THE INFLUENCE OF EASTERN RED CEDAR

ON TABANIDAE POPULATIONS

IN OKLAHOMA

By

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Abstract: Horse flies (Tabanidae) are commonly associated with pastured beef systems in the southern United States and are one of the most difficult fly pests to control. Tabanids are strong visual predators with a painful bite and can fly miles to find their hosts. Cattle are continually exposed to multiple species of tabanids throughout the summer, but little is known about the effect of landscapes and vegetation type on tabanid behavior. In the last few decades there has been an increase in Eastern Red Cedar (ERC), *Juniperus virginiana*, encroachment across Oklahoma. The majority of western Oklahoma is prairie, however, ERC is encroaching and altering the landscape. Biting fly populations as well as the diseases they transmit have been on the rise. The objectives of this study were to 1) determine habitat use by tabanidae in relation to ERC and 2) determine the abiotic factors that are associated with tabanidae use of ERC habitat. In 2017 and 2018 we employed two different styled traps into habitat classified by the percentage of ERC to monitor the *Tabanus*, *Chrysops* and *Hybomitra* in the area. Data was collected for 20 weeks and analyzed. The data was evaluated along with abiotic weather factors to study the effect on tabanid populations. Habitat association was significantly higher in Open Canopy Cedar habitat both years of the study, regardless of genus or trapping type. Abiotic factors influencing the abundance of tabanids varied by year, genus and species. This study provided support that tabanidae in Oklahoma are utilizing Open Canopy Cedar habitat and removing ERC from beef pastures could be a viable method to controlling tabanid populations.

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CHAPTER I

Literature Review

Tabanid Biology and Ecology

The Phylum Arthropoda is a unique and diverse group of organisms that includes everything from isopods to beetles to ticks, mites and spiders. Each class of arthropods has its own group of characteristics for which it is known. Class Insecta is specifically a group of six-legged insects that go through a form of metamorphosis. It is a broad class of organisms with a fair number of recognizable beneficials, pests and parasites. The Order Diptera, the true flies, are grouped together based on morphological and behavioral characteristics. These holometabolous insects go through a lifecycle comprised of four stages: egg, larva, pupae and adult. Typical adult flies will have one set of wings and one set of halters. Within this study we are interested in the family of biting flies known as Tabanidae (tabanids).

Nearly every genus in the family Tabanidae, is a recognized livestock pest(Stone 1938). The adult females require a blood meal in order to produce eggs. The males feed solely on plant pollen and nectar. Species are univoltine, producing one generation per year, although new adults emerge throughout the summer. Adult females readily feed from large warm-blooded animals such as cattle, horses, deer, elk, moose, goats, sheep, pigs, and even humans, Tabanids have been known to feed from dogs, cats and reptiles as well. After a female has fed sufficiently and mated, she will find a suitable egg laying site. Tabanid eggs are laid on vegetation overhanging standing water. When the larvae hatch, it will develop in the semiaquatic areas around water. This includes mud, wet leaf litter and effluent from livestock production (Schwardt 1936, Stone 1938, Webb and Wells 1942, Gingrich and Hoffman 2015). The larvae are voracious predators, eating soil-dwelling arthropods and nematodes, other dipteran larvae and even brood mates. The larvae exhibit high levels of cannibalism which makes it very difficult to rear horse flies in a laboratory setting and usually requires separating the eggs prior to hatching or isolating the larvae as soon as they hatch (Philip 1928, Webb and Wells 1942). Pupation will occur in slightly drier soils located near their larval habitats. In Oklahoma, emergence of adults begins in the spring and can last through the fall (Hollander and Wright 1980a). Adult tabanids are considered a warm weather pest, dying in the fall.

Historically, tabanids are associated with woody vegetation. Many studies have found that adult tabanids use the pasture and wooded habitat interface to ambush hosts as they transfer from one habitat to the next. Studies looking at the behavior of *Tabanus abactor* (Philip), have shown significant use of wooded habitat over open pasture by adults (Davis and Sanders 1981, Foil 1983, Moore et al. 1996, Slosser et al. 2000, Barros and Foil 2007). However, researchers were not able to find a significant difference in use between juniper or mesquite and juniper or oak habitats.

Eastern Red Cedar Ecology

Eastern Red Cedar (ERC) (*Juniperus virginiana* L.) is an evergreen conifer in the juniper family. It is found endemically in the eastern part of the United States. It is considered an early successional colonizer and if given the chance, will quickly establish and grow in disturbed or neglected areas (Holthuijzen and Sharik 1985). ERC's dense, cone-like canopy is comprised of needlelike leaves, called scales. ERC mainly produces pollen and seeds during the winter months.

ERC consumes large amounts of water from its surrounding area and can quickly change the surrounding soil and vegetation profile (Limb et al. 2010). Unlike many of the open grassland species in the Great Plains, ERC stores most of its carbon and nitrogen above ground (Norris et al. 2001). Since it is an evergreen tree, leaf litter production is reduced (Limb et al. 2010). The canopy has been shown to retain humidity and regulate temperature, staying cooler in the summer and warmer in winter (unpublished data). These biological adaptions have led to a quick change in the carbon, nitrogen and water cycles of its habitat (Norris et al. 2001, Limb et al. 2010, Pierce 2010). Yet its quick growth, hardiness and dense canopy also lends itself to human and animal use.

