MONITORING AND MANAGEMENT OF *CULICOIDES* SPP. (DIPTERA: CERATAPOGONIDAE) IN WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS*) PRODUCTION FACILITIES IN TEXAS, USA

A Dissertation

by

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ABSTRACT

Biting midges, *Culicoides* spp. (Diptera: Ceratopogonidae), are important ectoparasites which disturb white-tailed deer (*Odocoileus virginianus*) populations in Texas. Biting midges are vectors of disease agents causing epizootic hemorrhagic disease (EHD) and bluetongue (BT). These diseases are easily recognized and feared by white-tailed deer producers, yet there is limited literature on biting midge management. The objectives of this dissertation study aimed to identify entomological problems faced by white-tailed deer producers in Texas. Four objectives were designed to address the questions of which insects were present in the deer-breeding pens, insect abundance, specifically presence of which *Culicoides* spp., distribution of BTV and EHDV, identification of the common organic materials in deer-breeding facilities used by *Culicoides* spp. for development, and integrated pest management recommendations specifically designed for Texas deer breeders. Twenty-four ranches across Texas participated in a two-year sampling study of Ceratopogonidae near deer-breeding pens. Results showed that filth flies were the most abundant group measured throughout the experiment and up to eight ceratopogonids were sampled at a time for a specific location. The second objective was to identify specific *Culicoides* species present in East Texas. Five *Culicoides* species were identified from seven ranches. Three species tested positive for EHDV or BTV using a real- time PCR procedure. Results from this study will be used to target insect control to specific *Culicoides* spp. in Texas white-tailed deer-breeding facilities. Work conducted also aimed to identify common areas of *Culicoides*

sonorensis development in a deer facility. Samples of manure, deer feed, the USDA rearing medium and deionized water were used as a substrate foundation and *C. sonorensis* development. Results demonstrated immature *C. sonorensis* more successfully completed development in a feed-based substrate than the rearing medium and deionized water. This information was utilized to design integrated pest management (IPM) recommendations for Texas white-tailed deer producers. Management of these insects, particularly the biting flies, will take an integrated approach. Biological, chemical, physical, and cultural recommendations are commonly used for pest control in production systems. There has previously not been an IPM program designed for white-tailed deer producers. Results from this collection of *Culicoides* spp. studies will soon prove to be an asset to entomological researchers and deer producers.

DEDICATION

I dedicate my dissertation work to my family. Without their love and continued support, none of this could have been possible.

To my parents, Karl and Tracie Schoenthal, thank you for reminding me to persevere until I reached my dreams. Thank you for teaching me that God has helped me to get where I am and that He will continue to guide me through the greater plan. Thank you for the pep talks and continuous blessings. You are the greatest examples of hard work, respect and integrity. Words will never be able to express my gratitude for your support.

To my brother, Chance Schoenthal. Thank you for being my best friend. Throughout all of this, you have always reminded me to have fun. Your love and support throughout our lives means more than you will ever know. Don't ever stop reaching for your dreams. I believe in you and I always will.

To Ryan Krejci, thank you for endlessly supporting me through this journey. I will always be appreciative of your patience, advice and love. I look forward to a lifetime of happiness with you; the best is yet to come.

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To my committee members Drs. Jeff Tomberlin, Pete Teel, and Don Davis, thanks you for your service and direction as I have worked through my dissertation project. Thank you for your suggestions and encouragement that benefitted this research.

Thank you, Robert Puckett, for your help with the initial studies that helped to get this project off the ground, my experimental design and assisting me with statistical analysis of the data. I thank you for your patience and advice throughout my time here. Thank you to Pam Ferro with Texas Veterinary Medical Diagnostics Laboratory for her assistance with the virus isolation process. Thank you to Lee Cohnstaedt with USDA-ARS in Manhattan, KS, for his advice in *Culicoides* spp. rearing methods. Thank you to Laura Nelson for reviewing everything I have written in the past three years and being a source of wisdom when I have needed it. Thank you to my labmates Elly Espinoza, Phillip Shults, Janis Reed, Andrew Davitt, Taylor Wade, and Chris Keefer. Each one of you have encouraged me throughout this process and been there for a laugh when needed. I appreciate each of you and I am grateful to call you my friends.

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Thank you to Ryan Krejci for his expertise in Excel programming that saved me many nights of frustration. And finally, thank you to my parents, Karl and Tracie Schoenthal for allowing me to bounce ideas and dreams off of you for much longer than the last three years.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Introduction to *Culicoides* spp. Biology

Biting midges, *Culicoides* spp. (Diptera: Ceratopogonidae), are important ectoparasites that feed on white-tailed deer (*Odocoileus virginianus*) populations in Texas. Also referred to as "punkies, "no-see-ums," or "sand flies" (Wirth 1977), biting midges are known to be vectors of disease-causing pathogens of medical importance (Linely et al. 1983, Tabachnick 1996). *Culicoides* spp. are found worldwide, with the exception of Antarctica and New Zealand (Mellor et al. 2000), and are the primary vector of bluetongue virus (BTV) in North America (Jones et al. 1981). International trade regulations have been put into place that prohibits the trade of United States livestock to bluetongue-free countries. As a consequence, the United States livestock industry has at least $125 million per year in loss to the cattle and sheep trades (Tabachnick 1996). There have been no economic studies to conclude how much in economic loss has been suffered by the United States white-tailed deer industry due to hemorrhagic diseases, but there has been no shortage of disease incidences. Thus, the importance of research into *Culicoides* biology and vector potential continues to grow.

Culicoides spp. measure 1 to 3 mm in length (Mellor et al. 2000), and are most readily identified by the spotted or banded color pattern on the wings (Wirth 1977). *Culicoides* spp. adults are also characterized by absent ocelli, 13 antennal flagellomeres, plumose antennae on males, and serrate mandibles on females (Borkent 2005). Research

on *Culicoides* as pathogen vectors has been delayed because the insects are small in size and difficult to maintain in colony (Blanton and Wirth 1970).

Currently, there are 103 genera of biting midges, but only four genera contain vertebrate blood feeders (Borkent 2005). *Culicoides variipennis*(Coquillett) (Diptera: Ceratopogonidae) is the most common species in the family Ceratopogonidae and the most common biting midge in the United States. Previously, *C. variipennis* was divided into five subspecies in the subgenus *Monoculicoides*: *C. variipennis variipennis, C. variipennis sonorensis, C. variipennis occidentalis, C. variipennis austalis,* and *C. variipennis albertensis* (Tabachnick 1996). The *C. variipennis* group of subspecies has now been split into 3 separate species based on morphological characteristics: *C. variipennis, C. sonorensis,* and *C. occidentalis*.

Culicoides spp. males feed on nectar from flowering plants, while females take blood meals from mammal hosts. The blood meals are necessary for egg development (Wirth and Blanton 1974). The mating of *Culicoides* usually occurs during flight when the males form swarms. Females will fly through the swarms and are captured by males (Downes 1955). Jones (1966) found that *C. variipennis* females can mate repeatedly and store sperm for as many as three egg batches. Jones (1967) determined that *C. variipennis* deposited one egg batch for each blood meal taken. One *C. variipennis* female can live 44 d, depositing 243 eggs in a single batch, for a maximum of 1,143 eggs in seven batches, during her lifetime (Jones 1967). It is unknown how many generations of Culicoides are produced each year. The *Culicoides* spp. exhibits a holometabolous life cycle. The egg is approximately 0.26 mm long, cylindrical and

white. The egg will hatch approximately 24 h after being laid on a suitable moist substrate. Immatures can survive in nearly any environment that contains a suitable amount of moisture for development (Borkent 2005). Larvae are vermiform (Mellor et al. 2000), semiaquatic (Blanton and Wirth, 1970) and include four instars. Larvae will pupate in 10 to 14 d under optimum conditions. The pupae stage is brief, in comparison, only lasting two to four days. Pupae are found free-floating or attached to debris in the substrate (Mellor et al. 2000), but are always found near the surface of the substrate. Mullens and Rodriguez (1992) found that the significant majority of pupae are found in the first 1 to 2 cm of organic substrate. After eclosion, adult *Culicoides* spp. mate and search for a blood meal. Most species of Culicoides overwinter as fourth instar larvae (Mellor et al. 2000). It is estimated that Culicoides spp. produce 12 generations each year.

Hemorrhagic Diseases

An arbovirus, as defined by Mellor (2000) is, “a virus which in nature can infect hematophagous arthropods by their ingestion of infected vertebrate bloods. It multiplies in the arthropod’s tissues and is transmitted by bite to other susceptible vertebrates”. Pathogens transmitted by arthropods are known to cause significant human and animal morbidity and mortality worldwide (Tabachnick 1996). With more than 1400 species identified worldwide (Mellor et al. 2000), *Culicoides* have mostly been studied as the vectors responsible for the transmission of pathogens that cause bluetongue (BT). Specifically, *C. sonorensis* is known to be the primary vector of bluetongue virus (BTV)

to ruminants in the United States (Holbrook et al. 2000). Along with BTV in cattle and sheep, *Culicoides* spp. have also been found to transmit filarial worms, pathogens responsible for horsesickness, buttonwillow virus, malaria-like protozoa including *Haemoproteus* of birds, *Leucocutozoon* of chickens, *Hepatocyctis* of monkeys, and epizootic hemorrhagic disease (Wirth and Blanton 1974).

Bluetongue virus was first described by James Spruell (1905) in South Africa. The disease was first given the name Malarial Catarrhal Fever, but was soon found to not be a malarial infection (Spruell 1905). Bluetongue virus is the causative agent of bluetongue (BT) disease (Verwoerd and Erasmus 2004) and is classified in the genus *Orbivirus*, the family Reoviridae. There are 24 known serotypes of BTV in the world. Four serotypes (10, 11, 13, and 17) are widely distributed across North America, but BTV- 1 and 2 are common to southeastern regions of the United States (MacLachlan 2008). Bluetongue virus is common to tropical, subtropical and several temperate regions of the world (Maclachlan et al. 2009) and latitudes between 35°S and 50°N (Borkent 2005).

Bluetongue virus is transmitted by *Culicoides* spp. between ruminant hosts (Mellor 2000, Gerry et al. 2001) and is not contagious, but is infectious to susceptible hosts (Carpenter et al. 2011). Bluetongue, also known as ‘sore-mouth’ or ‘ulcerative stomatitis’ (Goltz 1978), is a disease of wild and domestic ruminants such as sheep, cattle, deer, buffalo, elk, and goats (OIE 2009). Cattle and goats are considered a natural reservoir for BTV. Clinical signs of a BT infection are more severe in sheep and deer than in cattle, and include fever, swelling of the buccal and nasal mucosa, salivation,

tongue swelling, hemorrhaging of the mucosal membranes of the mouth, oral lesions, hemorrhaging of the coronary bands, and death (Tabachnick 1996). There are currently no vaccines for BTV, though there have been attempts to create polyvalent vaccines. Preliminary vaccines have failed, in part, due to the variability in BTV serotypes.

Along with BTV, pathogens transmitted by *Culicoides* spp. have been found to cause epizootic hemorrhagic disease (EHD) in North American white-tailed deer populations. Like BTV, EHDV is caused by an *Orbivirus* in the Reoviridae family. These viruses are antigenically different, but the signs and symptoms of EHD and BTV in white-tailed deer are clinically indistinguishable. Epizootic hemorrhagic disease was first diagnosed in the United States in 1955 in New Jersey (Shope et al. 1960). Outbreaks failed to occur in the same area until it was identified in South Carolina in 1971, and spread throughout the fall season to seven other southeastern states (Prestwood et al. 1974). Currently, EHD is widespread across the United States, though there are no recent maps which delineate distribution.

Eight serotypes of EHDV have been identified (Mellor et al. 2000), including EHD- 1 through 7, and an Ibaraki strain common to Japan. EHDV occurs in Africa, Southeast Asia, Japan, Australia, and the Americas (Mellor et al. 2000). Infections of EHDV occur in domestic and wild ruminants often show sub-clinical symptoms, but are severe in white-tailed deer (*Odocoileus virginianus*). There have not been any vaccines developed for the prevention of EHD in ruminants and treatments is limited to supportive care.

Culicoides spp. distribution in the United States continues to spread with the movement of livestock. This gives opportunity to the spread of disease to virtually any area where *Culicoides* spp. can survive.

Current *Culicoides* spp. Control Methods

There are currently no integrated pest management (IPM) recommendations published for the control of *Culicoides* spp. in white-tailed deer-breeding operations. The basics of an IPM program include cultural, mechanical, biological, and chemical methods to control insect pest populations. Difficulties for the control of *Culicoides* spp. arises from their small size, complications with colonization, and diverse habitats.

Borkent (2005) describes that the beginning of systematic efforts to control *Culicoides* spp. began in the early 1900s and included methods for draining or filling insect development sites, and the use of crude oil in moist environments. Chlorinated hydrocarbons, including DDT, were used, but, much like other target pests, *Culicoides* spp. developed resistance (Borkent 2005). Though the use of pesticides has been found to be effective against *Culicoides* spp., the long term efficacy of the product has not been evaluated (Wirth 1977). There have been several studies conducted evaluating natural enemies of *Culicoides* spp., though none have been found to be highly effective (Borkent 2005). Wirth (1977) compiled a review of pathogens and parasites of biting midges in which a limited amount of literature was available. A study in West India found that only adult tiger beetles, *Cicindela suturalis*, would feed on adult and pupae forms of *C. phlebotomus* (Yaseen 1974). Several studies (Chapman et al. 1968, Clark and O'Grady

1975) reported the use of pathogenic viruses used to control tree hole species of *Culicoides* in Louisiana and California. Symbiotic bacteria of *C. nubeculosus* (Lawson 1951) and *C. salinarius* (Becker 1958) were identified, but neither was found to be useful for control. Four species of parasitoids acting as biological control agents for ceratopogonids have been recorded, but none have been found to be effective against species in the genus *Culicoides* (Wirth 1977).

It is understood that climate and weather have effects on *Culicoides* populations (Mellor et al. 2000), but several factors hamper the control of *Culicoides*. It is not fully known which species are abundant in the United States and developmental biology of the insect is poorly understood. Also, flight range and dispersal has only been associated with wind (Mellor et al. 2000) and more research is needed to enforce what is currently known.

Cultural control is usually considered the least expensive mode of insect control. Research has been conducted to investigate the areas in livestock production systems where *Culicoides* thrive in an effort to focus control efforts. Schmidtmann et al. (1983) used dairies and their assorted aquatic habitats in New York as a model to study the abundance of *C. variipennis*. Manure-polluted areas, such as lagoons, were identified as notable areas for *Culicoides* development. Mullens and Lii (1987) evaluated site variability based on season and water level for *Culicoides* development in California dairies. As an expansion on site importance, Mullens and Rodriguez (1992) found that 98.1% of *C. variipennis* pupae were found in the top 2 cm of mud near a dairy wastewater site in California. While there have been no published studies addressing

insect control on Texas white-tailed deer production facilities, management parallels are evident between other production facilities, such as dairies, and Texas deer ranches.

There have been no studies performed within the last decade that address the control of *Culicoides* spp. as vectors of disease-causing pathogens in production systems in the United States. The development of IPM tactics for livestock producers in the United States would not only benefit the animals, but also benefit international trade and agricultural economics.

History of the White-tailed Deer-breeding Industry

The white-tailed deer (*Odocoileus virginianus*) breeding industry is the fastest growing industry in rural America (Anderson et al. 2007b). White-tailed deer, or cervids, are members of the Cervidae family, and include white-tailed deer, elk, reindeer, axis, sika, red deer, fallow, and others (American Heritage Dictionary 2011). In 2006, the total number of cervid ranches in the United States was 7,828 with Texas and Pennsylvania claiming 1,000 ranches each as of 2007 (Frosch et al. 2008).

The cervid breeding industry is separated into three operational structures: breeding only, hunting only, and breeding and hunting (Frosch et al. 2008). Breeding only operations involve scientific breeding and rearing of cervids as a means to develop breeding stock and to harvest their by-products, such as venison and urine. Hunting-only operations purchase cervids from breeding operations as a way to stock their herd or improve antler size and then release them (Frosch et al. 2008). Hunting only operations also manage deer populations through selective harvest and nutritional supplements.

Breeding and hunting operations purchase breeding stock as well as implement breeding strategies to supplement the genetics within the herd (Frosch et al. 2008). Deer management within ranches varies greatly. Production of deer breeder stock is monitored through the deer management permit and scientific breeders permit issued by the Texas Parks and Wildlife Department (Baccus 2002). Breeder stock includes does and bucks, with values ranging from $1,500 (DeVuyst 2013) up to $1,000,000. The value of individual deer is determined by the market place, with those from desired genetic lines and high scoring potential being appraised with metrics equivalent to "Boone & Crockett Club" (B&C) scoring (Frosch et al. 2008).

The direct economic impact of the cervid production industry in the United States is estimated at $893.5 million (Anderson et al. 2007b). When combined with the $757 million contributed by hunters and consumers, the white-tailed deer industry generates over $3 billion in economic activity in the United States. The United States cervid industry, in turn, supports an estimated 29,199 jobs, most of which occur in rural areas (Anderson et al. 2007b). Texas is the nation's leader in the white-tailed deer-breeding industry with an estimated annual impact of $652 million and a direct economic impact of $318.4 million annually (Anderson et al. 2007a). Hunters and consumers of deer products generate an additional $129 million. Subsequently, the deer-breeding industry in Texas supports 7,335 jobs (Anderson et al. 2007a). Ninety-seven percent of Texas land area is privately owned where the majority of this hunting occurs (Baccus 2002), some of which are leased for the hunting season, or are "day hunted". Revenues from hunting and wildlife enterprises have become an important aspect of

economic viability for land owners in rural Texas (Baccus 2002). The initial investment in a small "Intensively Managed Hunting Operation" in Texas can cost approximately $200,000, not including the purchase of approximately 300 acres (Anderson et al. 2007a). In 2011, there were 1,261 "Deer Breeder" permits issued by the Texas Department of Parks and Wildlife, the state agency that regulates captive cervid production. The Texas Deer Association (TDA), which was established in 1999, has 2,600 members, the majority of whom are involved in some aspect of intensive deer management (TDA 2013).

According to Dr. James C. Kroll, Regents' Professor and Director of the Institute for White-tailed Deer Management & Research at Sam Houston State University, "deer-breeding is contributing to saving the family farm and ranch" (Ammoland 2011). Private land owners in Texas are constantly challenged with the fragmentation of farms and ranches due to economic stressors and increased costs associated with laws, taxes, world agricultural markets, and an overall increase in the cost to successfully operate a farm or ranch. The result of these stressors has led to the need for more economic output from non-traditional means, such as white-tailed deer-breeding operations (Baccus 2002). The increase in new types of confined animal feeding operations presents an interference of insect pests transmitting fatal pathogens in new environments with expensive animals. The severity of this disease cycle is only heightened without an integrated pest management (IPM) program in place to control pests. Disease outbreaks in animal production systems can cause significant costs through loss of productivity and death to livestock (Rich and Winter-Nelson 2007). The

development of captive production deer facilities has provided the same challenges that exist with confined operations of any and all livestock including management of manure and other wastes, control of insect pests, and protection from disease agents that adversely affect animal health, growth, phenotypic expression, and subsequent value. Deer held in captive operations produce wastes in the form of feces and urine, along with spoiled feed materials. The environment involving these waste products is conducive to the production of important vectors of cervid disease.

Other fly species known to be associated with deer confinement facilities include house flies *(Musca domestica) (*Diptera: Muscidae) (Linnaeus), stable flies (*Stomoxys calcitrans*) *(*Diptera: Muscidae) (Linnaeus), horn flies (*Haematobia irritans*) *(*Diptera: Muscidae) (Linnaeus), horse flies *(Tabanus* spp.) *(*Diptera: Tabanidae), and deer flies (*Chrysops* spp.) *(*Diptera: Tabanidae). These flies are not only a nuisance, but also reduce animal fitness and production, and are potential mechanical vectors of pathogens in captive deer populations.

While the association of biting flies to enzootic situations in deer has been discussed in the literature, there is much to be learned about favorable conditions that contribute to elevated population densities of these insects in and around captive deer management operations. In addition, information about the seasonal occurrence of the flies and the presence of the disease agents needs to be assessed. Deer breeders have asked for help in identifying conducive conditions which foster insect population growth; identification of which specific insects groups have the potential to irritate the penned deer; identification of the insects which are potential vectors of pathogens which

adversely affect deer health and production; correlation of insect numbers and species to evidence of hemorrhagic diseases in deer production units; and developing best management practices that will potentially reduce or limit insect problems.

CHAPTER II

SAMPLING OF BITING MIDGES (DIPTERA: CERATOPOGONIDAE) AND OTHER POTENTIAL PATHOGEN VECTORS IN TEXAS WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS*) BREEDING FACILITIES

Introduction

Biting midges, *Culicoides* spp. (Diptera: Ceratopogonidae), are important ectoparasites of white-tailed deer (*Odocoileus virginianus*) populations in Texas. Also referred to as "punkies, "no-see-ums," or "sand flies" (Wirth 1977), biting midges are known to be vectors of disease-causing pathogens and of medical importance (Linely et al. 1983, Tabachnick 1996).

Mellor (2000) defined an arbovirus as, "a virus which in nature can infect hematophagous arthropods by their ingestion of infected vertebrate bloods. It multiplies in the arthropod's tissues and is transmitted by bite to other susceptible vertebrates." Pathogens transmitted by arthropods are known to cause significant human and animal morbidity and mortality worldwide (Tabachnick 1996). With more than 1400 species identified worldwide (Mellor et al. 2000), *Culicoides* spp. have mostly been studied as the vector responsible for the transmission of pathogens that cause bluetongue (BT). Specifically, *C. sonorensis* is known to be the primary vector of bluetongue virus (BTV) to ruminants in the United States (Holbrook et al. 2000). Bluetongue virus is the causative agent of bluetongue (BT) disease (Verwoerd and Erasmus 2004) and is classified in the genus *Orbivirus* and the family Reoviridae. There are 24 known

serotypes of BTV in the world. Four serotypes (10, 11, 13, and 17) are widely distributed across North America and BTV- 1 and 2 are common to southeastern regions of the United States (MacLachlan 2008). Bluetongue, also known as 'sore-mouth' or 'ulcerative stomatitis' (Goltz 1978), is a disease wild and domestic ruminants such as sheep, cattle, deer, buffalo, elk, and goats (OIE 2009). Cattle and goats are considered a natural reservoir for BTV. Clinical signs of a BT infection are more severe in sheep and deer than in cattle and include fever, swelling of the buccal and nasal mucosa, salivation, tongue swelling, hemorrhaging of the mucosal membranes of the mouth, oral lesions, hemorrhaging of the coronary bands, and death (Tabachnick 1996).

Along with BTV, pathogens transmitted by *Culicoides* spp. have been found to cause epizootic hemorrhagic disease (EHD) in North American white-tailed deer (*Odocoileau virginianus*). Like BTV, EHDV is caused by an *Orbivirus* in the Reoviridae family. These viruses are antigenically different, but the signs and symptoms of EHD and BTV in white-tailed deer are clinically indistinguishable. Eight serotypes of EHDV have been identified (Mellor et al. 2000), including EHD- 1 through 7 and an Ibaraki strain common to Japan. EHDV occurs in Africa, Southeast Asia, Japan, Australia, and the Americas (Mellor et al. 2000). Infections of EHDV occur in domestic and wild ruminants often show sub-clinical symptoms, but are severe in white-tailed deer.

The white-tailed deer (*Odocoileus virginianus*) breeding industry is the fastest growing industry in rural America (Anderson et al. 2007b). White-tailed deer, or cervids, are members of the Cervidae family, and include white-tailed deer, elk, reindeer, axis, sika, red deer, fallow, and others (American Heritage Dictionary 2011). In 2006, the

total number of cervid ranches in the United States was 7,828 with Texas and Pennsylvania claiming 1,000 ranches each as of 2007 (Frosch et al. 2008). The direct economic impact of the cervid production industry in the United States is $893.5 million (Anderson et al. 2007b).

The increase in new types of confined animal feeding operations presents an interference of insect pests vectoring fatal pathogens in new environments with expensive animals. The severity of this disease cycle is only heightened without an integrated pest management (IPM) program in place to control the system. Disease outbreaks in animal production systems can cause significant costs through loss of productivity and death to livestock (Rich and Winter-Nelson 2007). The development of captive production deer facilities has provided the same challenges that exist with confined operations of any and all livestock including management of manure and other wastes, control of insect pests, and protection from disease agents that adversely affect animal health, growth, phenotypic expression, and subsequent value. Deer held in captive operations produce wastes in the form of feces and urine, along with spoiled feed materials. The environment involving these waste products is conducive to the production of important vectors of cervid disease.

While the association of biting flies to enzootic situations in deer has been discussed in the literature, there is much to be learned about favorable conditions that contribute to elevated population densities of these insects in and around captive deer management operations. In addition, information about the seasonal occurrence of the flies and the presence of the disease agents needs to be assessed.

The objective of this experiment was to determine *Culicoides* spp. and other dipteran vector population numbers on Texas white-tailed deer-breeding facilities. Climate data, including minimum and maximum temperature and total precipitation, and location were important parameters monitored. It is hypothesized that there will be a change in the number of possible pathogen vectors throughout the year. It is also expected that precipitation will be a significant factor influencing insect abundance.

Materials and Methods

Two independent deer producers located in the Brazos Valley, Texas area expressed interest in entomological research in 2011. They aimed to have their questions answered regarding which insects were present in the deer pens, how many of those insects were possible vectors of disease-causing pathogens, and the abundance of ceratopogonids in the facility. Ranch #1 was equipped with 20 lighted insect traps in the deer-breeding pens and five lighted insect traps in the buck pens. Ranch #2 deployed 21 traps to the fences surrounding their deer-breeding pens. The traps were designed by securing a glue trap (Masterline by Univar, #2475, Austin, TX) to a 25 x 25 cm piece of wood with two large binder clips (Acco, #72100, Lincolnshire, IL). Two holes were drilled into the top center ridge of the board and a solar light source (Malibu, #8501-0603-01) was secured to the board using a zip tie. Two additional zip ties were used to mount the board and light source to the fence (Fig. 1). The glue traps were replaced every two weeks and the following data were recorded from the traps: total number of all insects trapped, number of Ceratopogonidae, Muscidae, Culicidae, Simuliidae, and filth

flies. Total number of possible pathogen vectors was calculated by adding the total number of insects classified as Ceratopogonidae, Muscidae, Culicidae, Simuliidae, and filth flies. This preliminary study was conducted from 12 September 2011 until 29 March 2012.

This experiment was expanded to include 24 ranches across the state of Texas and was initiated in December 2012 and continued through November 2014. Insects were trapped over a 24 month sampling period. The Texas Deer Association had 1,114

Fig. 1. An insect trap with light source used in the preliminary study. Traps were mounted to the fence around deer pens with zip ties.

ranches registered as members as of October 2012. These ranches are unequally distributed among 8 regions (Fig. 2). The total ranches sampled in this study in each region were weighted as a percentage of the total ranches registered as members of the TDA. This percentage was applied to the 24 total ranches needed for this experiment in an attempt to use ranches that were evenly distributed across Texas based on total ranches per region. The total of 24 ranches was calculated as the maximum number of ranches that were practical for this replicated study. Cooperators from 22 ranches were initially found and two ranches were added after the start of the experiment. Figure 3 shows the statewide distribution of participating ranches.

Ten clipboards (Office Express, Inc. #BSN 16506) measuring 15.2 cm by 22.8 cm were installed at each ranch on fences surrounding the breeding pens where deer were confined (Fig. 4). There was no source of light attached to the clipboards. Initial proof of concept results showed total insects and number of Ceratopogonidae trapped was similar between traps with a light and those without. Therefore, traps were less expensive and required less maintenance for the two year experiment if they were deployed without light sources. Global positioning system (GPS) coordinates were recorded for each trap location. Cooperating ranches were asked to attach glue boards (Masterline by Univar, #2475, Austin, TX) to the clipboards for the first 5 d of each month. At the conclusion, cooperators bagged the traps in closable plastic bags and stored them in a freezer.

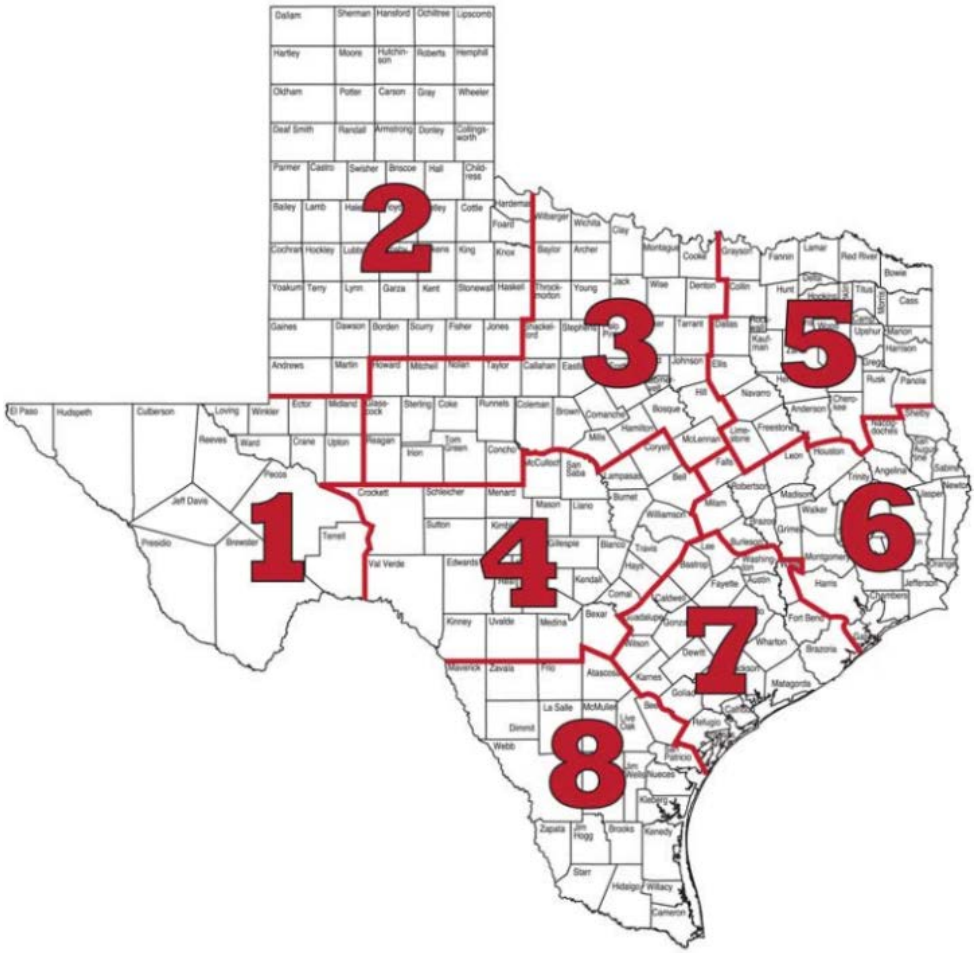

Fig. 2. The eight regions defined by the Texas Deer Association.

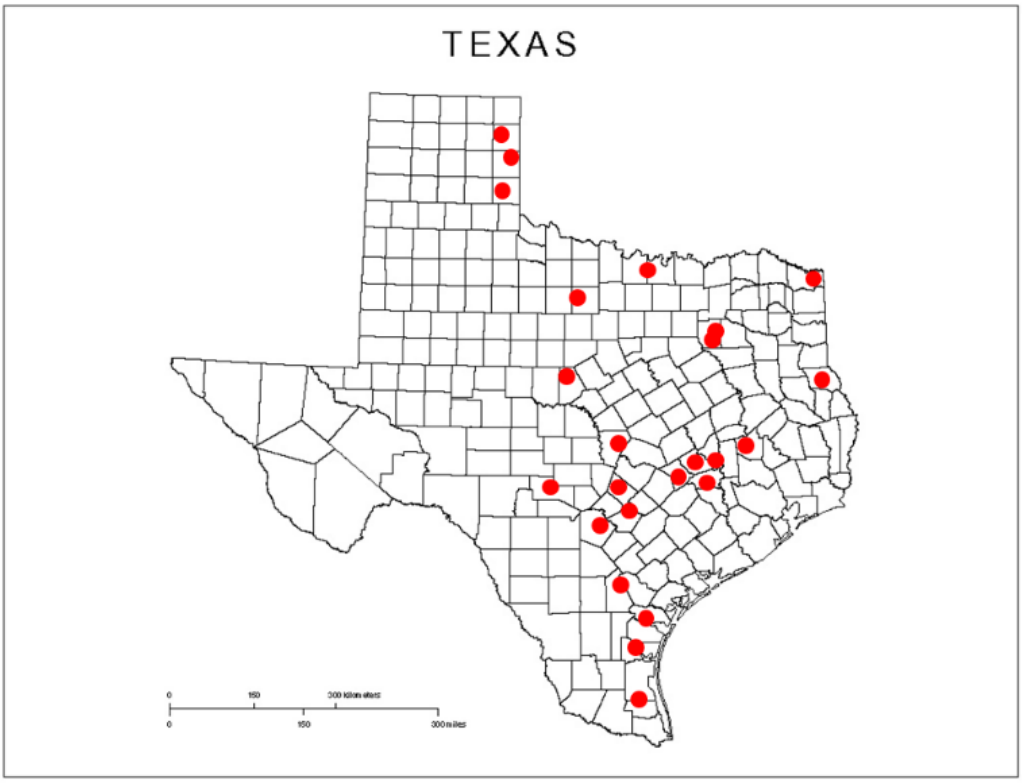

Fig. 3. A graphic display of ranch cooperators. Each location designated by a red dot on the map was sampled once per month over a 24 month period.

