The Effects of Black Objects and Carbon Dioxide on Horse and Deer Fly (Diptera: Tabanidae) Trap Success in Brazos County, Texas

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Edited by Shelby Kerrin Kilpatrick

Abstract: This study analyzed the effectiveness of traps for horse and deer flies (Diptera: Tabanidae) in Brazos County, Texas, U.S.A., using black objects and carbon dioxide (CO_2). Tabanid flies vector several important livestock diseases and can be pests to humans. Heliothis cone traps were modified and placed at four approximate locations on one site, the Riley Estate in Brazos County, Texas. Four different trap styles were randomly tested at the locations on eight days between 24 March and 14 April 2015. Seventeen individual tabanids representing two species, *Chrysops callidus* Osten Sacken and *Tabanus lineola* F., were collected and the former species was the most abundant. A one-way analysis of variance (ANOVA) calculation revealed that the combined black object and CO_2 trap had a positive trend for being the most effective style for capturing tabanid flies out of those tested. Trap location was determined to be insignificant compared to the number of tabanid flies captured. While more data is required to determine if the difference between the trap styles is significant, the traps have the potential to be used to collect behavioral data, measure biodiversity and relative abundance of tabanid flies, as well as control their populations in areas where they cause irritation and spread disease.

Keywords: Tabanid traps, Chrysops, Tabanus, dipterous disease vector, Texas

Flies in the family Tabanidae are commonly known as horse and deer flies. There are about 350 species represented in North America and the two most common genera in the region are *Chrysops* and *Tabanus* (Triplehorn and Johnson 2004). There are at least 38 species of Tabanidae recorded from Brazos County, including seven *Chrysops* species and 24 *Tabanus* species (Goodwin and Drees 1996). It is difficult to study the biology of tabanid flies for several reasons such as their long life cycle and the predatory to cannibalistic nature of the larvae (Goodwin and Drees 1996). The flies are associated with aquatic and moist habitats as well as flyways at the edges of forested areas (Goodwin and Drees 1996). The activity, behavior, and rate of trap success of adult tabanids including *Tabanus lineola* F. is correlated with weather patterns and a greater percentage of the population is active on days with higher maximum temperatures (Joyce and Hansens 1968). The adult females of most Tabanidae species are haematophagous and prefer warm-blooded animals, although a few will feed on cold-blooded vertebrates (Pechuman and Teskey 1981). Female *Chrysops callidus* Osten Sacken individuals have been known to double their body weight after engorging on cattle (Hollander and Wright 1980). Males feed on pollen and nectar from flowers and can be distinguished from females by their large, contiguous eyes (Triplehorn and Johnson 2004).

Most females are anautogenous and equipped with piercing-cutting style mouthparts that can cause painful bites and impact the health of animals and humans. Their adaption to blood feeding gives them the ability to biologically and mechanically transmit many pathogens, viruses, bacteria, protozoans, and helminths including the causative agents of equine infectious anaemia virus and western equine encephalitis (Krinsky 1976). It is notable that *Chrysops* species are the only insects able to support the developmental cycle of *Loa loa* (Cobbold) (Nematoda: Spirurida), a parasitic microfilarial worm in Africa that could potentially become introduced to non-endemic regions such as the U.S.A. (Foil 1989). Additionally, *C. callidus* transmits *Haemoproteus metchnikovi* (Simond) (Apicomplexa: Haemospororida), a turtle parasite (Degiusti et al. 1973).

Methods of collection of tabanids have been studied previously. One method for collecting tabanid flies is the Manitoba trap, composed of a black ball suspended under a cone on a tripod. The black ball provides a visual cue for the flies, and in one study, the Manitoba trap collected a wider variety and number of individual *Chrysops* species than the other traps that were tested (Thompson 1969). The traps were also selective for tabanids when compared to other insect groups (Thompson 1969). Olfactory attractants for tabanid flies have also been compared in trap designs. Dry ice sublimates to produce carbon dioxide (CO_2) gas that many blood-feeding insects are attracted to as it simulates CO_2 produced during the animal respiration process. The combination of CO_2 from dry ice and l-octen-3-ol, a chemical compound isolated from cattle, increases trap success by three times compared to the catch rate of CO_2 alone (French and Kline 1989).

