Journal of Economic Entomology, XX(XX), 2019, 1–9
doi: 10.1093/jee/toz243
Research

Behavioral Inhibition of the House Fly (Diptera: Muscidae) When Exposed to Commercial Equine Fly Repellents

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Subject Editor: Kimberly Lohmeyer

Received 17 April 2019; Editorial decision 8 August 2019

Abstract

House flies can have negative consequences on the welfare of horses and other equids. Fly repellents in the form of on-animal sprays, wipes, or spot-ons are the most commonly used fly control method for horses. Many products are available, but repellent efficacy and duration of effectiveness may influence repellent choice by horse owners. A better understanding of the efficacy of common fly repellent products will help guide repellent selection to reduce fly pressure on horses. To evaluate commercially available repellents, house fly behavioral inhibition after application of three products marketed as natural (Ecovet, Equiderma, and Outsmart) and four with synthetic pyrethroids as active ingredients (Bronco, Endure, UltraShield, and Optiforce) was compared at 100, 50, and 25% concentration and at 15, 30, 60, 240, 1,440, and 2,880 min. Time and product were significant at all tested concentrations. The natural products performed as well as or better than the synthetic products at all dilutions and times. Ecovet in particular retained over 75% inhibition of flies for >1 d at the 100 and 50% concentrations. Differences were seen among products with pyrethroids, suggesting that formulation differences significantly affect efficacy. Cost and application suggestions are discussed, and these results will aid horse owners in selecting fly repellents to meet their individual needs.

Key words: natural repellent, permethrin, pyrethroid, C8910, fatty acid

House flies (*Musca domestica* L.) are common on equine farms and can have negative consequences on the welfare of horses and other equids. Although house flies do not bite, they can cause physical irritation and are implicated in the transmission of parasites and pathogens that can cause habronemiasis (Amado et al. 2014), pigeon fever (Spier et al. 2004, Barba et al. 2015), and other conditions. Horse behavior may be altered in response to high fly populations, such as tail swishing, twitching, stamping, and shelter seeking (McDonnell 2003). Grazing time may be reduced with increased fly pressure, thus reducing forage intake, which may interfere with the growth and health of foals (Keiper and Berger 1982). Rider safety and comfort may also be compromised with high fly pressure even with short exposure times.

On-animal fly repellents in the form of sprays, wipes, or spot-ons are the most commonly used fly control method for horses. In 2015, 86.5% of equine owners reported using fly sprays (USDA 2017), and in Florida, 95.9% of surveyed horse owners reported using insecticides for fly control (Machtinger et al. 2015). An estimated $40 million is spent on pest control for horses and horse farms in the United States (Geden and Hogsette 2001). With the increase in available chemical and nonchemical control products, current and future expenditures could be much higher than reported. However, repellent efficacy and duration of effectiveness may significantly influence expenditures if some repellents are less effective and require additional applications. A better understanding of product efficacy needs to be established to mitigate these and other negative consequences associated with high fly populations.

There are some challenges with fly repellent use on equids for fly control. Most fly repellents marketed toward horse owners use pyrethroids as active ingredients. As in other animal facilities, consistent application of compounds with the same mode of action may increase fly resistance (Boxler and Campbell 1983, Kaufman et al. 2001, Butler et al. 2007, Kozaki et al. 2009, Memmi 2010). High levels of house fly resistance to permethrin were found consistently across the United States (Scott et al. 2013). This may lead to behavioral tolerance to the effects of compounds frequently marketed as fly repellents. In addition to resistance, formulation and application differences may also affect repellent performance. Duration of repellency might be affected by application method, animal sweat (Brown et al. 1997), or environmental considerations, such as dirt and rain.

Because of the perceived harmful effects of synthetic chemicals to health and the environment, the public has an increasing demand

for 'natural' ingredients in pest control products. Equine owners are no exception. In a survey of horse owners, over 80% requested more information on nontoxic pest control solutions (Machtinger et al. 2015). Current fly repellent products for equids marketed as 'natural' contain either essential oils and/or other plant extracts, or short-chain fatty acids, such as the C8910 complex (Zhu et al. 2014). There has been some research on the use of these compounds as house fly insecticides (Malik et al. 2007), but more limited research on the efficacy of these ingredients as repellents. Singh and Singh (1991) evaluated 31 essential oils for repellent properties against house flies, but repellency was evaluated by knockdown in an enclosed arena, and not by behavioral repellency or inhibition. Other evaluations of catnip oil (Zhu et al. 2009) and bergamot mint (Kumar et al. 2011) have demonstrated varying levels of efficacy, but none of the currently available equine fly spray products with natural ingredients contain these active ingredients.

Although use of fly repellents is common among horse owners, many fly repellent products are marketed with little to no public efficacy data. Thus, there is a need to evaluate currently available fly repellent products with both synthetic and natural ingredients. Although true repellent behavior is defined as arthropod orientation away from a substance or source of a substance (Klowden 1996), the objective of the present study was to assess house fly behavioral inhibition (hereafter referred to as 'inhibition'; Dogan et al. 1999) while passing through a treated area in response to seven common fly repellants labeled for use on equids at different doses and over time.

Materials and Methods

Fly Colonies and Rearing

A 6-mo-old (24–36 generations) colony of house flies was tested in these assays. Approximately 300 adult house flies were collected by sweep netting from mixed animal facilities in Clinton, Lycoming, and Centre Counties, Pennsylvania, in May 2018 and had been in culture for 4 wk at the onset of the study. The source population came from farms that had been exposed to pyrethroid and imidacloprid insecticides as part of routine fly control, as would be expected in most areas of the United States (Scott et al. 2013).

Adult flies were reared in a 32.5 cm^3 BugDorms (Megaview Science, Taiwan) and were provided water, and a mixture of dried egg yolk, milk powder, and sucrose ad libitum with both food and water replenished twice weekly. Immature flies were reared on wheat bran and calf manna hydrated to approximately 75% moisture at 25°C and 25% RH. The fly colony is currently kept in the Veterinary Entomology Lab at the Pennsylvania State University in University Park, PA.