Fig. 4. A glue board attached to a fence. The glue boards were secured to a clipboard that had been attached to fences surrounding the deer pens.

Traps were mailed to the Center for Urban and Structural Entomology, Texas A&M University, using mailing labels provided. A box of supplies was distributed to each ranch containing all materials needed for the 24 month survey, including replacement traps and mailing boxes. Once traps were received, a single random quadrant of each glue trap (Fig. 5) was evaluated through a dissecting microscope and a template measuring19.5 x 13 cm with the top quarter square (9.75 x 6.5 cm) cut out. The use of a template assured that the same size area would be evaluated for each trap. The following data were recorded: total number of all insects trapped, number of Ceratopogonidae, Muscidae, Culicidae, Simuliidae, and filth flies. Total number of possible pathogen vectors was calculated by adding the total number of insects classified as Ceratopogonidae, Muscidae, Culicidae, Simuliidae, and filth flies. Weather data, including the mean maximum and minimum temperature and total precipitation, was recorded for the 7 days before trapping and the 5 days during the trapping.

The objective of this experiment was to determine to what capacity all insects, along with possible pathogen vectors and known BTV and EHDV vectors, plagued white-tailed deer-breeding facilities. It was hypothesized that ceratopogonids and other possible pathogen-vectoring dipterans would be prolific. It was also expected that there would be a significant effect of seasonal precipitation and temperature on insect activity.

Fig. 5. Examples of low and high count glue trap. Glue traps were mailed to Texas A&M Center for Urban & Structural Entomology, College Station, Texas, by cooperating producers after sampling. Insects caught by the traps were counted by taxa and recorded as data.

Datum was analyzed using Analysis of Variance (ANOVA). For this experiment, the number of insects (total number of insects, number of Ceratopogonidae, Muscidae, Culicidae, Simuliidae, and filth flies) was totaled for all ten traps at a specific ranch on a specific date. Each of these insect parameters were considered dependent variables. The ranch, the date, the mean temperature and the total precipitation were considered independent variables. An ANOVA procedure was conducted using each independent variable as a factor against each dependent variable. Means separation was performed using Tukey's post-hoc analysis. Values were considered significantly different when $P < 0.05$. The statistical package IBM SPSS version 21.0 was used to perform the analysis (SPSS 2012).

Results

The results from preliminary data taken at Ranch #1 and Ranch #2 during the September 2011 to March 2012 trapping showed that the methods used to trap insects in these types of confined animal facilities were effective. The mean number of all insects trapped on both ranches was highest during the months of September and October 2011 and began to increase again in the month of March (Fig. 6). There were less possible pathogen vectors trapped on Ranch #2 than Ranch #1, but the seasonal trend of vector

abundance was similar to that of total trapped insects (Fig. 7). The trapping method was effective for trapping ceratopogonids, though there was not any evidence of ceratopogonids in the 2012 trapping season (Fig. 8). Figure 9 shows an increase in the number of ceratopogonids trapped in the months of September and October 2011. The data from the preliminary study was used to create distribution map of possible pathogen vectors in each ranch. Ranch #1 had a higher number of insects on traps number 7, 16, and 20 (Fig. 10) than on other traps on the same ranch, as shown by the enlarged size of the red balloons. The buck pens located on Ranch #1 had a larger number of possible pathogen vectors than that of the breeding pens. Traps #21, 23, and 25 showed evidence of a higher number of possible pathogen vectors than any other location surveyed on the ranch (Fig. 11). Ranch #2 is represented in Figure 12. Locations along the fence line that runs southwest to northeast provided more consistent evidence of possible vectors than that of the other trap location. However, trap #7 secured more possible vectors than that of any other location on Ranch #2.

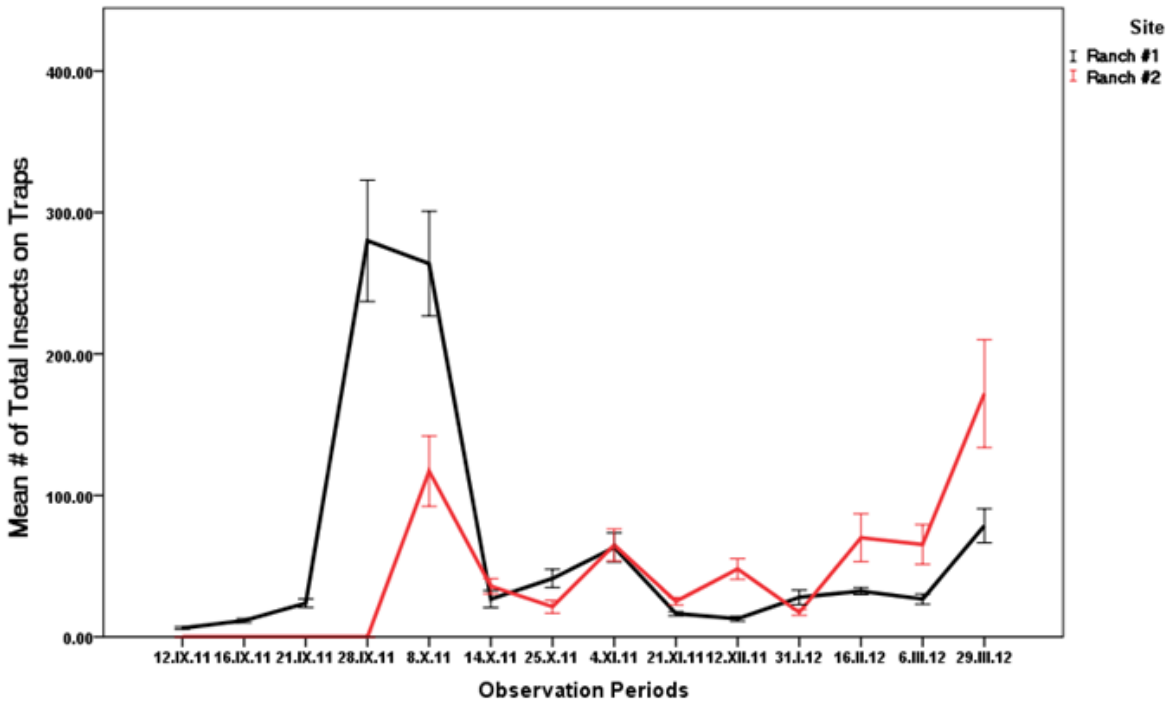

Fig. 6. The mean total number of insects trapped in the preliminary study. Data were collected from two ranches from 12 September 2011 to 29 March 2012. Observation periods were plotted on the x-axis and the mean number of total insects was plotted on the y-axis. The mean number of all insects trapped on both ranches was highest during the months of September and October 2011 and began to increase again in the month of March.

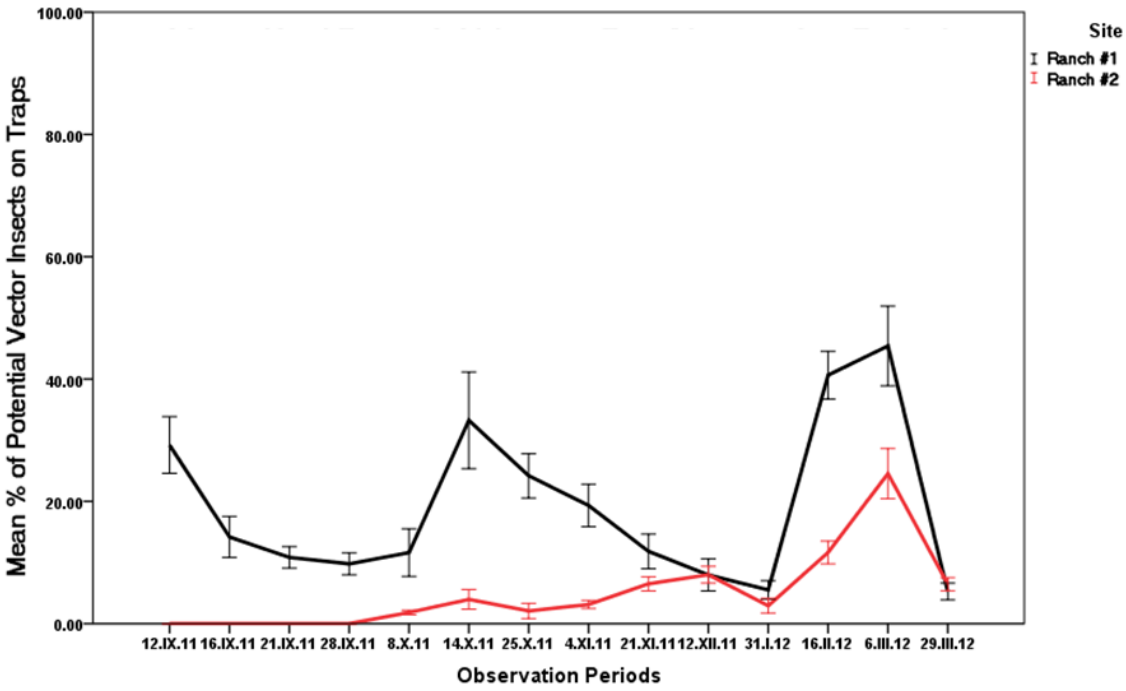

Fig. 7. The mean percent of possible insect vectors trapped in the preliminary study. Data were collected from two ranches from 12 September 2011 to 29 March 2012. Observation periods were plotted on the x-axis and the mean percent of potential vectors trapped was plotted on the y-axis. Possible vectors included a total from Ceratopogonidae, Simuliidae, Culicidae, Muscidae, and filth flies on each trap. Fruit flies, *Drosophila* spp., blow flies (Family: Calliphoridae), stable flies (Family: Sarcophagidae), and moth flies (Family: Psychodidae) were considered part of the filth fly group. There was less possible pathogen vectors trapped on Ranch #2 than Ranch #1.

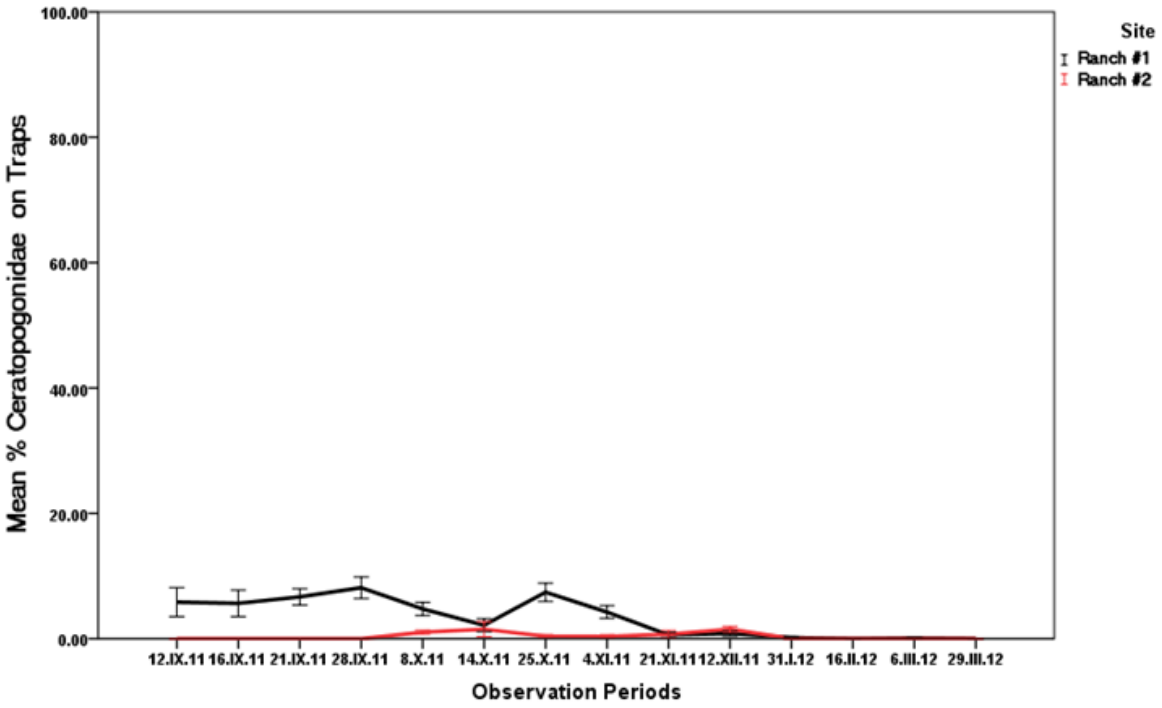

Fig. 8. The mean percentage of ceratopogonids trapped in the preliminary study. Data were collected from two ranches from 12 September 2011 to 29 March 2012. Observation periods were plotted on the x-axis and the mean percent of ceratopogonids trapped was plotted on the y-axis.

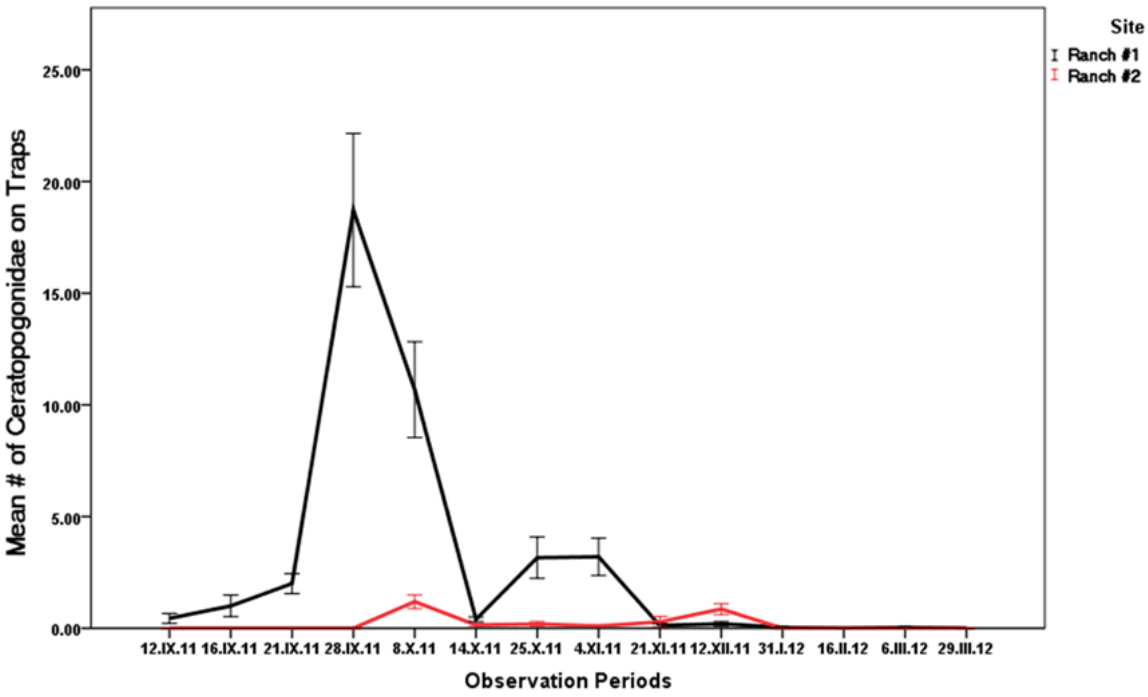

Fig. 9. The mean total number of ceratopogonids trapped in the preliminary study. Data were collected from two ranches from 12 September 2011 to 29 March 2012. Observation periods were plotted on the x-axis and the mean number of ceratopogonids trapped was plotted on the y-axis. There was an increase in the number of ceratopogonids trapped in the months of September and October 2011.

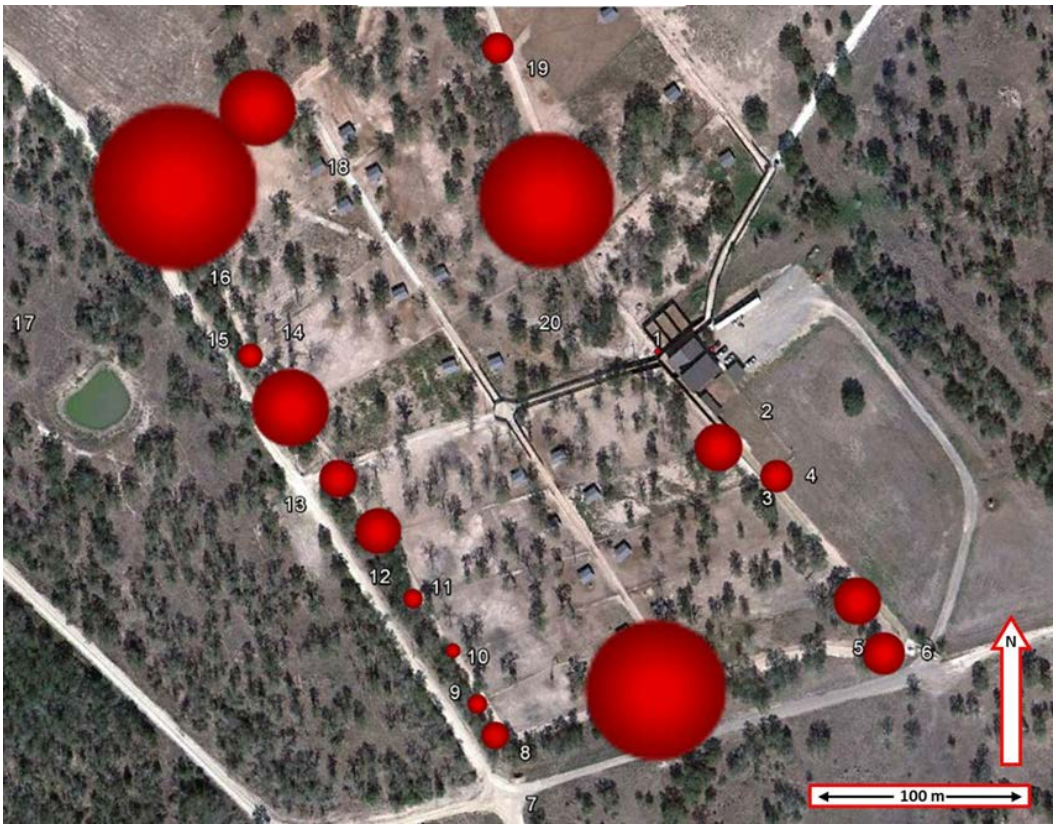

Fig. 10. The spatial distribution of Ceratopogonidae on Ranch #1. Data were recorded 12 September 2011 to 29 March 2012. Each trap location is denoted by a number and a red balloon. The red balloon size is representative of how many ceratopogonids were found in the location during the trapping period. Ranch #1 had a higher number of insects on traps number 7, 16, and 20, than on other traps on the same ranch.

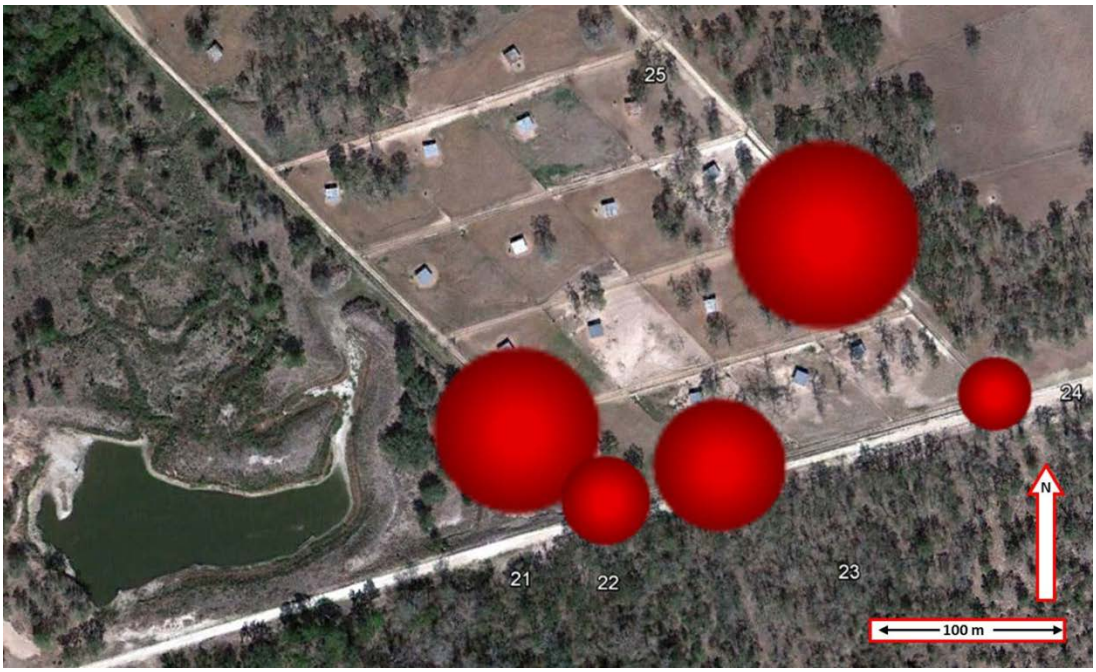

Fig. 11. The spatial distribution of Ceratopogonidae near the buck pens of Ranch #1. Data were recorded 12 September 2011 to 29 March 2012. Each trap location is denoted by a number and a red balloon. The red balloon size is representative of how many ceratopogonids were found in the location during the trapping period. The buck pens located on Ranch #1 had a larger number of ceratopogonids than that of the breeding pens. Traps #21, 23, and 25 showed evidence of a higher number of possible pathogen vectors than any other location surveyed on the ranch.

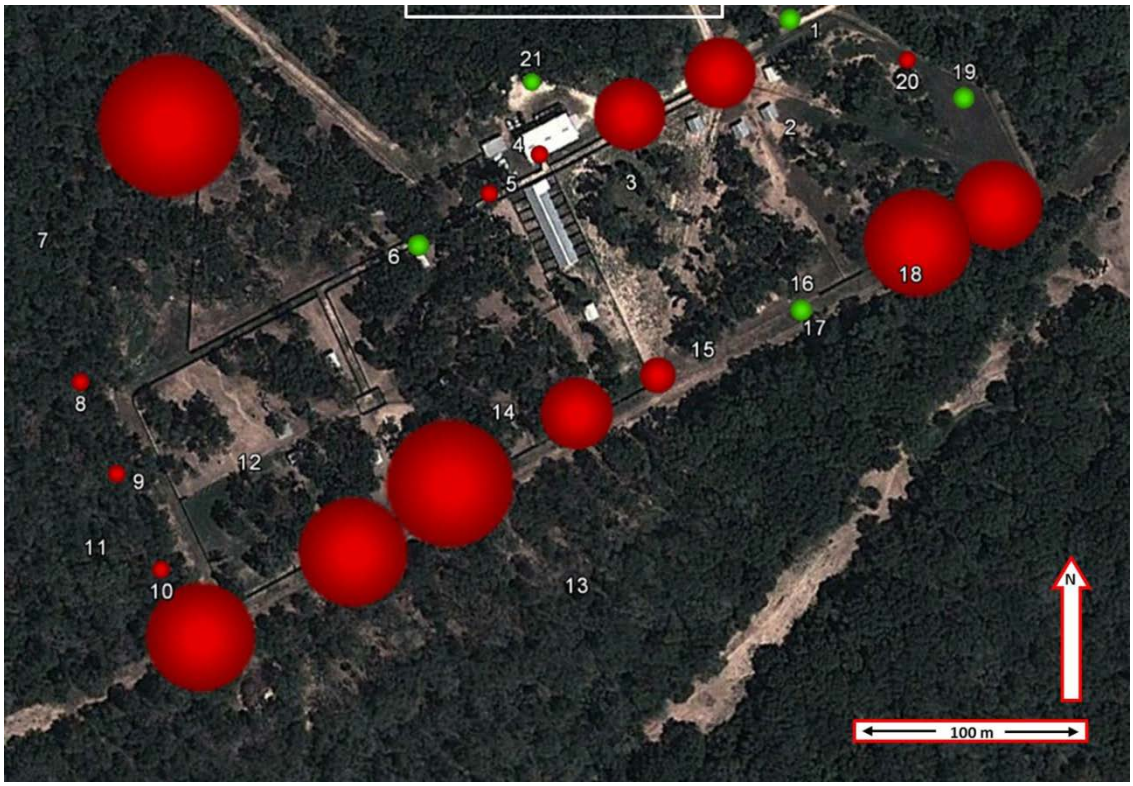

Fig. 12. The spatial distribution of Ceratopogonidae on Ranch #2. Data were recorded 12 September 2011 to 29 March 2012. Each trap location is denoted by a number and a red balloon. The red balloon size is representative of how many ceratopogonids were found in the location during the trapping period. Locations along the fence line that runs southwest to northeast provided more consistent evidence of ceratopogonids than that of the other trap location.

The experiment was expanded to include 24 ranches across the state of Texas in December 2012 and continued until November 2014. Five ranches did not complete the experiment correctly or failed to participate at all, and were not included in the results. The locations that fully participated in the study are displayed in Figure 13. Of the ranches included in these results, Table 1 shows the percentage of traps submitted and the ranches ranked by percent response. Of the ranches that were included in these results, a mean of 84.24% of the traps were received back for evaluation. The total number of traps read was 4,101 and a total of 28, 707 data points were recorded from the traps alone. Data presented in Table 2 shows the ranches ranked based on total number of insects in each category, as noted.

The first analysis conducted was the influence of ranch, or location, on total insects caught. There was a significant difference ($F = 4.033$; df = 18; $P < 0.001$) between ranches based on total insects caught. The post-hoc analysis showed that ranch BRR (n = 86.8) reported a significantly higher number of total insects caught than all other ranches, while L7W (n = 16.25) reported a significantly lower number of total insects caught than all other ranches.

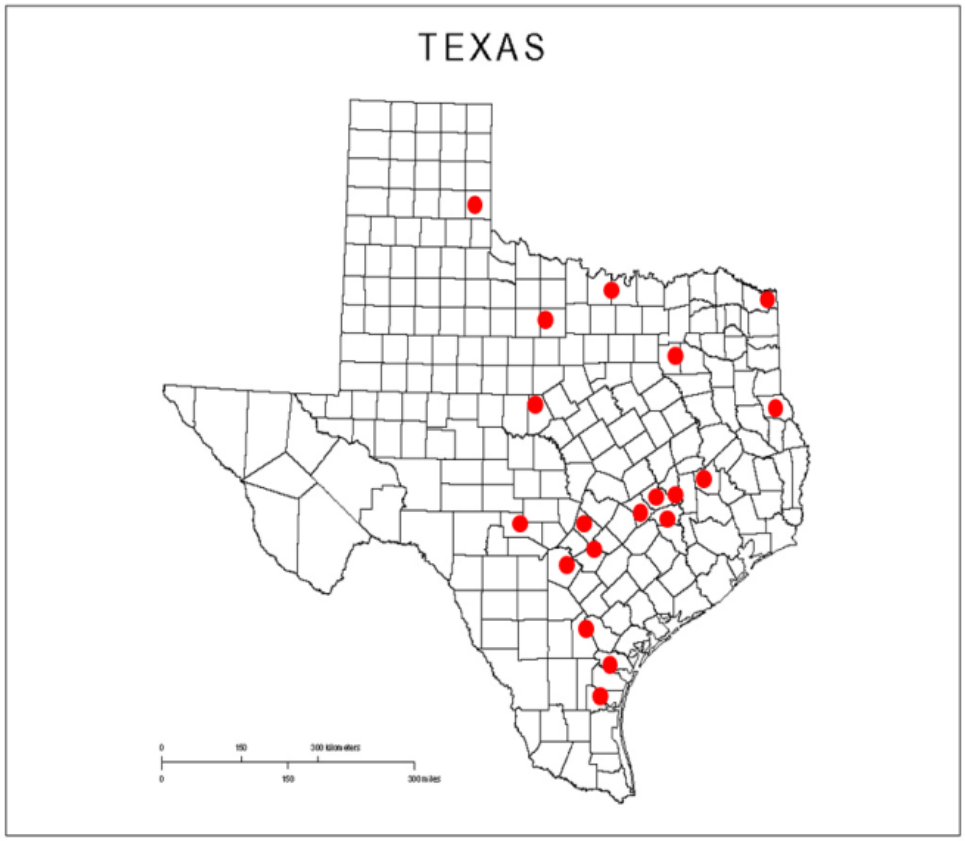

Fig. 13. Map of ranches included in data analysis. Each red dot represents a ranch that completed the experiment in its entirety.

Table 1. The response rate and ranking of cooperating ranches. Only ranches included in the data are listed. Total traps submitted were divided by a total of 240 traps expected. A mean of 84.24% of the traps were received back for evaluation. The total number of traps read was 4,101 and a total of 28, 707 data points were recorded from the traps alone.

Ranking	Ranch	Total Traps Submitted	% Response
1	8	240	100.00%
1	13	240	100.00%
1	16	240	100.00%
4	14	238	99.17%
5	22	237	98.75%
6	18	236	98.33%
7	12	232	96.67%
8	15	225	93.75%
9	17	217	90.42%
10	7	206	85.83%
11	23	203	84.58%
12	2	199	82.92%
13	19	185	77.08%
14	5	178	74.17%
15	24	157	71.36%
16	21	163	67.92%
17	4	151	62.92%
18	9	141	58.75%
19	6	139	57.92%

Table 2. Insect pest ranking by ranch and type. The total for an insect pest type (Ceratopogonidae, Muscidae, Culicidae, Simuliidae, and filth flies) was added up from the entire study. Ranches were ranked by number of total pests.

	Total Insects		Ceratopogonidae		Muscidae		Culicidae		Simuliidae		Filth Flies		Possible Vectors	
Ranch	Ranking	Count	Ranking	Count	Ranking	Count	Ranking	Count	Ranking	Count	Ranking	Count	Ranking	Count
BCR	18	642	17	1	14	65	13	7	14	11	8	119	9	203
3AR	17	704	11	2	17	42	15	4	16	10	18	39	18	97
TRW	14	841	12	2	9	75	8	16	6	18	12	75	11	186
MRW	15	769	18	0	8	80	16	2	18	9	17	50	15	141
KWMA	12	1021	19	0	13	68	19	1	1	27	9	94	10	190
TCW	9	1054	7	4	5	92	17	2	10	15	13	72	12	185
L7W	19	390	13	2	18	38	12	9	13	12	19	30	19	91
TPTW	6	1130	14	2	6	87	2	79	2	24	16	51	8	243
VC	11	1041	4	10	1	199	5	33	15	11	7	141	5	394
HW	10	1052	8	4	7	87	3	34	8	16	6	244	6	385
TJR	7	1085	9	4	16	48	9	16	17	10	14	62	16	140
WEDR	2	1660	2	18	2	178	4	34	4	20	3	343	3	593
FBR	4	1363	15	2	12	69	6	23	11	15	4	324	4	433
WTW	3	1366	5	5	11	70	11	15	19	9	5	283	7	382
RR	13	963	3	13	15	65	18	2	12	15	10	79	14	174
TAMUK	16	713	16	2	10	72	14	7	5	20	11	77	13	178
RLDF	8	1065	10	4	19	38	7	17	7	17	15	56	17	132
BRR	1	2083	1	19	3	161	1	121	9	16	2	442	1	759
CCBW	5	1343	6	5	4	161	10	16	3	22	1	487	2	691

Figure 14 shows the difference among all ranches. After looking at the total insects trapped, it was of interest as to how the ranch location played a role in the number of possible vectors trapped. Possible vectors included a total from Ceratopogonidae, Simuliidae, Culicidae, Muscidae, and filth flies on each trap. Fruit flies, *Drosophila* spp., blow flies (Family: Calliphoridae), stable flies (Family: Sarcophagidae), and moth flies (Family: Psychodidae) were considered part of the filth fly group. There was a strong significant difference ($F = 5.182$; df = 18; $P < 0.001$) in the number of possible vectors trapped on each ranch. Ranches BRR (n = 31.6) and CCBW (n = 28.8) were prone to more possible vectors than any other ranch, while ranches L7W (n= 3.79), 3R (n = 4.04), RLDF (n = 5.5), TJR (n = 5.83), and MRW (n = 588) provided data that showed they had a lower number of total possible vectors than any other ranches. Figure 15 shows the difference in possible vectors among all ranches in the study. After comparing the number of vectors, it was sensible to take a look at the total number of ceratopogonids sampled on each ranch with respect to ranch location. Again ranch BRR (n = 0.79) had a significantly ($F = 2.870$; df = 18; $P < 0.001$) higher number of ceratopogonids sampled than any other ranch. Ranches MRW (n = 0), KWMA (n = 0), and BCR (n = 0.04) had a significantly lower number of biting midges sampled than the other 16 ranches. The difference in number of ceratopogonids trapped on each ranch is expressed in Figure 16.