In this study, four trap styles were selected to test the effects of black objects and CO_2 on tabanid fly trap success in Brazos County, Texas. The positive hypothesis was that combining these two previously known attractants would result in a synergistic effect, causing the number of tabanid flies captured in the trap with the black object and CO_2 to be greater when compared to the other trap styles. The null hypothesis was that the trap style would have no effect on the number of tabanid flies collected.

Materials and Methods

Trapping Location

Field trapping was conducted on the Riley Estate (30.58849°N, 96.25366°W) at 1884 Harris Drive, College Station, Brazos County, Texas, U.S.A. The traps were set up at four different grassy, open approximate locations spread over three acres with an average of 78.4 meters between each of them on 13 March 2015 (Fig. 1, Google 2015). The trap at location number one was just outside of the range of an aerobic septic system spray zone and the trap at location number four was adjacent to a small pond. It is also notable that cattle, several species of exotic deer, and other large animals are kept on properties near the Riley Estate.

Trap Design

Four Heliothis cone traps were modified to model Manitoba traps and test the effectiveness of the black objects and CO_2 at attracting tabanid flies. The Heliothis trap bodies were large rounded cones made of light wire screen material. The traps measured 78.74 cm in diameter at the base with a 7.62 cm collar of mesh along the inside edge to encourage insects to fly upwards into the trap. The cone narrowed steadily towards the top and terminated with a 3.81 cm opening. A trap head fit over this opening and allowed specimens to enter the trap head, but inhibited them from escaping afterwards. The trap head consisted of a canister made of light

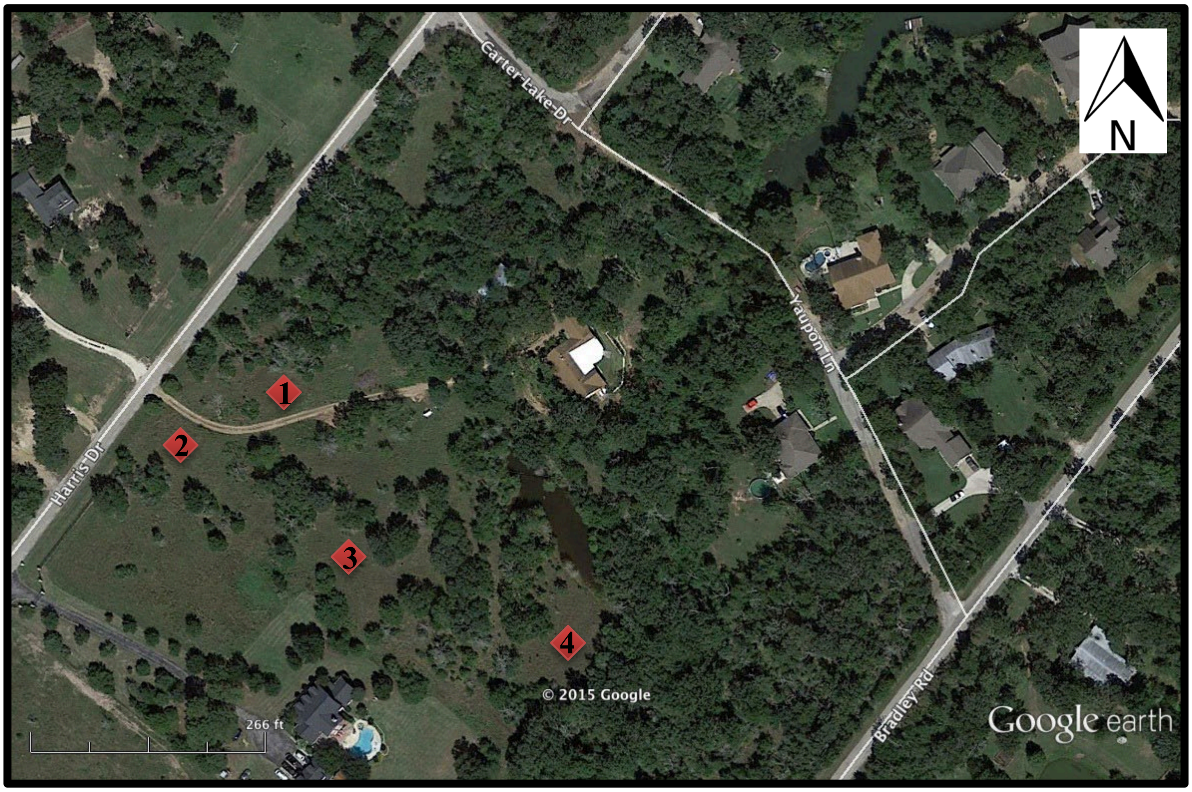

Fig. 1. Aerial image of the Riley Estate with the four trap locations marked (Google 2015).

wire screen material with a removable cap for ease of specimen collection and was secured to the trap body by stiff wire hooks on springs. A crossbar at the base of each trap was used to mount each of the cones centrally on metal polls about 172 cm above the ground.

