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Use of attractant and repellent semiochemicals to manage stable fly (*Stomoxys calcitrans* (L.)) populations on pastured cattle using a push-pull strategy

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Stable flies (Diptera: Muscidae) are major economic pests of pastured cattle. Their feeding activity causes billions of dollars of damage to the cattle industry. This project investigated the management of stable flies on pastured cattle using a novel Push-Pull treatment in field trials. The repellent Push treatment, a hydrogenated coconut oil containing approximately 70% of C8, C10, and C12 fatty acids in a starch-pectin water formulation, was applied to the animals weekly. The Pull component was a stable fly trap augmented with an attractant (m-cresol). Permethrin and untreated controls were used as comparisons. Both permethrin and Push-Pull treatments reduced stable fly numbers on cattle compared with the control treatment. Traps used in pastures as part of the Push-Pull treatment captured large numbers of stable flies. However, the contribution of the traps to the observed reduction in stable fly infestation levels needs further investigation. This project also investigated the effects of altering visual and olfactory components of a stable fly trap on capture rates. This involved evaluating the effects of changing the trap appearance, lure size, and dose of m-cresol on stable fly capture rates. Trap appearance had no effect on fly capture; white traps captured the same number of flies as striped traps. Traps with lures, particularly larger sized lures, captured more flies. Our results demonstrate that m-cresol lures captured stable flies, but more investigation is needed. Our research suggests that the use of a Push-Pull treatment to manage stable flies may provide an alternative control method to the traditional pesticide application.

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Introduction

Stable flies (Diptera: Muscidae, *Stomoxys calcitrans* (L.)) are blood feeding (Foil and Hogsette 1994) pests of cattle with a near-global distribution (Bishopp 1913, Brues 1913, Zumpt 1973). Their painful bites stress cattle and cause economic losses to all sectors of the U.S. cattle industry with an overall estimated value $2.2 USD billion per year (Taylor et al. 2012). Besides direct injury, stable flies can mechanically transmit viruses (West Nile fever virus and Rift Valley fever virus), bacteria (ex. (*Bacillus anthracis* (C.), *Francisella tularensis* (D.)), protozoa (several Trypanosoma (G.) species), and helminths (*Habronema microstoma* (D.) and *Dirofilaria repens* (R&H.) to their hosts (Baldacchino et al. 2013).

The purpose of this review is to discuss the biology, ecology, and management of stable flies with emphasis on the use of repellents and attractants within a push-pull integrated pest management strategy.

Biology

Stable flies have broad abdomens and piercing sucking mouthparts (Foil and Hogsette 1994). They are grey in color, with four black stripes on the thorax, and the abdomens are checkered in black (Foil and Hogsette 1994) (Fig. 1). Adult stable flies range in size from 4-7mm. Like all Dipterans, stable flies have a holometabolous lifecycle. Eggs hatch after 12-24 hours and develop through three instars, forming a coarctated pupa during the third instar (Rochon et al. 2021). Seven to fourteen days after pupariation, adults emerge and both males and females can take their first blood meal within 12-24 hours (Parr 1962). In the field, adult flies have an average life span of two weeks (Killough and Mckinstry 1965). Adults begin mating at 3 to 5 days old, and oviposition begins when females are 5-8 days old (Foil and Hogsette 1994). Females can lay anywhere from 60 to 800 eggs during their lifetime and will oviposit in an array of materials e.g., manure vegetation mix (Foil and Hogsette 1994, Killough and Mckinstry 1965), decomposing sea grass (Hansens 1951), decomposing pineapples (Solórzano et al. 2015), coffee (Broce et al. 2005), sugar cane (Broce et al. 2005), and other organic matter. Cook et al. (2018) investigated the diversity of developmental sites that could support larvae.

Stable flies are generalist blood feeders and feed on livestock, pets, wildlife, and humans (Bishopp 1913, Hansens 1951, Hogsette et al. 1987, Foil and Hogsette 1994). Stable flies congregate and feed on the lower portions of the host (Lyskk 1995) at temperatures between 10 to 20°C (Lysyk 1995). Female flies average 1.8 feeding events per day while males average 2.8 events per day (Harris et a. 1974). Stable flies consume 11–15 µL of cattle blood per meal (Baldacchino et al. 2013).

Besides blood loss, stable fly feeding causes cattle to express defensive behaviors that include bunching, migrating to windy areas, leg stomping, and tail flicking (Mullens et al. 2006). As potential mechanical vectors of viruses, bacteria, and protozoans, the impacts of stable flies as vectors of disease-causing pathogens are probably underestimated (Baldacchino et al. 2013).

Stable fly olfaction

Stable flies use olfactory cues to locate hosts and immature developmental sites. The sensory organs responsible for the perception of chemical odorants are the funicles of antennae (Sukontason et al. 2004, White and Bay 1980). The bosonic and clavate sensilla pore structures of the funicles have been suggested to provide stable fly olfactory functions (Tangtrakulwanich et al. 2011). The stable fly antennae sensory organs are known to respond to stimuli such as warmth, humidity, animal skin odors, ammonia, and carbon dioxide (Hopkins 1964, Gatehouse 1969, Lewis 1971).

Stable fly vision

Olfactory cues are not the only cues that stable flies utilize. Visual cues are also relied on to locate hosts and immature developmental sites (Jeanbourquin and Guerin 2007, Seenivasagan and Vijayaraghavan 2010, Zhu et al. 2016). Stable flies show strong peaks of spectral sensitivity at 360 nm (UV), 525 nm (blue-green), and at 635 nm (orange-red)

(Agee and Patterson 1983 and Zhu et al. 2016). The gathering of flies on surfaces with UV-reflecting properties correlates with mating and thermoregulation behavior (Allan et al. 1987). High contrast against backgrounds can influence the orientation and detection of stable flies to a host or an object (Allan et al. 1987). Low-intensity colors are more attractive to stable flies than high intensity colors (Allan et al. 1987) and capture rates are enhanced by the addition of olfactory lures (Allan et al. 1987).

Semiochemicals

The use and exploitation of olfactory cues has been investigated and much dedication has been made to identify and understand their relationship to the stable flies. These cues are chemicals called semiochemicals. Semiochemicals are behavioral and physiological modifying chemicals and acting as kairomones, repellents, or attractants (Logan and Birkett 2007). Semiochemicals occur naturally and need to be identified and isolated so the insect behavior can be linked to a particular compound. The process of isolating semiochemicals involves solvent washing, solid phase microextraction, vacuum distillation, and air entrainment. These isolation techniques are used to collect pheromones, plant, vertebrate and invertebrate volatiles (Logan and Birkett 2007). To identify and quantify these compounds high-resolution gas chromatography and coupled GC-mass spectrometry can be used (Logan and Birkett 2007). The next process is to identify an antennal response to semiochemicals using electroantennography (GC-EAG) or single-cell recordings (GC-SRC) (Logan and Birkett 2007). After semiochemicals are isolated and identified, bioassays can be conducted to evaluate their effectiveness.

For example, using semiochemical lures as part of a trapping systems (Logan and Birkett 2007).

Stable Fly Semiochemicals

Several semiochemicals have been successfully identified as able to manipulate stable fly behaviors. Host derived volatile semiochemicals that stimulate antennal responses and induce behaviors include 1-octen-3-ol (Logan and Birkett 2007), acetone (Logan and Birkett 2007), phenol, m-cresol, and p-cresol (Tangtrakulwanich et al. 2011). 1-octen-3-ol is associated with cattle breath (Hall et al. 1984), and phenol, m-cresol, and p-cresol are associated with cattle excrement (Hassanali et al. 1986, Bursell et al. 1988). These semiochemicals stimulate flight and directed movement towards the source (Birkett et al. 2004). Oviposition behaviors by gravid stable flies are also initiated by semiochemical stimuli and are influenced by the presence of conspecific and heterospecific larvae and parasites on the substrate (Baleba et al. 2020). Olfactometer and field-testing of blends of phenol and m-cresol or p-cresol baited traps attracted the most stable flies (Tangtrakulwanich et al. 2015). Raising the possibility that baited traps could be used to improve fly capture rates and assist in the management of stable flies (Tangtrakulwanich et al. 2015).

Control Tactics

Stable flies are controlled using many different types of methods and sometimes a combination of control methods is used. These methods include chemical, cultural, mechanical, and biological. Chemical control includes insecticides, systemic insecticides, insect growth regulators, and repellents (Cook 2020). Cultural control is the process of sanitation, which includes the removal of waste products that have the possibility of becoming developmental sites (Cook 2020, Cruz-Vazquez et al. 2007, Thomas et al. 1996), along with keeping vegetation and animal waste separate because moving food sources periodically can reduce stable fly development (Rochon et al. 2021). Mechanical or physical controls, including the wide array of traps that have been tested and evaluated throughout history, can be effective against adult stable flies (Rochon et al. 2021). Biological control involves natural predators, parasitoids, bacteria, fungi, and nematodes (Rochon et al. 2021). In this review the focus will be on mechanical (traps) and chemical (semiochemicals) control methods.

Traps

Many types of traps for monitoring and removal of stable flies have been proposed (Cook 2020). The first documented use of stable fly traps was in the early 1900s and involved the use of traps affixed to barn windows that relied on the insect's attraction to sunlight (Hodge 1913). Other early trap designs used a black painted shingles coated with “Deadline” adhesive (Hansens 1951) and plywood panels coated with Tack-Trap® (Bailey et al. 1973).

An early successful trap was the Williams trap (Williams 1973), made of panels of Alsynite (a fiberglass material) coated with Tack-Trap as an adhesive. Broce 1988 improved the design and capability of the trap by forming the Alsynite into a cylinder and using Olson “Sticky Stuff” adhesive. This trap was later marketed as the Olson trap and captured more flies per cm^2 than the flat panel Williams trap (Hogsette and Ruff 1990). Coroplast traps designed by Beresford and Sutcliffe (2006) were found to capture more flies than the Alsynite traps. Further research by Taylor and Berkebile (2006) compared the efficiency of six stable fly traps and found that BiteFree trap (multi-sided, clear plastic panels) coated with a non-drying glue was the most efficient at capturing stable flies. The white panel trap using corrugated plastic material Coroplast was later improved upon by the addition of olfactory lures using phenol, m-cresol, and p-cresol which increased trap captures of stable flies (Zhu et al. 2016).