Ranches were then classified by their location in ecoregions, as determined by the United State Environmental Protection Agency. The Level III ecoregions are shown

in Figure 17 (TPW 2011). There was a significant difference (P = 0.008) in the number of ceratopogonids trapped between ecoregions. A Duncan's post-hoc analysis shows

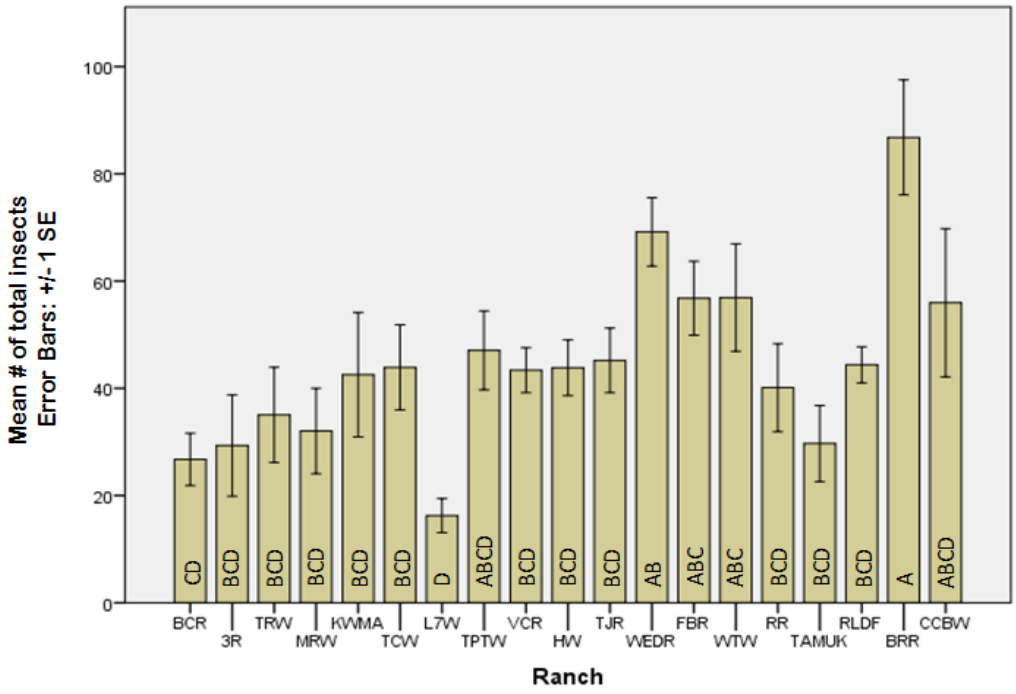

Fig. 14. A comparison of the total number of insects trapped based on ranch. The ranch name was listed on the x-axis and the mean number of all insects trapped is denoted on the y-axis. There was a significant (F = 4.033; df = 18; P < 0.001) difference between BRR and all other ranches. Ranch L7W had a significantly lower number of insects than all other ranches in the study. Means separation was determined by Tukey's LSD. Means with the same letter are not significantly different (α = 0.05).

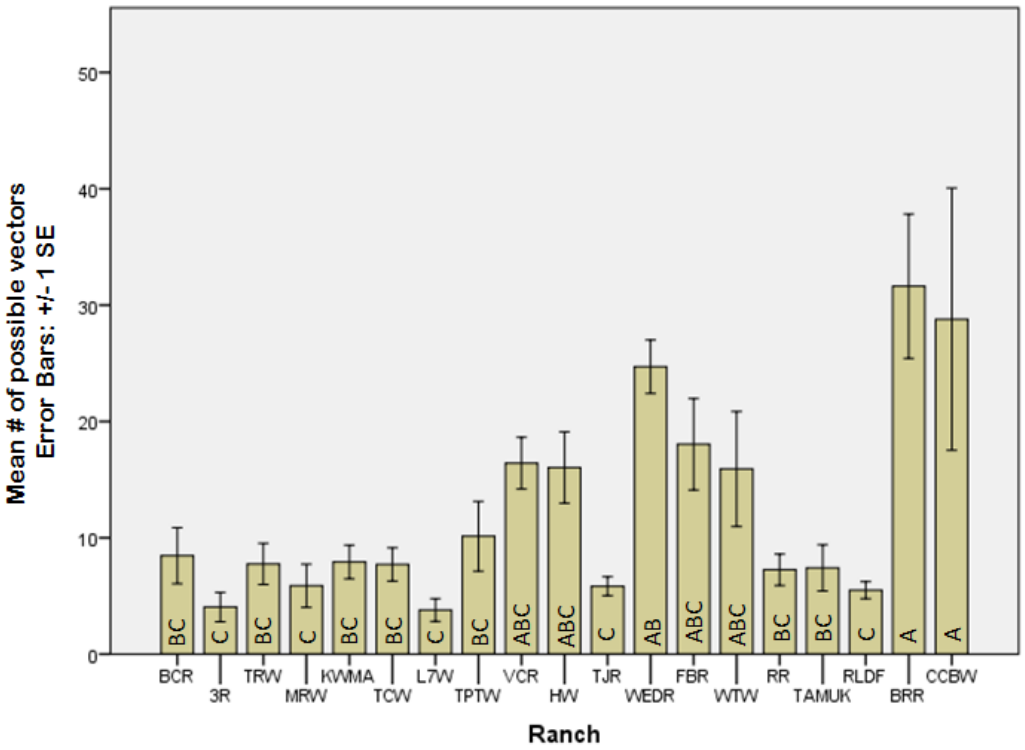

Fig. 15. A comparison of the total number of possible pathogen vectors trapped based on ranch. The ranch name was listed on the x-axis and the mean number of possible vectors trapped is denoted on the y-axis. Possible vectors included a total from Ceratopogonidae, Simuliidae, Culicidae, Muscidae, and filth flies on each trap. Fruit flies, *Drosophila* spp., blow flies (Family: Calliphoridae), stable flies (Family: Sarcophagidae), and moth flies (Family: Psychodidae) were considered part of the filth fly group. There was a significant (F = 5.182; df = 18; P < 0.001) difference between BRR and CCBW and all other ranches. Means separation was determined by Tukey's LSD. Means with the same letter are not significantly different ($\alpha = 0.05$).

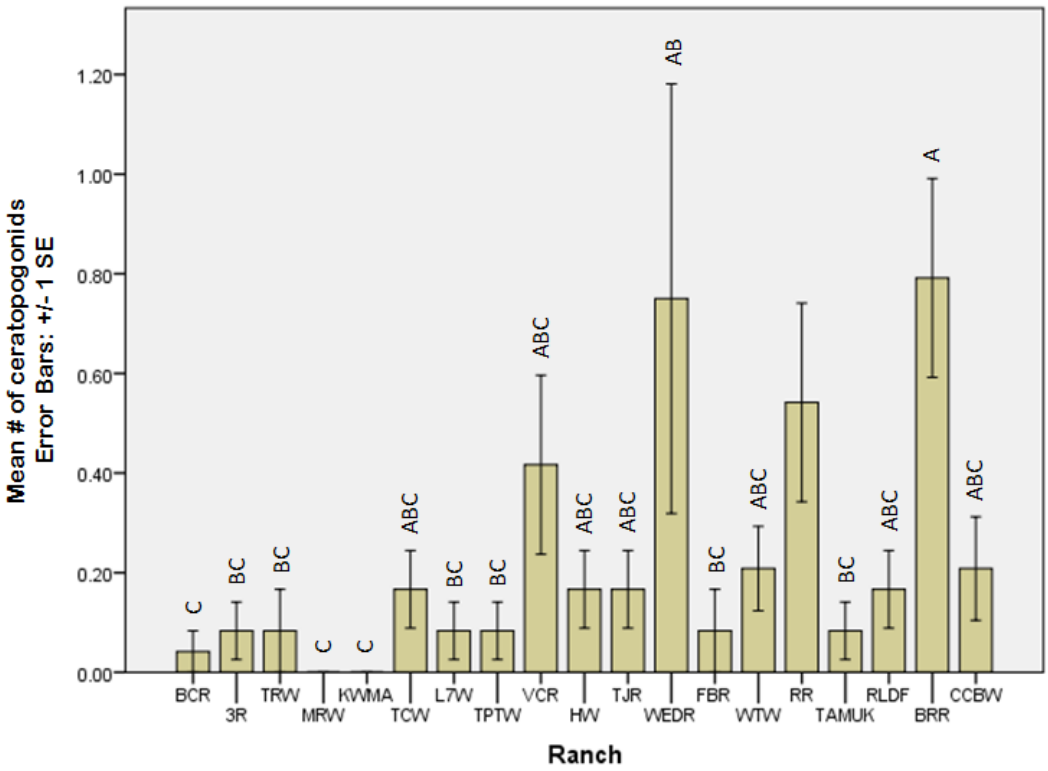

Fig. 16. A comparison of the mean number of ceratopogonids trapped on participating ranches over the 24 month sampling period. The ranch name was listed on the x-axis and the mean number of ceratopogonids trapped is denoted on the y-axis. There was a significant (F = 2.870; df = 18; P < 0.001) difference between BRR and all other ranches. Means separation was determined by Tukey's LSD. Means with the same letter are not significantly different (α = 0.05).

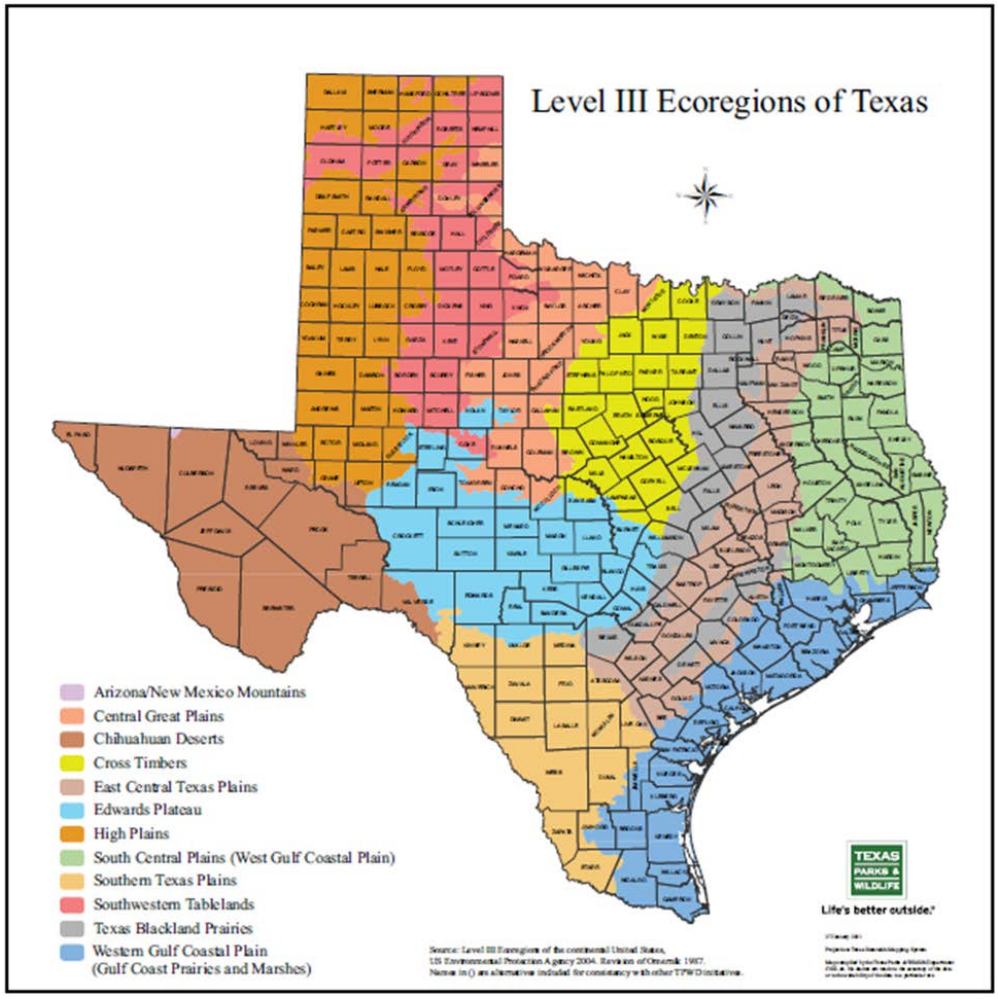

Fig. 17. Level III ecoregions of Texas (TPW 2011). Ranches were classified in an ecoregion by their location. Ranches occurred in the following ecoregions: Central Great Plains, Cross Timbers, East Central Texas Plains, Edwards Plateau, South Central Plains, Texas Blackland Prairies, and Western Gulf Coastal Plain.

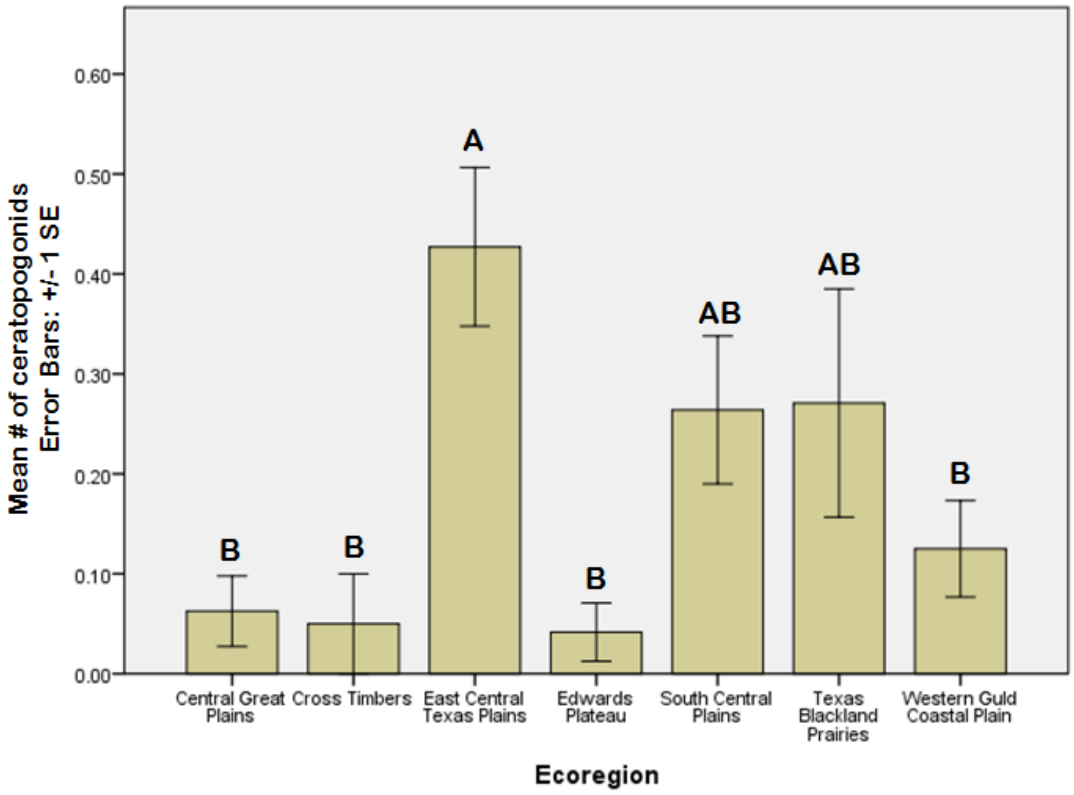

Fig. 18. Mean number of Ceratopogonidae trapped in each Texas ecoregion. The ecoregion was listed on the x-axis and the mean number of ceratopogonids trapped is denoted on the y-axis. There was a significant difference (P = 0.008) in the number of ceratopogonids trapped between ecoregions. Means separation was determined by Duncan's post-hoc analysis. Means with the same letter are not significantly different (α = 0.05).

there to be a higher mean number of Ceratopogonidae trapped in the East Central Texas Plains than other ecoregions (Fig. 18). Ranches BCR and 3R are classified in this ecoregion. Ranches CCBW, VC, and HW are located in the South Central Plains and ranches TPTW, WEDR, FBR, and TCW are located in the Texas Blackland Prairies. The mean number of ceratopogonids trapped in the South Central Plains and the Texas Blackland prairies was similar to that of the East Texas Plains. All other ranches were located in the Central Great Plains, Cross Timbers, Edwards Plateau, and Western Gulf Coastal Plain.

The next variable that was compared among the trapping variables was date. The time of year was first compared to the total insects trapped. The total numbers of Ceratopogonidae, Muscidae, Culicidae, Simuliidae, and filth flies from all ranches are compared to each other over time in Figure 19. The number of filth flies was consistently higher than all other types of flies, with the exception of muscid flies that had higher numbers in the winter of 2013. The number of Ceratopogonid flies trapped on all ranches was consistently lower than that of all other fly types recorded. As shown in Figure 20, May of 2013 had significantly more ($F = 6.587$; df = 23; $P < 0.001$) mean insects trapped (n = 105.2) than any other month. Alternatively, December 2013 and January 2014 had significantly fewer insects trapped than any other month at 19.9 and 17.5, respectively. When comparing the date of trapping to the total number of possible pathogen vectors, there was a significant difference ($F = 1.604$; df = 23; $P = 0.039$), though a Tukey's post-hoc analysis did not show separation between the groups (Fig. 21). Figure 22 is a comparison of the date of trapping with respect to the mean number

of ceratopogonids trapped. An ANOVA analysis showed that there was a significant difference (F = 1.699; df = 23; P = 0.024) between trapping months, a Tukey's post-hoc analysis did not show a separation of means.

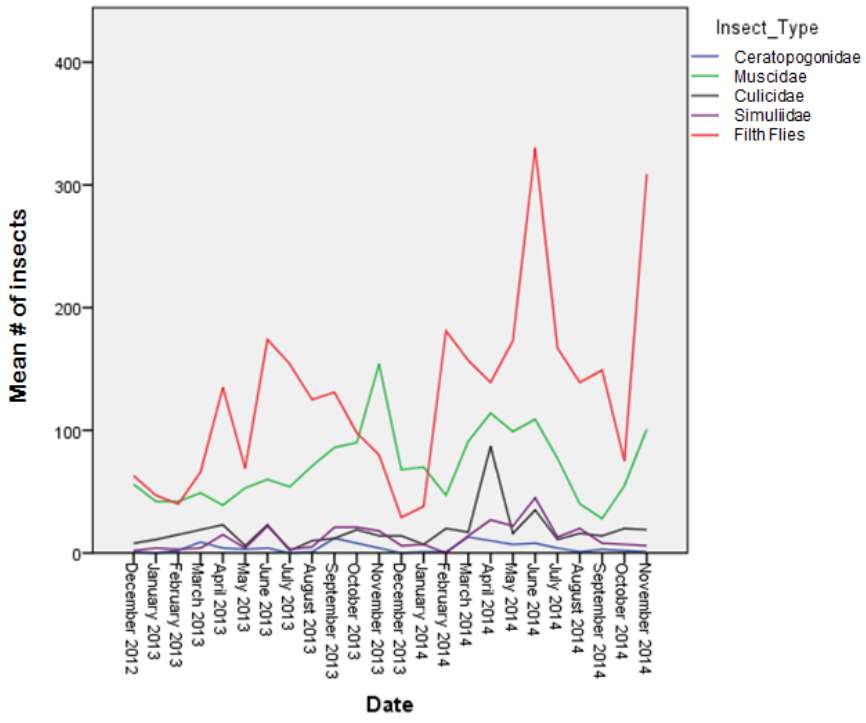

Fig. 19. Insect type by sample month. The total number of each insect type (Ceratopogonidae, Muscidae, Culicidae, Simuliidae, and filth flies) were compared across sampling months. On average, there were more filth flies present on ranches than any other fly sampled throughout the year. Fruit flies, *Drosophila* spp., blow flies (Family: Calliphoridae), stable flies (Family: Sarcophagidae), and moth flies (Family: Psychodidae) were considered part of the filth fly group.

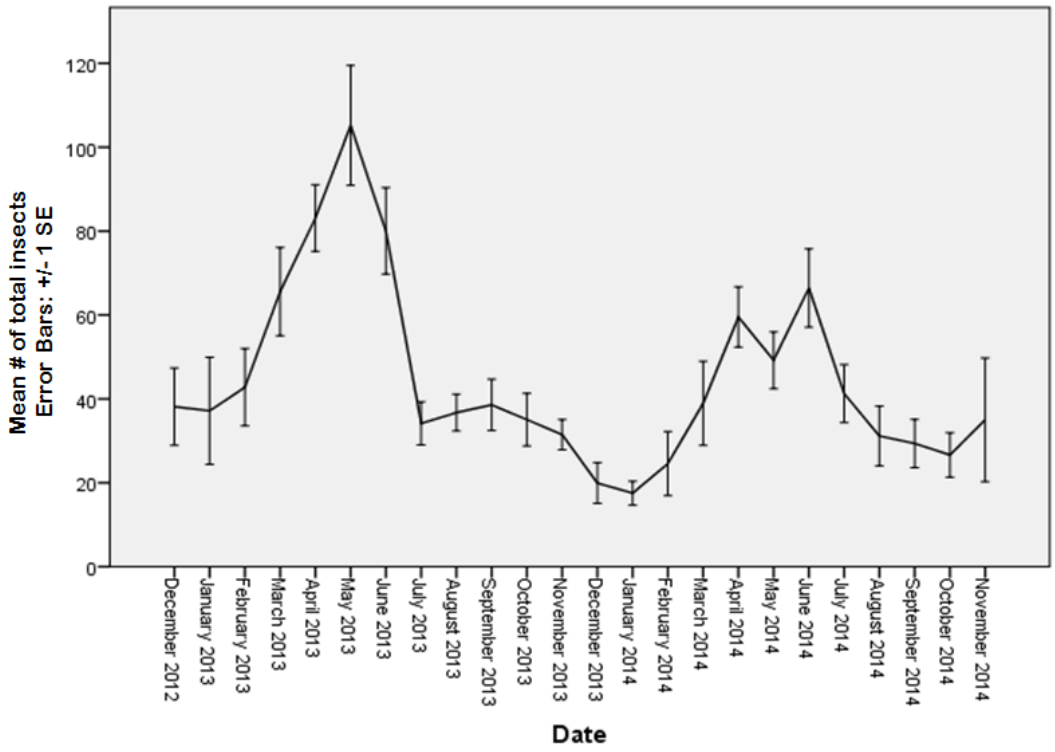

Fig. 20. A comparison of the mean total number of insects trapped on 19 cooperating ranches with respect to date. The trapping date is listed on the x-axis and the mean number of total insects is listed on the y-axis. May 2013 had significantly (F = 6.587; df = 23; $P < 0.001$) more insects trapped than any other month sampled.

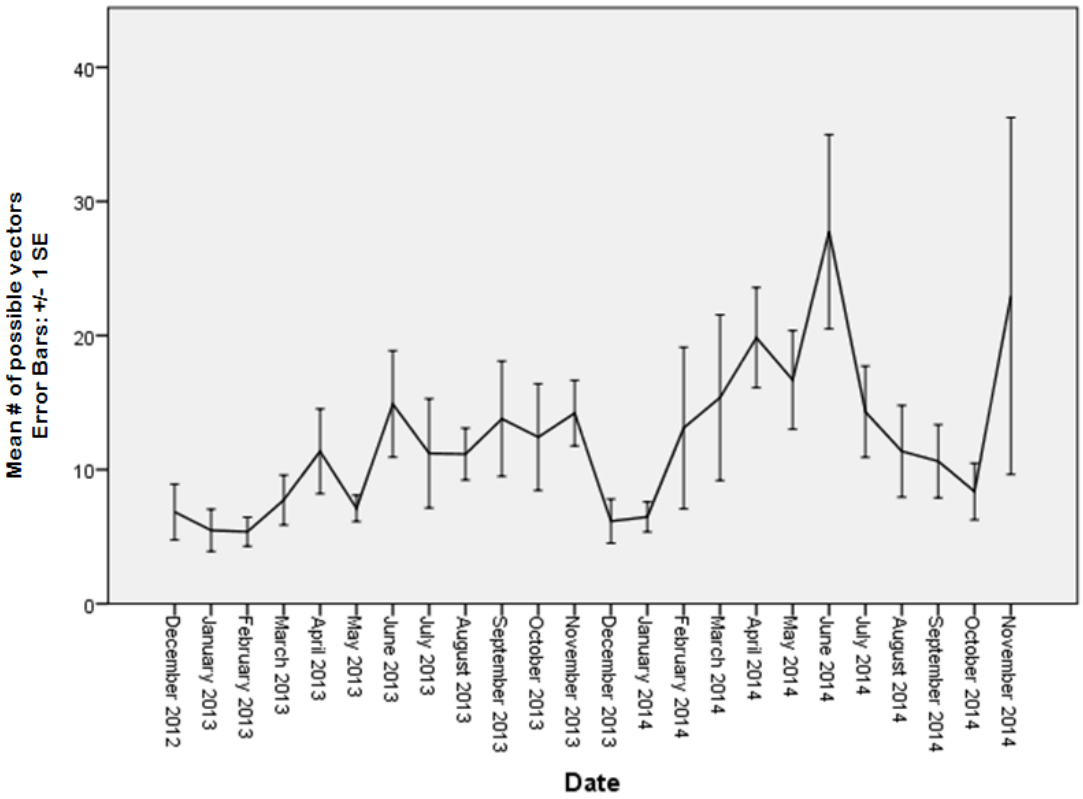

Fig. 21. A comparison of the mean number of possible pathogen vectors with respect to date. The trapping date is listed on the x-axis and the mean number of possible vectors is listed on the y-axis. Possible vectors included a total from Ceratopogonidae, Simuliidae, Culicidae, Muscidae, and filth flies on each trap. Fruit flies, *Drosophila* spp., blow flies (Family: Calliphoridae), stable flies (Family: Sarcophagidae), and moth flies (Family: Psychodidae) were considered part of the filth fly group. Tukey's HSD found no separation between means. ANOVA showed a significant difference ($F = 1.604$; df = 23; $P = 0.039$) in the mean number of possible vectors by date.

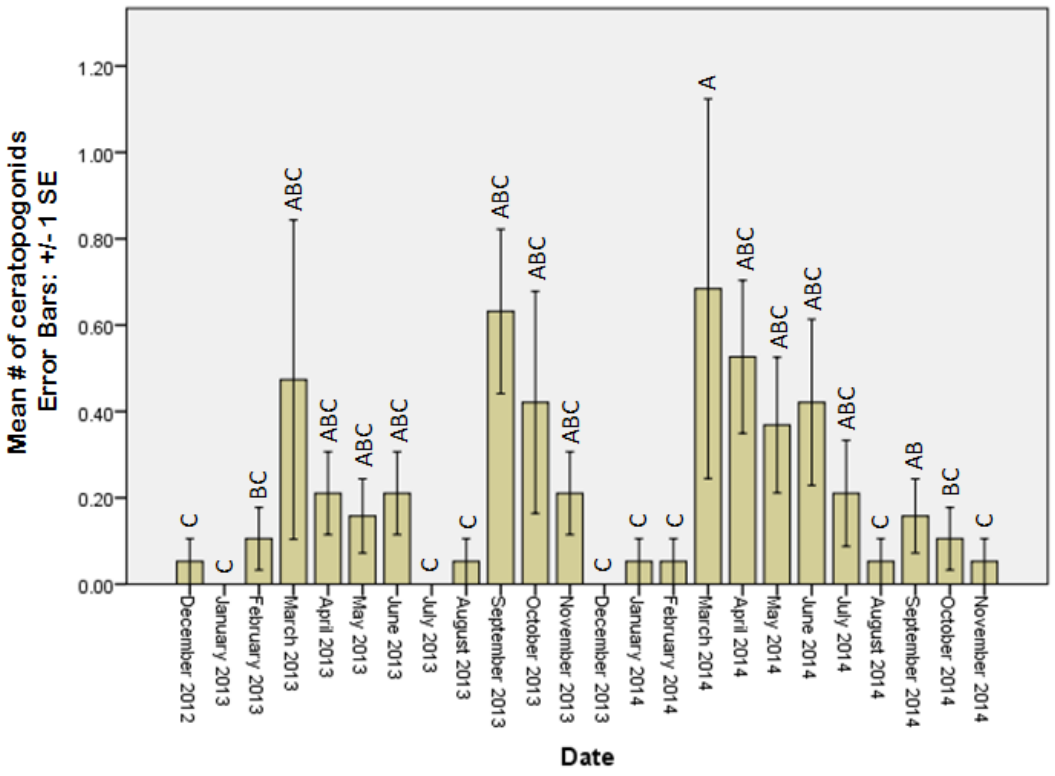

Fig. 22. A comparison of mean number of ceratopogonids trapped with respect to date. The trapping date is listed on the x-axis and the mean number of ceratopogonids is listed on the y-axis. ANOVA showed a significant difference (F = 1.699; df = 23; P = 0.024) in the mean number of ceratopogonids by date. Means separation was performed by Duncan's multiple range test. Means with the same letter are not significantly different (α = 0.05).

Climatological data were recorded for the duration of the experiment using the National Oceanic and Atmospheric Administration records. The maximum and minimum temperature for the 7 days before the trapping and the 5 days of trapping in each month was recorded. For this same time, the total precipitation was recorded. This datum was used to provide a curve estimation of dependent variables. Figure 23 is a curve estimation of the mean temperature based on total insects trapped. On average, the daily temperature was 19.32°C, and the mean number of insects trapped was 44.5 insects. This total is broken down to only include possible pathogen vectors (Fig. 24). The scatter plot with quadratic line shows the curve of the prediction line to have less slope than that of the Figure 23, meaning that there are less possible insects, on average, than total insects. The mean daily temperature was still 19.32°C, but the mean number of expected possible pathogen vectors was 12.3. When comparing mean daily temperature to the number of ceratopogonids trapped (Fig. 25), it is difficult to determine the slope of the quadratic line due to low trap numbers; however, it was evident that all ceratopogonids were trapped between -0.22 and 32.51°C.

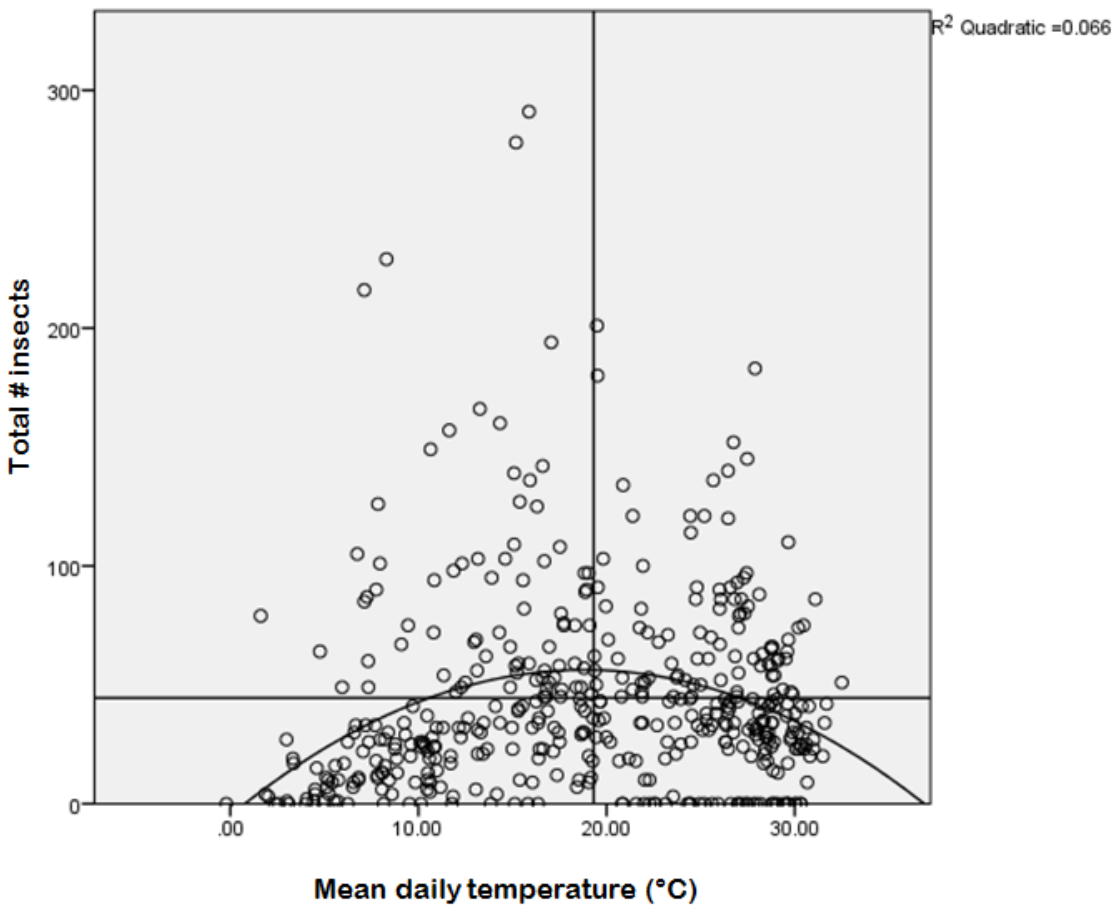

Fig. 23. A curve estimation of the total number of insects trapped with respect to mean daily temperatures. The maximum and minimum temperature for the 7 days before the trapping and the 5 days of trapping in each month was recorded and averaged. The mean daily temperature is displayed on the x-axis and y-axis shows the total number of insects. On average, the daily temperature was 19.32°C, and the mean number of insects trapped was 44.5 insects. Quadratic slope (R^2 = 0.066) of the line is $y = -4.95+6.51x-0.17x^2$.