The four different Heliothis cone trap styles were composed of a control trap with no attractants, a trap with a black object only, a trap with CO_2 only, and a trap with both a black object and CO_2 (Fig. 2). The black objects used in this study were three-gallon plastic flowerpots with slits cut into the bottoms so that they fit over the main trap poll. The plastic flowerpots were suspended

Fig. 2. From left to right, Heliothis cone traps modified with control, black object, CO_2, and black object and CO_2 treatments at the Riley Estate on 4-IV-2015.

Fig. 3. From left to right, dry ice after being cut and placed into the cooler and a foam box with three 5.08 cm diameter holes in the lid to allow CO_2 gas to escape.

with the open side down, using aluminum wire, directly under the crossbar of the cone. Dry ice was employed to sublimate and produce CO_2 gas. An average of 1.88 kg (4.15 lbs.) of dry ice was used in each of the two trap styles with CO_2. The dry ice was cut into large chunks and placed in a 3.81 cm thick Styrofoam box with a volume of 10.16 cm deep, 20.32 cm long, and 15.24 cm wide (Fig. 2). Three holes were drilled into the lid of each of the two boxes so that the CO_2 gas, which sublimated from the dry ice, could escape into the environment (Fig. 3). On 24-III-2015 the holes measured 1.27 cm in diameter. In order to increase the rate of sublimation and release more CO_2 gas, the size of the holes was enlarged to 1.27 cm by 3.81 cm on 29-III-2015, and again to 5.08 cm in diameter on 3-IV-2015 (Fig. 3). A piece of black duct tape was placed on short sides of each box to prevent the lids from blowing away. An average of 0.9 kg (1.98 lbs.) of dry ice was left in each box after each trapping period, because not all of it sublimated.

Trapping Procedures

Attractants were not placed in the traps in the time between trapping dates. Each trap location and trap design was assigned a number and these were randomly paired on each day of the study. The traps were baited with the control or attractant treatments on eight separate dates: 24-III-2015, 29-III-2015, 2-IV-2015, 3-IV-2015, 4-IV-2015, 11-IV-2015, 12-IV-2015, and 14-IV-2015. The treatments were applied between 9:30 A.M. and 9:45 A.M. and the trap heads were collected between 5:00 P.M. and 6:30 P.M.

Specimen Preservation and Identification

The trap heads were removed from the traps and placed in a freezer for approximately 5 min to temporarily subdue any specimens that were collected. The specimens were placed in labeled, quart size zip-locking plastic bags and frozen until they could be processed. The specimens were pinned and labeled following standard entomological guidelines. The tabanid flies were sorted from the other insects, determined as male or female, quantified, and identified to species using keys from *The Horse and Deer Flies (Diptera: Tabanidae) of Texas* (Goodwin and Drees 1996). The other insects that were collected were identified to the family level. Specimens were deposited in the Texas A&M University Insect Collection under voucher number 712 at the conclusion of the study to be used in future research.

Statistical Analysis

A one-way analysis of variance (ANOVA) calculation was used to compare the effect of trap style on number of tabanid flies caught (Lowry 2015). The effect of trap location on the number of tabanid flies caught was also compared (Lowry 2015). Additionally, ANOVA was used to determine if there was a significant difference between trap style and the individual species of tabanid flies it attracted (Lowry 2015).