Traps with targets that act as barriers and are impregnated with insecticide (Takken et al. 1986, Vreysen et al. 2013) can also be used. Targets have been used against stable flies. These targets lasted 3 months in a field setting and required the flies to get a toxic dose at 30 seconds which resulted them to be dead within a 20-minute period (Hogsette et al. 2008). Currently, a novel panel tape material impregnated with attractant is being developed (Zhu et al. 2021).

Another trap design that has been successfully used against the biologically similar tsetse fly is the blue-black cloth traps Nzi (Mihok 2002) and Vavoua (Mihok et al. 1995). These cloth traps have been modified and have shown to be effective against stable flies. Comparisons between the Nzi and Vavoua found that when baited with fermented cattle urine, stable fly capture was increased in both traps, with Vavoua outperforming the Nzi (Tunnakundacha et al. 2017). In North America, Nzi cloth traps performed well against Alsynite cylindrical traps for sampling Stomoxyinae (Mihok et al. 2006).

Another stable fly trap to consider is the Knightstick, a black and white cylindrical trap with an adhesive foam wrap (BugJammer, Inc., Stockton, NJ). Knightstick captured more stable flies than the Olson trap and is recommended as a replacement for the Olson trap (Hogsette and Kline 2017). The adhesive sticky wrap is highly attractive to stable flies and has the potential to lure stable flies to unattractive areas (Hogsette and Kline 2017). The sticky wraps also capture and hold stable flies over a wide temperature range (Hogsette and Kline 2017).

Insecticides

In many situations, conventional insecticides have been the most efficient and cost-effective strategy for managing flies (Foil and Hogsette 1994). Pyrethrin, dichlorvos, permethrin, diflubenzuron, spinosad, and tetrachlorvinphos are insecticides with multiple modes of action labeled for stable fly control (Gerry 2020) and can be applied as pour-ons, sprays, dusts, or delivered as feed supplements. To avoid the development of insecticide resistance, rotation through different modes of action classes should be followed (Sparks and Nauen 2015). Unfortunately, the overreliance on insecticides such as permethrin (Cilek and Greene 1994, Pitzer et al. 2010, Olafson et al. 2019) has led to insecticide resistance in both exposed and unexposed populations that were exposed and some populations that were not exposed to these insecticide classes. Cilek and Greene (1994) noted that use of the insecticide naled, which is converted to dichlorvos, can lead to stable fly populations resistant to dichlorvos. Immigration of insecticide resistant stable fly populations can also result in insecticide resistance in areas without a history of use of that insecticide (Cilek and Greene 1994); stable fly resistance to organophosphates has been shown to spread by immigration of resistant populations (Cilek and Greene 1994, Pitzer et al. 2010).

Biopesticides are a promising alternative to conventional insecticides and include a broad array of organisms (microbes, nematodes, viruses), organismal products (toxins and semiochemicals), and genes (e.g., *cry* and *Bt*) that can be used to manage insects (Copping and Menn 2000). Biopesticides are of increasing interest due to their often-reduced environmental impacts and by offering alternative modes of action (Copping and Menn 2000). For this review, the focus is on repellent (semiochemicals) and attractants.

Repellents can be an important component in preventing stress and discomfort that comes from biting insect infestations (Cook 2020). Plant essential oils such as catnip (Zhu et al. 2014), peppermint (Showler 2017), eucalyptus (Showler 2017), and lemongrass (Baldacchino et al. 2013) are repellent to stable flies for up to 1-2 days. Fatty acid mixtures also provide repellency to stable flies. In bioassays, up to two weeks of repellency has been reported (Roh et al. 2020, Hieu et al. 2015, Mullens et al. 2009). In the field, fatty acids protected cattle for up to 96 hours (Zhu et al. 2018).

Adding an attractant to traps can improve capture rates (Colvin et al. 1989). Attractants associated with host breath or urine and feces, including carbon dioxide, acetone, 1-octen-3-ol, and phenols are stable fly attractants (Logan and Birkett 2007). Tangtrakulwanich et al. (2015) compared phenol, m-cresol, p-cresol, and blends of phenol and cresol and found that m-cresol and a phenol + m-cresol blend were the most effective stable fly attractants.

Cook (2020) reviewed stable fly management and emphasized the need for control options that minimize pesticide use. IPM strategies have been successful against Muscid flies in different systems. In poultry, the combination of early-season manure removal and adult fly control using insecticide baited stations and multiple applications of insecticides inside and outside the poultry barns provided excellent stable fly control (Axtell 1970). For beef cattle, the importance of early sanitation to keep feed bunk areas clear of manure and feed residue is discussed (Campbell and McNeal 1980). In dairy operations, a multi-tactic strategy of using baited traps, releasing parasitoids, and calf pen clean-outs gave mixed results in reduction of stable flies (Miller et al. 1993). Taylor (2021) discussed the importance of area-wide management to reduce the stable fly problem because of the insect's high mobility and the difficulty of finding the flies' developmental sites.

With the large body of research on semiochemicals, especially repellents (Logan and Birkett 2007), and Muscid fly chemical ecology (Cook 2020, Zhu 2011), an IPM strategy of increasing interest is Push-Pull (Cook et al. 2007). Pyke et al. (1987) coined the term Push-Pull. A push-pull strategy involves the use of behavioral modifying stimuli that manipulate the distribution and abundance of beneficial and/or pest insects (Cook et al. 2007). The objective of the push-pull strategy is to reduce the abundance of pestiferous insects on a protected commodity, either a crop or animal (Cook et al. 2007). The push is the use of stimuli to mask host apparency or repel pest insects (Cook et al. 2007). The pull is an attractive stimulus that moves pest insects from the commodity to trap crops or physical traps where they can be managed (Cook et al. 2006).

Push-Pull strategies have been tested with biological related species of Hippoboscid flies with positive results. A combination of repellents and traps has been used to manage tsetse flies in Africa (Bett et al. 2015, Olaide et al. 2019). In both studies, the push-pull tactic involved the use of a repellent and an attractant. Skin odorants isolated from zebra (Olaide et al. 2019) and waterbuck (Bett et al. 2015) were used for the repellant. Saini et al. (2017) had success using a waterbuck repellent dispensed by slow releasing collars; the collars repelled tsetse flies and increased animal welfare. Ngu traps, consisting of blue and black fabric baited with cattle urine and acetone (Olaide et al. 2019) were the pull component. Both studies concluded that push-pull strategies can effectively manage tsetse flies on cattle and are promising strategies for alternative management of Muscid flies (Olaide et al. 2019, Bett et al. 2015).

Conclusion

In conclusion, stable fly olfactory (Hassanali et al. 1986, Bursell et al. 1988) and visual (Agee and Patterson 1983, Zhu et al. 2016) cues can be manipulated and used to manage them in different environments. The use of semiochemicals and traps have been shown to improve capture rates (Zhu et al. 2016, Zhu et al. 2021). Different designs of traps have been investigated and compared with each other. Coroplast traps captured more flies than Alsynite traps (Beresford and Sutcliffe 2006). The Nzi and Vavoua blue-black traps were shown to also outperform Alsynite traps (Mihok et al. 2006). The cylindrical trap known commercially as the Knightstick captured more stable flies than the Olson trap. The use of semiochemicals that show repellence against stable flies such as fatty acids can protect cattle up to 96 hours (Zhu et al. 2018). The combination of repellents and baited traps used in a Push-Pull strategy can be an alternative management for Muscid flies (Olaide et al. 2019, Bett et al. 2015).

Figure 1. Adult stable fly. Note the piercing mouthpart protruding forward from the head and the checkered coloration on the abdomen. Photo credit: Dr. Jim Kalisch.

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Chapter 2: Use of Semiochemicals in a Push-Pull Strategy to reduce Stable Fly (*Stomoxys calcitrans* (L.)) Populations on Pastured Cattle

Abstract

Stable flies (Diptera: Muscidae) are economically important pests of livestock, annually causing billions of dollars in losses to the livestock industry. Fly biting cause changes in cattle behaviors such as bunching and leg stomping that result in reduction in weight gain, increased stress, and reduced animal welfare. We tested a novel Push-Pull treatment in field trials to manage stable flies on pastured cattle. A repellent, Push treatment, C8, C10, and C12 fatty acid mixture attained from coconut oil, was applied to cattle weekly. The Pull component was a stable fly trap with both visual and odorant attractants. Untreated and permethrin treated groups were evaluated as comparisons. Permethrin and Push-Pull treatments provided similar levels of efficacy when compared with control. We also noted that the longevity of the push-pull treatment was similar to the permethrin treatment. Traps placed in pastures as part of the push-pull treatment captured large numbers of stable flies, however, their effect on reducing stable fly biting pressure on cattle requires further investigation. Our study supports the use of a Push-Pull treatment to manage stable flies in pastures as a low impact alternative to the use of standard insecticides.

Introduction

Stable flies (*Stomoxys calcitrans* (L)) are important pests of pastured cattle (Hall et al. 1982). Cattle being stressed by stable flies engage in defensive behaviors including tail flicking, leg stomping, moving into windy areas, and bunching (Mullens et al. 2006) that reduce productivity and cause economic loss. The economic impact of stable flies in the United States was estimated to be 2.2 billion USD per year (Taylor et al. 2012).

Stable fly economic thresholds have been developed for different production systems. The economic threshold for stable flies in a confined areas such as feed lots has been determined to be 14 flies per animal (Berry et al. 1983). In a pasture system an average of 3.64 stable flies per leg or more can cause an average of 0.20 kg per steer per day weight reduction (Campbell et al. 2001).