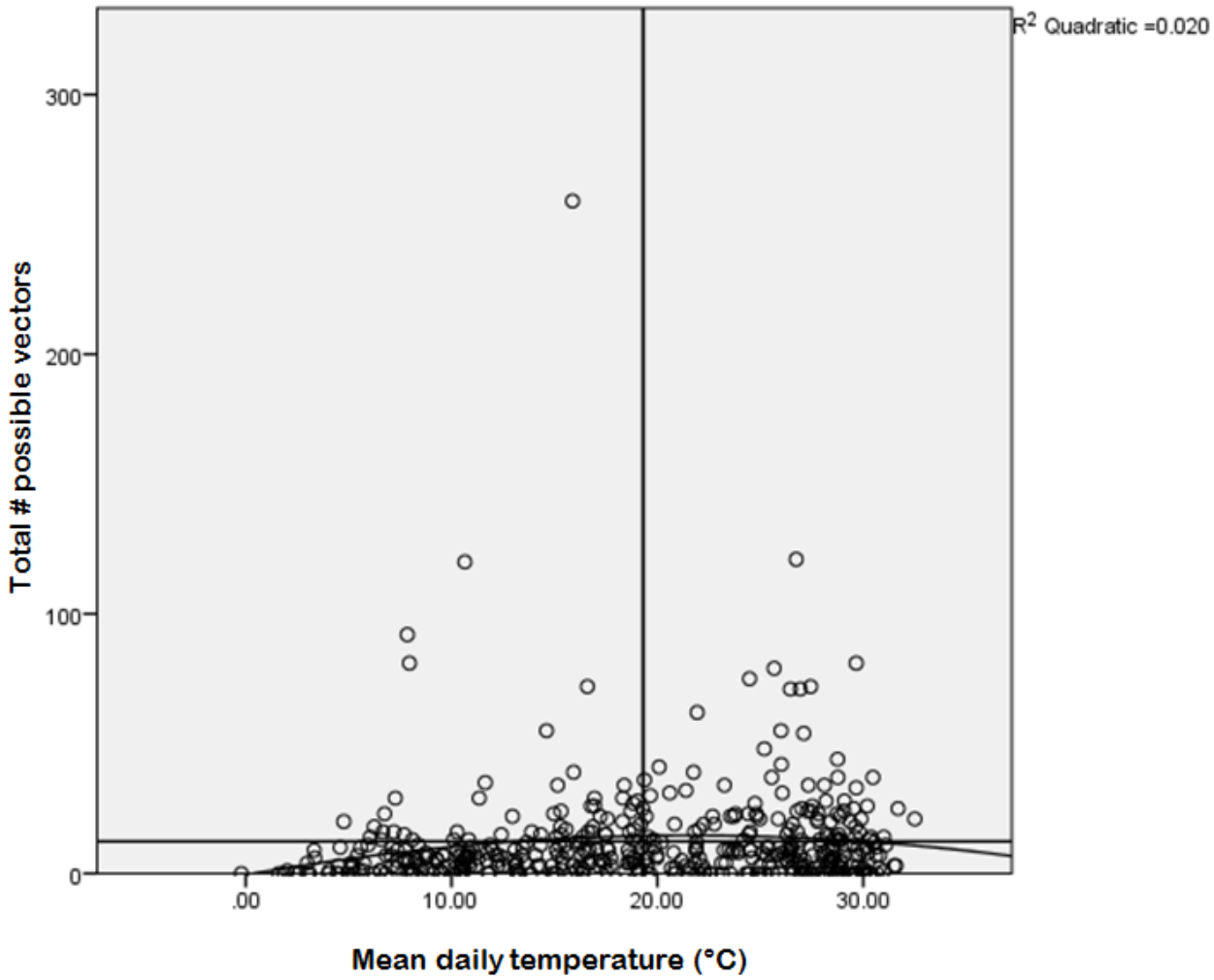

Fig. 24. A curve estimation of the possible pathogen vectors trapped with respect to mean daily temperatures. The maximum and minimum temperature for the 7 days before the trapping and the 5 days of trapping in each month was recorded and averaged. The mean daily temperature is displayed on the x-axis and y-axis shows the total number of possible vectors. Possible vectors included a total from Ceratopogonidae, Simuliidae, Culicidae, Muscidae, and filth flies on each trap. Fruit flies, *Drosophila* spp., blow flies (Family: Calliphoridae), stable flies (Family: Sarcophagidae), and moth flies (Family: Psychodidae) were considered part of the filth fly group. On average, the daily temperature was 19.32°C, and the mean number of insects trapped was 12.3 insects. Quadratic slope ($R^2 = 0.020$) of the line is $y = 0.54+1.41x-0.03x^2$.

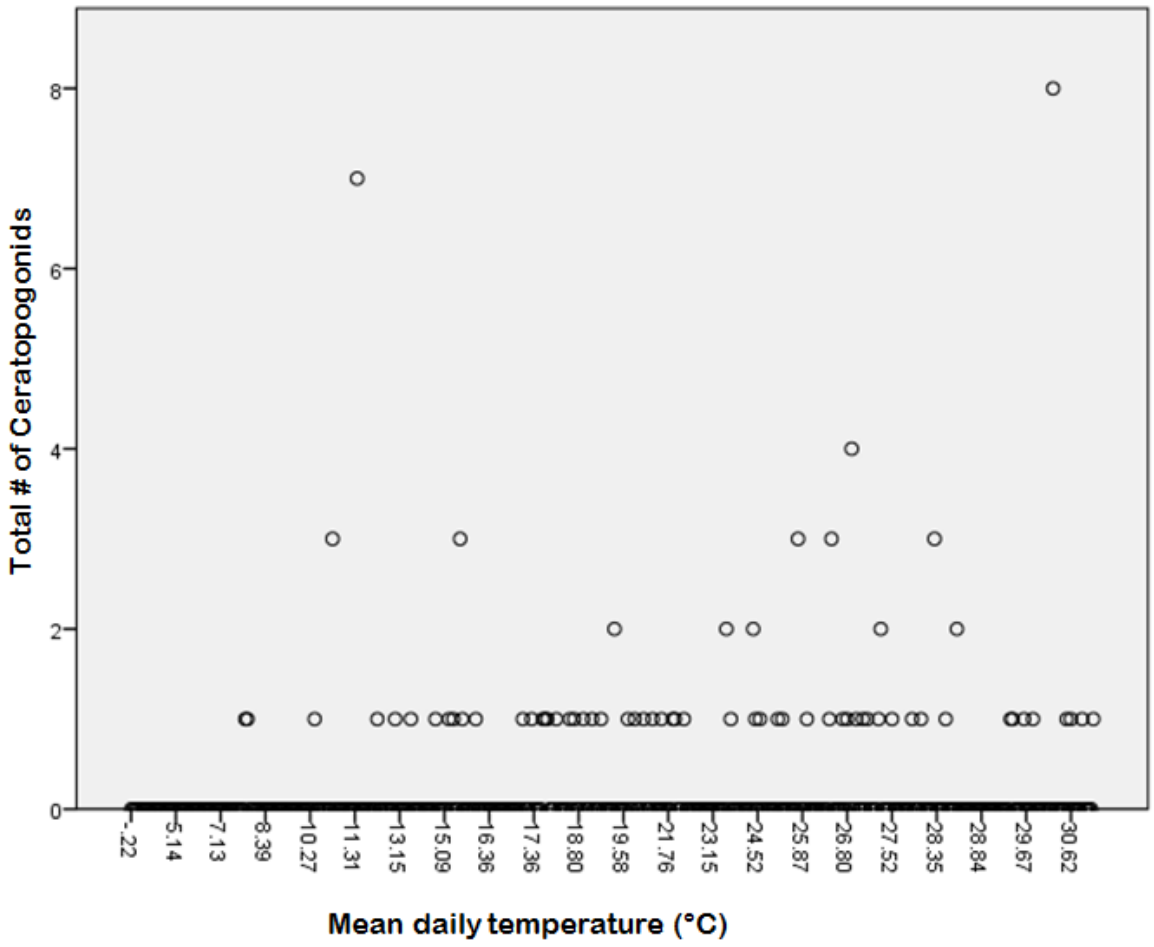

Fig. 25. Distribution of trapped ceratopogonids with respect to mean daily temperatures. The maximum and minimum temperature for the 7 days before the trapping and the 5 days of trapping in each month was recorded and averaged. The mean daily temperature is displayed on the x-axis and y-axis shows the total number of ceratopogonids. On average, the daily temperature was 19.32°C, and the mean number of insects trapped was 0.2 insects. All Ceratopogonids were trapped between -0.22 and 32.51°C.

The next comparison made among dependent variables was to the total precipitation throughout the collection time frame, and any precipitation deposited 7 d before trapping. Figure 26 shows a curve estimation of the total precipitation with relation to the total number of insects trapped. Mean precipitation received during the sampling period was 2.21 mm and mean number of total insects trapped was 44.5. Figure 27 shows only the total possible insect pathogen vectors trapped on the y-axis with total precipitation received on the x-axis. Mean total precipitation received when trapping possible pathogen vectors was 1.04 mm. On average, each ranch reported 12.7 possible insect vectors per month. Comparing the number of ceratopogonids trapped to total precipitation is shown in Figure 28. The mean number of ceratopogonids reported per trap was 0.2 with the same mean precipitation of 2.21 mm.

Discussion

The objective of this experiment was to determine Ceratopogonidae and other possible pathogen vector activity over the course of two years on Texas white-tailed deer-breeding operations. Of those insect sampled, the number of ceratopogonids, known BTV and EHD vectors, and other possible pathogen vectors in a certain location

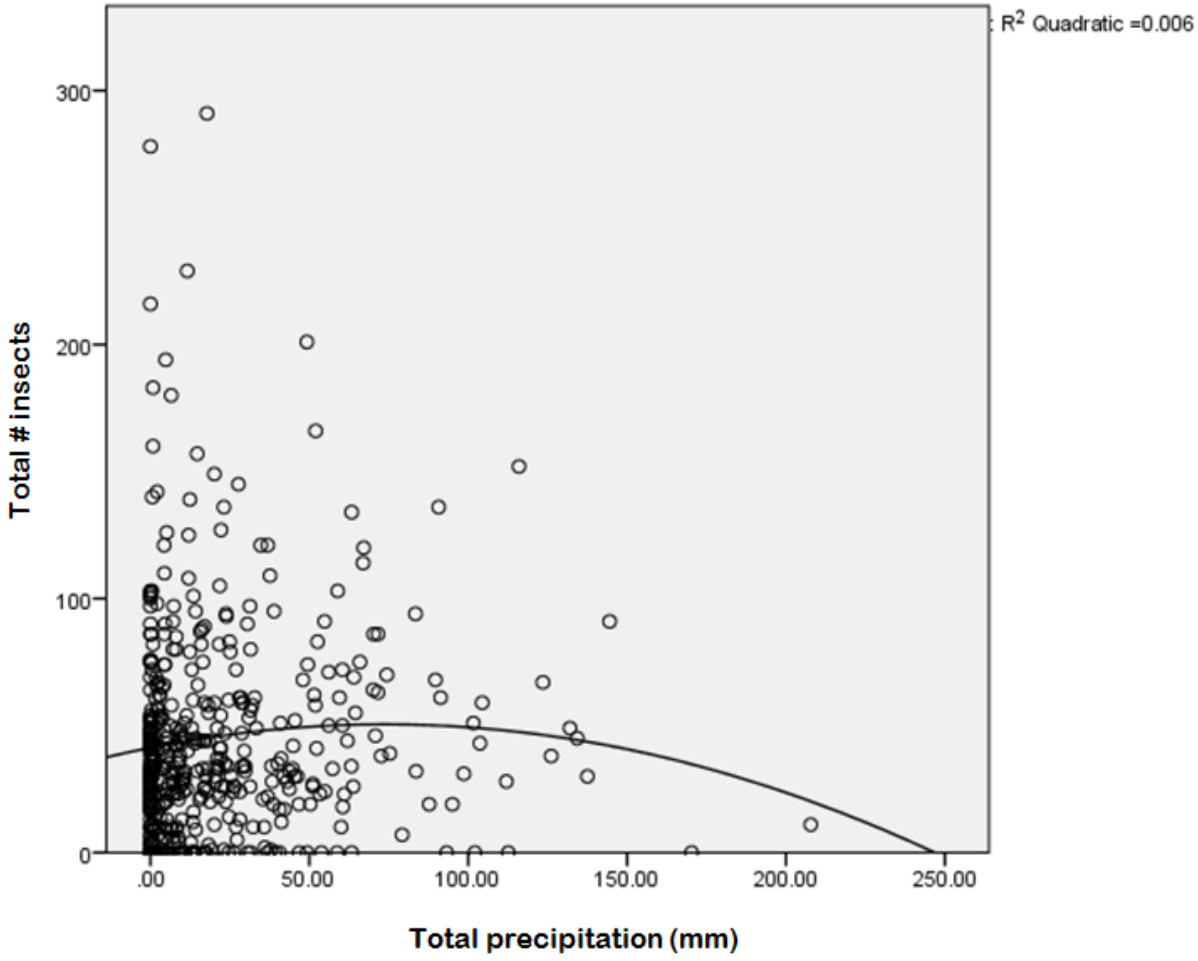

Fig. 26. Curve estimation of the total insects trapped with respect to the total precipitation received. Total precipitation for the 7 days before the trapping and the 5 days of trapping in each month was recorded. The total precipitation is displayed on the x-axis and y-axis shows the total number of insects. On average, the total sampling period precipitation was 2.21 mm, and the mean number of insects trapped was 44.5 insects. Quadratic slope (R^2 = 0.006) of the line is y = 41.29+0.25x-1.69E-3x^2.

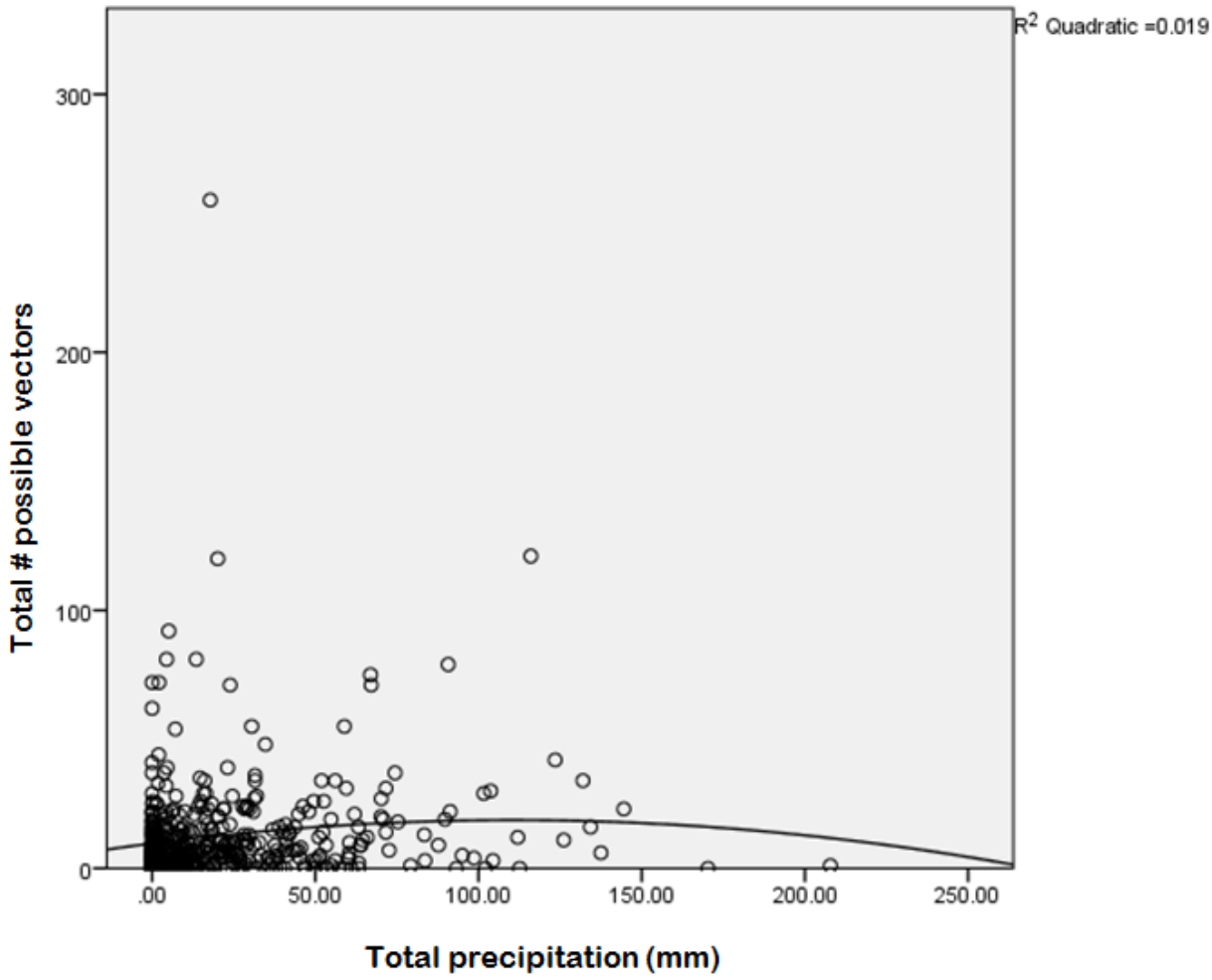

Fig. 27. Curve estimation of the total possible pathogen vectors trapped with respect to the total precipitation received. Total precipitation for the 7 days before the trapping and the 5 days of trapping in each month was recorded. The total precipitation is displayed on the x-axis and y-axis shows the total number of possible vectors. Possible vectors included a total from Ceratopogonidae, Simuliidae, Culicidae, Muscidae, and filth flies on each trap. Fruit flies, *Drosophila* spp., blow flies (Family: Calliphoridae), stable flies (Family: Sarcophagidae), and moth flies (Family: Psychodidae) were considered part of the filth fly group. The mean total precipitation during the sampling period was 2.21 mm, and the mean number of possible vectors trapped was 12.7 insects. Quadratic slope (R^2 = 0.019) of the line is y = 9.69+0.16x-7.36E-4x^2.

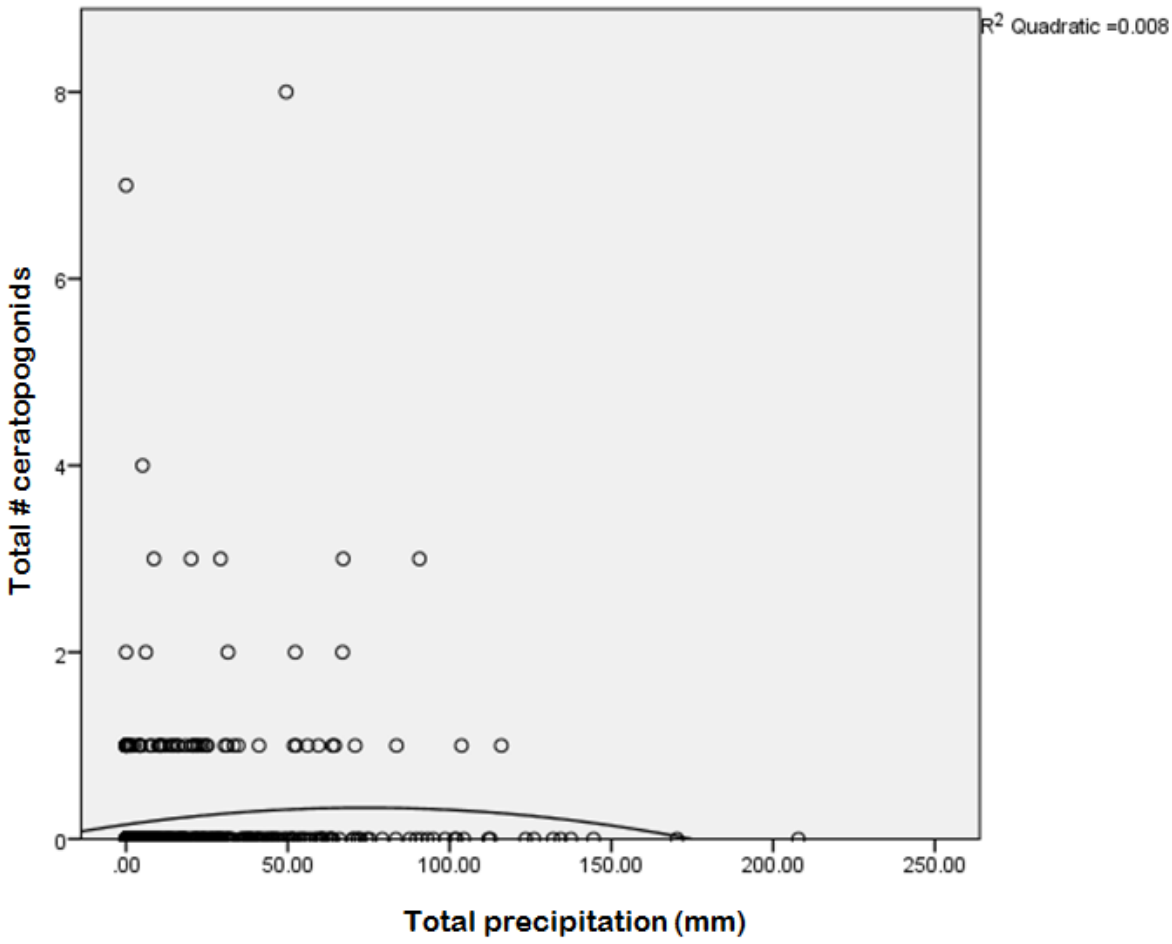

Fig. 28. Curve estimation of the number of ceratopogonids trapped with respect to the total precipitation received. Total precipitation for the 7 days before the trapping and the 5 days of trapping in each month was recorded. The total precipitation is displayed on the x-axis and y-axis shows the total number of ceratopogonids. On average, the total precipitation was 2.21 mm, and the mean number of Ceratopogonids trapped was 0.2 insects. Quadratic slope (R^2 = 0.008) of the line is y = 0.15+4.86E-3x-3.29E-5x^2.

could be calculated. Possible vectors included a total from Ceratopogonidae, Simuliidae, Culicidae, Muscidae, and filth flies on each trap. Fruit flies, *Drosophila* spp., blow flies (Family: Calliphoridae), stable flies (Family: Sarcophagidae), and moth flies (Family: Psychodidae) were considered part of the filth fly group. The results of the preliminary data proved that, at sometimes 40% of the total insect population, the presence of possible vectors on white-tailed deer ranches was a problem that needed to be addressed. ceratopogonids, the known vector of BTV and EHDV, were also trapped using the methods developed in the target production system. The results from the preliminary data were compounded into spatial maps that show possible vector distribution between trapping locations. The inflated marker balloons shown on the map were able to assist in developing conclusions about from where the ceratopogonids may be travelling. In Figure 10, the balloon for trap 17 is enlarged. This may be due to its proximity to the pond that is approximately 100 m away. As discussed, water sources are known breeding sources for ceratopogonids and other possible pathogen vectors. The ranch depicted in Figure 12 is unique in that there are not any large sources of water in close proximity (500 m) to the deer-breeding pens. However, there is evidence of vector activity depicted on the distribution map. It was found that there was a ditch that ran parallel to the fence line that held water after rain events and provided a conducive environment for insect development. This information was provided to the deer producers and was used to place deer in areas that were less conducive to insect activity.

The experiment was expanded to included ranches from across the state of Texas. Nineteen ranches were included in the study results out of the 24 that started in the

study. It was evident in the results that working with multiple locations out of range for conducting the experiment personally could be difficult. Table 2 show the ranches ranked by type of insect trapped. Ranch BRR ranked in the top three ranches for six of the seven insect counts taken, while L7W ranked consistently lower in insects counts than other ranches for all measurements.

The first variable evaluated was the ranch location. Ranch BRR reported the highest number of insects trapped on average. This location is within 100 m of the Brazos River, a slow moving water source that could be optimal for insect population growth. Also, the pens were surrounded by tall trees that provide prime insect habitats. Alternatively, The L7W ranch had the lowest mean number of insects. This ranch was in a much more rocky location, which may account for less overall insect activity, but also sent in one of the fewest number of traps (58.75%) which could explain the low insect count. The two ranches that had the highest mean number of possible vectors in those insects trapped were BRR and CCBW. It is expected that BRR be one of the highest because this location had a significantly higher number of insects overall, but CCBW, which sent in only 71.36% of the traps was surprising. It can be deducted that CCBW probably has a higher density of possible vectors than all other ranches, which would have been evident with a complete data set. In Figure 16, it was evident that ranch BBR is not only producing more insects than any other location, but it also more ceratopogonids. There was significantly more ceratopogonids trapped in the East Central Texas Plains ecoregion than any other ecoregion sampled. This region is made up of

irregular plains and post oak savannah vegetation (TPW2011), which may be beneficial to *Culicoides* spp. development in trees and low-lying areas.

A yearly trend for insect activity is shown in Figure 20. As expected, insect activity climbs in the spring months and decreases in the late summer to fall months in both years surveyed. The mean insects trapped totaled lower in 2014 than in 2013, but this could be attributed to more precipitation and lower overall temperatures statewide in 2013. The number of insect vectors were constant in 2013 and significantly different ($P = 0.039$) than in the 2014 season. The number of ceratopogonids trapped by date followed the same trend that was recorded in the proof of concept studies. The number of ceratopogonids was significantly ($P = 0.024$) higher in the spring and fall months and lower in the summer and winter months. This is likely due to the higher levels precipitation that occurs statewide throughout the year.

Because insects are poikilothermic, it was expected that temperature would play a role in insect activity, so the mean daily temperature and total precipitation for the seven days before trapping and the five days of trapping was recorded. The mean temperature recorded was 19.32°C and the mean number of total insects was 44.5. When the sample size was reduced to only possible vectors, the mean number of possible vectors trapped was 12.3 and the mean number of ceratopogonids trapped was 0.2. Unfortunately, there are not any conclusions that can be drawn from the temperature curve estimations for total insects, possible vectors, and ceratopogonids trapped because the R^2 values equaled 0.066, 0.020, and 0.013, respectively.

Along with placing traps and sampling the insect population, this study was an opportunity to talk to deer producers about the problems they were facing, as they are the individuals who see the deer every day. Many cited seeing their deer stomp their feet and shake their heads when being plagued by insects. These physical reactions are known to cause a decrease in animal health and subsequently, a decrease in production value and a loss of money by the producer. While talking with deer producers who participated in this study, it was easy to see that nuisance flies, such as house and stable flies, were prevalent. Sources for fly development, such as feed and hay on the ground, should be removed. Also, there are currently no manure management recommendations in place for deer producers. It was obvious when looking at some pens that deer health and fitness could be improved by removing manure or rotating deer through the pens to allow for the substrate to dry out.

The importance of the information gathered in this study was more than surveying an insect population in a system. White-tailed deer-breeding facilities are highly specialized in the way they manage an expensive commodity. The information provided by this study will be combined with what is known about integrated pest management in other commodity systems to provide treatment recommendations based on time of year, product efficacy, and sanitation practices. Over time and as more information is gathered, it is expected that the use of IPM techniques in this highly specialized industry will begin to become more prevalent.

The goal is to use the information gathered to provide each participating ranch a detailed explanation of the insect threats surrounding their deer pens. This information

can offer them guidance to decide which pens to place their deer, along with landscape management advice based on what is currently known about Ceratopogonid development. With continued support from the TDA and Texas white-tailed deer producers, the data can be used to create interactive maps that could simulate insect activity across Texas depending on weather, location and time of year.

CHAPTER III

THE PRESENCE OF HEMORRHAGIC DISEASE VIRUSES IN *CULICOIDES* SPP. (DIPTERA: CERATOPOGONIDAE) SAMPLED FROM TEXAS WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS*) RANCHES

Introduction

With more than 1400 species identified worldwide (Mellor et al. 2000), *Culicoides* have mostly been studied as the vector responsible for the transmission of *Orbivirus* spp. pathogens that cause bluetongue disease (BT). Specifically, *C. sonorensis* is known to be the primary vector of bluetongue virus (BTV) to ruminants in the United States (Holbrook et al. 2000). The biting midge, *Culicoides* spp. (Diptera: Ceratopogonidae), is an important ectoparasite disturbing white-tailed deer (*Odocoileus virginianus*) populations in Texas.

An arbovirus is a virus that, in nature, can infect hematophagous arthropods by their ingestion of infected vertebrate bloods. It multiplies in the arthropod's tissues and is transmitted by blood feeding to other susceptible vertebrates (Mellor 2000). Pathogens transmitted by arthropods are known to cause significant human and animal morbidity and mortality worldwide (Tabachnick 1996). Bluetongue virus is the causative agent of BT (Verwoerd and Erasmus 2004) and is classified in the genus *Orbivirus* and the family Reoviridae. There are 24 known serotypes of BTV in the world. Four serotypes (10, 11, 13, and 17) are widely distributed across North America but BTV- 1 and 2 are common to southeastern regions of the United States (MacLachlan 2008).

Bluetongue, also known as ‘sore-mouth’ or ‘ulcerative stomatitis’ (Goltz 1978), is a disease wild and domestic ruminants such as sheep, cattle, deer, buffalo, elk, and goats (OIE 2009). Cattle and goats are considered a natural reservoir for BTV. Clinical signs of a BT infection are more severe in sheep and deer than in cattle and include fever, swelling of the buccal and nasal mucosa, salivation, tongue swelling, hemorrhaging of the mucosal membranes of the mouth, oral lesions, hemorrhaging of the coronary bands, and death (Tabachnick 1996).

Along with BTV, pathogens transmitted by *Culicoides* spp. have been found to cause epizootic hemorrhagic disease (EHD) in North American white-tailed deer. Like BTV, EHD is caused by an *Orbivirus* in the Reoviridae family. These viruses are antigenically different, but the signs and symptoms of EHD and BTV in white-tailed deer are clinically indistinguishable. Eight serotypes of EHD have been identified (Mellor et al. 2000), including EHD- 1 through 7 and an Ibaraki strain common to Japan. EHDV occurs in Africa, Southeast Asia, Japan, Australia, and the Americas (Mellor et al. 2000). Infections of EHDV occur in domestic and wild ruminants often show sub-clinical symptoms, but are severe in white-tailed deer.

The increase in new types of confined animal feeding operations presents an interference of insect pests vectoring fatal pathogens in new environments with expensive animals. The severity of this disease cycle is only heightened without an integrated pest management (IPM) program in place to control the system. Disease outbreaks in animal production systems can cause significant costs through loss of productivity and death of livestock (Rich and Winter-Nelson 2007). The development of

captive production deer facilities has provided the same challenges that exist with confined operations of any and all livestock including management of manure and other wastes, control of insect pests, and protection from disease agents that adversely affect animal health, growth, phenotypic expression, and subsequent value. Deer held in captive operations produce wastes in the form of feces and urine, along with spoiled feed materials. The environment involving these waste products is conducive to the production of important vectors of cervid disease.

The objective of this study was to determine which *Culicoides* spp. were present in white-tailed deer-breeding facilities and the absence or presence of BTV and EHDV in trapped insects on white-tailed deer-breeding operations in Texas. The goal was to determine if multiple *Culicoides* spp. were present throughout the year, and if EHDV and BTV would be isolated in sampled insects. It was also hypothesized that climatological parameters would play a role in results.

Materials and Methods

Carbon dioxide traps (Bioquip # 2836BQ) were used to sample the *Culicoides* spp. population on seven Texas white-tailed deer-breeding facilities (Fig. 29). Monthly samples were taken from March 2013 through November 2014. Center for Disease Control (CDC) CO_2 traps (Bioquip # 2836BQ) were used to attract and capture insects. The CDC traps were secured to an inverted insulated container (Igloo, #00001795, Katy, TX). A 6.35 mm hole was drilled into the bottom of the container, a ring bolt was

screwed into the hole and a carabineer hook was attached. The carabineer hook was used to secure the entire trap to the fence. Six 6.25 mm holes were also drilled into the sides

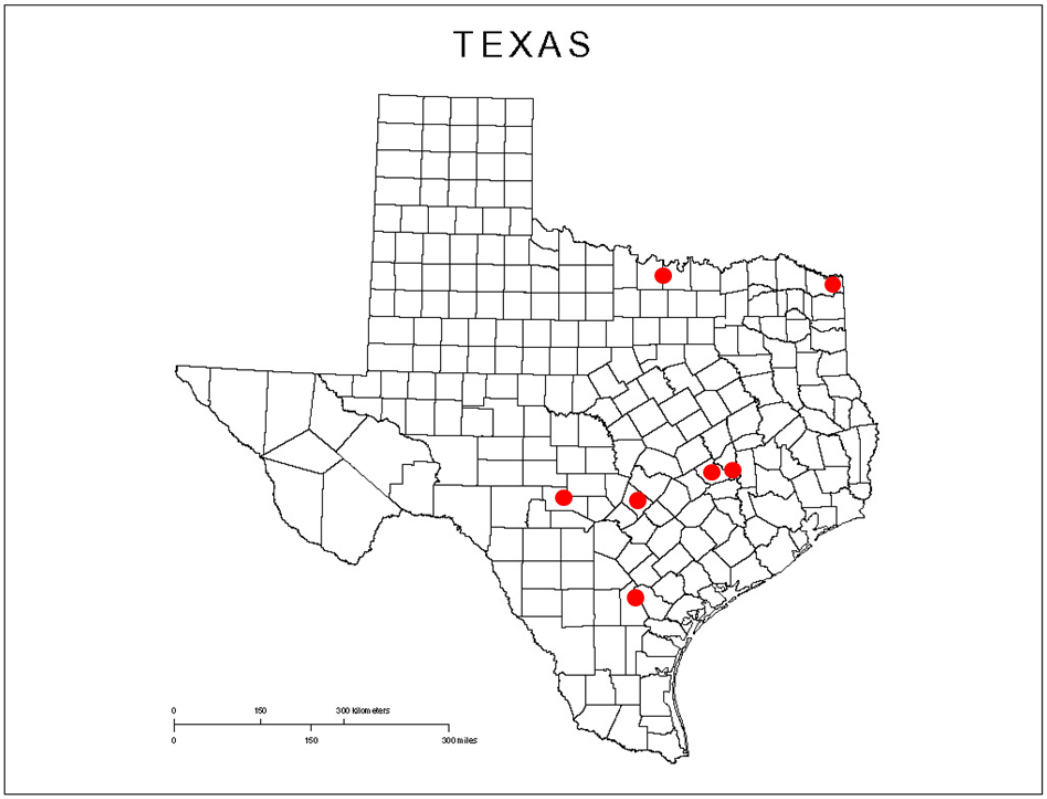

Fig. 29. A graphic display of the ranches that cooperated in the experiment. Each red dot represents a ranch.

of the Igloo container to allow CO_2 gas to escape at a controlled rate. Dry ice was packed into the Igloo container and the drink spout was opened (Fig. 30). A single trap was deployed overnight for approximately 17 h on participating ranches. Cooperators were instructed to deploy traps at approximately 4:00 p.m. and to retrieve the traps the following day at 9:00 a.m. Once traps were picked up, cooperators placed the collection cup in an insulated shipping container with ice packs and shipped them overnight to the Center for Urban and Structural Entomology, Texas A&M University, using mailing labels provided. Containers were stored at -80°C until the insects could be sorted. *Culicoides* spp. were separated from the total collection of insects and identified to species. All non-*Culicoides* spp. insects were discarded. Pools of five insects of the same *Culicoides* species were amalgamated into Seal-rite 2.0 mL microcenterfuge tubes (USA Scientific #1620-2700) and stored at -80˚C. The samples were then delivered to the Texas Veterinary Medical Diagnostics Laboratory (TVMDL), College Station, Texas.