Results

A total of 17 flies in the family Tabanidae were collected between 24-III-2015 and 14-IV-2015. The black object and CO_2 combination trap style captured the most tabanid flies having ten individuals collected, while the black object only trap caught the second most at five, the CO_2 only trap attracted two tabanids, and no tabanids were collected in the control trap. There was a not a significant effect of trap style on the number of tabanid flies collected at the $p<0.05$ level for the four conditions [$F(3,28) = 1.8$, $p = 0.17$] (Fig. 4; Lowry 2015). *Chrysops callidus* was the most prevalent species with 14 individuals captured. Of these, eight were in the black object and CO_2 combination trap style, four were captured in the black object only trap, and two were collected in the only CO_2 trap (Fig. 5). There was a not a significant effect of trap style on the number of *C. callidus* individuals collected at the $p<0.05$ level for the four conditions [$F(3,28) = 1.6$, $p = 0.21$] (Fig. 5; Lowry 2015). Three *T. lineola* specimens were also collected. Two were in the black object and CO_2 combination trap style and the third was collected by the black object only trap (Fig. 6). There was a not a significant effect of trap style on the number of *T. lineola* individuals collected at the $p<0.05$ level for the four conditions [$F(3,28) = 0.73$, $p = 0.54$] (Fig. 6; Lowry 2015). There was a not a significant effect of trap location on the number of tabanid flies collected at the $p<0.05$ level for the four conditions [$F(3,28) = 0.41$, $p = 0.75$] (Fig. 6; Lowry 2015). No tabanid flies were collected on the first, second, or eighth days of the study. *Chrysops callidus* was collected on days three through seven and *T. lineola* was only caught on day seven. Day seven was also the only day that tabanid flies were collected in the non-combination trap styles. All of the tabanid flies collected in the traps were females. In addition to tabanid flies, other insects were collected over the trapping period from the families Apidae, Megachilidae, Sphecidae,

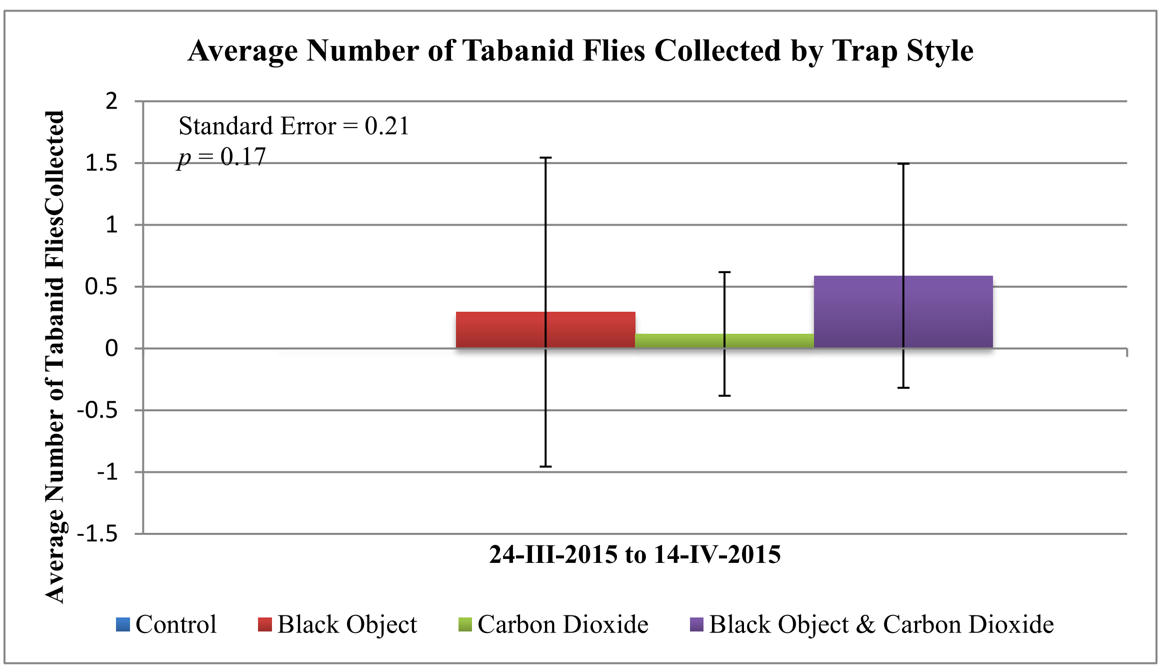

Fig. 4. The relationship between the four trap styles and the average number of tabanid flies collected by them between 24-III-2015 and 14-IV-2015 is shown.