Repellents

Seven commercially available fly repellents labeled for use on horses were tested, and deionized water was used as a control (Table 1). The three repellents with natural compounds were Ecovet (Natural A; Ecovet, Snohomish, WA), which used a fatty acid complex as active ingredients (C8910 complex of octanoic, nonanoic, and decanoic acid), Equiderma (Natural B; Telesis Animal Health, Inc., Greenville, FL) with neem, aloe, and eight essential oils as active ingredients, and Natural C (Natural C; SmartPak Equine LLC., Plymouth, MA), a plant-based repellent made from geraniol and peppermint oil. The four synthetic repellents tested were the permethrin-based Bronco (Pyrethroid A; Farnam Companies, Inc., Phoenix, AZ), the pyrethroid mixture Endure (Pyrethroid B; Farnam Companies, Inc., Phoenix, AZ) that included cypermethrin, butoxy polypropylene glycol, piperonyl butoxide and pyrethrins, Endure (Pyrethroid C; Manna Pro Products, LLC., Chesterfield, MO) that included cypermethrin as an active ingredient, and the permethrin-based UltraShiel Red (Pyrethroid D; W.F. Young, Inc., East Longmeadow, MA). These repellents were purchased in 'ready to use' concentrations and were chosen based on an informal survey of products used for fly control in the equine industry in the Mid-Atlantic region of the United States.

Cost per ounce of each fly repellent product tested was derived by averaging the online price of each product from four retailers including smartpakequine.com, valleyvet.com, statelinetack.com, and anddoversaddlery.com in March 2019. In some cases, products were not available from those online suppliers, so prices from Schneiders.com, Centerlinestyle.com, Jeffersequine.com, or equiderma.com were used. The average price was divided by the product amount in ounces to calculate the price per ounce.

Three concentrations of all repellents were tested in a serial dilution of 100, 50, and 25% with dilutions created with dH_2O. Water was used as a diluting agent as horses would commonly encounter it after repellent application in the form of rainwater, bathing, or partially in sweat. The dilutions were vortexed before application to ensure a homogenous emulsion.

Bioassay

Flies (5–8 d old) were removed from the colony via a hand-held aspirator (BioQuip Products, Inc., Rancho Dominguez, CA). Flies were anesthetized with CO_2 and sorted by sex. Females were placed in groups of 10 in each of eight 17.5 × 17.5 × 17.5 cm test arenas (BugDorms). Flies in test arenas were starved for 24 h prior to each test, but provided water ad libitum. The inhibition bioassay required starved house flies to pass through a treated area to reach an attractive food to represent passage through treated space, such as hair, to reach an animal. Males were not used because of initial challenges with mortality after starvation. The food consisted of a mixture of 1 oz milk powder (protein source), 1 oz sucrose (carbohydrate source), and 4.9 ml blue culinary food coloring (McCormick and Co., Hunt Valley, MD). At the end of the feeding trial, cages were frozen and fly abdomens were observed for blue coloration. Although sucrose is not considered attractive, carbohydrates are required as an energy source to prevent mortality. This mixture was placed in 200 ml Pyrex beakers (10 cm × 6 cm, Corning, Inc., Corning, New York; Fig. 1). Flies that were observed on the treated area or that passed through the treated area to the food, signified by having blue dye in the gut, were considered not inhibited. Flies with blue abdomens outside of the beaker was determined to be <2%.

A modified filter paper in the shape of an inverted cone was treated with the respective compound and placed above the food in the opening of the beaker. To create the inverted cones, Whatman #4 filter papers (90 mm, GE Healthcare, Little Chalfont, Buckinghamshire, United Kingdom) were folded in half (Fig. 2a and b). A point was marked at the intersection of where a 1.6-cm line would transect the edge of the filter paper from the center of the top margin. A line was drawn from the center of the filter paper to this previously marked point on the outside margin of the filter paper (approx. 2.0 cm). This was cut out of the filter paper and this top portion discarded. From the remaining portion, a semicircle was cut from the center with a measurement of 0.5 cm from each edge of the top portion of the circle, and a depth of 0.7 cm. This created a hole for the flies to access the food source when folded. A thin line of hot glue was used to connect the edges of the filter paper, which overlapped 0.5 cm, creating a cone that would sit in the top of the

Table 1. Commercial repellents labeled for use on horses that were used in this study to test house flies (*Musca domestica*) for behavioral inhibition, including manufacturer, active and inert ingredients, and cost per ounce to purchase the product

Repellent	Reference name	Manufacturer	Active ingredients	Inert ingredients	Cost per ounce[a]
Products marketed as natural repellents					
Ecovet	Natural A	Ecovet	5% Octanoic acid 5% Nonanoic acid 5% Decanoic acid	84% Silicone oil 1% fragrance	$1.19
Equiderma	Natural B	Telesis Animal Health, Inc.	Neem leaf tea Aloe vera gel Neem oil Red cedar oil Eucalyptus Lemongrass Citronella Lemon peel Tea tree Lavender	N/A	$0.72
Outsmart	Natural C	SmartPak Equine LLC.	2% Geraniol 2% Peppermint oil	Total 96%, Water Isopropyl alcohol Soap Glycerin Potassium sorbate	$0.62
Products with synthetic compounds					
Bronco	Pyrethroid A	Farnam Companies, Inc.	0.1% Permethrin 0.33% Prallethrin 0.5% Piperonyl butoxide	99.37%	$0.27
Endure	Pyrethroid B	Farnam Companies, Inc.	0.15%, Cypermethrin 0.20% Pyrethrins 1.60% Piperonyl butoxide 5% Butoxy polypropylene glycol	93.05%	$0.72
Optiforce	Pyrethroid C	Manna Pro Products, LLC.	1% Cypermethrin	99%	$0.62
UltraShield Red	Pyrethroid D	W.F. Young, Inc.	0.9% Permethrin 0.25% Tetramethrin 0.025% Pyrethrins 0.1% Cypermethrin 1% Piperonyl butoxide	97.73%	$0.59