Fig. 30. A photograph of the CO_2 insect trapping equipment. A CO_2 trap was secured to the fence surrounding the deer pens to attract and capture *Culicoides* spp. overnight.

The virus detection was conducted by the Texas A&M Veterinary Medical Diagnostic Laboratory (TVMDL) using real-time RT- PCR. Pools of five *Culicoides* specimens from the same species, trapping date, and location were placed in a single microcentrifuge tube (USA Scientific, #1620-2700, Ocala, FL) and stored at -80°C until processed. To determine absence or presence of BTV or EHD, a sterilized 3.96 mm gold plated tungsten bead and 200 µl homogenization buffer (cell culture media containing antibiotics, 50 µg/ml streptomycin, 50 U/ml penicillin, and 2.5 µg/ml amphotericin B) were added to each of the microcentrifuge tubes containing the insects. The samples were homogenized by mechanical agitation in a TissueLyser twice for 1 minute at 25 rotations per second and rack was flipped between the two agitations. After homogenization, the tubes were centrifuged at room temperature briefly to remove any liquid from the caps. Nucleic acid was extracted from a 50 µl aliquot of the sample using a magnetic bead extraction kit (MagMax AM1840, LifeTechnologies) and a magnetic particle processor (Kingfisher). Nucleic acid was tested for BT and EHD using previously published methods (Clavijo et al. 2010). All RT-PCR positive samples underwent virus isolation attempts. For bluetongue, intravenous inoculation of embryonated chicken eggs and inoculation of Vero cells methods were utilized and for EHD, BHK cells were used. Cell layers were monitored daily for cytopathic effects and eggs were examined daily for deaths. Suspect cultures were verified as positive by fluorescent antibody staining and/or real-time RT-PCR.

Data were analyzed using Analysis of Variance (ANOVA). The ranch location, the trapping date, the mean daily temperature, and the total precipitation were considered

independent variables. The *Culicoides* spp. sampled, the number of *Culicoides*, and the virus detected were considered dependent variables. An ANOVA procedure was conducted using each independent variable as a factor against each dependent variable. Means separation was performed using Tukey's post-hoc analysis. Values were considered significantly different when $P < 0.05$. The statistical package IBM SPSS version 21.0 was used to perform the analysis (SPSS 2012).

Results

The cooperator response to this experiment was lower than expected. Only three of the seven ranches completed the sampling each month, as shown by Figure 31. Ranch CBG was not added to the experiment until June 2014 and is reflected in Figure 31. Ranch BRR, TJR, and KWMA submitted 100% of the samples for the experiment. The following ranches submitted a percentage of the samples requested: CCBW (33.10%), RR (47.62%), TRW (14.29%), and CBG (33.33%).

There was not a significant difference ($F = 1.978$; df = 6; $P = 0.068$) in the species of *Culicoides* trapped at each location (Table 3).

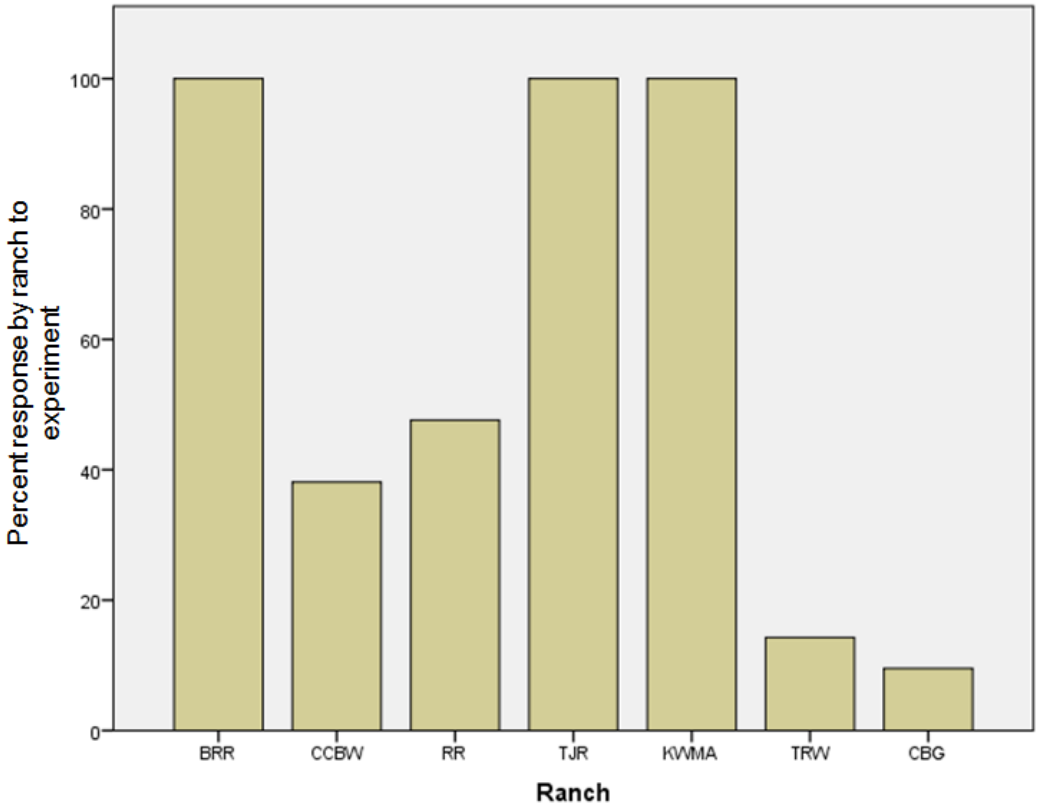

Figure 31. The response percentage for each sampling location over the duration of the experiment. Only three of the seven ranches completed the sampling each month. The following ranches submitted a percentage of the samples requested: CCBW (33.10%), RR (47.62%), TRW (14.29%), and CBG (33.33%).

Table 3. The total number of each *Culicoides* spp. sampled at each location.

		Insect					
		C. sonorensis	C. haematopopus	C. crepuscularis	C. multipunctatus	Unknown	C. butleri
		Count	Count	Count	Count	Count	Count
Ranch	BRR	42	5	7	11	1	2
	CCBW	10	1	2	0	0	0
	RR	9	0	1	1	0	0
	TJR	140	1	73	68	0	2
	KWMA	1	2	1	0	0	0
	TRW	1	2	1	0	0	0
	CBG	1	1	2	1	0	0

An ANOVA was conducted to determine the number of all *Culicoides* spp. sampled from ranches varied based on location. Figure 33 shows a significantly higher (F = 13.686; df = 6; P < 0.001) number of *Culicoides* spp. were sampled from ranch locations BRR, CCBW, RR, and TJR. Ranches TRW and CBG has significantly lower numbers of *Culicoides* spp. individuals trapped, while KWMA was similar to each set. There was not a significant difference (F = 0.992; df = 6; P = 0.430) in the type of virus sampled between ranches. Figure 33 shows that while most locations submitted insects that were negative for BTV or EHDV, only two locations (BRR and TJR) were positive for either virus through the duration of the experiment.

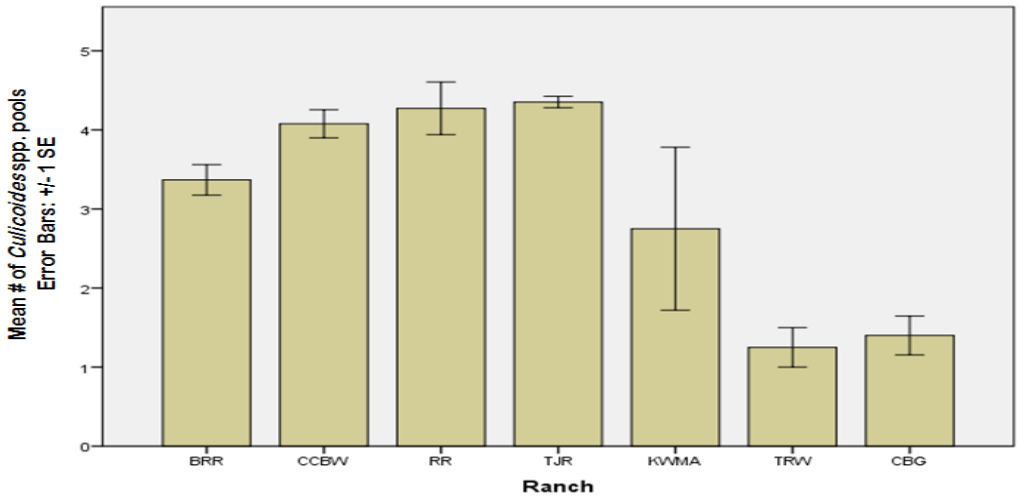

Fig. 32. *Culicoides* spp. sampled from each ranch location. There was a significantly higher (F = 13.686; df = 6; P < 0.001) number of *Culicoides* sampled from ranch locations BRR, CCBW, RR, and TJR than the other ranches surveyed.

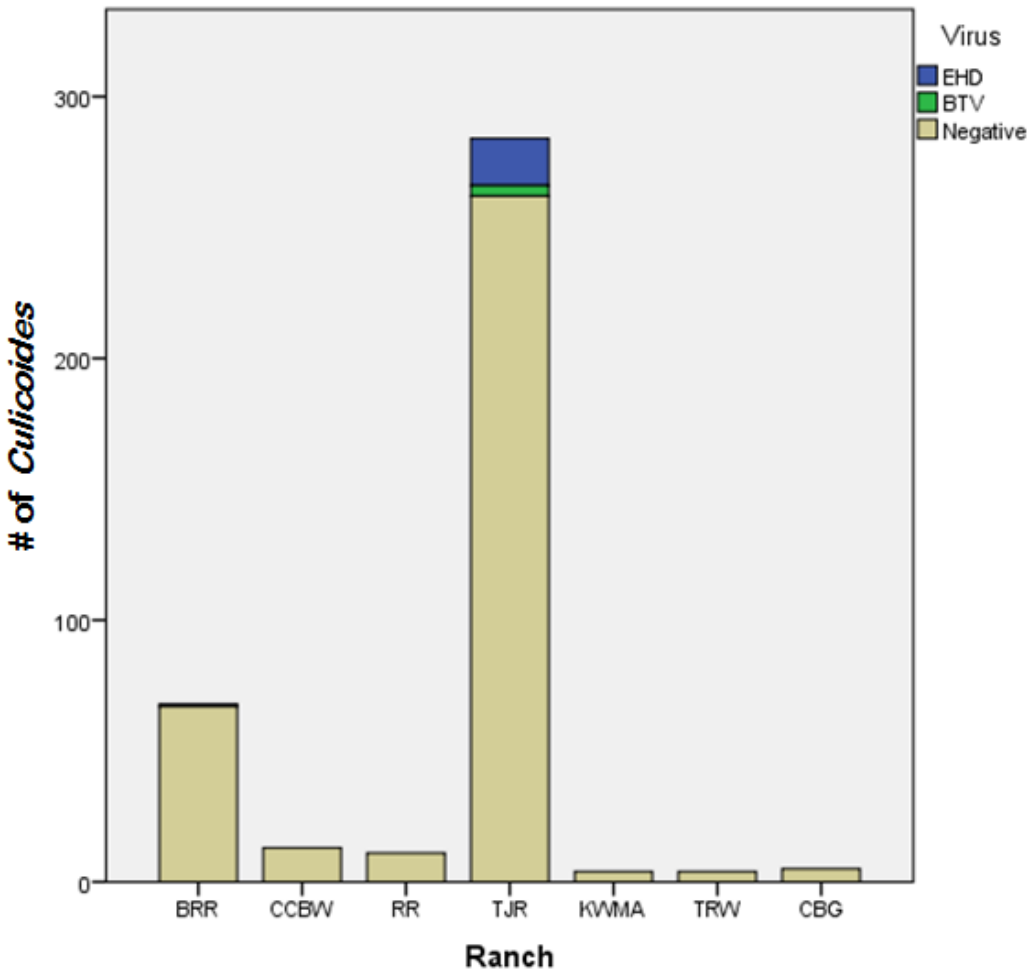

Fig. 33. Distribution of virus type by ranch location. Ranches were displayed on the x-axis and the numbers of *Culicoides* insects sampled by virus were located on the y-axis. There was not a significant difference ($F = 0.992$; df = 6; $P = 0.430$) in the type of virus sampled between ranches. Only two ranches (BRR and TJR) provided samples of insects that tested positive for BTV or EHDV.

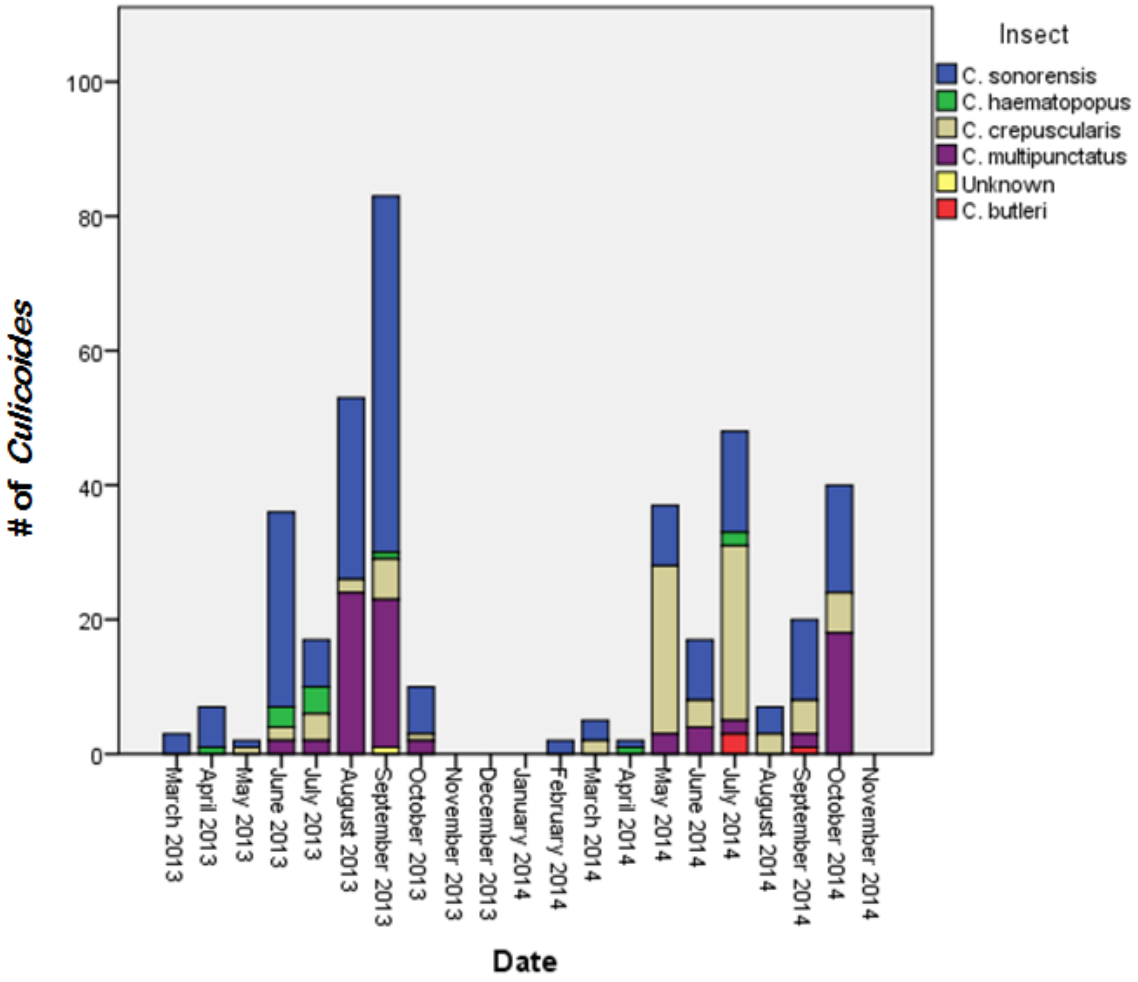

Fig. 34. Distribution of *Culicoides* spp. caught over the dates in the sampling period. Dates were displayed on the x-axis and the numbers of *Culicoides* insects sampled by species were located on the y-axis. The number of *Culicoides* sampled was significantly different ($F = 2.643$; df = 16; $P = 0.001$) between months, though Tukey's HSD found no separation between means.

Dates were the next independent variable to be analyzed. Date of insect capture was factored against the *Culicoides* spp. present using an ANOVA. Figure 34 shows the distribution of *Culicoides* spp. throughout the 21 months to be significantly different ($F = 2.643$; df = 16; $P = 0.001$) between months, though a Tukey's post-hoc analysis did not distribute the means to be separate. When comparing the number of *Culicoides* spp. individuals trapped to the date, there was significant ($F = 5.527$; df = 16; $P < 0.001$) difference between months. Figure 35 shows that February 2014 had significantly more *Culicoides* spp. trapped than any other month. Alternatively, May 2013 and April 2014 were significantly lower in *Culicoides* spp. count than any other month. Table 4 shows the Tukey's post-hoc analysis of these results. The date of sampling was compared to the virus detected using an ANOVA. There was no significant difference ($F = 1.227$; df = 16; $P = 0.245$) in the date in which virus was detected (Fig. 36).

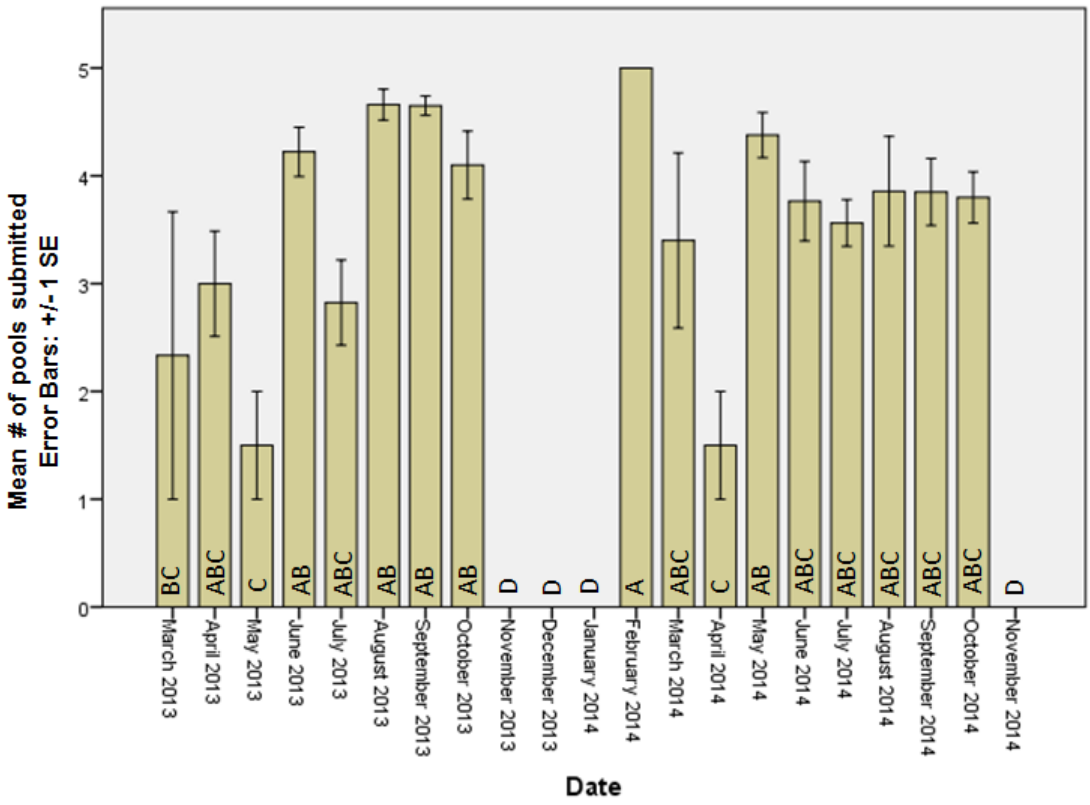

Fig. 35. Distribution of the mean number of *Culicoides* spp. caught over the dates in the sampling period. Dates were displayed on the x-axis and the numbers of *Culicoides* spp. pools submitted to TVMDL were located on the y-axis. February 2014 had significantly more ($F = 5.527$; df = 16; $P < 0.001$) *Culicoides* spp. trapped than any other month. Alternatively, May 2013 and April 2014 were significantly lower in *Culicoides* spp. count than any other month. There were not any *Culicoides* collected in the months of November 2013 to January 2014 or in November 2014. Means separation was determined by Tukey's LSD. Means with the same letter are not significantly different ($\alpha = 0.05$).

Table 4. Tukey's post-hoc analysis of the distribution of the total number of *Culicoides* spp. caught over the dates in the sampling period. Means with the same letter were not significantly different ($\alpha = 0.05$)

Sum

Tukey HSD[a,b]

Date	N	Subset for alpha = 0.05		
		C	B	A
May 2013	2	1.50		
April 2014	3	1.67		
March 2013	3	2.33	2.33	
July 2013	19	2.89	2.89	2.89
April 2013	7	3.00	3.00	3.00
March 2014	5	3.40	3.40	3.40
June 2014	19	3.58	3.58	3.58
July 2014	60	3.68	3.68	3.68
October 2014	43	3.70	3.70	3.70
August 2014	7	3.86	3.86	3.86
October 2013	11	3.91	3.91	3.91
September 2014	23	3.91	3.91	3.91
May 2014	39		4.21	4.21
June 2013	36		4.22	4.22
September 2013	83		4.65	4.65
August 2013	54		4.67	4.67
February 2014	2			5.00
Sig.		.065	.090	.207

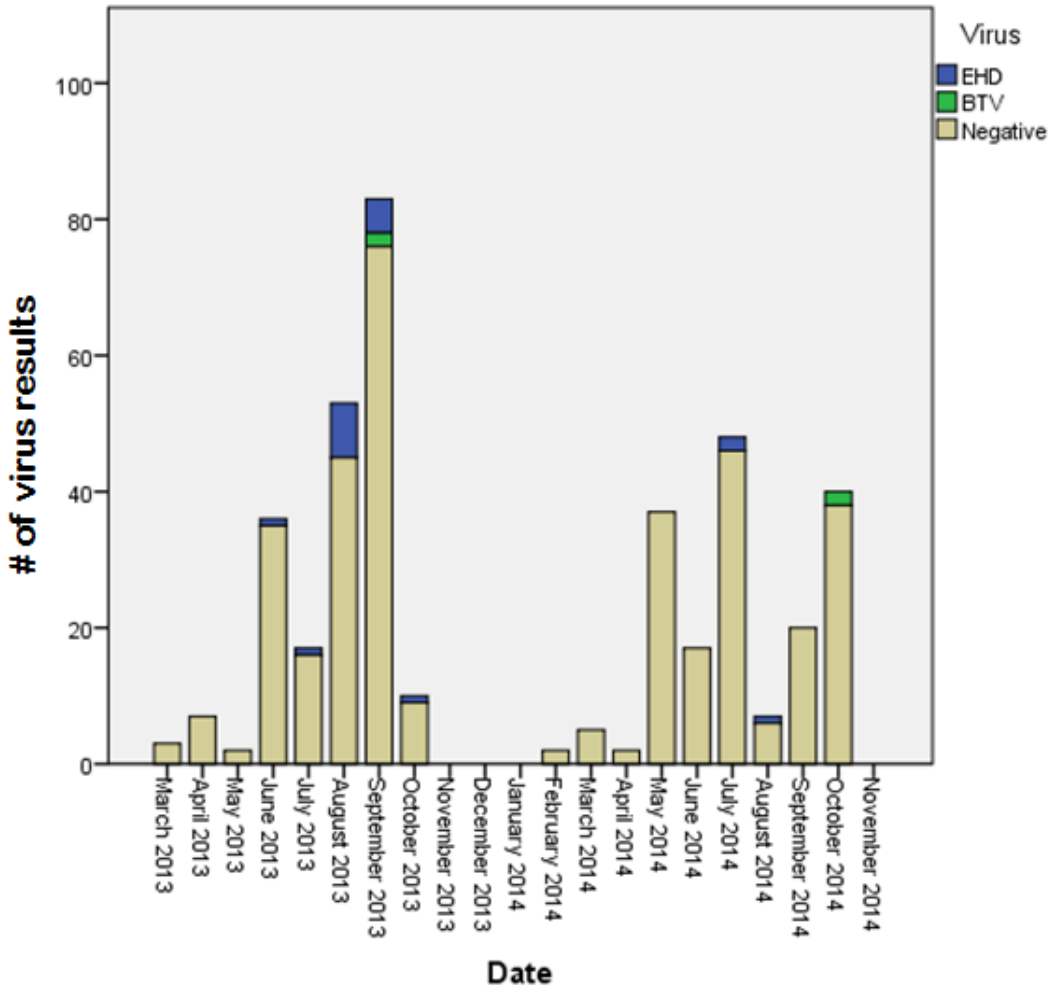

Fig. 36. Virus (BTV/EHDV) detected with respect to date of *Culicoides* spp. sampling. Dates were displayed on the x-axis and the numbers of virus results were located on the y-axis. There was no significant difference (F = 1.227; df = 16; P = 0.245) in the date in which virus was detected.

The next step was to determine if climatological factors played a role in which insect was captured, how many were trapped, and which virus was detected. Climatological data were recorded for the duration of the experiment using the National Oceanic and Atmospheric Administration records. Mean temperature for the experimental period was calculated and factored against *Culicoides* spp. sampled. There was not a significant difference ($F = 0.875$; df = 145; $P = 0.738$) in the species of *Culicoides* caught, but mean temperature did play a significant ($F = 0.832$; df = 145; $P < 0.001$) role in how many *Culicoides* spp. individuals were trapped (Figure 37). A quadratic line ($y = 27.1 - 4.82x + 0.19x^2$) ($R^2 = 0.128$) was placed on Figure 37 that shows the number of *Culicoides* present based on temperature. Along with temperature, total precipitation was monitored during sampling. There was not a significant effect ($F = 1.091$; df = 130; $P = 0.348$) between the species of *Culicoides* sampled based on precipitation, but there was a significant effect ($F = 6.756$; df = 130; $P < 0.001$) on the number of *Culicoides* sampled based on precipitation acquired (Fig. 38). A quadratic line ($y = 23.93 - 6.76E{-}3x + 5.78E{-}6x^2$) ($R^2 = 0.013$) shows the relationship between precipitation and the number of *Culicoides* spp. sampled by this survey. There was no significant effect ($F = 0.983$; df = 130; $P = 0.542$) on the virus detected due to total precipitation.

Unfortunately, while virus absence or presence in submitted samples is known, the attempt to identify BTV/EHDV serotype was hampered by the loss of samples in a failed freezer. There were three species of *Culicoides* that tested positive for BTV and EHDV (Table 5).

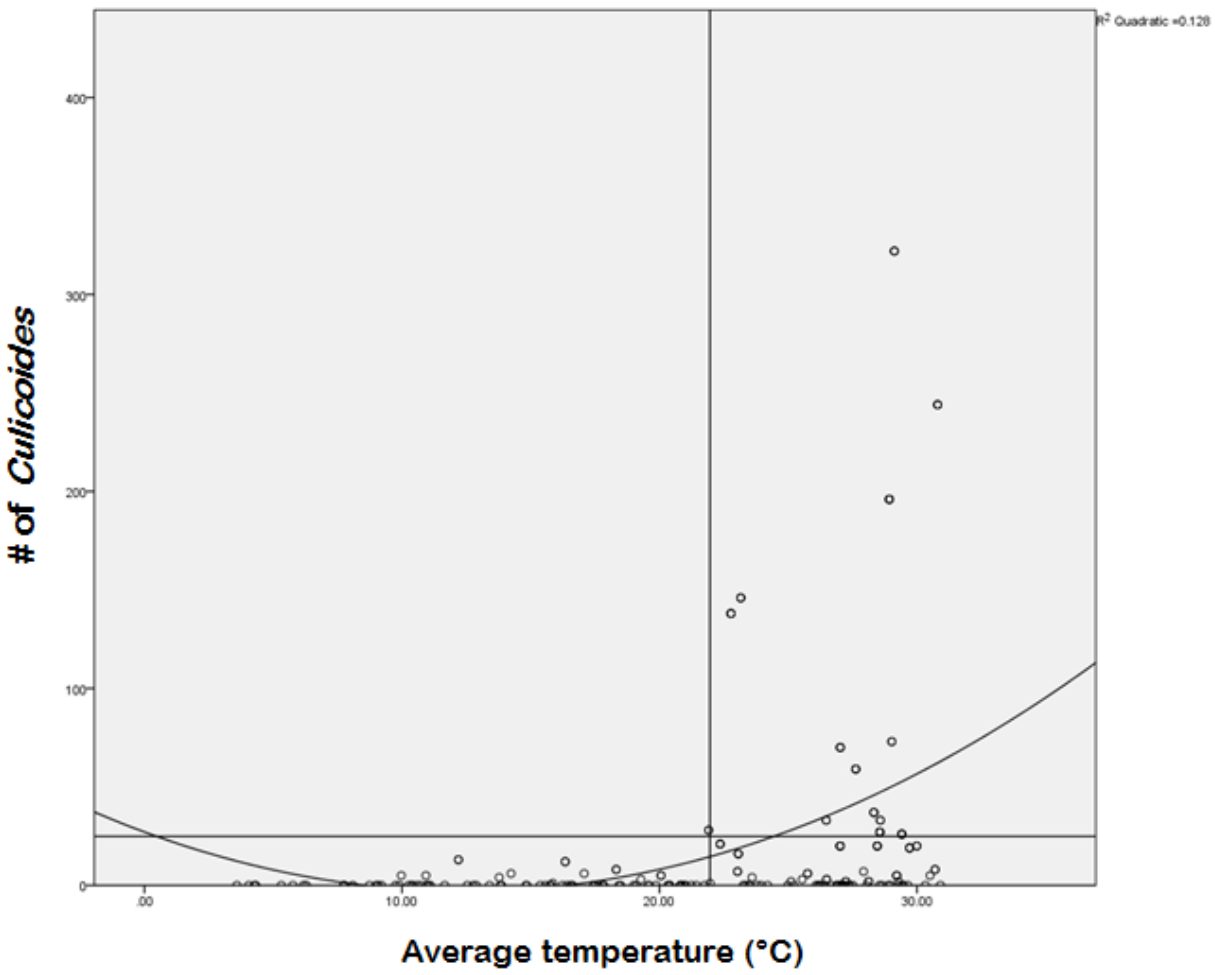

Fig. 37. Mean temperature measured compared to total *Culicoides* spp. trapped. The maximum and minimum temperature for each day of the month was recorded and averaged. The mean daily temperature is displayed on the x-axis and y-axis shows the total number of insects. Mean temperature (24.8°C) plays a significant (F = 0.832; df = 145; P < 0.001) role in how many *Culicoides* spp. are trapped (mean = 24.8). The curve estimation is provided by the quadratic line (R^2 = 0.128), y = 27.1+-4.82x+0.19x^2.