The ideal concept of stable fly control would be to manage the sources of development sites, but these can be difficult to locate, therefore, adult management methods are needed. In these situations, chemical control is the most convenient and cost-effective option for stable fly control (Foil and Hogsette 1994). Insecticides with different modes of action labeled for stable fly control in the United States for cattle include pyrethrin, DDVP, permethrin, diflubenzuron, spinosad, and tetrachlorvinphos (Gerry 2020, see also Table 1) and can be applied using pour-on, spray, or dust applications or as feed supplements. To preserve insecticide efficacy, rotating insecticide mode of action classes should be done (Sparks and Nauen 2015, see also Table 1). Insecticide resistance is caused by the overreliance on synthetic insecticides like permethrin and organophosphates (Cilek and Greene 1994, Pitzer et al. 2010).

Because of the potential for insecticide resistance, alternatives are needed. Semiochemicals have the possibility to be an alternative product. Some of the notable semiochemicals show pesticidal activity against stable fly activity these include catnip, geranium, and short chain fatty acids (Mullens et al. 2009, Zhu et al. 2015, Roh et al., 2020). A trial with a catnip oil in a starch wax solution showed promising spatial repellency (Zhu et al. 2010). Mullens et al. 2009 observed antifeedant and repelling behaviors in stable flies in bioassays using a C8C9C10 fatty acid mixture. In a field study, Zhu et al. (2018) found that on-animal application of a coconut oil derived fatty acid mixture was effective for 96 hours in the field which was the longest protection reported for a natural repellent. In field and lab trials, the coconut fatty acid biopesticide was also shown to be a strong antifeedant and to have toxicity against stable flies (Roh et al. 2020). Fatty acid biopesticides have a novel mode of action affecting the AW1 neuronal cell lines by inducing apoptosis (Ren et al. 2019) and are valuable options for rotation in an insecticide resistance management plan

Stable fly trapping is a management option with a long history of use. Early stable fly traps relied on visual attraction to fiberglass (Williams 1973), Alsynite (Broce 1988), or Coroplast (Beresford and Sutcliffe 2006) panels, cards or cylinders coated with an insect adhesive (Zhu et al., 2016). Agee and Patterson (1983) tested 30 different surfaces and Alsynite was the most attractive to stable flies. This result was explained by the emission spectra Alsynite in the 365 nm to 640nm range that is attractive to stable flies (Agee and Patterson 1983). Hogsette and Kline (2017) tested Knightstick (BugJammer, Inc., Stockton, NJ) traps and found that the Knightstick trap caught 3-5 times more flies than Alsynite Olson traps.

While visual cues are important in stable fly attraction, stable flies also use host associated odors to locate cattle (Cilek and Greene 1994, Kristensen and Sommer 2000). When phenol, p-cresol, and m-cresol lures were added to Coroplast traps, more stable flies were captured on traps with m-cresol than the other odorants (Zhu et al. 2016). The development of an impregnated adhesive tape material found that m-cresol treated material was able to capture and reduce stable fly numbers enough to reduce cattle stress (Zhu et al. 2021).

Push-Pull strategies integrate both attraction and repellency in an insect management tactic (Pyke et al. 1987, Cook et al. 2007) to modify insect behaviors and cause a change in the distribution and abundance of beneficial and/or pest insects (Cook et al. 2006). The first animal system push-pull project used novel repellents and dispensing mechanisms to successfully reduce tsetse fly transmission of trypanosomes which cause trypanosomiasis in cattle (Saini et al. 2017). Extension of this strategy to other blood feeding flies was identified as a research priority by the national USDA research committee, S1076 Fly Management in Animal Agriculture Systems and Impacts on Animal Health and Food Safety (USDA NIMSS 2021).

Objectives

The goal of this study was to determine the efficacy of a Push-Pull Strategy on stable fly infestation levels on pasture cattle. Our objectives were to repel stable flies from cattle using a repellent and capture them on traps with attractive visual and odor cues. The expected outcome is a novel stable fly management program using a design with two management tactics instead of one and that will lessen reliance on conventional insecticides for stable fly management.

Materials and Methods

For the 2018 field season, testing was done using 24 yearling cattle (steers and heifers) for a 6-week period from July 3rd to August 10th. Cattle were randomly sorted into four treatment herds of six animals each that remained together through the testing period. Each herd was randomly assigned a treatment. Treatments rotated with the herds and each treatment had 2 herds. Treatment herd is defined as the group of animals that were in each treatment, permethrin, and coconut FFA (repellent) and were replicated weekly. For both treatments, a volume of 500 ml was applied to each animal. During the study, treatment herds were randomly rotated through pastures each week following treatment reapplication.

Pastures included one irrigated and three upland pastures. With the irrigated pasture being 0.0436 km^2 and the no-irrigated being 0.068 km^2 in size.

The 2018 treatments were:

1. Positive control, permethrin (10% EC, applied at 0.05%)

2. Coconut oil repellent (FFARC) (Acme-Hardesty; Blue Bell, PA, applied at 24.7g)

The coconut oil repellent consisted of 14.20 % solid contents and was composed of 45.0 % coconut fatty acid, 55.0 % starch and 85.80 % water.

2018 Stable Fly assessments. The total number of stable flies on all four legs and belly of each cow were counted and expressed as the total number of stable flies per animal. Fly counts were made between 1300 and 1600. Counts were done on days 1 through 4 post-treatment.

For the 2019 and 2020 field seasons testing was done using 30 yearling cattle (steers and heifers) each year. The cattle were randomly sorted into six treatment herds of five animals that remained together through the testing period. Herds were randomly assigned to a treatment (treatment herds). Treatment herds were maintained throughout the duration of the study to avoid possible confounding effects from residues if treatments were rotated among herds. Treatment herds were randomly rotated weekly through a pasture complex of three upland and four irrigated pastures following each study week's retreatment.

The pastures were located on the West Central Research and Extension Center, North Platte, Nebraska. The irrigated pastures were 0.0109 km^2 each and could support 5 cattle per week. The non-irrigated pastures were 0.068 km^2 and could support up to 10 cattle per week. The irrigated pastures each had a water tank while the non-irrigated pastures shared a central watering tank but fencing prevented the herds from co-mingling.

Cattle were treated weekly through each year's study period. In 2019 the study period lasted 5 weeks and the 2020 study period lasted 6 weeks. Three treatments were used.

1. Push-Pull. A two-component treatment

a) Push component - Coco FFA, a repellent, fatty acid based biopesticide (USDA-ARS National Center for Agricultural Utilization Research, Bio-Oils Research Unit, Peoria, IL) consisting of a coconut fatty acid 6.855%, waxy starches, pectin 8.47%, and distilled water 84.4% mixture.

b) b. Pull component - stable fly traps integrated in pastures with PP treatment herds. Trap design and placement is described below.

2. Negative Check. Water

3. Positive Check. Permethrin (ProZap Insectin® X 10% permethrin Lot 10312578)

See Figure 1 for a diagram of treatment components and objectives. The three treatments were replicated twice each study week.

Coco FFA and Permethrin Application Procedures

Applications of permethrin and Coco FFA was made on day 1 of each study week. At treatment, herds were moved into separate holding pens and rotated through a cattle chute in this order; control herds, push-pull herds, and then permethrin treatment herds (Figure 2).

Cattle in permethrin treatment herds were treated with 500 ml of a 0.05% (AI) solution. However, because of the low flowability of Coco FFA, the mixture was diluted 1 to 1 with water warmed to 26.6°C and a volume of 1000 ml was applied to cattle to achieve the 0.05 % AI.

Treatments were applied evenly onto the cattle in a secure cattle chute. Following use, the sprayers were cleaned with a 1% concentration of ammonia, ca. 38 ml per 3.78 l of water and then rinsed with water.

The stable fly traps consisted of a white, 30cm long 10.8 cm diameter pipe, a Knightstick® sticky wrap (http://www.bugjammer.com) secured to the pipe with binder clips. An olfactory lure (Chemglass, CG-3022-95, 8mm) bait dosed with 1 mg of m-cresol (Sigma-Aldrich, C85727-100G, Batch #: 08608DJ, 99% m-cresol) was suspended in a screened holder suspended in the middle of the pipe. The pipe was anchored to the ground using steel shepherd hooks (Figure 3). To prevent cattle from damaging the traps, they were enclosed in steel wire mesh (0.76 m tall, 1.98 m long, and 0.58 m diameter) (Figure 4). The basic trap design was used because of its demonstrated effectiveness (Hogsette and Kline 2017) and stability in windy conditions.

Within a study week, daily stable fly trap counts were made by removing them from sticky wraps with forceps and transporting them to the lab for later counting. Sticky wraps were replaced at the start of each study week.

The total number of stable flies on all four legs and belly of each cow were counted and expressed as the total number of stable flies per animal or fly load.

Statistical Analysis

For both the 2018 and the 2019/2020 studies, group was considered the experimental unit, so the average number of flies was calculated for each day by group and was the response variable analyzed. The data analysis for this paper was generated using the GLIMMIX procedure in the SAS/STAT software, Version 9.4 of the SAS System for Windows. For all analyses, a Kenward-Rogers degree of freedom adjustment was used. An alpha level of 0.05 was used for determining significance.

Statistical Analysis for 2018

The study design was a repeated measures, cross-over, where each of four groups received both treatments over the 6 periods and were measured for five days during each treatment period. The initial model for these analyses included the following terms: fixed effects of pasture, treatment (Permethrin, Coconut Oil), period (1-6), day (1-5), treatment by period and treatment by day interactions and random effects of group, group by period and residual. Pasture, treatment, period and the treatment by period interaction effects were tested over the group by period random effect, while the other effects were tested over the residual. An AR(1) covariance structure was used for the repeated measurements over days. The final model dropped out the non-significant pasture and interaction terms.