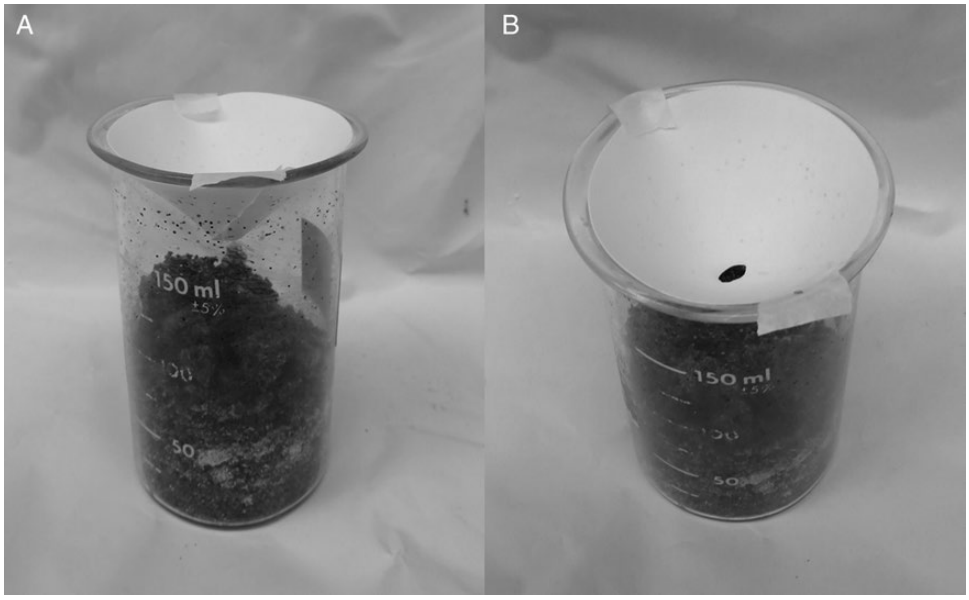

Fig. 1. Pyrex beakers with modified filter paper cone to assess house fly (*Musca domestica*) behavioral inhibition in response to seven commercially available fly repellents labeled for use on horses (Natural B, Natural A, Outsmart, Optiforce, Endure, Pyrethroid D, and Bronco). Repellents were tested at 100, 50, and 25% which made for concentrations of 10.2, 5.1, and 2.6 μl/cm^3, respectively, on the cone treatment area.

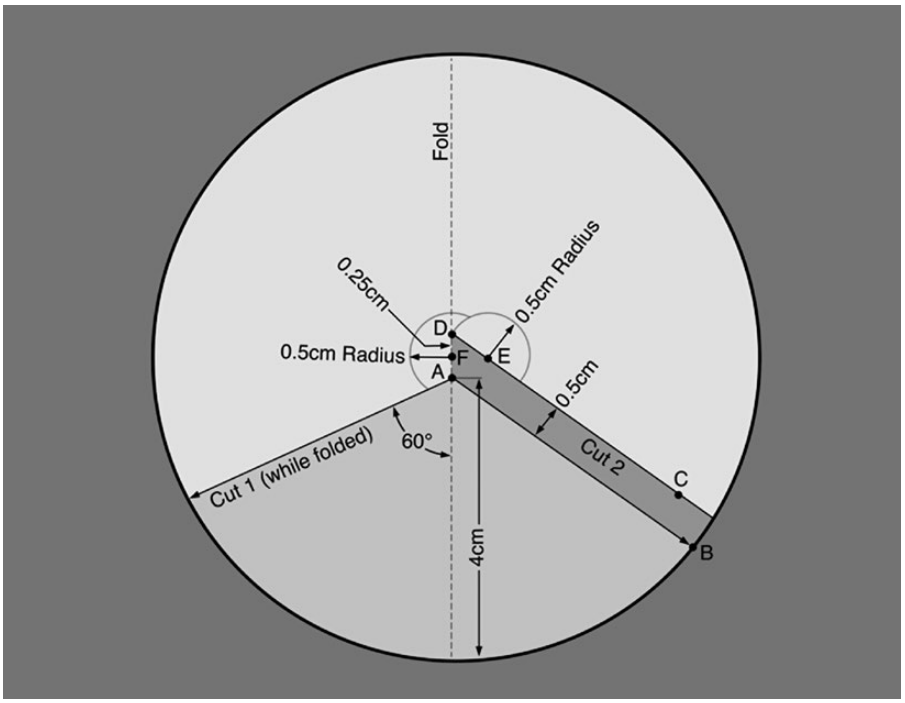

Fig. 2. Filter paper design used for house fly (*Musca domestica*) inhibition assays; fold filter paper in half and from point A, mark a point along the edge (point B) that creates a 60° angle and cut along that line (cut 1 while folded). The second cut at point C is a line 0.5 cm from the line at point B and terminates at the center fold point D. Two semicircles are removed from the center. At point E, the first is a semicircle with a 0.5 cm radius 0.5 cm from point D, and the second is a semicircle with a 0.5 cm radius drawn from point F which is 0.25 cm from point D.

beakers (Fig. 1). Total surface area was 105.0 cm^2 and volume was 49.2 cm^3.

Filter paper cones were labeled and placed in individual petri dishes for repellant application. To apply repellent, 500 µl of the product and respective concentration being tested (100, 50, or 25%) was pipetted on each cone and given 10 min to dry and spread to complete coverage over the filter paper. Dilutions were tested to because current product application methods are typically applied with a spray action, thus not completely saturating and/or covering the animal. Therefore, it is likely that the amount of active ingredient per unit skin area is not 100%. Equiderma, Optiforce, and Pyrethroid B were applied with a small paintbrush for full coverage as these formulations were too viscous to pipette on to the cones. This made for repellent concentrations of 10.2, 5.1, and 2.6 µl/cm^3, respectively. Pipette tips and paintbrushes were replaced or cleaned in acetone between applications. After drying, treated cones were placed tip down into each beaker. A small (1.8 cm × 1 cm) piece of masking tape was used to secure the cone to the beaker on opposite sides to prevent alternative routes of fly entrance to the food source. Beakers were placed individually in random order in the test arenas (one arena for each treatment and one for the control), and arenas were held at 23°C and 50% RH with 10:14 (L:D) h.