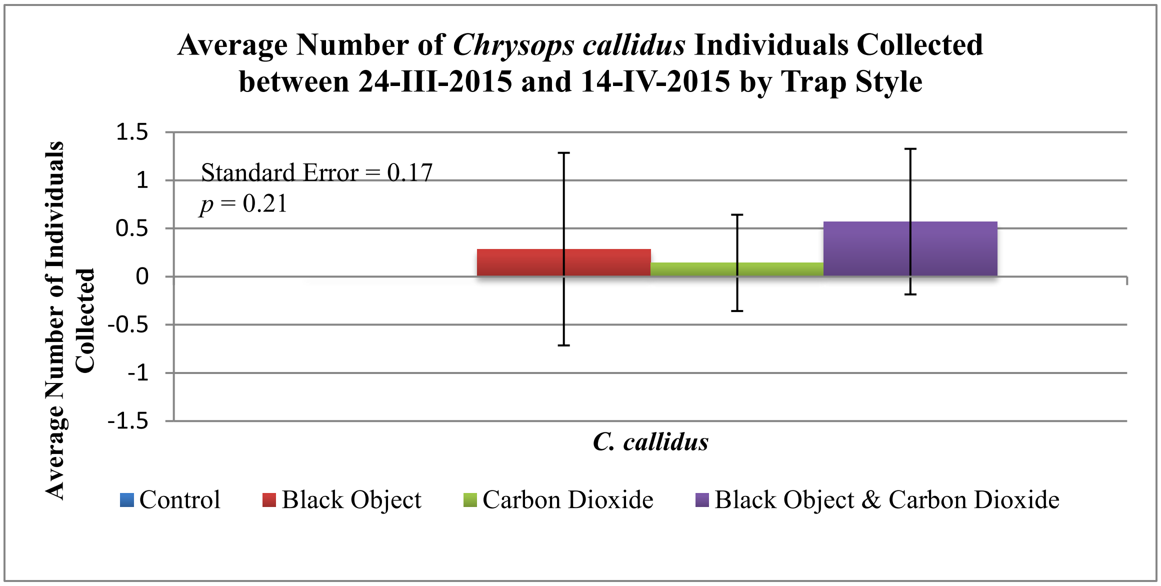

Fig. 5. The relationship between the four trap styles and the average number of *Chrysops callidus* individuals collected by them between 24-III-2015 and 14-IV-2015 is shown.

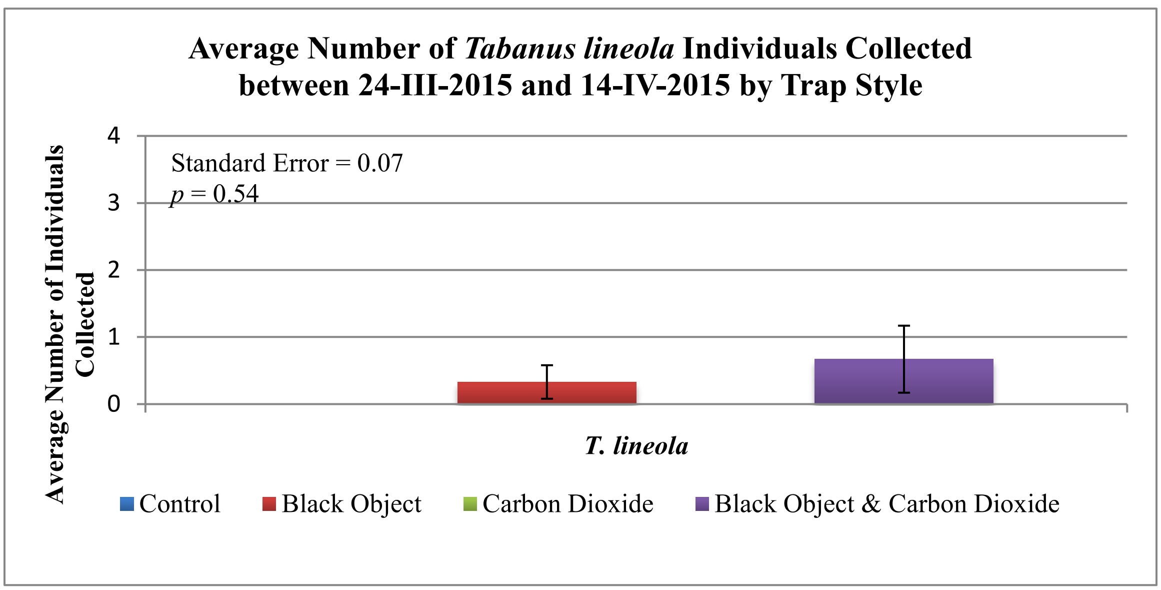

Fig. 6. The relationship between the four trap styles and the average number of *Tabanus lineola* individuals collected by them between 24-III-2015 and 14-IV-2015 is shown.