Statistical Analysis for 2019/2020

The study design was a repeated measures, random complete block, where each of six groups was randomly assigned to one of three treatments (C, I, PP). There were two sets of repeated measures. The first was week (1-5) and the second was day (1-4) nested within week. The initial statistical model included the fixed effects of year, treatment, week, day, and all two-, three, and four-way interaction terms and the random year by group nested within treatment, year by period by group(treatment) and year by week by group nested within treatment. The error term for year, treatment and the year by treatment interaction was the random year by group nested within treatment random effect. The error term for week was the random year by week by group nested within treatment using an AR(1) covariance structure to account for repeated weeks. The error term for day and all interaction effects including day was the year by day by period by week by group nested within treatment term using an AR(1) covariance structure to account for repeated days nested within week. Year was involved in several significant interactions. Year 2020 was a very dry year, which contributed to very few flies. Year 2019 was a high moisture year, which contributed too many flies. Because of these differences, it was decided to analyze the years separately. The final model for each year included treatment, week, day, and all two- and three-way interaction terms and the random group nested within treatment and period by group nested within treatment effects. Treatment was tested over the random group nested within treatment random effect and period and treatment by period were tested over the random period by group nested within treatment random effect. The rest of the effects were tested over the residual error.

Results

In 2018 no difference was observed in fly load for both permethrin and coco FFA (F=0.02, df= 1, p=0.8974, Fig. 5). The fly loads (±SEM) on the coconut FFA were 18.09 (±2.43) and 17.21 (±1.29) for the permethrin fly loads.

For 2019 and 2020 both study years differed by temperature and rainfall with 2019 being wetter and cooler than 2020 (Table 3). This resulted in 2019 and 2020 to be different (F=800.23, df=1, p>0.0001).

In 2019 testing was done over a five-week period. In weeks 1 – 4, fly loads on control herds were higher post treatment than on push-pull and permethrin herds and fly loads on the push-pull and permethrin treatment herds were similar. In week 5, control herd fly load declined to values near those of the push-pull and permethrin treatment herds and results were mixed. Refer to Table 4 for statistics and Figure 6A for plotted data.

In 2020, control herd fly loads were higher post treatment than those on permethrin and push-pull treatment herds except for week 1 when control and push-pull fly loads did not differ. In week 1, control and push-pull fly loads were higher than on the permethrin herd (Table 4 and Figure 6B).

In 2019, there was a significant treatment by day interaction ($F=3.83$; $df=45$, $p=0.0036$) (Fig. 7A) and we compared differences by day. Fly loads on control treatment herds were significantly higher all days (1 to 4) compared to those of the push-pull and permethrin herds. However, fly loads on the push-pull and permethrin treatment herds were very similar and did not differ (Table 5 and Fig. 7A).

The 2020, as in 2019, treatment by day interaction was significant ($F=7.03$; $df=42$, $p<0.0001$) and we again analyzed treatment effects by day. Control fly load was higher during days 1 and 2 when compared to push-pull but on day 3 there was similar fly loads. On day 3, permethrin had a lower fly load when compared to control (Table 5 and Fig. 7)

Significant interaction effects were not observed in either 2019 or 2020 for treatments by all days (week and day in weeks) (2019: $F=1.38$; $df=24$; $p=0.1720$; 2020: $F= 1.39$; $df= 22$, $p=0.1742$ and Fig. 8). This indicates that fly loads, regardless of treatments, varied together (increased up or down together) through each fly season (year).

The pull strategy of the push-pull treatment herds captured large numbers of stable flies in both study years. In 2019, traps caught a total of 13,638 stable flies and in 2020, when stable fly populations were lower, a total of 5,359 flies were captured.

Discussion

Our intent was to conduct a three-year study using a fatty acid repellent in a push-pull design to manage stable flies in pasture settings in the Great Plains of North America. However, because of poor pasture condition in 2018 we were limited to comparing repellent and permethrin treatments only. We found that cattle in both treatments had similar fly loads throughout the study period (Figure 5) suggesting that the fatty acid repellent is as effective as permethrin in managing stable flies on pasture cattle.

In 2019 and 2020 conditions improved and 5- and 6-week pasture studies were done with a full treatment regime. Fly loads on control cattle were on average 5 times higher in 2019 than in 2020 and therefore the two years were analyzed separately. However, despite the environmental differences between the years, the 2019 and 2020 results were consistent, and the push-pull and permethrin treatments significantly reduced fly loads compared to the control values. We also found the push-pull and permethrin treatments did not differ significantly from each other both years.

Campbell et al. (2001) investigated the relationship between fly load and average daily weight gain in pastured yearling steers in Nebraska and using Berry et al. (1983) conversion factor of 2.8 stable flies per front leg. This number of stable flies per front leg can reduce the daily weight gain by 0.2 kg per day and when the cattle were moved to a feedlot, compensatory gain did not occur (Campbell et al. 2001). Using this conversion factor of 2.8 stable flies per leg, we found that in 2019 the control herds averaged 12.9

per leg while the permethrin and push-pull averaged 12.4 these are 4 times more than the Berry et al. (1983) conversion factor. Although we counted stable flies on all legs compared to the front legs as in Berry et al. 1983, our control herds in 2019 likely had reductions in average daily weight gain. When we look at the 2020 herds the fly numbers on control, permethrin, and push-pull cattle were lower at 4.4 and 3.0 per leg, resulting in a difference of 1.4 which may have been too small to affect the average daily weight gain (Campbell et al. 2001)

A perceived shortcoming of repellents is their period of residual efficacy. Several repellent compounds have been found that repel stable flies; however, their efficacy period is short, <4 d (Rehman et al. 2014) so frequent reapplications are required to retain efficacy. However, we found that the median chain coconut oil fatty acid repellent tested lasted relatively long with longevity equal to that of permethrin. Fatty acid repellents have benefits requested by producers, such as an expected low cost and environmental safety (listed as “generally regarded as safe” (GRAS) by the US Environmental Protection Agency, Zhu et al. 2018).

As the pull component of the push-pull treatment we used a modified trap with a PVC tube wrapped with a sticky foam sheet and added 1-mg m-cresol as an attractant lure. We chose this trap design because a similar trap was shown to be more effective than Olson fly traps in capturing stable flies (Hogsette and Kline 2017) and because the round design is stable in windy environments. While we found that the traps captured stable flies, however, the impact of the traps on reducing on-animal fly load is unknown.

Further investigation of the contribution of traps to reducing fly load and deployment tactics (numbers and placement) are needed.

For a trapping program to be successful you need to trap ≈ 40% of the adult population (Weidhaas and Haile 1978). While it is difficult to estimate absolute stable fly population levels in pastures, the 40% capture rate offers a general guideline needed to analysis trap efficacy.

Our study demonstrated that a push-pull strategy is as effective as a conventional insecticide application in managing stable flies on pasture cattle. However, practical considerations remain to improve acceptability to producers. Weekly gathering of cattle for retreatment with repellents (or insecticides) can stress cattle and may lessen the benefit of stable fly population reduction (Campbell et al. 2001) and be too costly for some pasture systems. Mist blower or automated spray systems that cattle pass through as part of their routine behavior would be, after cattle acclimation, less stressful and may be acceptable alternatives for some production systems. Continued trap improvement to increase attraction and lengthen their effective period are needed to improve the impact of the pull component of the design.

In conclusion, this paper is the first use of a push-pull integrated pest management strategy to manage stable flies and only the second application to a Muscid fly (Olaide et al. 2019). As such, it represents a low-impact alternative to conventional insecticides for management of stable flies on pastured cattle.

Table 1. List of insecticides for stable fly management used in the United States.

Insecticides	**Mode of Action and IRAC Classification**	**Application Methods**	**Reported Insecticide Resistance**
Pyrethins and Permethrin	Sodium Channel Modulators, 3A	Pour on, Spray, Aerosol, and Dust	(Cilek and Greene 1994, Pitzer et al. 2010)
DDVP (2,2-dichlorovinyl dimethyl phosphate)	Acetylcholinesterase (ACHE) inhibitors, 1B	Spray	(Cilek and Greene 1994)
Diflubenzuron	Inhibitors of chitin biosynthesis affecting CHS1, 15	Feed supplement larvicide	None
Spinosad	Nicotinic Acetylcholine receptor (NACHR) allosteric modulators-site 1, 5	Spray	None
Tertacholvinphos	Acetylcholinesterase (ACHE) inhibitors, 1B	Larvicide	None

Table 2. Relative amounts of coconut fatty acids in coco FFA.

Fatty Acid	Relative amounts (%)
Caprylic acid ($C_{8:0}$)	6.85$\pm$0.03
Capric acid ($C_{10:0}$)	7.33$\pm$0.02
Lauric acid ($C_{12:0}$)	52.68$\pm$0.11
Myristic acid ($C_{14:0}$)	17.14$\pm$0.04
Palmitic acid ($C_{16:0}$)	8.44$\pm$0.03
Stearic acid ($C_{18:0}$)	1.29$\pm$0.01
Oleic acid ($C_{18:1}$)	6.02$\pm$0.10
Linoleic acid ($C_{18:2}$)	0.34$\pm$0.01

Table 3. The monthly average air temperatures and total rainfall for the 2019 and 2020 study season. Weather data from National Oceanic and Atmospheric Administration (https://www.noaa.gov/)

Year	Month	Air temperature (°C)	Rainfall (cm)
2019	May	12.11	15.72
	June	20.02	9.12
	July	24.38	14.71
	August	22.55	13.67
		Mean = 19.76	Sum = 53.22
2020	May	14	9.55
	June	23.8	4.29
	July	24.77	10.94
	August	24.11	1.11
		Mean = 21.67	Sum = 25.89

Table 4. 2019 and 2020 Study by week ANOVA table from the Simple Effect Comparisons of Treatment*Week Least Squares Means by Period Adjustment for Multiple Comparisons: Tukey.