Fly position in each cage was recorded at 15, 30, 60 (1 h), 240 (4 h), 1,440 (24 h), and 2,280 (48 h) min. During the time checks, the number of flies on the filter paper, in the beaker with the food source, and out of the beaker was recorded. If a fly died outside of the inverted cone trap, it was considered inhibited as it was often observed that with the more effective products some flies would die before passing through the treated cone, likely from the effects of starvation. After 2,280 min, the beaker was removed from the test arena and the remaining flies were aspirated from the cages and anesthetized to count numbers with blue food coloring in their gut. Between replicates, beakers were washed with 70% ethanol, rinsed with dH_2O, and air dried. Test arenas were washed and air dried between replicates.

Statistical Analysis

Five replicates of each of the three concentrations of the seven products and the control were conducted using a different cohort of adult house flies and different product solutions for each. PA-mixed colony flies were tested at all three concentrations (100, 50, and 25%, respectively).

All statistical analyses were completed using the R statistical programming language (R Core Team 2018). A binomial generalized linear mixed-effects model (GLMM) with logit link was fitted using the lme4 package (Bates et al. 2015). GLMMs are the appropriate choice for the analysis of data that are non-normal and require random effects (Bolker et al. 2008). The final data set included five replicates for each unique combination of two factors (8 levels of product code and 3 levels of concentration = 24 unique combinations), resulting in 120 cages with observations recorded at six time points in each cage for the wild colony only. The repeated observations within each cage imply both temporal autocorrelation and within-cage correlation. As a result, a random effect was included for cage such that repeated measurements taken at consecutive time points were nested within each cage. The response variable was the proportion of inhibited flies and explanatory variables included time, concentration, and product code (main effects) as well as relevant interactions. Time was rescaled from minutes to days and concentration was rescaled from percentage to proportion to assist with optimization. Two models were fitted and compared

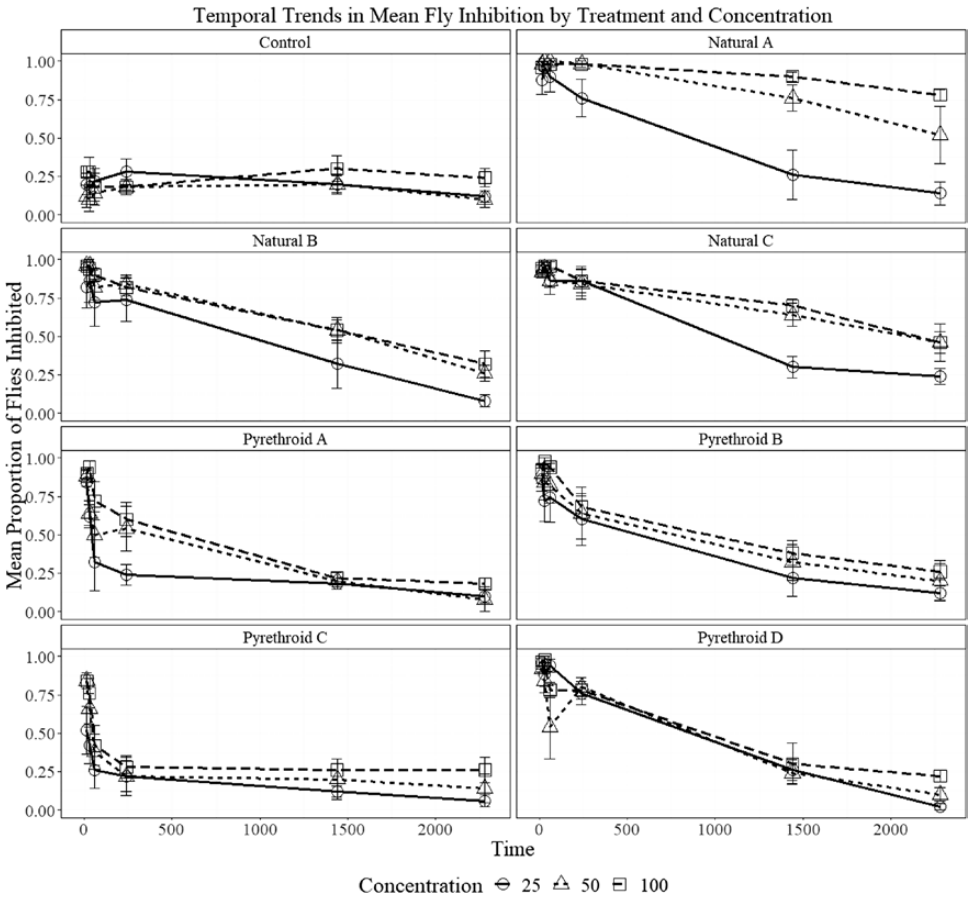

Fig. 3. Temporal trends in mean house fly (*Musca domestica*) inhibition to equine fly repellents by product and concentration (25, 50, and 100%).

via both Akaike information criterion (AIC) and Bayesian information criterion (BIC). These were the simplest models constructed that still addressed all the research questions. The first included all main effects and interactions for 1) product code and time, 2) time and concentration, and 3) product code and concentration. The second model included all main effects and only interactions (1) and (2). Tests for overdispersion were conducted by calculating the estimated overdispersion parameter and testing whether the sum of squares of the standardized residuals followed a χ^2 distribution (Qian 2017) and using the R package DHARMa (Hartig 2019). Post hoc pairwise comparisons were computed using the R package emmeans (Lenth 2019). Effects displays were constructed using the R package effects (Fox 2003, Fox and Weisberg 2019).

Results

The AIC and BIC for the model with the main effects time, concentration, and product code and interactions for 1) product code and time, 2) product code and concentration, and 3) time and concentration were 2,527.446 and 2,651.085, respectively. The AIC and BIC for the model with main effects time, concentration, and product code and interactions 1) product code and time, and 2) time and concentration were 2,522.045 and 2,613.630, respectively. The latter model was selected since it had both the lowest AIC and BIC. The estimated overdispersion parameter of the selected model was 0.433 (χ^2 = 303.1, P = 1.0) and additional residual diagnostics did not show any model misspecifications.