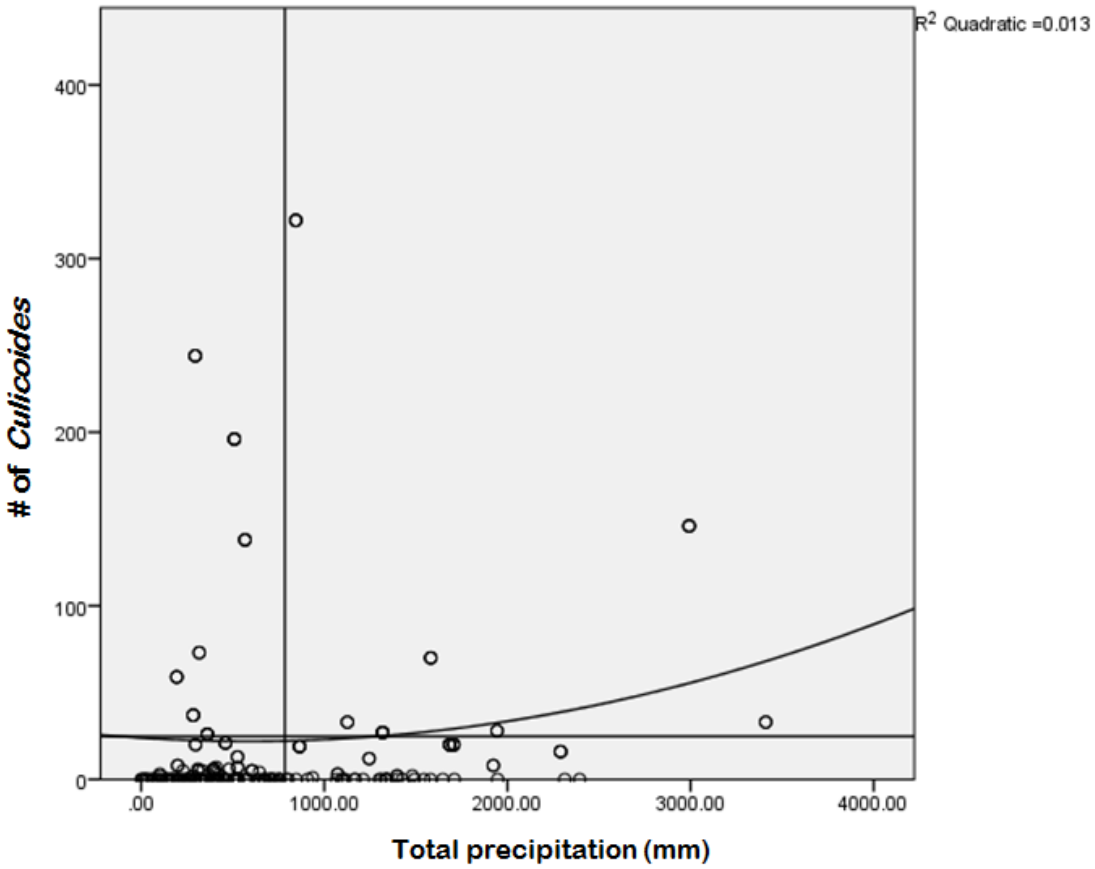

Fig. 38. Total precipitation measured compared to total *Culicoides* spp. trapped. The total precipitation for the month was recorded. The total precipitation is displayed on the x-axis and y-axis shows the total number of *Culicoides* sampled. Mean precipitation (784.6 mm) plays a significant (F = 6.756; df = 130; P < 0.001) role in how many *Culicoides* spp. were trapped (mean = 24.8). The curve estimation is provided by the quadratic line (R^2 =0.013), y = 23.93-6.76E-3x+5.78E-6x^2.

Table 5. The species of *Culicoides* that tested positive for hemorrhagic disease viruses. Virus types are listed in columns, while species are listed in rows. The number denotes how many times the specific serotype was identified. Vials contained as few as 1, but as many as 5 infected *Culicoides* spp.

	EHDV Serotype					BTV
Species	1	2	1 & 2	6	Unknown	Unknown
C. sonorensis	1	5	2	1	4	3
C. crepuscularis	0	0	0	2	0	0
C. multipunctatus	2	0	0	0	2	1

Discussion

The objective of this research was to assess the abundance of *Culicoides* spp. and the presence of virus pathogens in sampled insects on white-tailed deer ranches. The limiting factor of this experiment was the response rate by ranches. There were two ranches that were handled personally due to their location to experimental headquarters, and the third location (KWMA) was a government facility. These three locations had a 100% submission rate of samples during the course of the experiment. Unfortunately, results were submitted at less than 50% for the other four participating ranches. While there was not a significant difference in species of *Culicoides* trapped based on ranch location, *C. sonorensis* was the species most collected. All ranch locations were positive for *Culicoides* spp. However, though ranch locations RR and CCBW submitted fewer samples than requested of this experiment, the number of *Culicoides* harvested was similar to that of TJR and BRR, two ranches that submitted 100% of the samples. This could mean that *Culicoides* population numbers were higher in these locations than other sampled ranches.

There were a total of 23 detected positive samples of BTV or EHD in the 416 submitted samples. Of the 23 positives, there could have been as few as one positive *Culicoides* spp. or as many as five positive insects per sample. There were three species of *Culicoides* that tested positive for EHDV or BTV, including: *C. sonorensis*, *C. multipunctatus*, and *C. crepuscularis*. The two other species sampled, *C. haematopotus* and *C. butleri*, were not found to be positive for BTV or EHDV in the course of this study. *C. sonorensis* was positive for EHDV-1, 2, 6 and an unknown BTV serotype.

Also, *C. sonorensis* was positive for both EHDV-1 and 2 in the same sample on two occasions. *C. crepuscularis* was positive for EHDV-6 on two occasions and *C. multipunctatus* was positive for EHDV-1, an unknown EHDV serotype, and BTV. There was virus detected at only two of the seven locations; however, *C. multipunctatus* was found to be positive for BTV in Burleson County, Texas. Vector competency was demonstrated and would be capable of transmitting the virus in other locations. The date of capture did not have a significant effect on the number of *Culicoides* spp. harvested, but the trend of insects captured follows that seen in previous studies; there were a larger number of insects collected in the spring and fall months. This is likely due to mild temperatures and more precipitation in the spring and fall months than in other sampled months. There was not a significant correlation between mean temperature and precipitation recorded for sampling periods and the number or species of *Culicoides* spp. collected. This is likely due to low sample size on the seven ranches.

Never before has there been a survey of the *Culicoides* spp. population in Texas. The results of this experiment satisfy the principles of Koch's postulate: the virus has been found in three species of the vector in Texas, the virus was isolated from the vector and identified to serotype, and, based on interviews with deer producers where infected insects were sampled, deer are suffering from virus infection. The information gathered about species abundance in Texas white-tailed deer-breeding facilities will be useful when determining which methods to recommend for use in control. Also, information about species abundance will be useful in *Culicoides* spp. development studies. It is expected that not all *Culicoides* spp. develop in the same way. As information comes

available about species distribution, recommendations can be made to producers of deer, cattle, and sheep about recommended control options. While many studies have been conducted that evaluate the vector potential of *C. sonorensis*, this may be the first report of *C. multipunctatus* and *C. crepuscularis* sampled from white-tailed deer facilities in Texas and testing positive for identified serotypes of EHDV and BTV.

CHAPTER IV

MEASUREMENT OF *CULICOIDES SONORENSIS* DEVELOPMENT IN COMMON SUBSTRATES FOUND IN WHITE-TAILED DEER-BREEDING FACILITIES

Introduction

The biting midges, *Culicoides* spp. (Diptera: Ceratopogonidae), are important ectoparasites disturbing white-tailed deer (*Odocoileus virginianus*) populations in Texas. Biting midges are vectors of disease agents causing epizootic hemorrhagic disease (EHD) and bluetongue (BTV). These diseases are easily recognized and feared by white-tailed deer producers, yet there is limited literature on biting midge management.

Culicoides spp. males feed on nectar from flowering plants, while females take blood meals from mammal hosts. The blood meals are necessary for egg development (Wirth and Blanton 1974). The mating of *Culicoides* usually occurs during flight when the males form swarms. Females will fly through the swarms and are captured by males (Downes 1955). Jones (1966) found that *C. variipennis* females can repeatedly mate and store sperm for as many as three egg batches. It has also been reported by Jones (1967) that *C. variipennis* deposited one egg batch for each blood meal taken. One *C. variipennis* female can live 44 d, depositing 243 eggs in a single batch, or a maximum of 1,143 eggs in seven batches during her lifetime (Jones 1967). The egg will hatch approximately 24 h after being laid on a suitable moist substrate. Immatures can survive in nearly any environment that contains a suitable amount of moisture for development (Borkent 2005). Larvae are vermiform (Mellor et al. 2000), semiaquatic (Blanton and

Wirth, 1970) and will pupate in 10 to 14 d under optimum conditions. The larvae stage includes four instars. The pupae stage is brief, in comparison, only lasting two to four days. Pupae are found free-floating or attached to debris in the substrate (Mellor et al. 2000), but are always found near the surface of the substrate. Mullens and Rodriguez (1992) found that the significant majority of pupae were found in the first 1 to 2 cm of substrate. After eclosion, adult *Culicoides* spp. will mate and search for a blood meal. Most species of *Culicoides* overwinter as fourth instar larvae (Mellor et al. 2000).

Information surrounding biting midge development in the environment is limited. Because of their small size and the incomplete knowledge of their developmental history, successful laboratory colonization of *Culicoides* spp. is limited. There is a need for foundational developmental information about *Culicoides* in order to develop environmental control measures. The objective of this experiment was to determine the rate at which *C. sonorensis*, the most abundant *Culicoides* spp. in North America, developed in common substrates found in white-tailed deer-breeding facilities. It was hypothesized that there would be no difference in the development of *C. sonorensis* to adulthood in all of the substrates tested to rear *C. sonorensis* in the laboratory, including: 1.) deionized water, 2.) USDA substrate, 3.) manure, and 4.) feed.

Materials and Methods

To determine possible substrates for C*ulicoides sonorensis* development in white-tailed deer-breeding facilities, developmental progress of *C. sonorensis* from egg to adult stages was monitored. Eight treatments were identified: deionized (DI) water, DI water (flood), USDA growth medium, USDA growth medium (flood), manure, manure (flood), deer feed, and deer feed (flood).

To prepare the deionized water medium, 1800 ml of deionized water was poured through a funnel lined with organza fabric into an Erlenmeyer flask. A graduated cylinder was then used to measure 180 mL of the filtered water, which was poured into each of five 150 x 26 mm petri dishes (Fisher Scientific, #401412, Rochester, NY). To make the deionized water (flood) medium, 1800 ml of deionized water was retrieved from the laboratory deionized water tap and stored in an incubator set at 28°C, 65% RH and 13:11 [L:D] for seven days. After this time, the water was poured through a funnel lined with organza fabric into an Erlenmeyer flask. A graduated cylinder was used to measure 180 mL of the filtered water, which was poured into each of five 150 x 26 mm petri dishes.

The USDA growth medium was prepared by mixing 1 mL nutrient broth, 1 mL bacterium inoculum, and 0.20 g of Kalf media with 1800 mL DI water (USDA 2014). The nutrient broth used in the USDA mixture was shipped with the *C. sonorensis* eggs and was prepared by researchers at the USDA-ARS facility in Manhattan, Kansas. Dried nutrient broth was mixed with DI water and heated on a hot plate. Once completely mixed, the solution was transferred to jars and autoclaved for 30 minutes. The nutrient

broth was cooled and refrigerated until use (USDA 2014). The bacterial inoculum used in the USDA mixture was shipped with the *C. sonorensis* eggs and prepared by researchers at the USDA-ARS facility in Manhattan, Kansas. To make the bacterium inoculum used in the USDA mixture, bacteria collected from a former sample site was mixed with autoclaved water, and nutrient broth. The solution was stored in the refrigerator until use. The Kalf medium used in the USDA mixture was shipped with the *C. sonorensis* eggs and prepared by researchers at the USDA-ARS facility in Manhattan, Kansas. The Kalf media was developed by mixing 140 g ground high protein supplement, 135 g alfalfa herb powder, 10 g brain heart infusion, 10 g powdered yeast, and 10 g albumin (USDA 2014). The growth medium mixture was poured through a funnel lined with organza fabric into an Erlenmeyer flask. A graduated cylinder was used to measure 180 mL of the filtered water, which was poured into each of five petri dishes. To make the USDA growth medium (flood), 1 mL nutrient broth, 1 mL bacterium inoculum, and a teaspoon of Kalf media with 1800 mL DI water and stored in an incubator set at 28°C, 65% RH and 13:11 [L:D] for 7 d. After this time, the mixture was poured through a funnel lined with organza fabric into an Erlenmeyer flask. A graduated cylinder was used to measure 180 mL of the filtered water, which was poured into a petri dish. This was repeated for a total of five petri dishes.

To develop the manure medium, manure was collected from Big Rack Ranch in Navasota, Texas. A 600 g collection was made by using gloved hands to collect fresh manure from the ground in a large breeder doe pen. Fresh manure was chosen because it was less likely to have previously been selected by other nuisance flies as a place to lay

eggs. Once the manure was brought back to the laboratory, the collection was mixed, by hand, to create a homogenous mixture for use in the experiment. A sample of 300 g manure was mixed with 1800 ml of DI water. The manure mixture was poured through a funnel lined with organza fabric into an Erlenmeyer flask. A graduated cylinder was used to measure 180 mL of the filtered water, which was poured into a petri dish. This was repeated for a total of five petri dishes. The manure (flood) treatment was prepared by mixing 300 g fresh deer manure with 1800 ml DI water and stored in an incubator set at 28°C, 65% RH and 13:11 [L:D] for 7 d. After this time, the mixture was poured through a funnel lined with organza fabric into an Erlenmeyer flask. A graduated cylinder was used to measure 180 mL of the filtered water, which was poured into each of five petri dishes.

The deer feed selected was a formulation commonly used by white-tailed deer producers. Antlermax Breeder Professional 16% (Purina Animal Nutrition, Shoreview, MN) was used in this experiment because it is widely used and is not compounded with antibiotics. The feed medium was prepared by mixing 300 g deer feed with 1800 ml DI water. The feed and water mixture was poured through a funnel lined with organza fabric into an Erlenmeyer flask. A graduated cylinder was used to measure 180 mL of the filtered water, which was poured into a petri dish. This was repeated for a total of five petri dishes. To mix the feed (flood) substrate, 300 g deer feed with 1800 ml DI water and stored in an incubator set at 28°C, 65% RH and 13:11 [L:D] for 7 d. After this time, the mixture was poured through a funnel lined with organza fabric into an Erlenmeyer flask. A graduated cylinder was used to measure 180 mL of the filtered

water, which was poured into a petri dish. This was repeated for a total of five petri dishes.

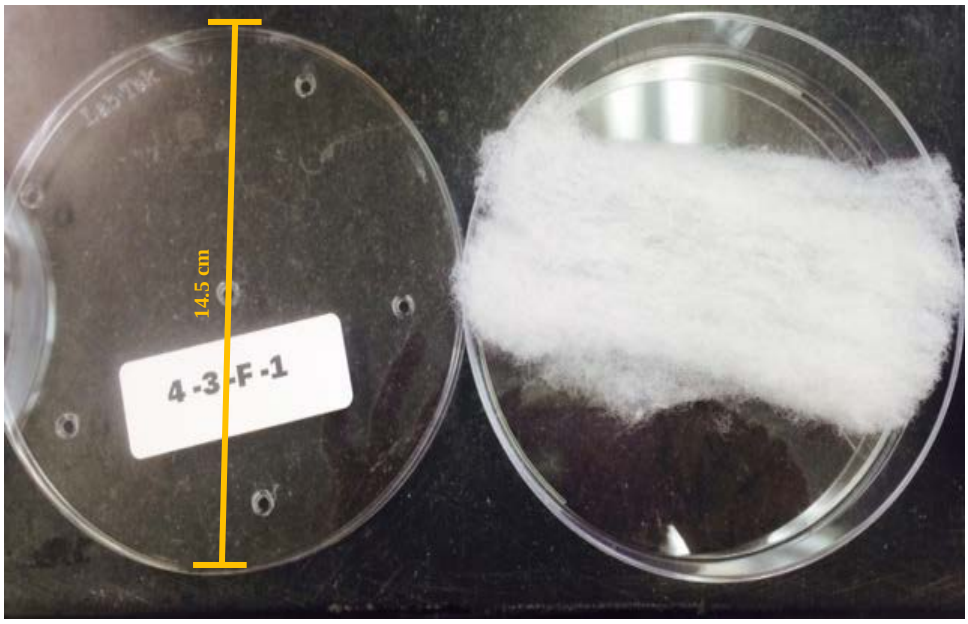

Fig. 39. Petri dishes were equipped with a dacron island to avoid *C. sonorensis* eggs from drowning. Six holes were melted in the lids to allow oxygen to reach the developing flies.

The petri dishes had been manipulated to have 6 holes in the lid to allow air flow to developing *C. sonorensis* (Fig. 39). Also, a 10 cm x 5 cm x 2.5 cm piece of dacron (Online Fabric Store, #142750-DECK, West Springfield, MA) fabric was placed in the dish. Once all the dishes had been prepared and filled, five – 50 mL samples of the substrate were saved in separate glass vials for evaluation of pH and ammonium levels. This process was replicated once the flood treatments of substrates were prepared.

Culicoides sonorensis eggs were shipped on ice overnight from the USDA Agricultural Research Service in Manhattan, Kansas. The *C. sonorensis* colony was established in 1973 from Owyhee County, Idaho. There have been no additions to the colony since its origin. Eggs were used within 6 h of arriving at the laboratory. A small spatula was used to count and separate 50 eggs from the shipped batch. These eggs were placed on a dacron island in each of five petri dishes for each variable. The petri dishes were covered with lids and placed in an incubator set at 28°C, 65% RH and 13:11 [L:D]. The petri dishes were checked after 24 h and egg hatch rate was calculated. The petri dishes were checked every day at the same time for 30 d. The number of pupae or adults present each day was recorded. The adults were removed each day they were present.

The five – 50 mL samples that were taken in the experimental preparation process were used when measuring pH and ammonium concentration (Fig. 40). These levels were taken no more than 24 h after the experiment started. To measure pH, a pH meter (Denver Instrument, Model UB-5, Arvanda, CO) probe was submerged in the solution, per equipment instructions, and the value was recorded. To validate the pH

meter, a pH litmus paper test (Hydrion, #9400, Brooklyn, NY) was conducted for each of the vials. A pH litmus strip was quickly submerged in the solution, then removed and

Fig. 40. Samples of each growth medium treatment were reserved for pH and NH_4 tests.

allowed to process. The value, as noted by the test, was recorded. To calculate ammonium levels, a nitrate nitrogen tablet kit (LaMotte Company, #3354-01, Chestertown, MD) was used. A 5 mL sample of the substrate was mixed with a single nitrate #1 tablet from the test kit in the provided vial. After the tablet had dissolved, a second tablet of nitrate #2 from the test kit was added and shaken until dissolved. The vial was then held up to the color scale provided by the kit and the ammonium level for the sample was recorded. This was repeated for all five replications of each time and substrate treatment.

The entire experiment was repeated five times. To monitor the level of oxygen available to developing *C. sonorensis*, a dissolved oxygen (DO_2) meter (Milwaukee, #MW600, Rocky Mount, NC) was used. During the fifth replication of this experiment, the DO_2 meter was submerged in the growth mediums of each of the five petri dishes of all medium and time treatments each day (Fig. 41). The parts per million (ppm) of oxygen available in the solution was recorded daily.

Data were analyzed using Analysis of Variance (ANOVA). For this experiment, the number of pupae and the number of adults counted each day, pH, NH_4, and the dissolved oxygen content were considered dependent variables. The substrate preparation, the substrate, and time were considered independent variables. An ANOVA procedure was conducted using each independent variable as a factor against each dependent variable. Means separation was performed using Tukey's post-hoc analysis. Values were considered significantly different when $P < 0.05$. The statistical package IBM SPSS version 21.0 was used to perform the analysis (SPSS 2012).

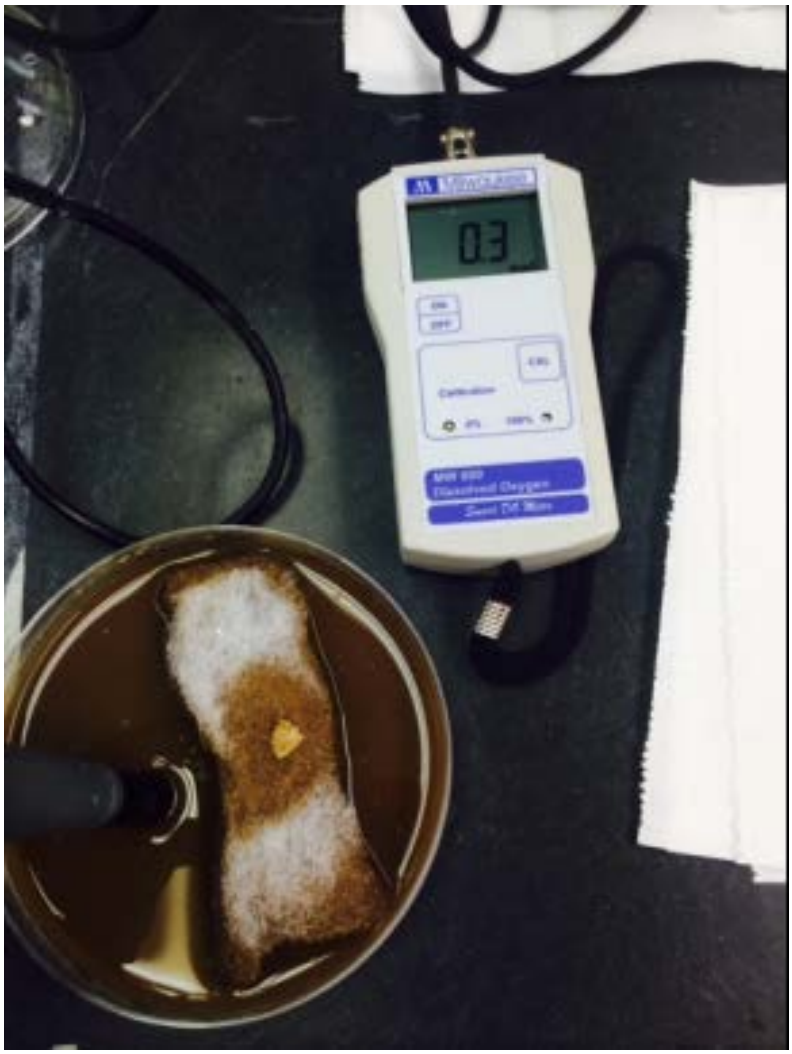

Fig. 41. Each replication was evaluated for dissolved oxygen content once per day for 30 d. To monitor the level of oxygen available to developing *C. sonorensis*, a dissolved oxygen (DO_2) meter (Milwaukee, #MW600, Rocky Mount, NC) was used. During the fifth replication of this experiment, the DO_2 meter was submerged in the growth mediums of each of the five petri dishes of all medium and time treatments each day. The parts per million (ppm) of oxygen available in the solution was recorded daily.

Results

The percent hatch of *C. sonorensis* eggs was calculated 24 h after they were deposited in the treatment substrate. There was no significant difference ($F = 2.612$; df = 1; $P = 0.114$) in the percentage of eggs that hatched from the run-off and flood preparations (Fig. 42). There was, however, a difference ($F = 5.063$; df = 3; $P = 0.005$) in the mean percentage of *C. sonorensis* eggs hatched in the different treatment substrates. A Tukey's post-hoc analysis showed that approximately 69.4% of eggs deposited in water hatched, which was significantly higher than other substrates in the experiment. Manure and feed substrates had a significantly lower number of egg hatch at 60.04% and 60.56%, respectively. The USDA treatment effect was similar to both the manure and the water and reported a hatch of 67.56% (Figure 43).

The number of *C. sonorensis* to pupate in each replication was recorded for 30 days. There were significantly more ($F = 48.984$; df = 1; $P < 0.001$) pupae that developed in the run-off preparations than in the flood preparations (Fig. 44). When comparing the effect of treatment to the number of pupae counted, there was a significant ($F = 7.701$; df = 3; $P < 0.001$) difference between treatments. A Tukey's post analysis showed that the feed treatment produced significantly more pupae (n = 240.3) than the manure (n = 105.8) and water (n = 0.6) treatments. The USDA treatment was similar (n = 60; $P = 0.058$) to the feed treatment and the manure treatment. Figure 45 shows the effect of treatment on the mean number of pupae counted in each replication. Figure 46 shows the distribution in pupae counts over the 30 d experiment. There was a

significant (F = 5.2.007; df = 29; P = 0.003) effect between the number of days and the number of pupae. The treatments follow the same statistical trend as in Figure 45.

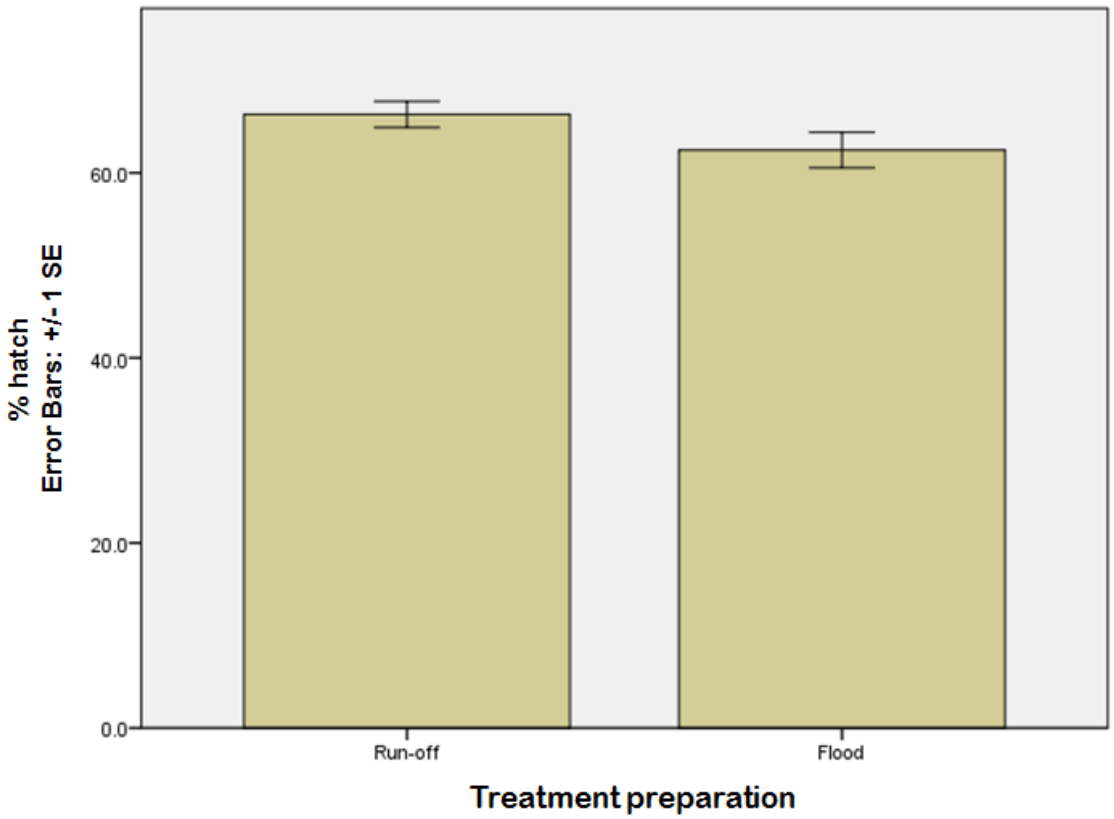

Fig. 42. The effect of substrate preparation on percent hatch of *C. sonorensis* eggs. The treatment preparation is displayed on the x-axis and the percent hatch of the *C. sonorensis* eggs is displayed on the y-axis. There was not a significant difference (F = 2.612; df = 1; P = 0.114) in the percentage of eggs that hatched from the run-off and flood preparations.

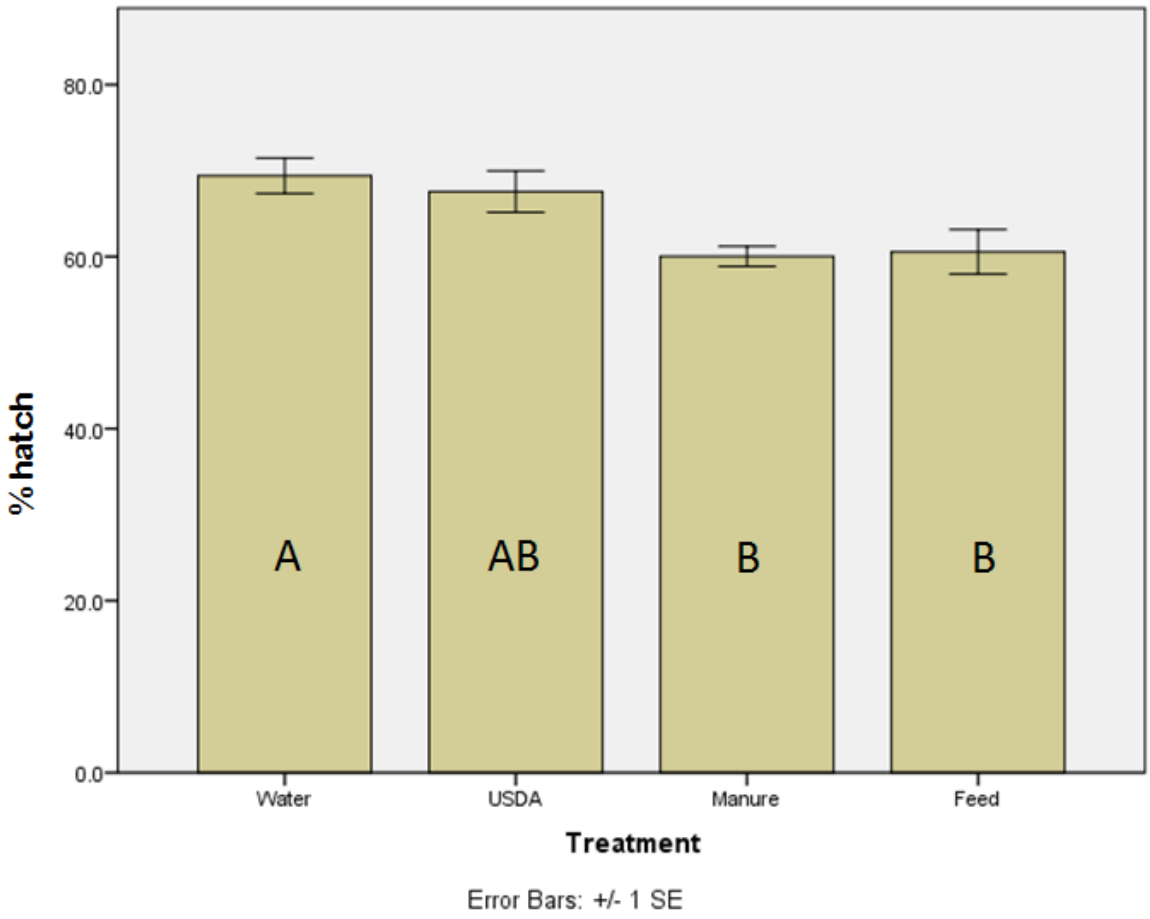

Fig. 43. The effect of treatment on percent hatch of *C. sonorensis* eggs. The treatment is displayed on the x-axis and the percent hatch of the *C. sonorensis* eggs is displayed on the y-axis. There was a significant difference ($F = 5.063$; df = 3; $P = 0.005$) in the mean percentage of *C. sonorensis* eggs hatched in the different treatment substrates. A Tukey's LSD analysis showed that approximately 69.4% of eggs deposited in water hatched, which was significantly higher than other substrates in the experiment. Manure and feed substrates had a significantly lower number of egg hatch at 60.04% and 60.56%, respectively. The USDA treatment effect was similar to both the manure and the water and reported a hatch of 67.56%. Means with the same letter are not significantly different ($\alpha = 0.05$).

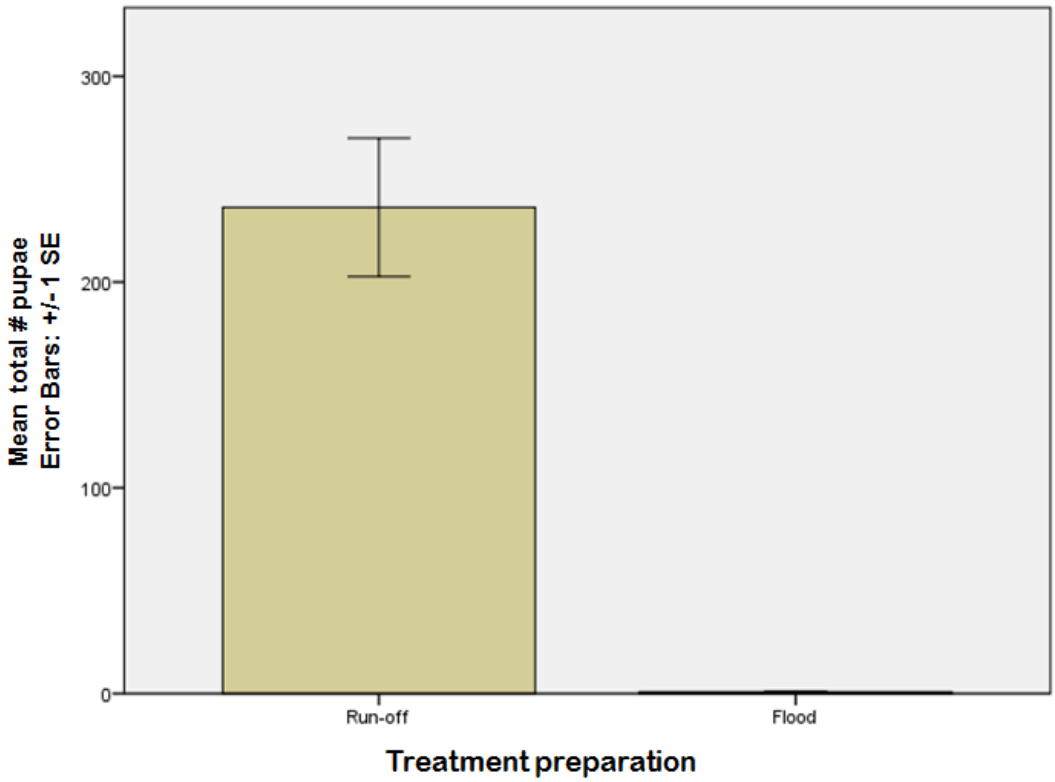

Fig. 44. The effect of treatment preparation on the number of reared pupae. The treatment preparation is displayed on the x-axis and the mean total number of pupae is displayed on the y-axis. There were significantly more (F = 48.984; df = 1; $P < 0.001$) pupae that developed in the run-off preparation than in the flood preparation.