Comparison	Week	Mean (+SEM)	t-test	p value
		2019		
Control vs Push-Pull	1	13.79(3.27)	4.22	0.0031
	2	13.94(3.27)	4.27	0.0029
	3	16.57(3.27)	5.08	0.0007
	4	12.87(3.27)	3.94	0.0051
	5	4.54(3.27)	1.39	0.3763
Control vs Permethrin	1	17.70(3.27)	5.42	0.0004
	2	12.52(3.27)	3.83	0.0024
	3	16.07(3.27)	4.92	0.0009
	4	10.48(3.27)	3.21	0.0190
	5	7.44(3.27)	2.28	0.0979
Push-Pull vs Permethrin	1	-3.91(3.27)	-1.20	0.476
	2	1.42(3.27)	0.44	0.9012
	3	0.50(3.27)	5.08	0.987
	4	2.39(3.27)	0.73	0.749
	5	-2.90(3.27)	-0.89	0.657
		2020		
Control vs Push-Pull	1	1.28(0.924)	1.38	0.381
	2	3.88(0.924)	4.19	0.003
	3	3.64(0.924)	3.93	0.005
	4	3.66(0.924)	3.96	0.004
Control and Permethrin	1	3.89(0.924)	4.20	0.003
	2	5.58(0.924)	6.03	0.0001
	3	4.75(0.924)	5.14	0.0006
	4	3.99(0.924)	4.313	0.002
Push-Pull and Permethrin	1	-2.61(0.924)	-2.83	0.037
	2	-1.70(0.924)	-1.83	0.198
	3	-1.11(0.924)	-1.20	0.473
	4	-0.325(0.924)	-0.351	0.934

*Degrees of freedom = 12 for all t values.

Table 5. 2019 and 2020 study Simple Effect Comparisons of Treatment*day Least Squares Means by Day Adjustment for Multiple Comparisons: Tukey

Comparison	**Day**	**Mean (+SEM)**	**t-test**	**p value**
		2019		
Control vs Push-Pull	1	19.025(2.88)	6.61	<0.0001
	2	12.58(2.88)	4.37	0.0002
	3	9.13(2.88)	3.17	0.0076
	4	8.64(2.88)	3.01	<0.0119
Control vs Permethrin	1	18.73(2.88)	6.51	<0.0001
	2	13.57(2.88)	4.71	<0.0001
	3	10.19(2.88)	3.54	0.0027
	4	8.88(2.88)	3.08	<0.0096
Push-Pull vs Permethrin	1	0.293(2.88)	0.10	0.9943
	2	-0.99(2.88)	-0.34	0.7325
	3	-1.06(2.88)	-0.37	0.9281
	4	-0.24(2.88)	-0.08	0.9962
		2020		
Control vs Push-Pull	1	6.74(0.83)	8.15	<0.0001
	2	2.39(0.83)	2.89	0.0164
	3	1.32(0.83)	1.60	0.2586
Control vs Permethrin	1	8.52(0.83)	10.30	<0.0001
	2	4.1(0.83)	4.96	<0.0001
	3	2.12(0.83)	2.56	0.0367
Push-Pull vs Permethrin	1	-1.78(0.83)	-2.15	0.9170
	2	-1.71(0.83)	-2.07	0.1090
	3	-0.80(0.83)	-0.97	0.6014

*Degrees of freedom = 45 for 2019 and 42 for 2020.

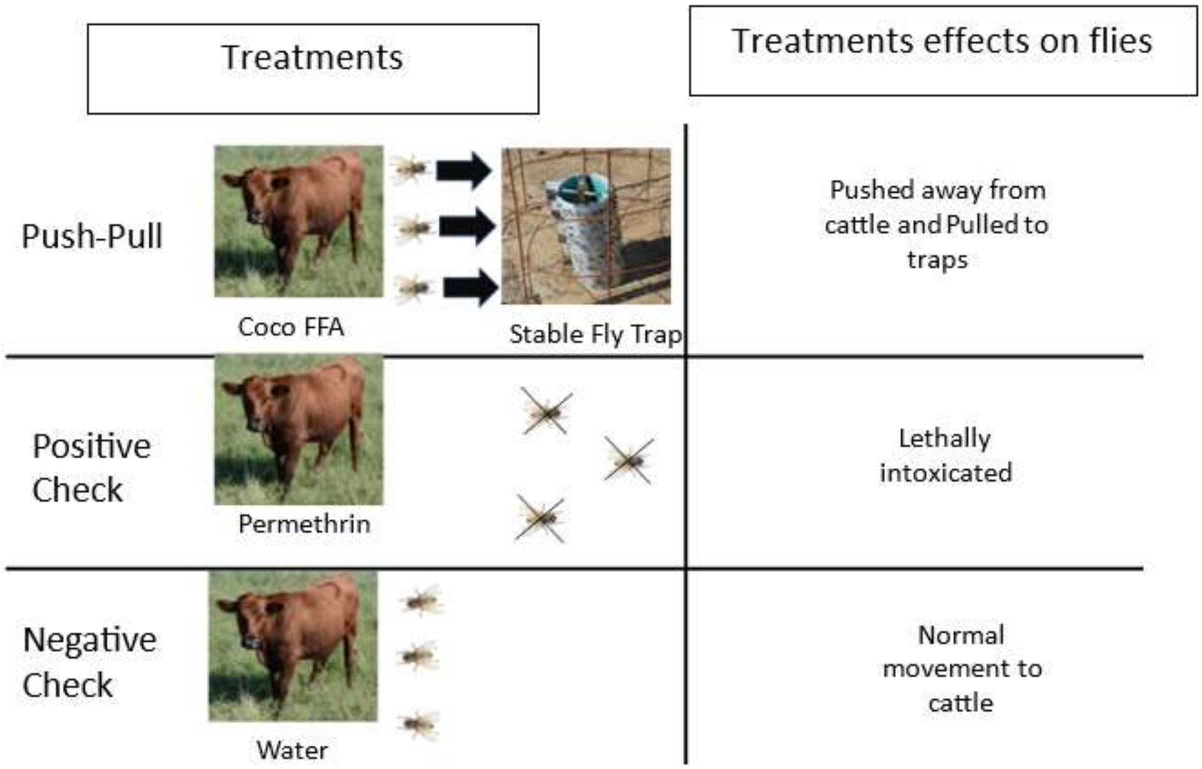

Figure 1. Diagram of anticipated interaction of stable flies to treatment applications. Push-Pull was a two-component treatment.

Figure 2. Weekly applications of coco FFA or permethrin were done in a cattle alleyway. After treatment, each herd was released into their randomly assigned pasture.

Figure 3. Stable fly trap consisting of a white pipe surrounded with a clear sticky wrap and with an olfactory lure suspended on the inside. Steel anchors driven into the ground held the trap in place. Wraps and lures were replaced weekly.

Figure 4. A stable fly trap in the field inside protective fencing to minimize cattle damage.

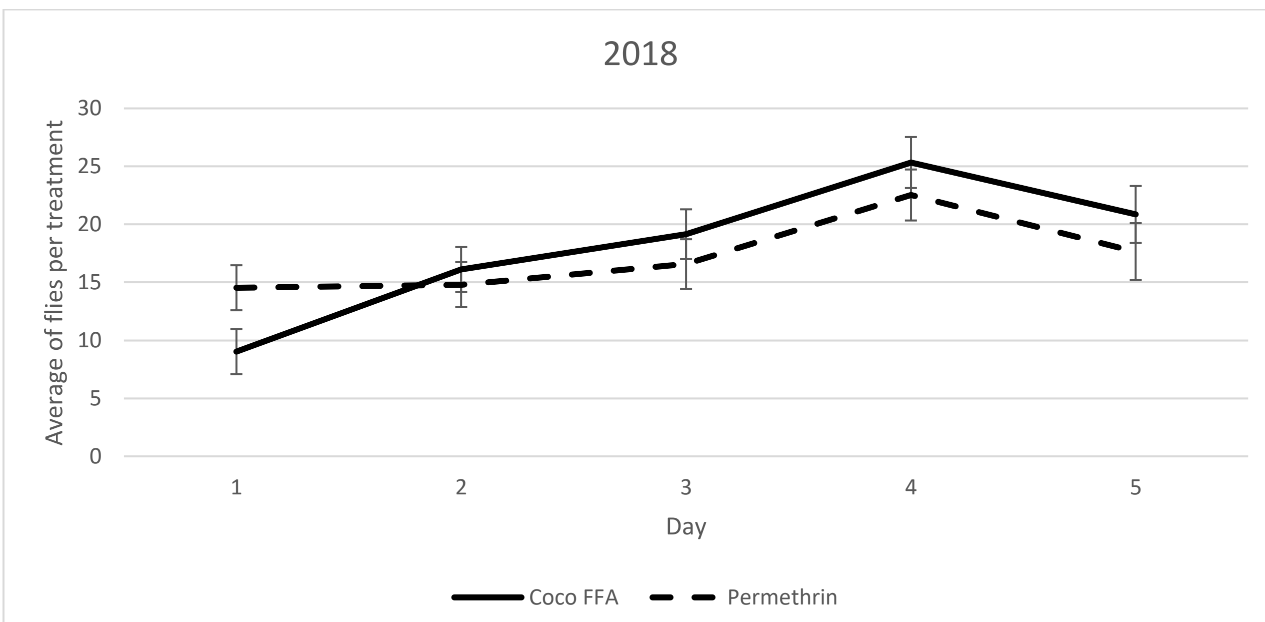

Figure 5. Mean (+SE) number of stable flies per treatment by day.