Individual product performance differed by concentration and time, as would be expected. Natural A was the only product with >50% inhibition at 100 and 50% concentration at the full duration of the trial (2 d; 2,000 min). Natural products A, B, and C were the only products with >50% inhibition at 1 d (1,000 min) at 100 and 50% concentrations (Fig. 3). At 8 h (500 min), all natural products and pyrethroid B and D were over 50% inhibition at 100 and 50% concentrations, but all pyrethroids were under 75%, while all natural products were near or over 75% inhibition. Pyrethroid A was over 50% inhibition at 100 and 50% concentrations at 4 h (250 min), but declined rapidly at subsequent time points. Pyrethroid B and D were similar to each other in efficacy over time, remaining over 50% at all dilutions at 4 h (250 min), but by 1 d (1,000 min), all were under 50% inhibition at all concentrations. Pyrethroid C inhibition efficacy dropped significantly after initial application to 25% inhibition in all concentrations, similar to control levels.

Fixed effects estimates, 95% confidence limits, SE, and z- and P-values are provided in Table 2. The estimated variance of the random intercept for cage was 0.64 (SD ±0.80), and observation within cage was 0.66 (SD ±0.81). There was some separation in pairwise comparisons with Natural A and C performed at similar levels, but Natural B and Natural C also performed similar to Pyrethroid B and D. Pyrethroid A inhibition efficacy was not different from the other pyrethroids, although it did not compare with the natural products. Pyrethroid C was not different from the control in the model.

Tables 3 and 4 summarize mean inhibited proportions of house flies depending on product and time (Table 3) and time and concentration (Table 4). The efficacy of the products declined with time (z = −1.86, P = 0.063, Table 2) and increased with increasing

Table 2. Binomial generalized linear mixed-effects model estimates and 95% confidence limits on the logit and odds ratio scales with associated SE and *P* values for comparison of commercial fly repellents labeled for use on horses

	Logit scale		Odds ratios					
Fixed effects	Estimate	95% confidence limits	Estimate	95% confidence limits	SE	*z*-value	*P* value	Product pairwise comparisons[a]
Intercept	−2.26	(−2.89, −1.63)	0.10	(0.06, 0.2)	0.32	−7.03	2.09E-12	
Time	−0.49	(−1.01, 0.03)	0.61	(0.36, 1.03)	0.27	−1.86	6.31E-02	
Concentration	1.04	(0.43, 1.65)	2.82	(1.53, 5.21)	0.31	3.32	8.97E-04	
Natural A[a]	5.60	(4.74, 6.46)	270.94	(114.58, 640.66)	0.44	12.76	2.82E-37	Natural C
Natural B[a]	4.20	(3.44, 4.96)	66.45	(31.05, 142.22)	0.39	10.81	3.10E-27	Natural C, Pyrethroid B and D
Natural C[a]	4.51	(3.74, 5.28)	91.05	(42.09, 196.98)	0.39	11.46	2.13E-30	Pyrethroid B and D
Pyrethroid A[a]	2.55	(1.83, 3.28)	12.86	(6.25, 26.45)	0.37	6.94	3.85E-12	Pyrethroid B, C, D
Pyrethroid B[a]	3.74	(2.99, 4.49)	42.09	(19.9, 89.03)	0.38	9.78	1.32E-22	Pyrethroid D
Pyrethroid C[a]	1.67	(0.95, 2.38)	5.29	(2.58, 10.84)	0.37	4.56	5.22E-06	Control
Pyrethroid D[a]	3.89	(3.14, 4.63)	48.81	(23.15, 102.89)	0.38	10.22	1.66E-24	
Time: Concentration	0.71	(0.21, 1.21)	2.04	(1.24, 3.36)	0.26	2.79	5.25E-03	
Time: Natural A[a]	−2.69	(−3.36, −2.01)	0.07	(0.03, 0.13)	0.34	−7.83	4.95E-15	
Time: Natural B[a]	−2.61	(−3.22, −2)	0.07	(0.04, 0.14)	0.31	−8.35	6.76E-17	
Time: Natural C[a]	−2.23	(−2.83, −1.63)	0.11	(0.06, 0.2)	0.31	−7.27	3.54E-13	
Time: Pyrethroid A[a]	−2.22	(−2.83, −1.61)	0.11	(0.06, 0.2)	0.31	−7.09	1.33E-12	
Time: Pyrethroid B[a]	−2.71	(−3.33, −2.09)	0.07	(0.04, 0.12)	0.32	−8.53	1.44E-17	
Time: Pyrethroid C[a]	−1.54	(−2.15, −0.94)	0.21	(0.12, 0.39)	0.31	−4.98	6.32E-07	
Time: Pyrethroid D[a]	−3.09	(−3.72, −2.46)	0.05	(0.02, 0.09)	0.32	−9.58	9.42E-22	

The last column provides the results of post hoc pairwise tests comparing product codes over a fixed time.

[a]Product pairs formed by fixed effect and those listed are not considered significantly different based on comparison of estimated marginal means at time = 0.47 d using an approximated Tukey's HSD (α = 0.05).

[b]Control (distilled H_2O) was the reference contrast for all terms involving products. *P* values (determined by Wald's test) indicate significance of effect in comparison to the control.

Table 3. Behavioral inhibited proportions of house flies (*Musca domestica*) in response to commercial repellents labeled for use on horses depending on product and time

	Mean proportion of flies inhibited (lower 95% confidence limit, upper 95% confidence limit)				
	Time (d)				
Product	0.01	0.4	0.8	1	2
Control	0.161 (0.103, 0.243)	0.157 (0.103, 0.231)	0.153 (0.099, 0.229)	0.151 (0.095, 0.231)	0.141 (0.069, 0.268)
Natural A	0.981 (0.962, 0.990)	0.945 (0.906, 0.968)	0.850 (0.771, 0.906)	0.766 (0.657, 0.848)	0.171 (0.084, 0.316)
Natural B	0.925 (0.877, 0.956)	0.813 (0.726, 0.877)	0.598 (0.475, 0.709)	0.465 (0.341, 0.593)	0.056 (0.026, 0.116)
Natural C	0.945 (0.906, 0.968)	0.874 (0.807, 0.920)	0.734 (0.628, 0.819)	0.635 (0.511, 0.743)	0.148 (0.076, 0.269)
Pyrethroid A	0.707 (0.593, 0.799)	0.496 (0.380, 0.612)	0.282 (0.191, 0.394)	0.199 (0.126, 0.300)	0.024 (0.010, 0.056)
Pyrethroid B	0.887 (0.821, 0.931)	0.726 (0.619, 0.811)	0.464 (0.344, 0.588)	0.332 (0.226, 0.458)	0.030 (0.013, 0.066)
Pyrethroid C	0.499 (0.378, 0.621)	0.347 (0.249, 0.459)	0.217 (0.143, 0.316)	0.167 (0.104, 0.258)	0.038 (0.017, 0.086)
Pyrethroid D	0.901 (0.842, 0.939)	0.725 (0.618, 0.811)	0.426 (0.309, 0.552)	0.283 (0.186, 0.404)	0.016 (0.007, 0.038)

concentration (z = 3.32, P = 0.0009, Table 2), the remaining explanatory variables held fixed. Natural A, B, and C outperformed Pyrethroid A–D, and all products outperformed the control (Table 3).