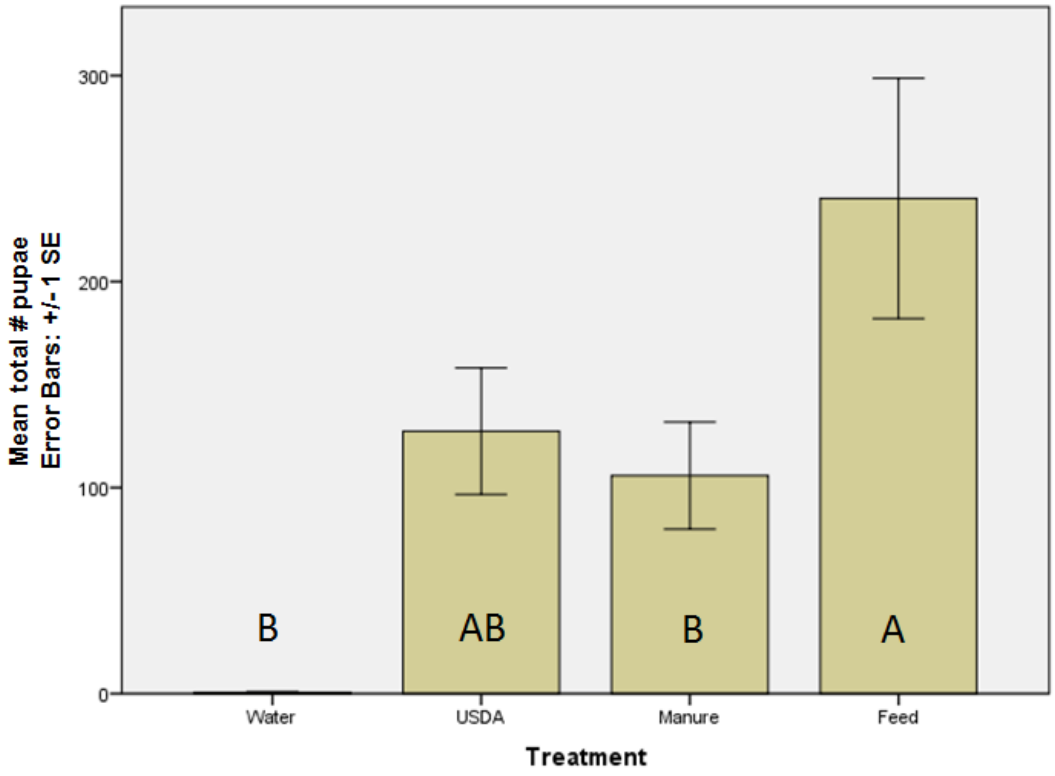

Fig. 45. The effect of treatment on number of reared pupae. The treatment is displayed on the x-axis and the mean total number of pupae is displayed on the y-axis. There was a significant (F = 7.701; df = 3; P < 0.001) difference between treatments. A Tukey's post analysis showed that the feed treatment produced significantly more pupae (n = 240.3) than the manure (n = 105.8) and water (n = 0.6) treatments. The USDA treatment was similar (n = 60; P = 0.058) to the feed treatment and the manure treatment. Means separation was determined by Tukey's LSD. Means with the same letter are not significantly different (α = 0.05).

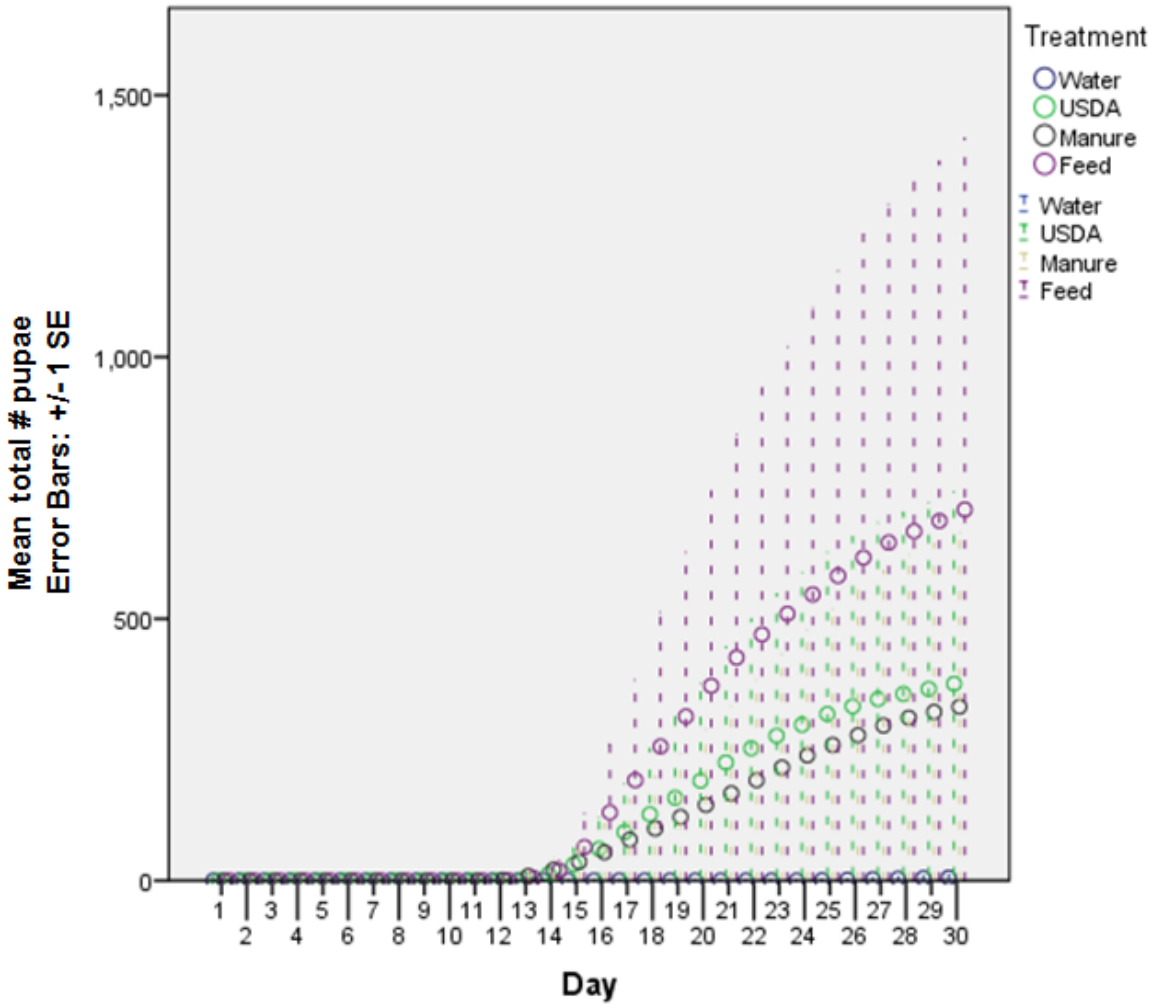

Fig. 46. The effect of time on number of pupae reared by treatment. The experimental day is displayed on the x-axis and the mean total number of pupae is displayed on the y-axis. There was a significant (F = 5.2.007; df = 29; P = 0.003) effect between the number of days and the number of pupae. A Tukey's post analysis showed that the feed treatment produced significantly more pupae (n = 240.3) than the manure (n = 105.8) and water (n = 0.6) treatments. The USDA treatment was similar (n = 60; P = 0.058) to the feed treatment and the manure treatment.

The same analysis was conducted on of the number of emerged adults (Fig. 47). As in the pupae data, the run-off treatment preparations produced significantly (F = 218.921; df = 1; $P < 0.001$) more adults than the flood treatments. The treatment factor was compared to the number of adults counted in Figure 48. Again, the means were separated using Tukey's and showed a significant (F = 7.603; df = 3; $P < 0.001$) difference in the number adults reared from the treatments. The feed treatment produced a mean of 57.7 adults, while the water treatment produced an mean of 0.1 adults. The manure and USDA treatments were similar to the feed and water treatments, respectively. The distribution of the mean number of adults reared over the 30 d experiment period was significantly (F = 54.326; df = 3; $P < 0.001$) different as time progressed through the experiment, as illustrated in Figure 49. Information gathered from percent hatch, pupae counts and adult emergence was consolidated into a life table (Fig. 50).

To determine differences for *C. sonorensis* pupae and adult development rates in differing substrates, secondary measurements of the nitrate concentration, pH, and dissolved oxygen concentration were measured in each of the treatments. Figure 51 shows the effect of substrate preparation on nitrate concentration. There was no significant difference (F = 1.197; df = 1; P = 0.281) in nitrate between the run-off and flood time treatment. Next, the effect of treatment on nitrate concentration was analyzed. There was a significant (F = 37.125; df = 3; $P < 0.001$) difference between treatment groups and a post-hoc analysis showed that the manure treatment has a higher mean concentration of nitrates (1.20 ppm) than all other treatments (Fig. 52). The effect of

substrate preparation on pH is illustrated in Figure 53. There was not a significant difference (F = 2.662; df = 3; P = 0.111) in pH between the run-off and flood preparations. Alternatively, when comparing the effect of treatment on pH (Figure 54), there was a significant difference (F = 43.087; df = 3; P < 0.001) between treatments. The post-hoc analysis showed that water had a significantly higher pH than other treatments at a mean of 7.60. The feed treatment was significantly more acidic with a mean of 4.73. The manure and USDA treatments fell in between these on the pH scale with means of 6.36 and 6.74, respectively.

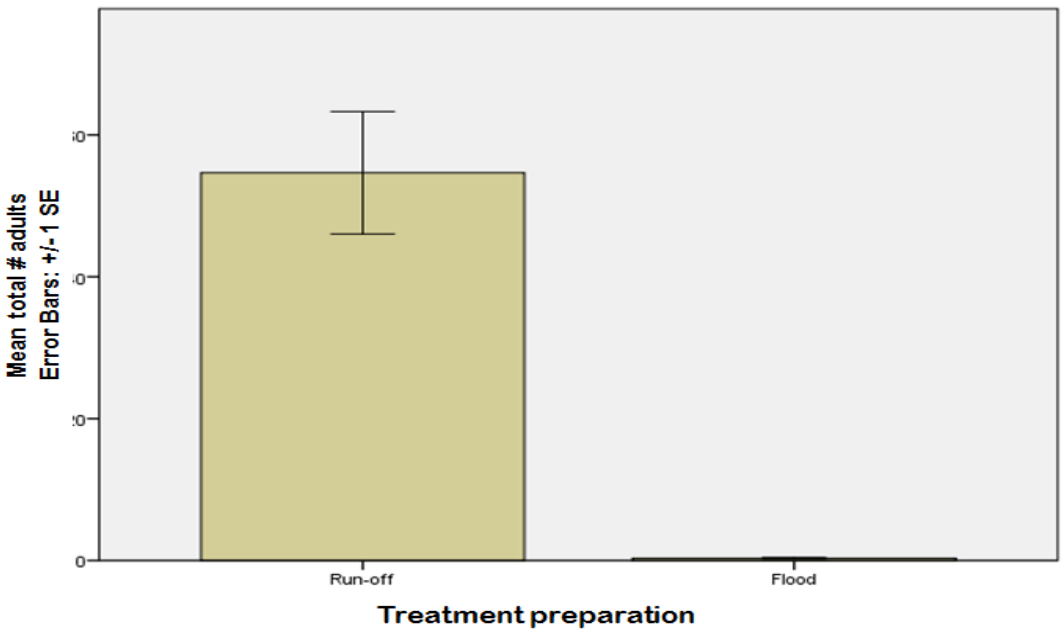

Fig. 47. The effect of treatment preparation on the number of reared adults. The treatment preparation is displayed on the x-axis and the mean total number of adults is displayed on the y-axis. The run-off treatment time produced significantly (F = 218.921; df = 1; P < 0.001) more adults than the flood treatments.

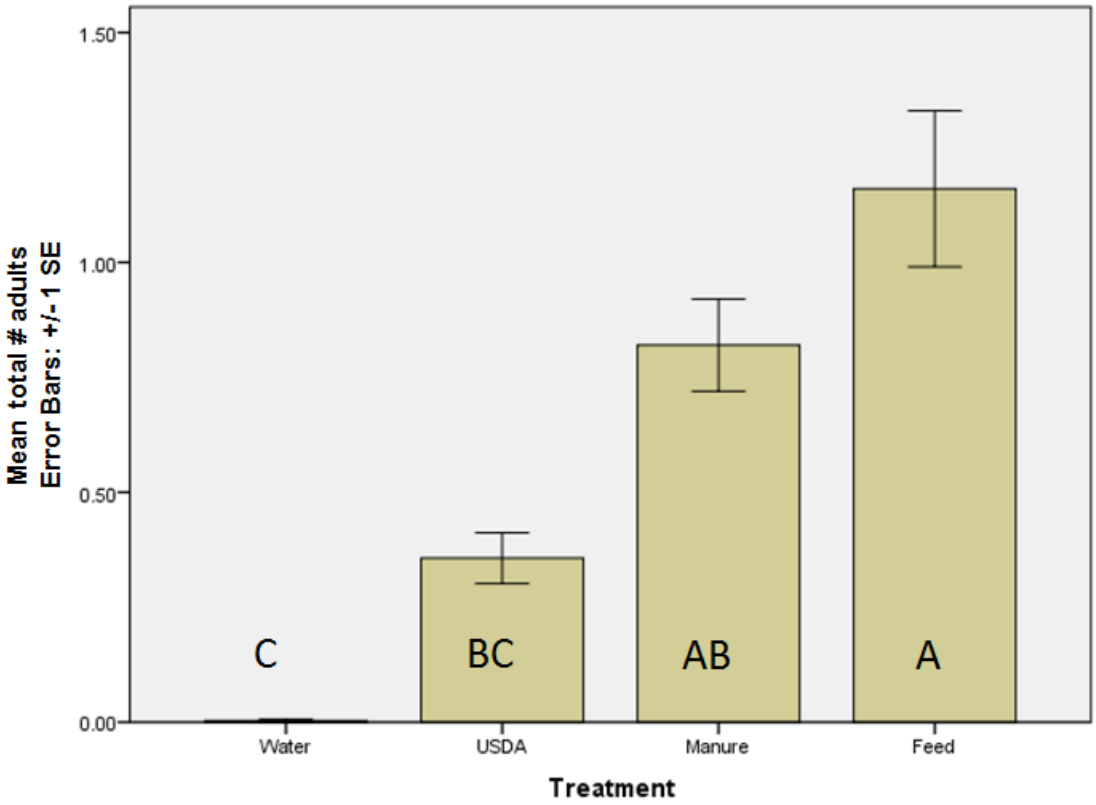

Fig. 48. The effect of treatment on number of reared adults. The treatment is displayed on the x-axis and the mean total number of adults is displayed on the y-axis. The means were separated using Tukey's and showed a significant (F = 7.603; df = 3; $P < 0.001$) difference in the number adults reared from the treatments. The feed treatment produced and mean of 57.7 adults, while the water treatment produced a mean of 0.1 adults. The manure and USDA treatments were similar to the feed and water treatments, respectively. Means with the same letter are not significantly different ($\alpha = 0.05$).

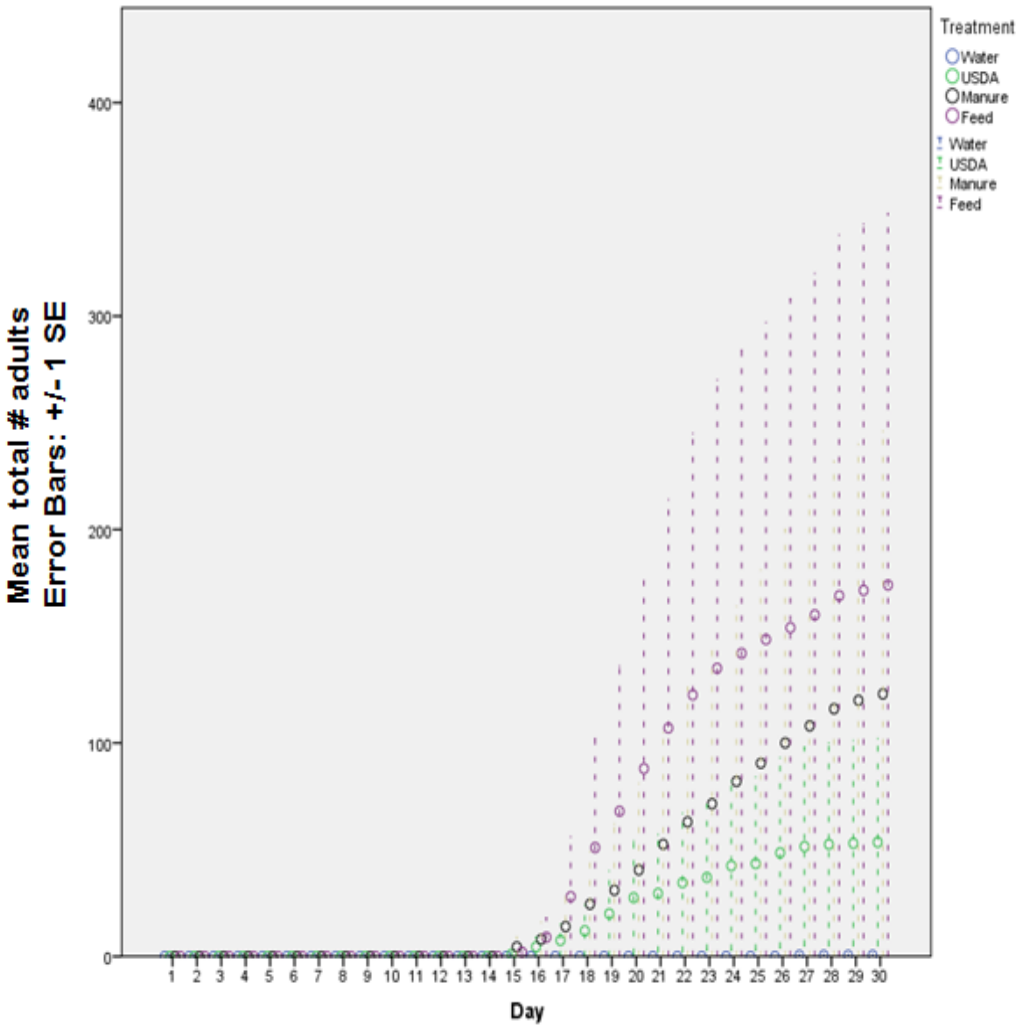

Figure 49. The effect of time on number of adults reared by treatment. The experimental day is displayed on the x-axis and the mean total number of adults is displayed on the y-axis. The distribution of the mean number of adults reared over the 30 d experiment period was significantly (F = 54.326; df = 3; $P < 0.001$) different as time progressed through the experiment.

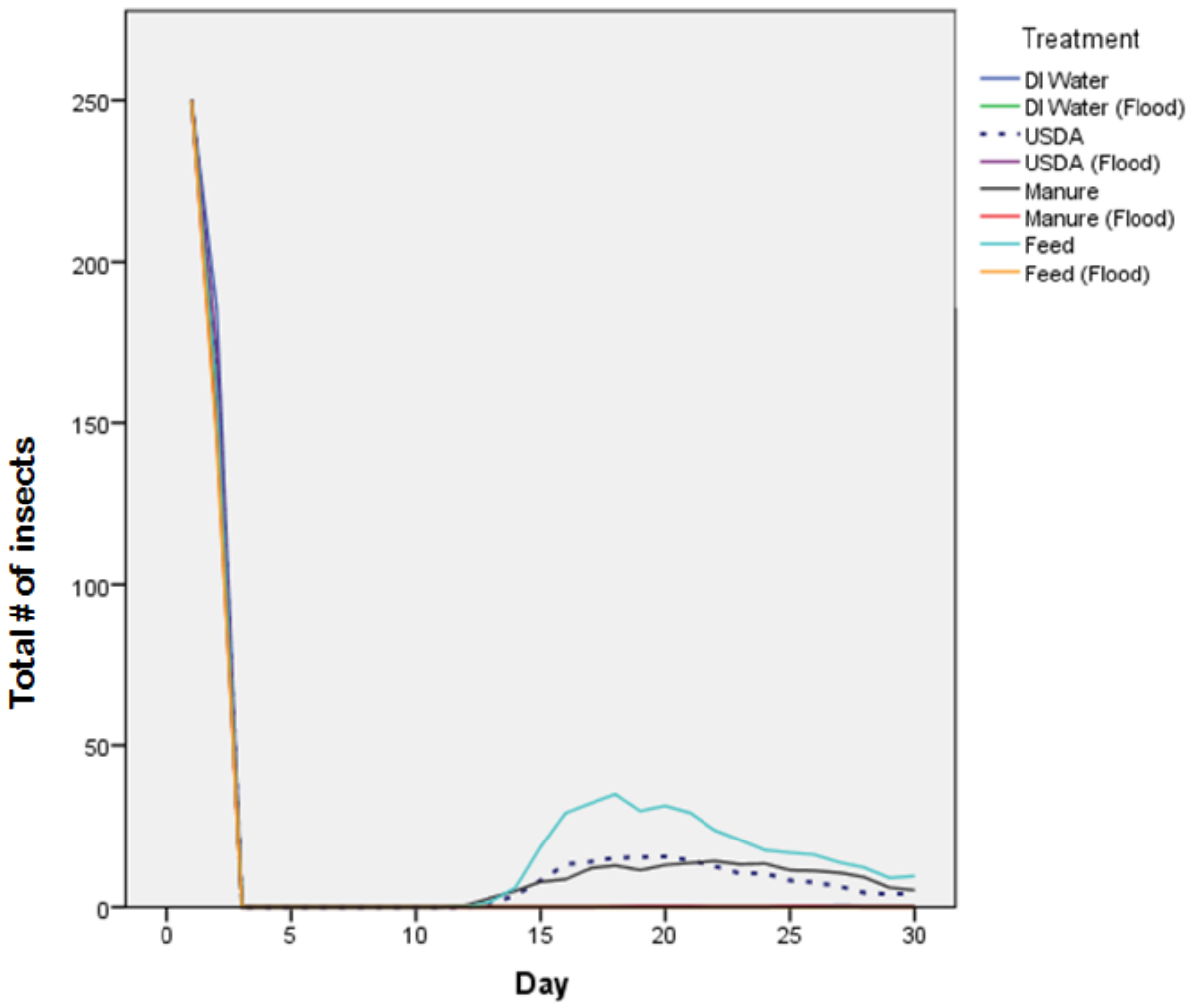

Fig. 50. Information gathered from percent hatch, pupae counts and adult emergence was consolidated into a life graph. The experimental day is displayed on the x-axis and the mean total number of insects is displayed on the y-axis. Only three substrates produced *Culicoides* during the course of development: feed, USDA mixture, and manure. None of the flood preparations or water substrates allowed for *C. sonorensis* development.

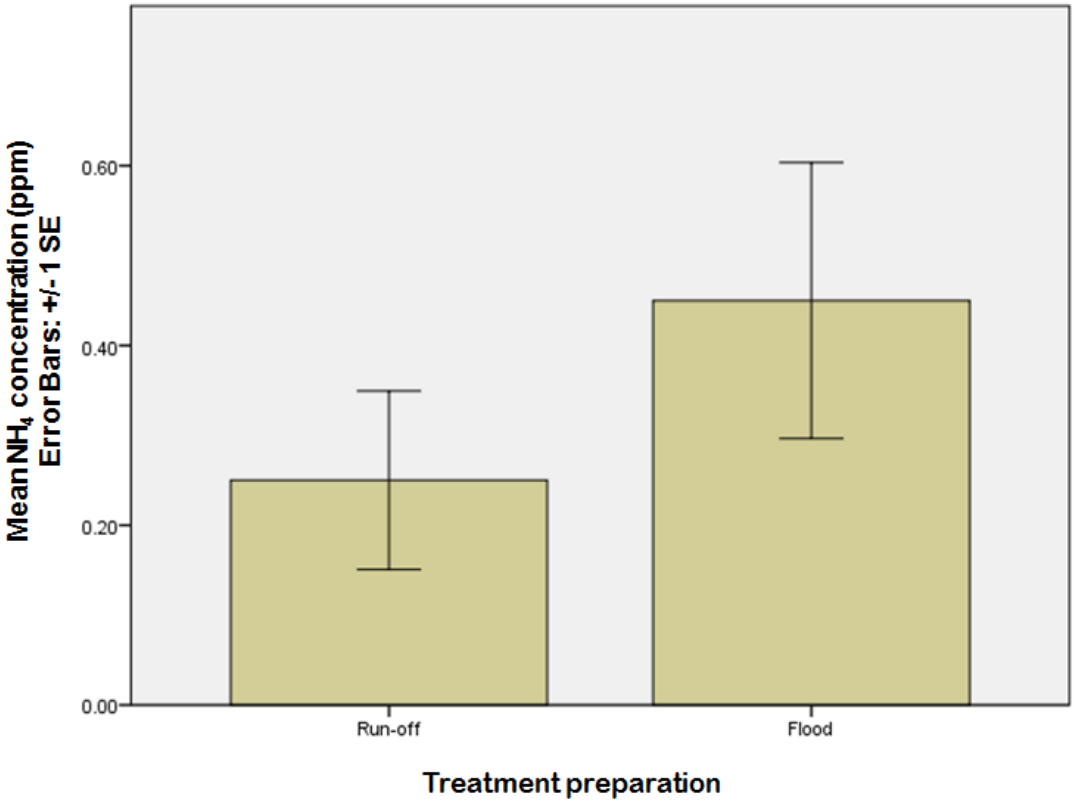

Fig. 51. The effect of substrate preparation on nitrate concentration. The treatment preparation is displayed on the x-axis and the mean NH_4 concentration is displayed on the y-axis. There was no significant difference (F = 1.197; df = 1; P = 0.281) in nitrate between the run-off and flood treatment preparations. The NH_4 concentration was measured at the beginning of the 30 d experiment.

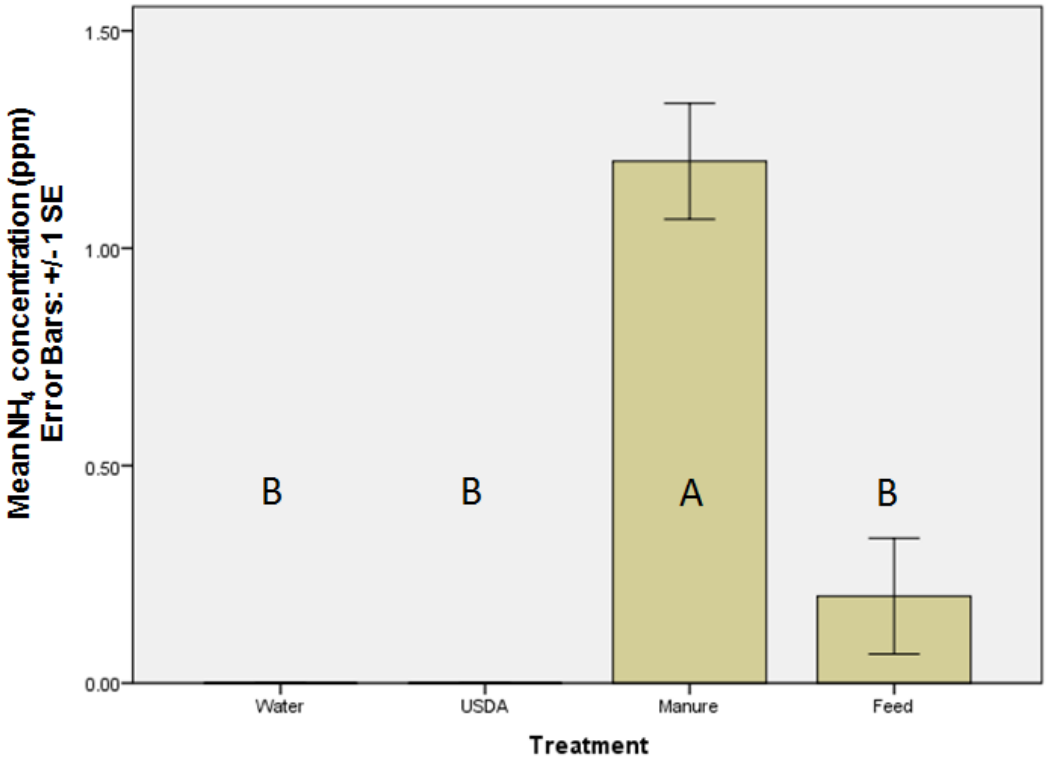

Fig. 52. The effect of treatment on nitrate concentration. The treatment is displayed on the x-axis and the mean NH_4 concentration is displayed on the y-axis. There was a significant (F = 37.125; df = 3; P < 0.001) difference between treatment groups and a post-hoc analysis showed that the manure treatment has a higher mean concentration of nitrates at 1.20 ppm than all other treatments. The NH_4 concentration was measured at the beginning of the 30 d experiment. Means with the same letter are not significantly different (α = 0.05).

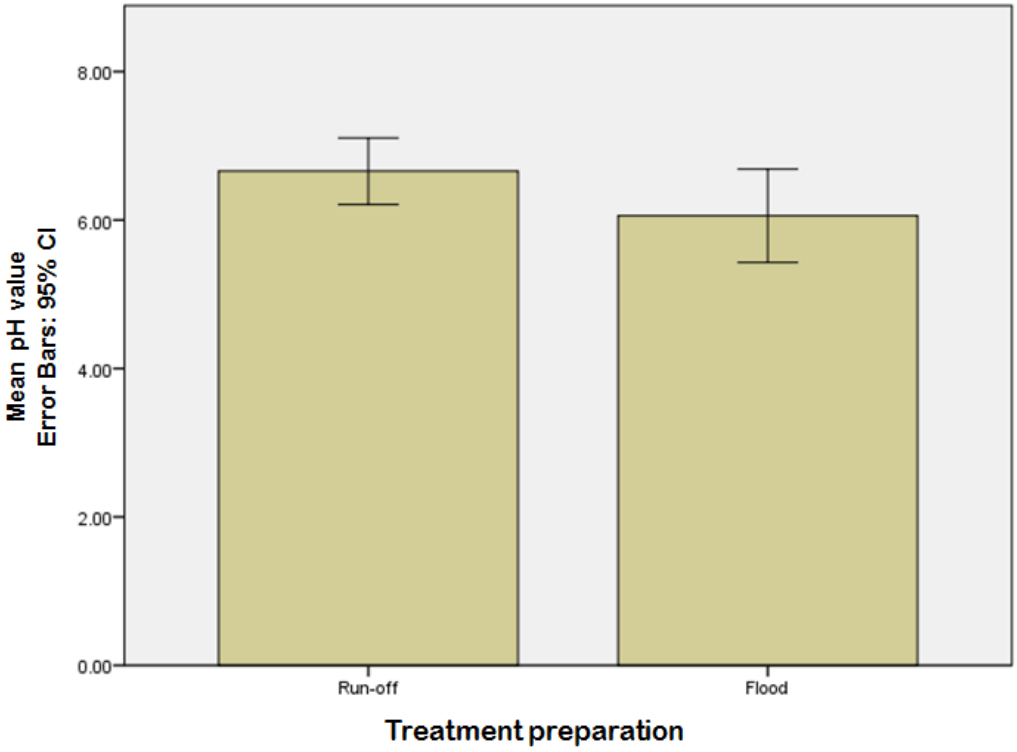

Fig. 53. The effect of treatment preparation on pH (water, USDA, manure or feed). The treatment preparation is displayed on the x-axis and the mean pH value is displayed on the y-axis. There was not a significant difference (F = 2.662; df = 3; P = 0.111) in pH between the run-off and flood preparations. The pH value was measured at the beginning of the 30 d experiment.

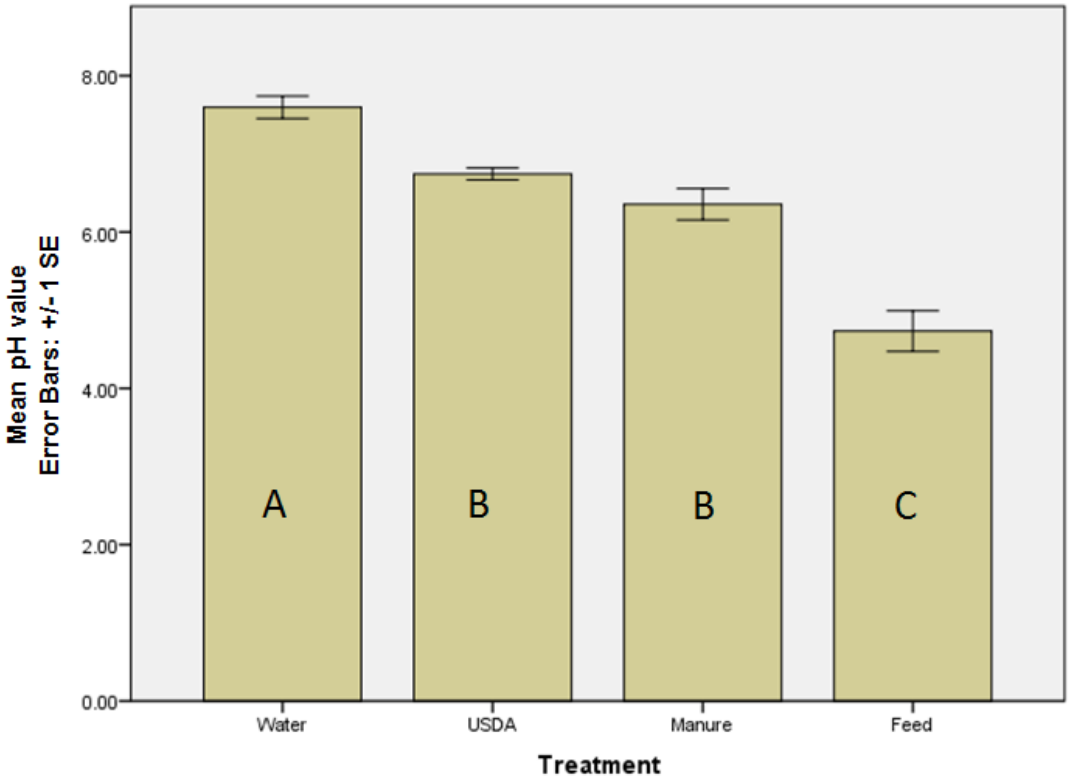

Fig. 54. The effect of treatment on pH value. The treatment is displayed on the x-axis and the mean pH value is displayed on the y-axis. There was a significant difference (F = 43.087; df = 3; P < 0.001) between treatments. The post-hoc analysis showed that water had a significantly higher pH than other treatments at a mean of 7.60. The feed treatment was significantly more acidic with a mean of 4.73. The manure and USDA treatments fell in between these on the pH scale with means of 6.36 and 6.74, respectively. The NH_4 concentration was measured at the beginning of the 30 d experiment. Means with the same letter are not significantly different (α = 0.05).