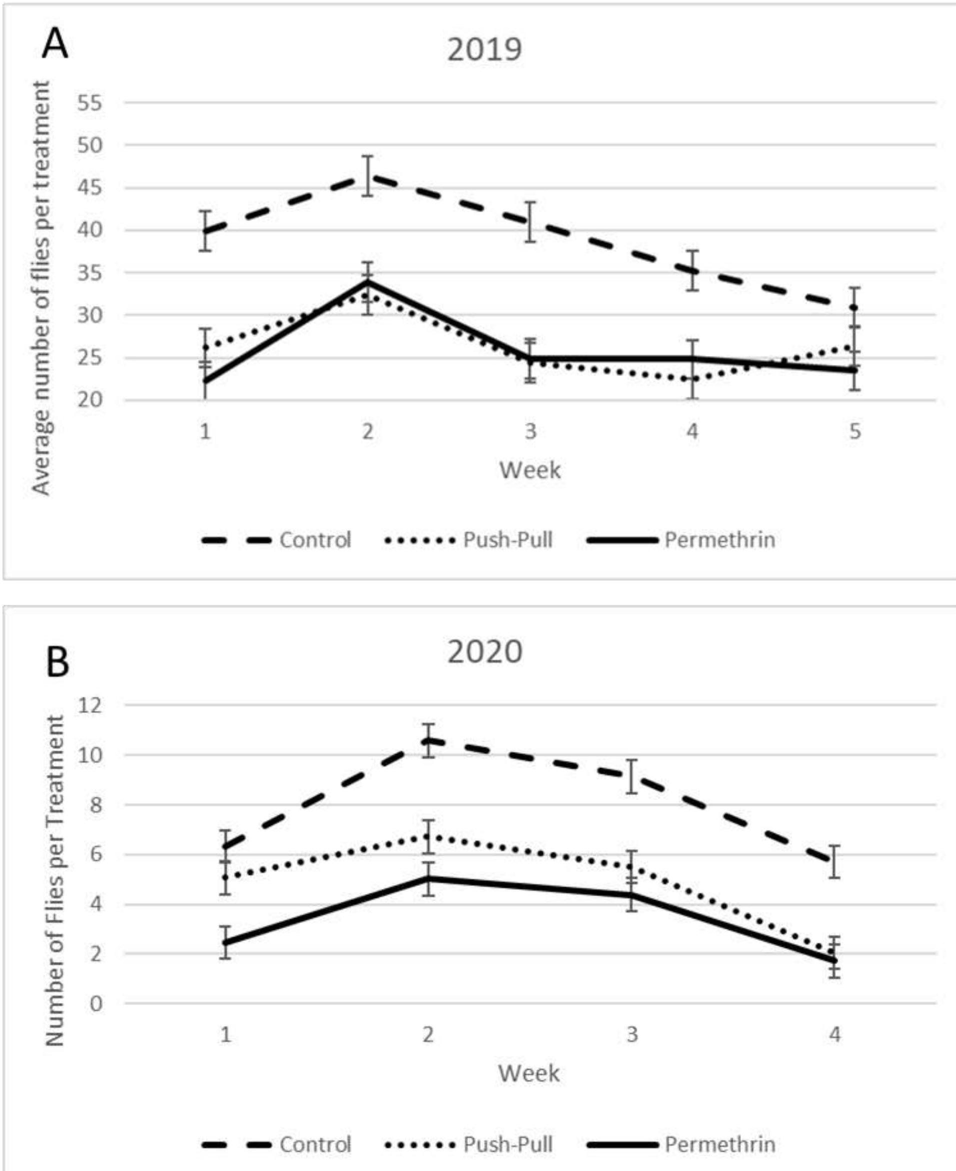

Figure 6. Mean (±SE) fly load per treatment by week.

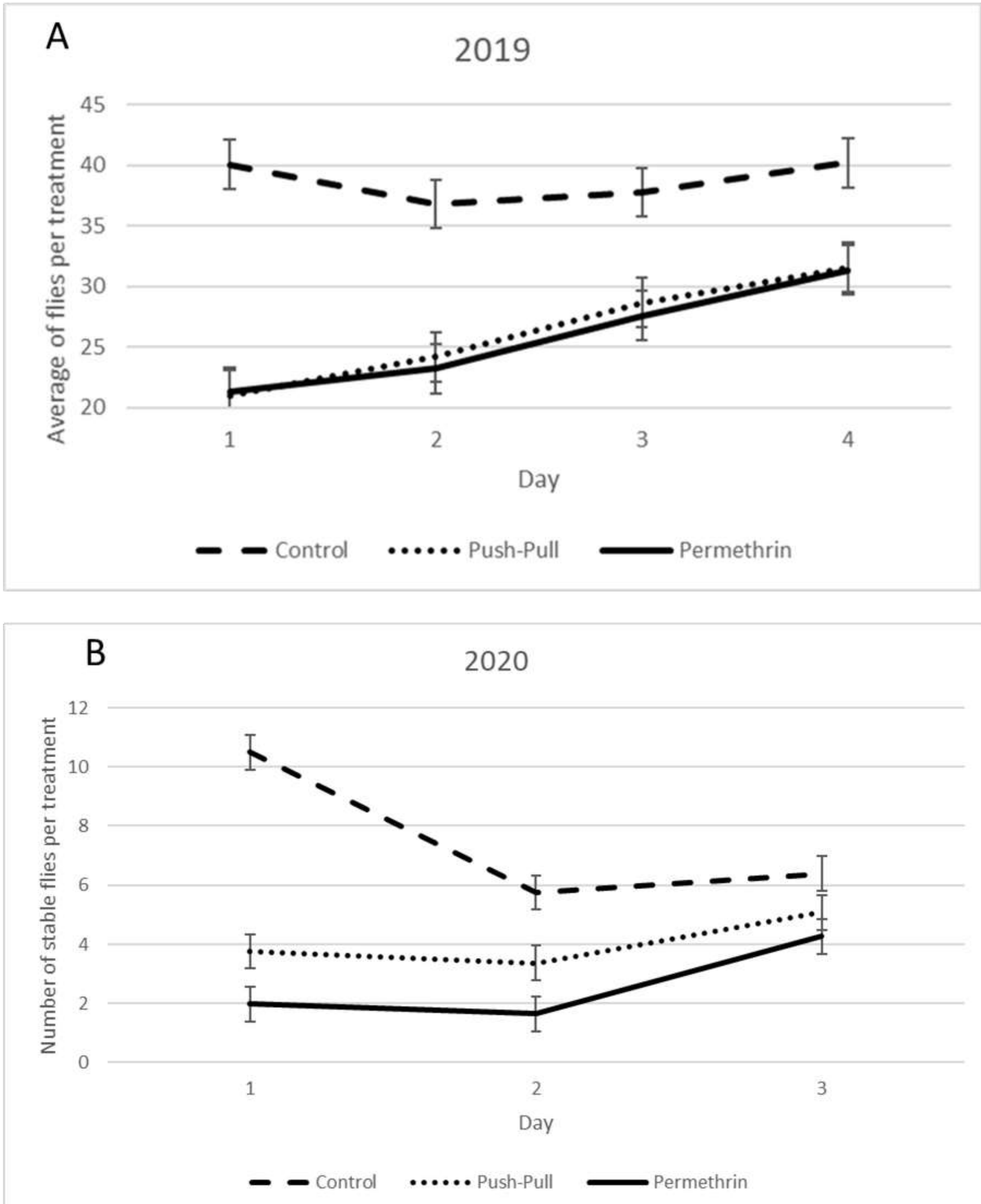

Figure 7. Mean (+SE) number of stable flies per treatment by day.

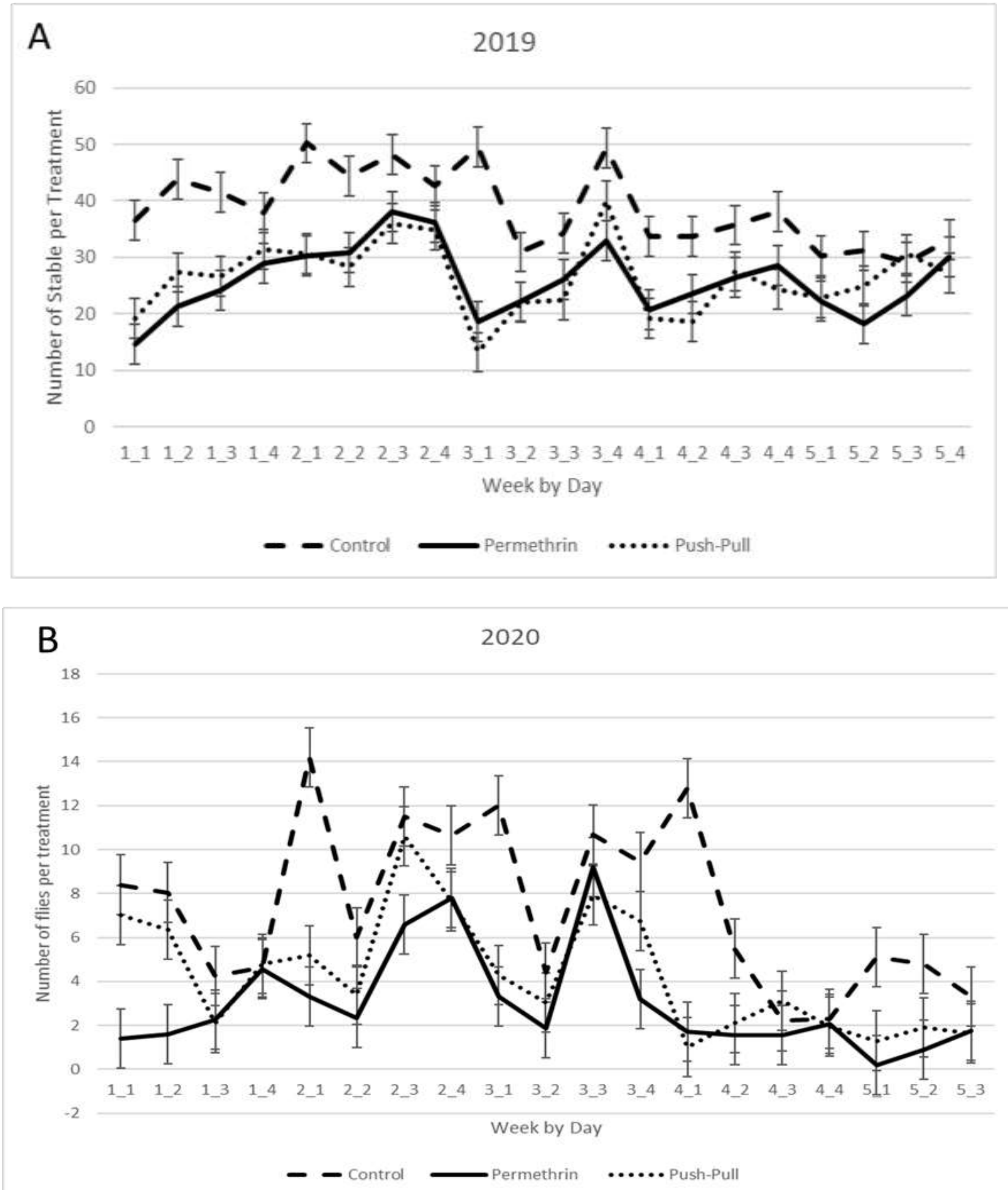

Figure 8. Mean (±SE) fly loads on all sample week and days.