Discussion

Although many products are currently marketed as fly repellents for equids, public efficacy results are limited. In the present study, the behavioral inhibition of house flies passing through an area treated with commercial products to represent product application to animal hair was tested.

All products marketed as fly repellents decline in inhibiting flies with time. Natural A appeared to inhibit flies longer followed by Natural C. Although not previously compared in these formulations, many of the ingredients in the tested natural products have shown efficacy in house flies and other filth fly species repellency. Geraniol found in Natural C is known to be toxic to male house flies (Gallardo et al. 2012), and the C8910 fatty acid complex used in Natural A has demonstrated efficacy against horn flies in pastured cattle (Mullens et al. 2017, 2018). Although specific plant species used for oils in the commercial products were not listed by the manufacturers, oils from lavender (*Lavandula angustifolia*), citronella (*Cymbopogen nardus*), eucalyptus (*Eucalyptus globulus*), lemongrass (*Cymbopogon citratus*), and peppermint (*Mentha piperita*) have all been found to have knockdown or repellent properties against house flies (Kumar et al. 2011, Morey and Khandagle 2012, Sinthusiri and Soonwera 2014), and synergy among several oils has also been found (Chauhan et al. 2018).

The decline in inhibition response by house flies over time may have been due to chemical properties such as volatilization, or house fly responses to starvation or the continuous exposure to active ingredients during these trials. As would be expected with

Table 4. Behavioral inhibited proportions of house flies (*Musca domestica*) in response to commercial repellents labeled for use on horses depending on time and concentration

	Mean proportion of flies inhibited (lower 95% confidence limit, upper 95% confidence limit)		
	Concentration (as proportion)		
Time (d)	0.25	0.5	1
0.01	0.777 (0.725, 0.821)	0.819 (0.787, 0.847)	0.884 (0.846, 0.914)
0.4	0.572 (0.510, 0.633)	0.651 (0.608, 0.691)	0.783 (0.729, 0.829)
0.8	0.334 (0.278, 0.395)	0.429 (0.384, 0.475)	0.626 (0.556, 0.692)
1	0.235 (0.188, 0.290)	0.323 (0.281, 0.367)	0.533 (0.457, 0.608)
2	0.026 (0.017, 0.040)	0.047 (0.035, 0.063)	0.144 (0.097, 0.209)

newly established colonies, there was some variability in inhibition response. The flies tested probably have some resistance to pyrethroids as a result of historical exposure of the founding flies from farms known to use pyrethroids for fly management. House flies can develop localized resistance to incorrectly applied or overused active ingredients (Abbas et al. 2015), and it is unknown to what extent the individuals collected for colony establishment had been exposed to active ingredients in the tested products. Alternatively, inhibition variability at these later time checks may be a result of physiological need for carbohydrates. Flies require a source of sucrose to provide energy for survival. When deprived of carbohydrates, sugar feeding has been cited as taking precedence over other nutritional needs (Greenberg 1959) and may direct other behaviors. Although repellency to boric acid led to starvation and death in other house fly trials (Balme et al. 2013), starved flies in the current experiment may have decreased sensitivity to active ingredients in the treatments and passed through treated areas in lieu of death which may have decreased the realized inhibition of some treatments. Future tests of compound longevity may mitigate for this potential response by evaluating naive flies at each time point.

Availability and cost are generally taken into consideration by animal owners when choosing pest control products, as with other commodities and pest management situations (Mumford and Norton 1984). Natural B and Natural C were comparable to many of the synthetic products tested in cost per ounce. Based on performance, this supports their use as alternatives to the synthetic products. Natural A was by far the highest priced at $1.19/oz, which may represent the cost of the materials because they are still relatively new to the market or that this product is manufactured with short-chain fatty acids in the C8910 complex, which are known and defined constituents. However, Natural A continued to perform at over 75% inhibition at 1 d post-application, even at 50% dilution. Testing the fatty acid complex at lower doses or in synergy with other natural ingredients, such as geraniol (Mullens et al. 2017, 2018), to reduce materials cost may make this product more competitive with other established products with lower prices. However, pest management decisions are not exclusively based on cost, but also take into consideration goals and behavior (Mumford and Norton 1984). With the desire for more natural products in equine pest control (Machtinger et al. 2015), consumers may be willing to pay more for an effective product. In contrast, Pyrethroid A was priced significantly lower than all the other tested products, consumers will need to decide if the product will meet their needs for fly control. Because this product only showed >50% efficacy at 4 h at 100% concentration, if longer periods of protection are required this treatment may not be suitable. However, Pyrethroid A may be acceptable if protection during limited turnout or short rides is desired.

Overall, higher doses of all products were more effective at inhibiting flies than lower doses, as would be expected. In individual product cases, at 100 and 50% dilutions Natural A, B, and C demonstrated over 50% inhibition around 1 d (1,500 min), which was not seen in any of the Pyrethroid products. It is important to emphasize that applications of diluted products are not recommended, but instead that the results presented demonstrate efficacy of products that may not be applied at 100% concentration universally across the body of the animal, or that may be diluted with sweat, rain, or rubbed off on soil or bedding. These results may aid equine owners in choosing products that will suit their duration and active ingredient needs, while also considering cost.