Vespidae, Achilidae, Noctuidae, Coccinellidae, Scarabaeidae, Muscidae, and Tachinidae.

Discussion

While the ANOVA statistical analysis did not determine a significant difference in the number of tabanid flies or individual species of tabanid flies collected by the different trap styles in the study, the data collected shows a positive trend that the combined black object and CO_2 trap is the most effective style for capturing tabanid flies. The location of the traps was not significant compared to the number of tabanid flies collected in them, which indicates that trap style is an important factor for trap success. It is notable that the black object only and CO_2 only traps were successful at trapping tabanids on one day late in the study period. However, due to the fact that tabanid flies were collected in these traps, it is evident that both of these items are attractive to the flies and contribute to trap success. The positive trend of the combined trap's success may be the result of a synergistic effect when the attractants are used together. This supports the positive hypothesis of the study. Regarding the slightly greater number of tabanid flies collected in the black object only trap compared to the CO_2 only trap, it is possible that the angle of the fly's landing site, such as the black object, to the trap entrance must be within a particular range for the fly to be captured upon taking flight. Therefore, it is also possible that more flies were attracted to the CO_2 only trap, but were not collected as a result of reduced landing site proximity to the trap entrance.

According to Goodwin and Drees (1996), *C. callidus* has been previously recorded in Brazos County for all of the months between March and September. Additionally, *T. lineola* has been collected as early as April and as late as October in Brazos County (Goodwin and Drees 1996). These previous accounts match with the months the specimens were collected during this project. As male tabanids are not blood feeding insects and therefore not attracted to CO_2, it was not surprising that they were not collected during the study. Additionally, the observation that tabanid flies were not collected on certain days of the study, late March in particular, is likely related to the cool weather during the time of the year the traps were set that would have reduced tabanid activity and potentially had an impact on adult emergence. The small hole size in the Styrofoam lids during the first two days of the study may have caused the CO_2 output by the two trap styles to be so low that active flies were not attracted to it.

The limitations of this study included that tabanid flies are not necessarily equally distributed on the Riley Estate. It was impossible to eliminate any effects that resulted from variations in the habitat near each trap site. Randomly pairing the trap location and trap design on each trapping day aided in compensating for these inconsistencies. The dates the study was conducted on were restricted by adverse weather conditions, transportation availability, and student schedules. It is also probable that the CO_2 sublimation rate was inconsistent between trapping days as a result of changes in the ambient temperature, humidity, and wind speed and direction. This variation may have had an impact on the ability of the CO_2 to be detected by tabanids in the environment.

Additional data should be collected in order to determine any significant differences between the trap styles and the total number of tabanid flies captured. Increasing the number of trapping days as well as extending the time period the traps are active per day are methods that would likely improve the results of this study. There may be a potential for genera and species preference between trap styles, and because of this, more data comparing these variables should be analyzed. Similarly, information about the other types of insects that these trap styles may be successful at collecting should be examined. There are various studies that could be conducted in the future using these trap designs, specifically the most statistically successful one if and when it is determined. Potential future studies include year-round trapping to collect behavioral data as well as measure the biodiversity and relative abundance of tabanid flies at the

Riley Estate, within other parts of Brazos County, or in any given locality. The traps could also be utilized for the control of tabanid populations to reduce irritation rates and the spread of diseases.

Acknowledgments

I would like to thank Edward Riley for providing instrumental support and guidance throughout this project as well as allowing for the use of Texas A&M University Insect Collection resources for the identification and preservation of specimens. Additionally, the Riley Family is recognized for the use of their property as the study site. I would like to thank Adrienne Brundage for supervising my project and for providing advice on statistical methods and scientific paper formatting. I would like to recognize my research team, Robin Callahan, Andrew Graf, Jakalynne Gosnell, and Nicole Poland, for committing their time and effort to all aspects of this project as well as for providing necessary transportation. I am grateful for the expertise Phillip Shults and James Woolley shared which aided in identifying the flies and hymenopteran insects collected over the course of the project respectively, for the identification literature that John Oswald loaned me, and for the additional information on representing data that Loriann Garcia provided. I would like to thank William Scott Kilpatrick and Susan Kilpatrick as well as the Texas A&M University Writing Center staff for taking the time to provide feedback on this paper. Special thanks to Christopher Parker and the United States Department of Agriculture Agricultural Research Service for lending me the Heliothis cone traps used in this study.

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