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Chapter 3: Effects of Altering Visual and Olfactory Traits of a Stable Fly Trap on Capture Rates

Abstract

Stable flies (Diptera: Muscidae) are a major pest of pastured cattle. Their feeding activity causes cattle to express stress related behaviors. We tested multiple types of visual and olfactory trap designs to improve trap efficacy. These traits included changes in appearance, lure size, and lure dose of m-cresol. In 2019, we found that traps without lures captured more flies, trap appearance didn't influence fly capture, and that lure size influences stable fly capture. In 2020 and 2021 the dosage study we found that olfactory lures often improved trap efficacy. We found that m-cresol wasn't biased towards female flies and that a white m-cresol baited trap is recommended. Further research in the dosages, trap design and the number of traps still needs to be done.

Introduction

Stable flies (Diptera: Muscidae) *Stomoxys calcitrans* (L.) are an important pest of pastured cattle (Hall et al. 1982). Their painful bites are unpleasant and stressful for cattle and the cattle display multiple defensive behaviors that include tail switching, leg stomping, congregating to windy and bodies of water, and kicking (Mullens et al. 2006). These behaviors reduce weight gains and productivity. The economic losses have been valued at $2.2 billion per year to the cattle industry in the United States (Taylor et al 2012).

Visual attract and kill stable fly traps that use insect adhesives as the killing agent have a long history of use in pastures, dairies, and feedlots. Some of the well-known examples include the Williams cross-configuration fiberglass trap (Williams 1973), cylindrical fiberglass trap (Broce 1988), and the Coroplast trap (Beresford and Sutcliffe 2006). The Knightstick® trap is a more recent addition (Hogsette and Kline 2017). Adhesives were either applied to the trap surface (Williams 1973, Beresford and Sutcliffe 2006) or as pre-applied sticky plastic sleeves secured to the trap (Hogsette and Ruff 1990, Hogsette and Ose 2017). In comparison with Olson traps, Knightstick® traps were 3 times more effective (Hogsette and Kline 2017).

Attractants

To improve visual stable fly trap capture rates, attractive lures emitting odors associated with cattle hosts or oviposition sites have been added (Cilek and Greene 1994, Kristensen and Sommer 2000). When host associated attractants phenol, p-cresol, and m-cresol were tested in olfactometer assays and in field trials with white panel traps, more stable flies were attracted to m-cresol and m-cresol blends than p-cresol, phenol, and a no lure control trap (Zhu et al. 2016). Tangtrakulwanich et al. 2015 noted that the attraction of stable flies to phenols, and cresols could provide an enhancement in mass trapping for a push-pull management strategy.

Objectives

The expected outcomes of this study are to improve practicality and efficiency of stable fly traps for use in pastures and other locations.

Materials and Methods

A series of field trials were done at the University of Nebraska, West Central Research and Extension Center, North Platte, Nebraska, USA or at nearby cooperator cattle operations. Locations were either in or near pastures with cattle and were chosen based on a history of stable fly populations. See Table 1 for specific locations for the studies.

The standard trap design consisted of PVC pipe (30 cm long and 10.8 cm in diameter) painted glossy white (Rust-Oleum®, RPM International, Vernon Hills, IL, USA) with a sticky wrap and an attractant lure. Each trap was enclosed inside a wire fence to prevent damage from cattle (Figure 1). Sticky wraps (Bugjammer, Stockton, NJ, USA; https://www.bugjammer.com) were wrapped around the pipe. Lures were 8 mm rubber septa (ChemGlass, Vineland, NJ, USA; CG-3022-95, chemglass.com) treated with 1 mg of m-cresol (99% Sigma Alpha, batch number 08608DJ) dissolved in 50 ml of absolute ethanol (Decon labs, 200 proof, CAS Number 64-17-5), air dried, and stored in a standard refrigerator for less than 2 weeks. Lures were suspended in the interior of the pipe for protection and as a slow-release device.

2019

In 2019, we ran a series of trials using pairwise comparisons in time analysis of variance with 3 weekly replicates. Traps were placed 1 m apart in a line next to a centralized water tank. This was later identified as a mistake and the distance was increased to 10 m for the 2020-2021 field season. Sticky wraps and lures were replaced weekly. See Table 1 for 2019 study details.

2020

The 2020 study compared 0.0, 0.5, 1.0, and 3.0 mg doses of m-cresol odorant lures. Sticky wraps were replaced daily to provide trap uniformity from day to day. The study ran for 3 weeks (July 8 to August 2) and was rerandomized each week. Testing was done in a complex of four abutting pastures (NW, NE, SW, SE; see Figure 2) with cattle during the testing periods and provide an even source of stable flies.

The experimental designed was a 4x4 (dose X distance) Latin Square design with columns positioned in a radial pattern (instead of the standard square) and with a 10 m spacing (Figure 2) extending out from a central loafing area with water tanks where cattle gathered several times daily. Data was analyzed by day within weeks, weeks, and all weeks as well as rows (distance from the water tanks) and columns (corresponding to pastures).

We expected cattle to be present in all four quadrants (pastures) of the 2020 radial Latin Square design. However, cattle were only located in one of the pastures each study week. To even out the fly pressure in each quadrant, data from pastures with cattle were omitted and the analysis adjusted using a normal distribution in a randomized complete block design.

2021

The 2021 study compared 0, 0.5, 1, and 3 mg doses of m-cresol odorant lure. Sticky wraps were replaced weekly, and flies were removed daily to provide daily stable fly capture rate. The study ran for 4 weeks (July 28 to August 22) and was rerandomized each week. Testing was done at the same location as 2020 but only utilized the SE pasture. The herd of cattle stayed in the SE pasture during the study period.

The experimental design was Random Complete and the traps were spaced 10 m apart (Figure 3).

Statistical Analysis

The data analyses for this paper were generated using the GLIMMIX procedure in the SAS/STAT software, Version 9.4 of the SAS System for Windows. An alpha level of 0.05 was used for determining significance. A Negative Binomial distribution was assumed for count data, because of large over-dispersion parameters under a Poisson distribution for some of the analyses. A Binomial distribution was assumed for the analyses investigating the differences in the proportions of male and female flies trapped.

Statistical Analysis for 2019

The three separate studies conducted in 2019 were analyzed separately. Each study was a split plot analysis in time (two measures two weeks apart), where the whole plot was location (Funston, West Place and South) by replication (3) nested within treatment. The model included location, treatment, week and treatment by week interaction effects. Location and treatment effects were tested over the whole plot error, while the week and treatment by week effects were tested over the residual error. Non-significant treatment by week effects were dropped and the analyses rerun. The odds ratio of the odds of a fly being female over the odds of a fly being male was analyzed using the same model under a Binomial distribution.

Statistical Analysis for 2020

Cattle were only located in one of the pastures each study week (NE, SW, SE, and NW). To even out the fly pressure in each quadrant, data from pastures with cattle were omitted from the analyses. For the first rep, the cattle were located in the SW pasture, while for reps 2 and 3, the cattle were located in the SE pasture. Therefore, the first rep was analyzed separately from reps 2 and 3. Because of weather, no flies were counted on day two of rep 3. Therefore, the mean fly count over days measured was the response analyzed. The model included pasture, trap_location and dose. The odds ratio of the odds of a fly being female over the odds of a fly being male was analyzed for both analyses using the same model under a Binomial distribution.

Statistical Analysis for 2021

The sum of the fly count over days was the response variable. The model included trap_location, dose and week. The odds ratio of the odds of a fly being female over the odds of a fly being male was analyzed using the same model under a Binomial distribution.

Results

In 2019 Study 1, comparing traps with and without lures, there was no difference in stable fly capture rates (F=0.75, df=1, p=0.4003). However, there were significant differences among trap locations (pastures) (F=42.41, df=2, p<0.0001) and study weeks (F=7.41, df=1, p=0.0174). There were also no differences among the dosage levels in the proportion of female and male flies captured. In 2019 Study 2, comparing white traps and white with vertical black stripes traps, there was no difference in stable fly capture rates (F=1.62, df= 1, p=0.2237). However, there was a significant difference in trap location (F=26.00, df=2, p<0.0001). While study week was not significant (F=0.34, df=1, p=0.5686). There were also no differences among the dosage levels in the proportion of female and male flies captured.

In 2019 Study 3, comparing traps with 8 mm standard lure and 5 mm lure with 1 mg of m-cresol applied to the lure, there was a difference in stable fly capture rates (F=7.04, df=1, p=0.018), with the 8 mm standard lure capturing more flies than the 5 mm lure (12.7 vs 6.3 flies). Both trap location (F=14.87, df=2, p=0.0003) and study week (F=6.05, df=1, p=0.024) were significant. There were also no differences among the dosage levels in the proportion of female and male flies captured.

In 2020 different dosages of m-cresol were tested. These dosages were 0 mg, 0.5mg, 1 mg, and 3 mg. For the rep 1 analysis, there were no differences among the dose levels for the sum of the fly counts over days. For the rep 2 and 3 analysis, there were no differences among the dose levels for the mean of the fly counts over days. There were also no differences among the dosage levels in the proportion of female and male flies captured.