Although none of the products induced 100% inhibition response across all time and dilution measures, the products marketed as 'natural' performed as well or better than all the synthetic products tested at earlier time points. There are some challenges with the use of natural ingredients in arthropod control. Maintaining strict quality control can be difficult with essential oils due to natural differences in plant metabolism, growing conditions, and location (Koul et al. 2008). The manufacturing protocols for each of the tested products were not known, but products with numerous ingredients and essential oils face greater potential for variable efficacy if ingredients are not consistent. This is less likely with products containing fatty acids or geraniol as these are specific chemical compounds and not oil mixtures. However, the added potential for inert ingredients to influence efficacy in terms of duration, applicability, and other factors strongly supports vigorous quality control during production of all products.

House fly resistance to pyrethroids has been demonstrated in many regions of the United States (Scott et al. 2013), but active and inert ingredient formulation differences may influence efficacy. Previous fly protectant research has mainly focused on products that containing pyrethroids, as they are the most common active ingredients in equine fly repellents. Although Schmidtmann et al. (2001) demonstrated 85–90% suppression of biting flies on horses after applications of permethrin-based fly sprays, Mottet et al. (2018) did not find any reduction in annoyance behaviors exhibited by horses pressured by stable flies after pyrethroid application. In the present study, although all contained cypermethrin, different inhibition responses were observed among all tested pyrethroid products. Pyrethroid products declined in efficacy by concentration and over time, but different inhibitions were seen among these products, suggesting house flies may be more inhibited by formulation differences or synergistic effects of the other active ingredients in the two former products than the latter. It is important to note that animal cues may differ from feeding cues associated with the present study, and on-animal evaluations of repellents may elucidate additional differences in efficacy. The overall lack of inhibition duration and, in some cases, poor performance of the synthetic products emphasize the need for

development of new modes of action and new formulations for equine fly control.

In addition to fly response to active ingredients, efficacy of repellents is related to the amount of active ingredient per unit skin area (Schmidtmann et al. 2001). Duration of repellency is influenced by animal sweat (Brown and Hebert 1997), dirt, rain, grooming, or other factors. Although these were not directly tested in the present study, it is important for consumers to recognize that even after full and ubiquitous application of a product to a surface, dilutions of that product will reduce efficacy. It is recommended that care is taken to properly apply repellents according to label directions, which recommend applying full coverage to a clean animal. In addition, applications using a sponge or a mitt to all body surfaces may increase coverage to the animal as opposed to the sprayer method, where the product lightly coats the hair or do not come in contact with the animal at all.

Continued investigation of efficacy on equids and ideal application methods is needed. Efficacy testing of equine fly repellent products and active ingredients on biting pest flies such as stable flies and horse and deer flies should be considered, as well as on animal assays. However, the results presented herein can assist equid owners in selecting fly repellents that will meet their needs for fly control to reduce house fly pressure on horses and other equids.

Acknowledgments

We thank Jessica Brown and Alexandra Pagac for their help with fly counts. In addition, we thank Hannah Greenberg, Karen Poh, and Chris Geden for their review of early drafts of this manuscript, and Nick Sloff at Penn State for his assistance with imagery. We thank Greg Martin for his assistance with farm identification for fly collection and Maria Wojakowski for her extensive help with statistics. This work was funded in part by the U.S. Poultry and Egg Association under award no. 196040 and the USDA National Institute of Food and Animal Health Appropriations under Project No. PEN04635 and Accession No. 1014569.

References Cited

Abbas, N., S. A. Shad, and R. M. Shah. 2015. Resistance status of *Musca domestica* L. populations to neonicotinoids and insect growth regulators in Pakistan poultry facilities. Pakistan J. Zool. 47: 1663–1671.

Amado, S., A. K. Silveira, F. D. Vieira, and D. Traversa. 2014. *Habronema muscae* (Nematoda: Habronematidae) larvae: developmental stages, migration route and morphological changes in *Musca domestica* (Diptera: Muscidae). Exp. Parasitol. 136: 35–40.

Balme, G. R., S. S. Denning, and D. W. Watson. 2013. Boric acid aversion in adult house flies, *Musca domestica* L. J. Agric. Urban Entomol. 29: 85–92.

Barba, M., A. J. Stewart, T. Passler, A. A. Wooldridge, E. van Santen, M. F. Chamorro, R. C. Cattley, T. Hathcock, J. A. Hogsette, and X. P. Hu. 2015. Experimental transmission of *Corynebacterium pseudotuberculosis* biovar *equi* in horses by house flies. J. Vet. Intern. Med. 29: 636–643.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67: 1–48.

Bolker, B., M. E. Brooks, C. J. Clark, S. W. Geange, J. R. Poulsen, M. H. H. Stevens, and J. S. White. 2008. Generalized linear mixed models: a practical guide for ecology and evolution. Trends Ecol. Evol. 24: 127–135.

Boxler, D. J., and J. B. Campbell. 1983. Survey of resistance by house fly, *Musca domestica* (L.) (Diptera: Muscidae), to dichlorvos in Nebraska feedlots. J. Kans. Entomol. Soc. 56: 159–163.

Brown, M., and A. A. Hebert. 1997. Insect repellents: an overview. J. Am. Acad. Dermatol. 36: 243–249.

Butler, S. M., A. C. Gerry, and B. A. Mullens. 2007. House fly (Diptera: Muscidae) activity near baits containing (Z)-9-tricosene and efficacy of commercial toxic fly baits on a southern California dairy. J. Econ. Entomol. 100: 1489–1495.

Chauhan, N., A. Malik, and S. Sharma. 2018. Repellency potential of essential oils against housefly, *Musca domestica* L. Environ. Sci. Pollut. Res. Int. 25: 4707–4714.

Dogan, E. B., J. W. Ayres, and P. A. Rossignol. 1999. Behavioural mode of action of deet: inhibition of lactic acid attraction. Med. Vet. Entomol. 13: 97–100.

Fox, J. 2003. Effect displays in R for generalized linear models. J. Stat. Softw. 8: 1–27. (http://www/jstatsoft.org/v08/i15/).

Fox, J., and S. Weisberg. 2019. An R companion to applied regression, 3rd ed. Thousand Oaks, CA. (http://tinyurl.com/carbook).

