C. Glaser, and J.R. Miller. 2001. West Nile virus infection in mosquitoes, birds, horses, and humans, Staten Island, New York, 2000. *Emerging Infectious Diseases*. 7(4): 722-725.
- Lang, J.T., C.E. Schreck, and H. Pamintuan. 1981. Permethrin for biting fly (*Deptera: Muscidae; Tabanidae*) control on horses in central Luzon, Philippines. *J. of Med. Entomol.* 18(6): 522-529.
- Magnarelli, L.A., J.F. Anderson, and A.G. Barbour. 1986. The etiologic agent of Lyme disease in Deer flies, Horse flies, and Mosquitoes. *Journ. Inf. Dis.* 154(2): 355-358.
- Matowo, J., M.A. Kulkarni, F.W. Mosha, R.M. Oxborough, J.A. Kitau, F. Tenu, and M. Rowland. 2010. Biochemical basis of permethrin resistance in *Anopheles arabiensis* from Lower Moshi, north-eastern Tanzania. *Malaria Journal*. 9:193.
- Menard, R.J., K.W. Hanks, B.C. English and K.L. Jensen. 2010. Staff Paper SP10-01Tennessee's Equine Industry: Overview and Estimated Economic Impacts. UT Institute of Agriculture Department of Agricultural Economics. <http://aimag.ag.utk.edu/pubs/equine.pdf>. Accessed March 6, 2013.
- Muzari, M.O., L.F. Skeratt, R.E. Jones, and T.L. Duran. 2010. Alighting and feeding behaviour of tabanid flies on horses, kangaroos and pigs. *Vet. Par.* 170(1): 104-111.
- PBI/ Gordon Corp. 2013. Permethrin-10 Livestock and Premise Spray. <http://www.pbigordon.com/pdfs/Permethrin-10-SL.pdf>.
- Petersen, J.M., P.S. Mead, M.E. Schreifer. 2008. *Francisella tularensis*: an arthropod-borne pathogen. *Vet. Res.* 40(2): 7.

- Pitzer, J.B., P.E. Kaufman, C.J. Geden, and J.A. Hogsette. 2011. The ability of selected pupal parasitoids (*Hymenoptera: Pteromalidae*) to locate stable fly hosts in a soiled equine bedding substrate. *Env. Entom.* 40(1): 88-93.
- Ratnadass, A., P. Fernandes, J. Avelino, and R. Habib. 2012. Plant species diversity for sustainable management of crop pests and diseases in agroecosystems: a review. *Agronomy for Sustainable Development.* 32(1): 273-303.
- Sadeghi-Hashjin, G., M.K. Koohi, and F. Fallah. 2010. Influence of permethrin and cypermethrin on behavior in the mouse. *Int. J. of Vet. Res.* 5(2): 119-124.
- Sarkar, M., I. Baruah, R.B. Srivastava, A. Borkotoki, and I.K. Bhattacharyya. 2010. High-throughput approach to detection of knockdown resistance (kdr) mutation in mosquitoes, *Culex quinquefasciatus*, based on real-time PCR using single-labelled hybridisation probe/melting curve analysis. *Pest. Man. Sci.* 67(2): 156-161.
- Schmidtman, E.T., J. E. Lloyd, Sr., R. J. Bobian, R. Kumar, J. W. Waggoner, Jr., W. J. Tabachnick, and D. Legg. 2001. Suppression of mosquito (Diptera: Culicidae) and black fly (Diptera: simuliidae) blood feeding from Hereford cattle and ponies treated with permethrin. *J. Med. Entom.* 38(5): 728-734.
- Sutherland, D.J. and W.J. Crans. 2009. Mosquitoes in your life. New Jersey Agriculture Experiment Station Publication SA220-5M-86. <http://www-rci.rutgers.edu/~insects/moslif.htm>. Accessed February 4, 2013.
- Tennessee Equine Veterinary Research Association (TEVRO). No Date. TEVRO. <http://www.vet.utk.edu/research/TEVRO/index.php>. Accessed March 5, 2013.
- Turell, M.J. and G.B. Knudson. 1987. Mechanical transmission of *Bacillus anthracis* by stable flies (*Stomoxys calcitrans*) and mosquitoes (*Aedes aegypti* and *Aedes taeniorhynchus*). *Amer. Soc. Micro.* 55(8): 1859-1861

Appendix

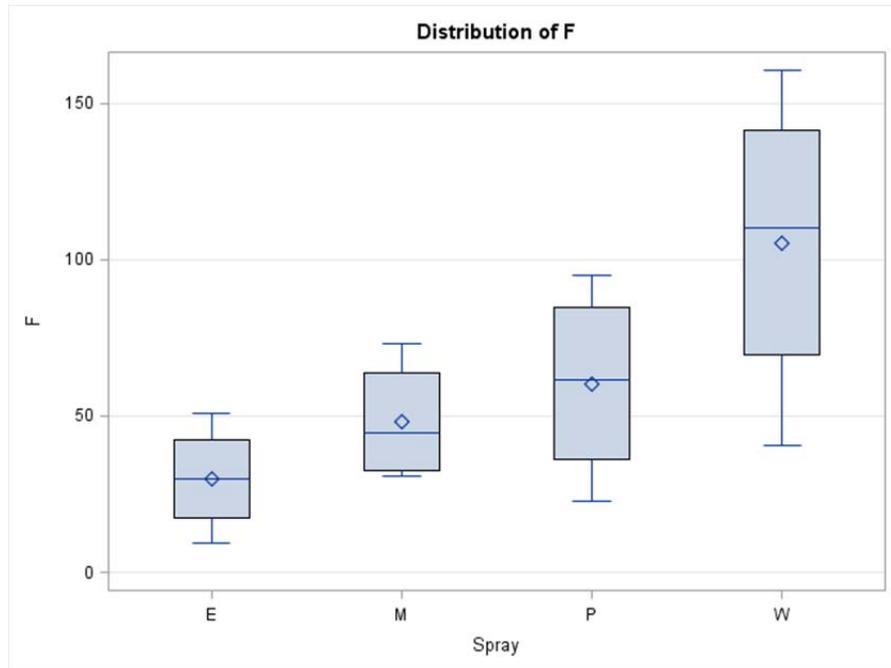


Figure A1. SAS boxplot for the mean number of flies by spray type.