Like nitrate concentration and pH, mean dissolved oxygen content was compared to the effects of substrate preparation and substrate treatment. Figure 55 show that there was a significant difference ($F = 29.537$; df = 1; $P < 0.001$) between the run-off and flood treatment preparations with respect to dissolved oxygen content. An ANOVA was conducted to compare the effects of the different treatments to dissolved oxygen content. The results are displayed in Figure 56. A Tukey's post-hoc analysis showed that each treatment varied significantly from each of the others (Table 6). The difference in mean dissolved oxygen content (ppm) among treatments as expressed by Tukey's LSD is shown in Table 7. Each treatment is significantly different. The dissolved oxygen content was tracked over the 30 d experiment (Figure 57). Each line is representative of each separate treatment and reflects the significant mean separation described in Table 6.

Table 6. Tukey's post-hoc analysis of the effect of treatment on dissolved oxygen content.

Tukey HSD[a]

Treatment	N	Subset for alpha = 0.05			
		D	C	B	A
Manure	60	3.1592			
Feed	60		4.3481		
USDA	60			7.1043	
Water	60				8.2393
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 60.000.

Table 7. Significant differences in Tukey's HSD comparison of treatments. Multiple comparisons showed significant differences between all treatments as noted in the column labeled, "Sig."

Multiple Comparisons

Dependent Variable: ppm

Tukey HSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Water	USDA	1.13500*	.32979	.004	.2817	1.9883
	Manure	5.08012*	.32979	.000	4.2268	5.9334
	Feed	3.89127*	.32979	.000	3.0380	4.7446
USDA	Water	-1.13500*	.32979	.004	-1.9883	-.2817
	Manure	3.94512*	.32979	.000	3.0918	4.7984
	Feed	2.75627*	.32979	.000	1.9030	3.6096
Manure	Water	-5.08012*	.32979	.000	-5.9334	-4.2268
	USDA	-3.94512*	.32979	.000	-4.7984	-3.0918
	Feed	-1.18885*	.32979	.002	-2.0422	-.3355
Feed	Water	-3.89127*	.32979	.000	-4.7446	-3.0380
	USDA	-2.75627*	.32979	.000	-3.6096	-1.9030
	Manure	1.18885*	.32979	.002	.3355	2.0422

*. The mean difference is significant at the 0.05 level.

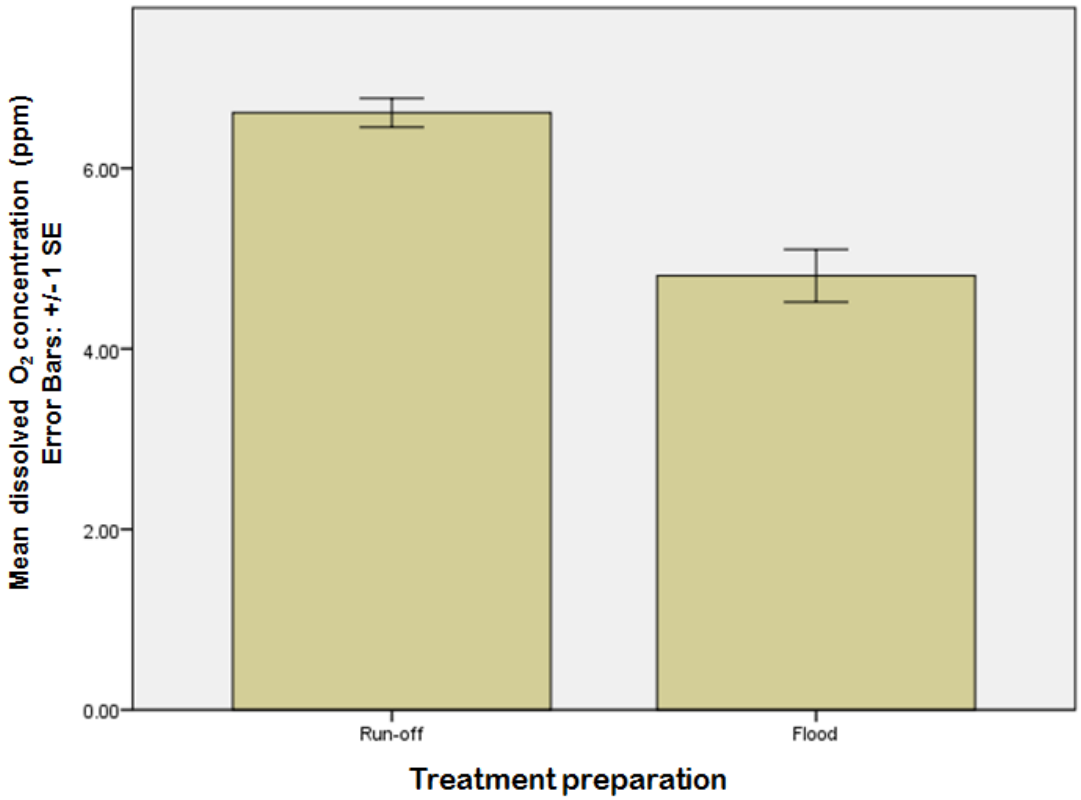

Fig. 55. The effect of treatment preparation on dissolved oxygen content. The treatment preparation is displayed on the x-axis and the mean dissolved O_2 concentration is displayed on the y-axis. There was a significant difference (F = 29.537; df = 1; $P < 0.001$) between the run-off treatment and the flood treatment preparations with respect to dissolved oxygen content (ppm).

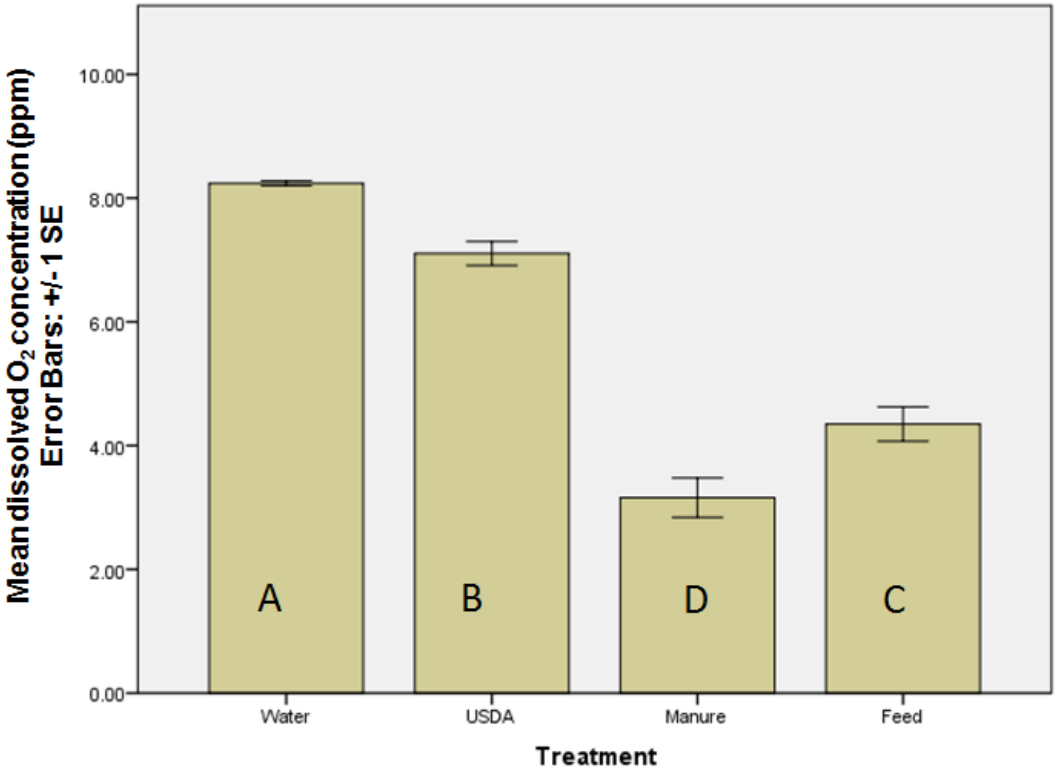

Fig. 56. The effect of treatment on dissolved oxygen content. The treatment is displayed on the x-axis and the mean dissolved O_2 concentration is displayed on the y-axis. Dissolved oxygen content was measure every day for 30 d for each treatment. Means separation was determined by Tukey's LSD. Means with the same letter are not significantly different ($\alpha = 0.05$).

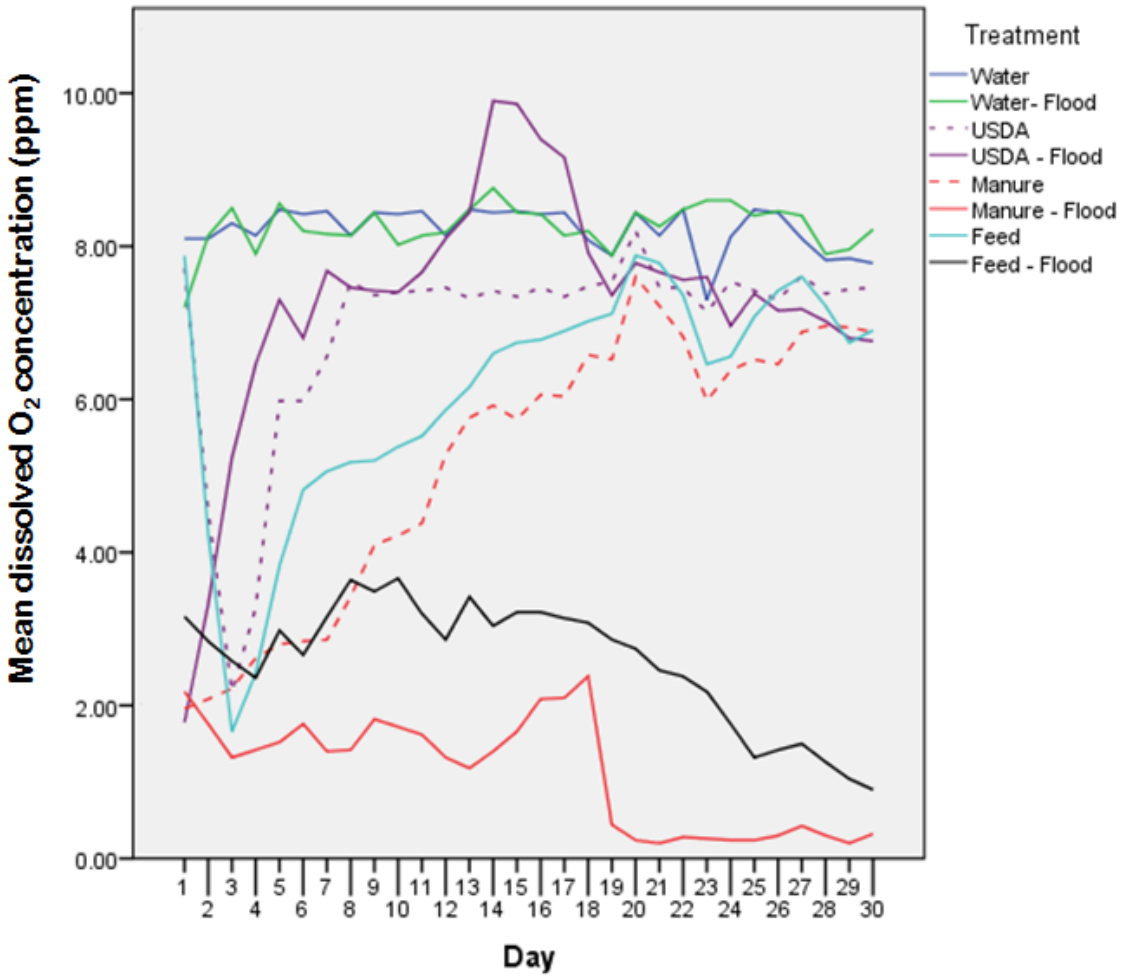

Fig. 57. Dissolved oxygen content over time by treatment. The experimental day is displayed on the x-axis and the mean dissolved O_2 concentration is displayed on the y-axis. The dissolved oxygen content was tracked over the 30 d experiment. Each line is representative of each separate treatment and reflects the significance.

Discussion

There is a need for information into *C. sonorensis* development, as it is the most abundant vector of BTV and EHDV in the United States. More specifically, details are needed into resources *C. sonorensis* utilize for develop in and around Texas white-tailed deer-breeding operations. The objective of this experiment was to determine the effectiveness of common substrates found in deer pens that would be used by *C. sonorensis* to complete their development. The hypothesis tested was that there would be no significant difference in the substrates used for *C. sonorensis* development.

The percent hatch of *C. sonorensis* eggs was calculated 24 h after the eggs were deposited on the substrates. After this time, the eggs had turned brown and would not have hatched if they had not done so already. The egg hatch datum was recorded to create a *C. sonorensis* life table for this experiment. The information provided by Figure 50 can be used to estimate *C. sonorensis* development under optimal conditions in common substrates found in white-tailed deer-breeding operations.

Two substrate preparations were tested: run-off and flood. These preparations were applied to all substrates (water, USDA mixture, manure, and feed) to mimic a rainfall event. The idea was that if a female *C. sonorensis* found a stagnant water source in a deer pen, such as a flooded corner of manure or a feed bucket filled with rain, they may try to lay eggs in such substrate. The time period tested for the flood treatment was 7 d. The run-off preparation was designed to mimic water running off the landscape, either with little rainfall or a spilled water bucket. There was a significant effect of preparation on both pupae and adult development. Essentially, almost no *C. sonorensis*

eggs developed in the flood treatment in any of the repetitions. The flood preparation was designed to mimic water settling in a substrate. The results from the first few repetitions of flood preparations spurred the question of why *C. sonorensis* was unable to develop. A dissolved oxygen meter was used to monitor oxygen levels in the last repetition for both the flood and the run-off treatments of all substrates tested (Fig. 57). It became obvious that, while the water and USDA substrates stayed steady at around 8.00 ppm of oxygen for the 30 d experiment, the manure and feed substrates became anaerobic within a few days. This would explain the absence of pupae development and was likely due to the absence of oxygen.

There were significantly more pupae counted in the feed substrate tested than in the other treatments. As expected, pupae did not develop in the water substrate. This is likely because of missing nutritional components needed by *C. sonorensis* during development. The USDA medium produced a similar number of pupae to the feed treatment, but did not surpass the other medium tested. As shown in Figure 46, the feed treatment started producing pupae earlier than any other treatment and continued showing signs of pupal activity for the duration of the experiment.

The effect of treatment on adult development was again significantly higher in the feed substrate than any other tested. Since there was no pupal activity in the water substrate, there was also no adult development. As seen in Figure 49, the manure treatment surpassed the USDA treatment in adult development, as it had previously been lower during the pupal development. Again, the feed treatment produced more adults over a longer time period than any of the other tested substrates.

The question of why the substrates were successful arose during the experiment planning process, so the steps of measuring the nitrate and pH of the substrates were added. There was a significant difference in the nitrate concentration of the manure substrate than all other substrates tested. Alternatively, there was not a significant difference in nitrate concentrations between the two time treatments of all substrates tested. This proves that any substantial ammonium that would be released into the substrate will do so immediately.

The pH of the substrates and treatment preparations were measured to determine at which rate *C. sonorensis* eggs developed in slightly acidic or basic mediums. There was not a significant difference in relative pH between the run-off and flood preparation, though the run-off preparation was consistently more basic than the flood preparation. The water substrate was significantly more basic than any other substrate, while the feed substrate was significantly more acidic. The USDA and manure substrates were similar in pH values. This information is becomes important when compared to both the pupae and adult development rates. When comparing pH and *C. sonorensis* development trends, the results show that control of this insect could lie in the ability to manipulate the pH of substrates.

It is known that *C. sonorensis*, along with other *Culicoides* species, are the primary vectors of BTV and EHDV in the United States. Information about the control of the biting midge in Texas white-tailed deer-breeding operations is limited, with most producers relying on applications of chemicals to control *Culicoides* spp. Through this study, it became evident that common organic materials in the deer pens can supply

sufficient nutritional needs for *C. sonorensis* and can produce significantly more insects than diets used for use in laboratory colonies. Integrated pest management practices for white-tailed deer producers to utilize in confined operations would be of great assistant to the battle of pathogen-vectoring biting midges. Practices that promote sanitation of deer pens and utilization of common IPM tactics would save money for the deer producer and improve the health of the white-tailed deer through the use of non-chemical methods. The information discovered by this experiment is only a small piece in the large project of insect control, but a step in the right direction towards control of an insect of which little is known developmentally.

CHAPTER V

INTEGRATED PEST MANAGEMENT RECCOMENDATIONS FOR TEXAS WHITE-TAILED DEER PRODUCERS

Introduction

Hunting white-tailed deer (*Odocoileus virginianus*) has an estimated annual economic value over $3 billion in the State of Texas (Anderson et al. 2007b). Private land owners in Texas are challenged with the fragmentation of farms and ranches due to economic perils associated with inheritance laws, taxes, down turns in world markets for agricultural products, and the increased costs associated with all aspects of farming and ranching. These factors have placed an economic strain on these landowners. The end result has been greater pressures to increase economic outputs from non-traditional means such as hunting and wildlife enhancement. One specific methodology that landowners have found useful is the development of captive deer farms and ranches (Baccus 2002). According to Dr. James C. Kroll, Regents' Professor and Director of the Institute for White-tailed Deer Management & Research at Sam Houston State University, "Deer breeding is saving the family farm or ranch in Texas" (Ammoland 2011)..

Texas is the nation's leader in the white-tailed deer-breeding industry with an estimated annual impact of at least $700 million (Anderson et al. 2007a). The initial investment in a small "Intensively Managed Hunting Operation" in Texas can approximate $200,000 associated with an average of 684 acres (cost of land not

included) (Anderson et al. 2007a). In 2011, there were 1,261 "Deer Breeder" permits issued by the Texas Department of Parks and Wildlife, the state agency that regulates captive cervid production. The Texas Deer Association (TDA), which was established in 1999, has 2,600 members, the majority of whom are involved in some aspect of intensive deer management (TDA 2013). The level of deer management on private property in Texas can vary tremendously, including "open range" (no specific management format other than Texas rules and regulations on numbers of deer harvested per license holder), traditional "hands off" operations with high fenced acreages (no culling or genetic manipulation), "low intensity management" (culling, but no genetic manipulation)," high intensity management" (culling, and genetic manipulation), and the production of "breeder stock" through the Deer Breeder Permit system (TDA 2013). Breeder stock, including does and bucks, can be sold to other deer breeders or can be released in high fenced management areas for stocking or eventual harvest. The value of individual deer is determined by the market place, with those from desired genetic lines and high scoring potential being appraised with metrics equivalent to "Boone & Crockett Club" (B&C) scoring. In addition to the genetics (genotype), nutrition plays a major role in production of the phenotypes of greatest worth to breeders and hunters alike.

The development of captive production deer facilities has provided the same challenges that exist with confined operations of any and all livestock including: management of manure and other wastes; control of insect pests; and, protection from disease agents that adversely affect animal health, growth, phenotypic expression, and value. Of particular concern are viruses which cause epizootic hemorrhagic disease

(EHD) and Bluetongue (BT) in North American deer. Both diseases are caused by viruses (*Orbivirus* spp.) (Verwoerd and Erasmus 2004), which are antigenically very different, with several serotypes of each. The signs and symptoms of EHD and BT are clinically indistinguishable. The hemorrhagic disease agents are moved in nature through populations of biting flies including *Culicoides sonorensis* and other related species (Mellor 2000, Gerry et al. 2001). While the association of biting flies to enzootic situations in deer has been discussed in the literature; there is much to be learned about conducive conditions that favor these insects in and around captive deer management operations. In addition, information about the seasonal occurrence of the flies and the presence of the disease agents needs to be assessed.

Other fly species known to be associated with deer confinement facilities include: house flies *(Musca domestica),* stable flies (*Stomoxys calcitrans*), horn flies (*Haematobia irritans*), horse flies *(Tabanus* spp.), and deer flies (*Chrysops* spp.). These flies are not only a nuisance, but reduce animal fitness and production, and are potentially mechanical vectors of pathogens in captive deer populations.

Deer held in captive operations produce wastes in the form of feces and urine, along with spoiled feed materials. The environment involving these waste products is rife with the potential for fly production. Deer breeders have asked for help in many areas of insect identification, but also to developing best management practices that will potentially reduce or limit insect problems.

Materials and Methods

The goal of this objective was to create an informational pamphlet for distribution by various extension and education organizations in Texas. This information was made available to white-tailed deer producers to aid them in protecting their deer from potential disease – causing pathogens vectored by dipterans, such as *Culicoides* spp.

To develop insect population management techniques to be utilized by white-tailed deer producers, data from the three previous objectives has been summarized. Along with field experience, interviews with producers, and collaboration with the TDA, the data collected from the three previous objectives will provide insight to important components of an IPM program for these flies. As of now, there have not been recommended IPM guidelines provided specifically for white-tailed deer breeders in Texas. The information gathered was included in four separate categories of the IPM informational flyer: description of the problem, identification of the pest, biology of the pest, and control recommendations (cultural, physical, biological, and chemical). Recommendations were made as to when *Culicoides* spp. and other possible pathogen vectors are active, along with guidelines for deer pen sanitation. Data presented outlined which species of *Culicoides* spp. and which BTV and EHDV serotypes were present throughout the State of Texas during the 2013-2014 CO_2 trapping experiment. The goal of this objective was to make the information gathered by this dissertation study easily accessible to white-tailed deer producers.

Results and Discussion

Integrated pest management (IPM) is the ecological approach to the control of target pests in which all available control techniques are systematically considered and integrated into a program that reduces economic damage and adverse side effects (Smith and Reynolds 1966). To implement an IPM program in a confined white-tailed deer facility, there are several steps that must be taken, beginning with surveying the pest population. Visual acknowledgement that there is a pest problem on the deer is an easy, qualitative way to survey the insect damage. Questions involving head shaking, movement of deer away from resources due to insect nuisances, and other physical reactions by the deer should be asked. Quantitative measurements, such as using glue board traps or CO_2 traps can provide data over time about fluctuations in the insect population numbers.

After the problem is identified, an IPM program can be identified. For *Culicoides* spp. in Texas white-tailed deer-breeding operations, the goal should be to control the insect in and around pens without disturbing the deer, as they are often prone to stress. Control actions should take into consideration that the deer are not often worked through a chute and will need to be dart tranquilized for elaborate insect control procedures, which is not ideal. The outcome of an effective integrated pest management program could include healthier deer and subsequently more profits for the deer-breeding operation.

Cultural control, or sanitation, is the least expensive and often the most effective method for insect management (Stern et al. 1959). In the deer-breeding industry, this

most often will require the removal of manure and other organic waste from the environment to suppress the pest population. Deer feed and hay should be kept off the ground, as biting midges and other filth flies will use these as a source of developmental nutrients. Manure often aggregates around trees, in corners, or under the protective shed in the deer pen. Though deer manure will dry out fairly quickly, accumulated manure in these areas can be prime locations for development of *Culicoides* spp. It is recommended that areas of accumulated waste in the deer pens be raked and removed, along with discarded feed and hay which is common underneath feed troughs.

Physical control employs the use of barriers to alter insect development. Physical control of insects in the deer pen could include draining water from low lying areas or repairing damage to a leaking water trough (Stern et al. 1959). Within the deer pen, water can often accumulate due to leaking water troughs. Antlered bucks can cause damage to water float systems and cause water accumulation. It has been found that this water will aid in the development of *Culicoides* spp. and other filth flies. Outside of the deer pens, it is important to keep water flowing. Stagnant creeks or ponds are key locations for *Culicoides* spp. development. If possible, deer pens should be situated away from stagnant water sources and on top of a hill. The placement of pens on a hill will allow any water accumulation, whether accidental or natural, to run off from the pens. Also, while only a small amount is known about *Culicoides* spp. flight, it is known that *Culicoides* spp. are carried by the wind. For pens on the top of a hill, the wind may carry more insects away from the deer pens than if they were located on a low-lying plane. Another advantage of pens being built on a hill would be that there would be less tree

cover. There are many species of *Culicoides* that are known to breed and develop in tree holes. It is unknown if all *Culicoides* can use trees for development, but as trees collect water during a rain event, it is suspected that tree holes could be an option.

Biological control uses the concepts of natural enemies, such as bacteria, fungi, or predacious arthropods to control the insect population (Stern et al. 1959). There are currently no biological control recommendations published for the use in regulating *Culicoides* spp. in white-tailed deer-breeding operations. Though there have been several studies conducted evaluating natural enemies of *Culicoides* spp., none have been found to be highly effective (Borkent 2005). Wirth (1977) compiled a review of pathogens and parasites of biting midges in which a limited amount of literature was available. A study in West India found that only adult tiger beetles, *Cicindela suturalis*, would feed on adult and pupae forms of *C. phlebotomus* (Yaseen 1974). Several studies have reported the use of pathogenic viruses used to control tree hole species of *Culicoides* in Louisiana and California. Symbiotic bacteria of *C. nubeculosus* (Lawson 1951) and *C. salinarius* (Becker 1958) were identified, but neither was found to be useful for control. Four species of parasitoids acting as biological control agents for ceratopogonids have been recorded, but none have been found to be effective against species in the genus *Culicoides* (Wirth 1977).

Chemical control employs the use of pesticides to manipulate insect population numbers and is often considered an effective option (Stern et al. 1959). Though the use of pesticides has been found to be effective against *Culicoides* spp., the long term efficacy of the product has not been evaluated (Wirth 1977). Borkent (2005) described

that the beginning of systematic efforts to control *Culicoides* spp. began in the early 1900s and included methods for draining or filling insect development sites and the use of crude oil for control. Chlorinated hydrocarbons were used, but, much like other target pests, *Culicoides* spp. developed resistance (Borkent 2005). Pyrethrums, which have a similar modes of action to chlorinated hydrocarbons, are widely used by white-tailed deer producers to control insects, though there has not been any products labeled for use in confined white-tailed deer-breeding operations. Interviews with deer producers have reported many to be fogging or spraying pyrethrum chemicals on deer up to seven times per day in an effort to control the insect problems. Another chemical that may be useful to control *Culicoides* spp. is methoprene, an insect growth regulator often used as a mosquito larvicide. Though more research needs to be done to determine chemical efficacy, the same larvicides used to control mosquitos may be effective against *Culicoides* spp. because of their developmental similarities. While chemicals may initially control the insect population, it is important to remember that insects can quickly develop resistance to a chemical and that they should be used sparingly.

The key to success in an integrated pest management program is to continue observing pest populations, implement the entire plan, and to keep records of outcomes due to IPM tactics. Based on results, the IPM plan may need to be adapted between seasons, but it will always be important to have a system for insect monitoring and control in place. With the help of these tools and their implementation, the deer may be able to avoid the painful bites of *Culicoides* spp. and the possible contraction of BTV and EHD in the process.

CHAPTER VI

SUMMARY AND CONCLUSIONS

The objective of this dissertation project was to address entomological issues faced by white-tailed deer producers in Texas. The Texas Deer Association proposed an issue to which very few answers were known and it was a goal to provide them with answers. This set of dissertation objectives was designed with a funneling effect in mind.

First posed was the largest question of which entomological threats plagued white-tailed deer in Texas breeding operations. This question was addressed by sampling ten locations on 24 ranches in separate regions of Texas. The response from deer producers was overwhelming and appreciated. The decision to use the 24 ranches in this study came from their location within a region and the ability to process approximately 240 insect traps per month. Though the number was manageable, I believe that the study would have benefitted from more ranches in the experiment and a longer survey period, but this could be said about any study. Unfortunately, there were a handful of ranches that failed to participate to the capacity needed for results, or not at all. The results of this study proved what was expected; that there are nuisance flies present in deer-breeding facilities and that ceratopogonids are numerous as well. It was interesting to see the distribution of fly taxa around individual deer-breeding facilities. The association of filth flies and other Muscid flies to water and filth sources was overwhelming. The deer producers will be receiving feedback from the two year study conducted on their

ranches. It is hopeful that this information will be helpful to them in deciding where to focus IPM tactics and in which pens to place deer.

The second question proposed dealt with *Culicoides* spp. presence and the abundance of BTV and EHDV in insects trapped in white-tailed deer-breeding pens. The first question of this dissertation study focused on all insects in the system, while this question aimed to determine to what effect *Culicoides* spp. where abundant and which viruses they were positive for. Both EHDV and BTV were sampled from a total of three species of *Culicoides* and a total of five species were sampled from ranch locations. Unfortunately, hemorrhagic disease datum was not collected from ranches outside of TDA Region 6, but that is likely due to lower response rates from ranches that had to ship their samples overnight. It is believed that more sampling times, possibly weekly instead of monthly, would have yielded more results. A total of five species of *Culicoides* (*C. sonorensis*, *C. haematopotus*, *C. crepuscularis*, *C. multipunctatus*, and *C. butleri*) were collected during the study. Of 416 samples of *Culicoides* submitted for viral isolation, 23 tested positive for hemorrhagic disease. Three species (*C. sonorensis*, *C. multipunctatus*, and *C. crepuscularis*) tested positive for BTV or EHDV. Bluetongue serotypes were not identified, but EHDV 1, 2, and 6 were isolated from these species. The missing serotypes in the data were a result of a failed freezer in the TVMDL laboratory during the experimental process. The procedure to isolate BTV and EHDV from *Culicoides* spp. is expensive, so funding to continue this research is a necessity. Sampling procedures were successfully developed over the 21 month course of the experiment and the data collected can be used to determine *Culicoides* species

distribution in Texas. For example, *C. multipunctatus* tested positive for BTV in Burleson County, Texas, so it can be deducted that, if found in other locations, this species has vector competence for BTV pathogens in other locations.

After determining which insects were present, which *Culicoides* spp. were abundant, and to what degree BTV and EHD were present in Texas white-tailed deer ranches, the third objective posed by focused around control. There is little known about *Culicoides* spp. development due to their small size and difficult colonization history, so control recommendations are few. The white-tailed deer-breeding industry is relatively new and highly specialized, so control of an insect to which very little is known biologically is a large project. To focus this large project down to directly benefit white-tailed deer producers, known locations for filth fly development were tested as sources for development for *C. sonorensis*, the most prolific *Culicoides* spp. in North America. In discussions about *Culicoides* spp. development prior to this study, it was unknown if common substrates such as feed and manure played a role in development. This hypothesis was tested and it was discovered that, given the opportunity and the right amount of moisture, it was possible for *C. sonorensis* to grow from egg to adult in less than 20 d. Each of the treated substrates was chosen based on their abundance in the deer pens. It was not expected that they would surpass the growth potential of the USDA substrate, a mixture used for rearing *C. sonorensis* in the laboratory. However, results concluded that natural elements found in deer pens are capable of acting as a larval food source and developmental substrate for *C. sonorensis*. This is important information that can be used in IPM recommendations for white-tailed deer producers. Cultural control is

built on sanitation concepts and the data found in this study proves that removal of manure sources and housekeeping practices for deer feed and hay could lead to improved deer health and safety.

After gathering all of the information about development, distribution, and vector capacity of *C. sonorensis*, it was a natural decision to focus the last objective of this dissertation on IPM recommendations for Texas white-tailed deer producers. The information gathered will be used to propose cultural, physical or mechanical, and possible chemical practices for use in the deer-breeding facilities. The aim of the Texas A&M AgriLife Extension Service is to provide research-based educational programs and solutions for all Texans. The information gathered by this dissertation study can be distributed as an informational bulletin in order to assist all producers who are battling *Culicoides* spp. and the pathogens they vector in their production system. The recommendations could be of help to cattle and sheep producers also, as they suffer the effects of infected *Culicoides* spp.

The future of *Culicoides* spp. research as vectors of pathogens should be directed towards discovering more about their biology and flight capabilities, as well as pesticide efficacy and safety, and natural enemies for insect control. A focus should be placed on educating people whom are affected by *Culicoides* spp., about insect control and monitoring efforts. Overall, this collection of dissertation objectives aimed to provide relief for an industry battling an insect capable of vectoring disease-causing pathogens. There was little information previously available to white-tailed deer producers, but the result of these studies is a step in the direction of assistance. The goal of any livestock

producer is to have healthy animals and with the discovery of this foundational information, the suppression of BT and EHD in white-tailed deer-breeding facilities is on the horizon.

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