For 2021 the same concentrations of m-cresol were used from the 2020 field season. Since a single location was used the effects of location was eliminated. Dosage levels (F=1.15, df=3, p=0.3378) were not significantly different. However, trap (F=9.30, df=3, p<0.0001) and week (F=9.79, df= 3, p<0.0001) had significant effects on the number of flies trapped. There were also no differences among the dosage levels in the proportion of female and male flies captured.

Discussion

Our intent was to improve trap efficacy by testing of traps that varied in appearance, lure size, and lure dose of m-cresol. In 2019, we found that traps that had a lure, white, and had an 8 mm lure performed the best. The issue of the trap spacing of 1 m may have interfered and caused issues with getting these results. The color of traps didn't matter in our research like some of the color patterns used in tsetse fly traps (Mihok 2002, Mihok et al. 2006) The Knightstick sticky wraps themselves may be major source of visual attraction for stable flies (Hogsette and Kline 2017). Other studies investigating color found that white and clear materials captured more stable flies (Cook 2020, Beresford and Sutcliffe 2006) than black and blue. We investigated lure size (lure surface area) for effect on stable fly capture rates but did not see any difference.

For 2020 we focused on using the standard trap design to compare lure attractant concentration. Because cattle were rotated among pastures during the study, arms of the radial Latin square design closest to the pastured cattle tended to have higher fly capture rates, regardless of treatment, leading to skewed results. Therefore, we adjusted the data analysis as described in the methods. For week 1 the cattle were in the SW pasture while in week 2 and 3 the cattle were in the SE pasture. The traps in those pastures and week many more flies than traps in the other pastures and skewed the results. After removing these pastures from our results, we were able to get a clearer representation of lure dosage effect on stable fly capture rates.

Using the 2020 modified experimental design, we found that in week 1 capture rates were highest at the 1 mg dose, in the NW pasture, and closest to the loafing area. In weeks 2 and 3, capture rates at trap doses 0.5 and 1 were the highest but not significantly different from 0 and 3 mg. Captures were highest in the SW pasture and those closest to the loafing area. That pastures varied in capture rates and that the pasture with the highest capture rate differed among weeks suggests location variability for stable fly presence.

Trap placement is important to capture the maximum number of stable flies and the importance of placing the traps within areas of cattle congregate has been identified. When the Williams trap was used within the pasture and by the areas of watering and loafing areas the traps captured more flies than the traps placed along the pasture perimeter (Hogsette et al. 1987). With the Knightstick® traps, Hogsette and Ose (2017) found that the traps placed closer to the animals captured 6-9 times more stable flies than the ones placed further away. We only saw this occur in 2019 but our trap placement was an issue. In 2020 and 2021 we didn't see a difference in trap placement.

Tangtrakulwanich et al. (2015) found that flies in an olfactometer were more attracted to lower concentrations of m-cresol when compared to higher concentrations. However, we didn't observe this in any of our experiments. Our 2019 comparisons found that using m-cresol didn't change the counts and we only observed that the size of the lure influenced capture rates. In 2020 and 2021 we found that the dosage levels of m-cresol didn’t influence capture rates of stable flies. Further investigation is needed to see if the material of the lure influenced capture rates.

In conclusion, we found that olfactory lures often improve trap efficacy and although we recommend using a lure with 1 mg m-cresol dose our results were inconsistent. Traps have the possibility of being low impact and highly mobile we must recognize that further trap improvement and a better understanding of trap contribution to the effects on stable fly populations are needed so this tactic can be a viable and have the possibility of being adopted by producers to control stable fly outbreaks and increase animal welfare.

Year & Study	Comparisons	*Replicates	Location and Week	Dates
2019 1	Standard lure and No lure	3	1.1	July 10-July 17
			1.2	July 17-July 24
			2.1	July 11-July 19
			2.2	July 19-July 26
			3.1	July 11-July 19
			3.2	July 19-July 26
2019 2	Standard tube and Striped tube	3	1.1	July 24-July 31
			1.2	July 31 -August 07
			2.1	July 26- August 01
			2.2	August 01- August 08
			3.1	July 26- August 01
			3.2	August 01- August 08
2019 3	8 mm lure and 5 mm lure	3	1.1	August 07-August 14
			1.2	August 14-August 21
			2.1	August 08-August 15
			2.2	August 15-August 22
			3.1	August 08-August 15
			3.2	August 15-22
2020 4	Lure dosages (4)	3	3.1	July 08- August 02
			3.2	
			3.3	
2021 5	Lure dosages (4)	4	3.1	July 28 to August 22
			3.2	
			3.3	
			3.4	

Table 1. 2019 and 2020 study details. * Replicates within locations

Figure 1. Standard trap with cattle fence.

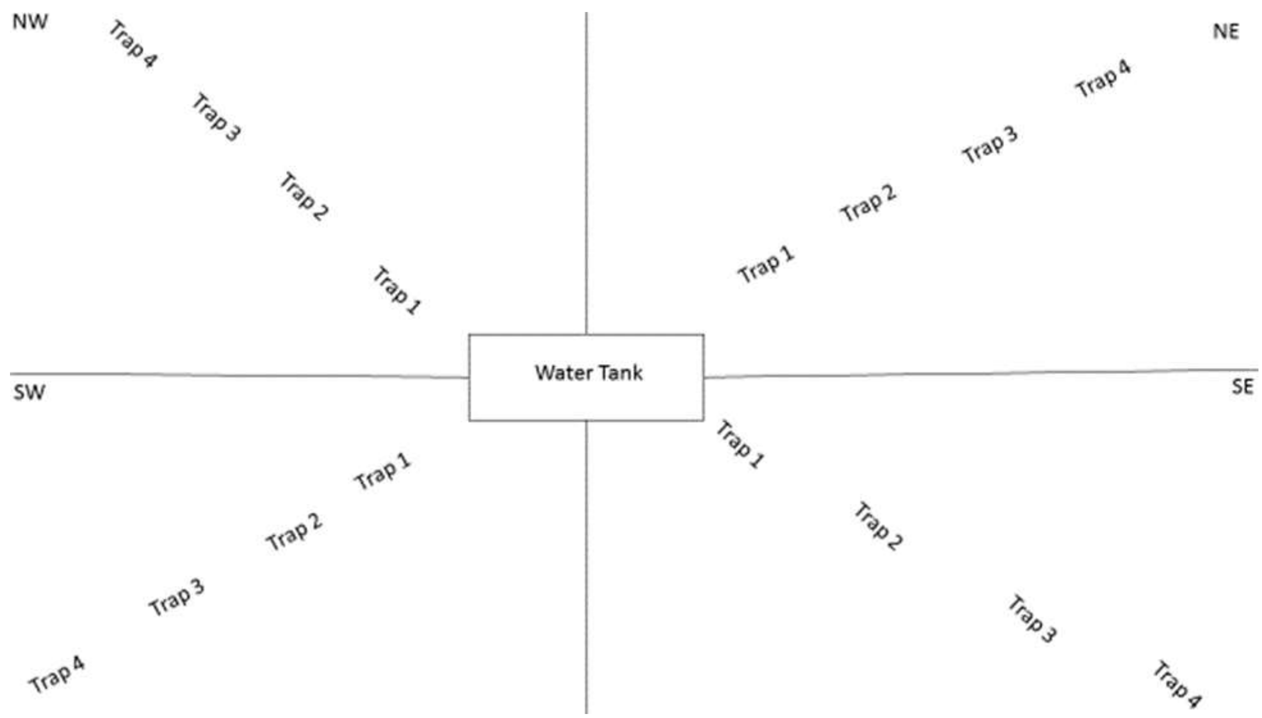

Figure 2. 2020 Latin Square study plot design. Traps in each radial arm were 10 m apart from one another with water tanks located in the center.

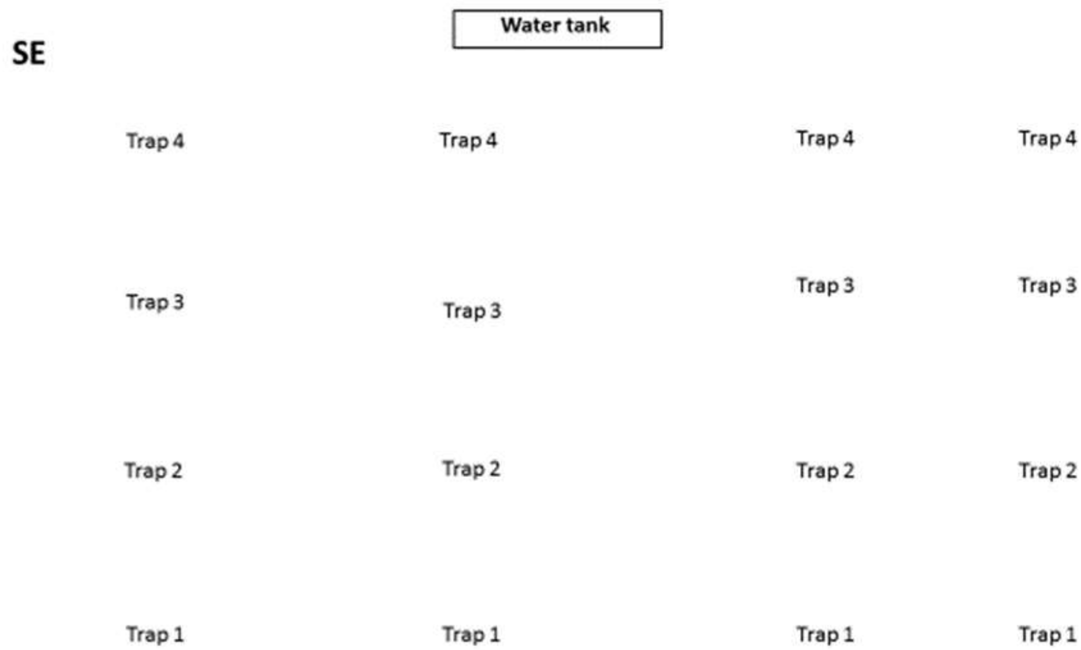

Figure 3. 2021 Random Complete Block study plot design. Traps in each column were 10 m apart from one another with the water tanks located to the north.

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