Table A1. ANOVA for fly numbers.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F Value	Pr>F
Week	3	4049.3	1349.8	11.97	0.0061
Horse	3	7818.1	2606.0	23.11	0.0011
Spray	3	12376.0	4125.3	36.59	0.0003
Error	6	676.5	112.8		
Total	15	24919.9			

Table A2. ANOVA for muscle twitches.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F Value	Pr>F
Week	3	520337.1	173445.7	2.93	0.1217
Horse	3	3302235.5	1100745.2	18.59	0.0019
Spray	3	340529.7	113509.9	1.92	0.2281
Error	6	355299.2	59216.5		
Total	15	4518401.5			

Table A3. ANOVA for tail movement

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Pr>F
Week	3	86499.6	28833.2	0.89	0.4978
Horse	3	686373.7	228791.2	7.07	0.0214
Spray	3	200140.5	66713.5	2.06	0.2068
Error	6	194148.7	32358.1		
Total	15	1167162.5			

Table A4. ANOVA for leg movement

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Pr>F
Week	3	4934.3	1644.8	1.46	0.3173
Horse	3	20138.4	6712.8	5.94	0.0314
Spray	3	7516.1	2505.3	2.22	0.1867
Error	6	6775.2	1129.2		
Total	15	39364.1			

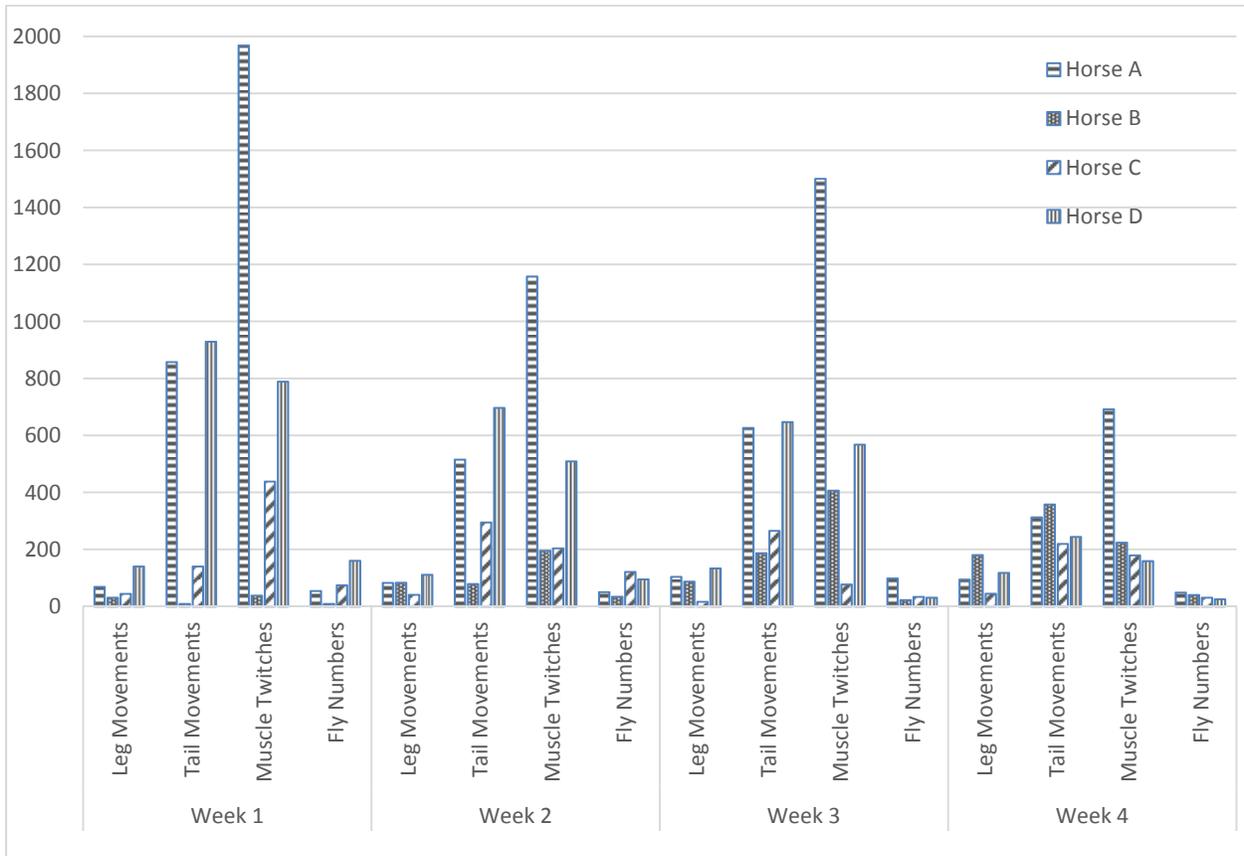


Figure A2. Means for all variables for all horses by week.