Gallardo, A., M.I. Picollo, P. González-Audino, and G. Mougabure-Cueto. 2012. Insecticidal activity of individual and mixed monoterpenoids of geranium essential oil against Pediculus humanus capitis (Phthiraptera: Pediculidae). J. Med. Entomol. 49: 332–335.

Geden, C. J., and J. A. Hogsette. 2001. Research and extension needs for integrated pest management for arthropods of veterinary importance. *In* Proceedings, Workshop in Lincoln, 12–14 April 1994, Nebraska. Last updated October 2001. Center for Medical, Agricultural, and Veterinary Entomology USDA-ARS, Gainesville, FL.

Greenberg, B. 1959. House fly nutrition. I. Quantitative study of the protein and sugar requirements of males and females. J. Cell. Comp. Physiol. 53: 169–177.

Hartig, F. 2019. SHARMa: residual diagnostics for hierarchical (multi-level/mixed) regression models. R package version 0.2.4. (https://CRAN.R-project.org/package=DHARMa).

Kaufman, P. E., J. G. Scott, and D. A. Rutz. 2001. Monitoring insecticide resistance in house flies (Diptera: Muscidae) from New York dairies. Pest Manag. Sci. 57: 514–521.

Keiper, R. R., and J. Berger. 1982. Refuge-seeking and pest avoidance by feral horses in desert and island environments. Appl. Anim. Ethol. 9: 111–120.

Klowden, M. J. 1996. Vector behavior, pp. 34–50. *In* B. J. Beaty and W. C. Marquardt (eds.), The biology of disease vectors. University Press of Colorado, Niwot, CO.

Koul, O., S. Walia, and G. S. Dhaliwal. 2008. Essential oils as green pesticides: potential and constraints. Biopestic. Int. 4: 63–84.

Kozaki, T., S. G. Brady, and J. G. Scott. 2009. Frequencies and evolution of organophosphate insensitive acetylcholinesterase alleles in laboratory and field populations of the house fly, *Musca domestica* L. Pestic. Biochem. Physiol. 95: 6–11.

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.

Lenth, R. 2019. emmeans: estimated marginal means, aka least-squares means. R package version 1.3.4. (https://CRAN.R-project.org/package=emmeans).

Machtinger, E. T., N. C. Leppla, and C. Sanders. 2015. Pest management perceptions and practices for equine farms in North and Central Florida. Department of Entomology and Nematology, UF/IFAS Extension. University of Florida, Gainesville, Florida.

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

McDonnell, S. M. 2003. The equid ethogram: a practical field guide to horse behavior. Eclipse Publications, Lexington, KY.

Memmi, B. K. 2010. Mortality and knockdown effects of imidacloprid and methomyl in house fly (*Musca domestica* L., Diptera: Muscidae) populations. J. Vector Ecol. 35: 144–148.

Morey, R. A., and A. J. Khandagle. 2012. Bioefficacy of essential oils of medicinal plants against housefly, *Musca domestica* L. Parasitol. Res. 111: 1799–1805.

Mottet, R. S., R. D. Moon, M. R. Hathaway, and K. L. Martinson. 2018. Effectiveness of stable fly protectants on adult horses. J. Equine Vet. Sci. 69: 11–15.

Mullens, B. A., D. W. Watson, A. C. Gerry, B. A. Sandelin, D. Soto, D. Rawls, S. Denning, L. Guisewite, and J. Cammack. 2017. Field trials of fatty acids and geraniol applied to cattle for suppression of horn flies, *Haematobia*

irritans (Diptera: Muscidae), with observations on fly defensive behaviors. Vet. Parasitol. 245: 14–28.

Mullens, B. A., D. Soto, A. C. Gerry, F. E. Fowler, and A. N. Diniz. 2018. Effects of fatty acid and geraniol repellent-oil mixtures applied to cattle on blood feeding and reproductive parameters in field populations of *Haematobia irritans* (Diptera: Muscidae). J. Med. Entomol. 55: 408–416.

Mumford, J. D., and G. A. Norton. 1984. Economics of decision making in pest management. Annu. Rev. Entomol. 29: 157–174.

Qian, S. S. 2017. Environmental and ecological statistics with R, 2nd ed. Chapman & Hall/CRC, Boca Raton, FL.

R Core Team. 2018. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. (https://www.R-project.org/).

Schmidtmann, E. T., J. R. Lloyd, R. J. Bobian, R. Kumar, J. W. Waggoner, 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. Entomol. 38: 728–734.

Scott, J. G., C. A. Leichter, F. D. Rinkevich, F. D. Rinkevihc, S. A. Harris, C. Su, L. C. Aberegg, R. Moon, C. J. Geden, A. C. Gerry, *et al*. **2013**. Insecticide resistance in house flies from the United States: resistance levels and frequency of pyrethroid resistance alleles. Pestic. Biochem. Physiol. 107: 377–384.

Singh, D., and A. K. Singh. 1991. Repellent and insecticidal properties of essential oils against housefly, *Musca domestica* L. Insect Sci. Appl. 12: 487–491.

Sinthusiri, J., and M. Soonwera. 2014. Oviposition deterrent and ovicidal activities of seven herbal essential oils against female adults of housefly, *Musca domestica* L. Parasitol. Res. 113: 3015–3022.

Spier, S. J., C. M. Leutenegger, S. P. Carroll, J. E. Loye, J. B. Pusterla, T. E. Carpenter, J. E. Mihalyi, and J. E. Madigan. 2004. Use of a real-time polymerase chain reaction-based fluorogenic 5′ nuclease assay to evaluate insect vectors of *Corynebacterium pseudotuberculosis* infections in horses. Am. J. Vet. Res. 65: 829–834.

(USDA) U.S. Department of Agriculture, (APHIS) Animal and Plant Health Inspection Service, (VS) Veterinary Services, and (NAHMS) National Animal Health Monitoring System. 2017. Equine 2015, equine management and select equine health conditions in the United States, 2015. USDA–APHIS–VS–CEAH–NAHMS, Fort Collins, CO.

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

Zhu, J. J., G. J. Brewer, D. J. Boxler, K. Friesen, and D. B. Taylor. 2014. Comparisons of antifeedancy and spatial repellency of three natural product repellents against horn flies, *Haematobia irritans* (Diptera: Muscidae). Pest Manage. Sci. 71: 1553–1560.