The Spread of Eastern Red Cedar

Eastern Red Cedar has been colloquially referred to as ‘The Green Glacier’ and is considered to be more ecologically devastating than the Dust Bowl (Engle et al. 2008). Ecologically this juniper species is endemic to parts of Oklahoma. However, it has been encroaching at a high rate into the Great Plains(Engle and Kulbeth 1992, Bidwell et al. 2008, Engle et al. 2008, Scholtz et al. 2018). Stands of eastern red cedar encroaching into unmanaged or abandoned farmland, can experience exponential canopy growth approximately 10 years (Holthuijzen and Sharik 1985, Engle and Kulbeth 1992). The increase of ERC has been linked to several mechanisms for its introduction and spread. As the Central Plains of the United States of America were colonized, much of the prairie was turned into farmland. Cultivation of row crops and sequential fragmentation of the prairie into smaller homesteads led to fire suppression which allowed this woody plant to gain a foothold. ERC is still planted as a windbreak and snowbreak for row crops, homes and livestock (Capel 1988, Ciblis et al. 2014). Many local and state government agencies helped further the spread by providing seedlings to farmers who wanted to help the landscape recover after the dust bowl as well as augment their farmland with refuge for wildlife. In urban areas, ERC is still planted as a living privacy fence and habitat for birds. This is somewhat counterintuitive. ERC does provide refuge for smaller mammals and particular avian species, as well as providing food for deer and other fruit eaters during the winter. Management for these more recognizable species has come at a cost to the native specialists of the grasslands. Specifically ground nesting birds have been in a steady population decline for decades (Coppedge et al. 2001, Pierce and Reich 2010). Increased fragmentation is correlated with higher nest mortality and higher nest parasitism by brown-headed cowbirds (*Molothrus alter* Boddart) (Herkert et al. 2003). Multiple researchers have found that ERC fruit and seeds are dispersed mainly by fruit eating birds as well as smaller rodents (Hasselschwert et al. 1993, Horncastle et al. 2004). ERC seedlings are randomly dispersed in nature however there is a higher percentage found along fence lines and other resting sites used by birds (Holthuijzen and Sharik 1985, Coppedge et al. 2001). As new ERC grow, more perches are provided for certain types of birds, which allows the ERC to expand its range into new areas.

Management of Eastern Cedar

Management of ERC after establishment is not easily accomplished with current control methods. Mechanical and chemical control can be time-consuming and costly. If not applied correctly chemical control has been proven quite ineffective for woody plant encroachment specifically ERC (Scholtz et al. 2018). However chemical application followed by a frequent prescribed fire regime can help the native grassland plant species by decreasing ERC presence (Scholtz et al. 2018). Studies have shown, cedar trees one meter in height have nearly 100% mortality with the prescribed fire utilizing at least a year’s worth of dead vegetation as fuel (Engle and Kulbeth 1992). If the trees are allowed to grow to a height over two meters, mortality after prescribed fire drops significantly (Engle and Kulbeth 1992). Using prescribed fire is a complex social issue (Wilcox et al. 2018). Many people do not have the knowledge, experience or time in order to use prescribed fire correctly. Surveys show a hesitance to use prescribed fire because of the landowner's perception of risk and the lack of experience (Kreuter et al. 2014, 2016, Toledo et al. 2014, Joshi et al. 2019). The general public's perception is influenced by extreme weather events leading to outbreaks of wildfire, as well as anti-fire propaganda (Twidwell et al. 2013). Much of the Great Plains is starting to understand the need for fire as an ecological tool. Prescribed burn associations are local cooperatives were landowners help each other plan and preform prescribed burns with the correct equipment and expertise (Toledo et al. 2014, Joshi et al. 2019). The increased utilization of prescribed burn associations is slowing the expansion of woody plant encroachment, especially the ERC, in areas of Oklahoma.

Eastern Red Cedar and Vector Ecology

Just as ERC has increased wildlife habitat for some species, it has also increased habitat for arthropod disease vectors. Mosquito, tick and tabanid populations have all been correlated with wooded habitat (James et al. 2015). Recent studies have shown higher populations of disease vectors are found in conjunction with ERC trees (O'Brien and Reiskind 2013, Noden and Dubie 2017). The canopy's ability to temper extreme temperatures and retain humidity, along with the close association of host species such as mice and birds, make this a wonderful habitat for host-seeking vectors (Masters 2014). Mosquitoes have been found in the fragmented prairie close to ERC guilds, where competent vectors were not previously found in high populations in typical prairie grasslands (O'Brien and Reiskind 2013). Also, ticks of all life stages have been found in high numbers under ERC canopy and in its leaf litter (Noden and Dubie 2017). Tabanids have been found using ERC as resting sites and mating sites. The tabanid *T. abactor*, has such a definitive use of another juniper species, the red berry juniper (*Juniperus pinchotii* Sudw.), it is colloquially known as the "Cedar fly" in the Rolling Plains Region of Texas (Davis and Sanders 1981). Many studies in the rolling plains have found *T. abactor* adults to use wooded habitat, but no significant difference in use between red berry juniper or mesquite dominated habitat. *T. abactor* larvae are found in higher numbers in the red berry juniper (*Juniperus pinchotii* Sudw.) leaf litter (Slosser et al. 2000, Wiedenmann et al. 2005). This tabanid species frequently uses leaf litter as larval habitat, making it the perfect candidate to dominate the drier prairies (Slosser et al. 2000). Few tabanid species have larval stages documented in leaf litter (Schwardt 1936, Schomberg 1952, Schomberg 1955). Schwardt 1936 study notes *Tabanus annulatus* Say, as having a larval habitat in rotten logs in Arkansas (Schwardt 1936). Schomberg notes *Tabanus equalis* Hine, being found in drier grass, under elm trees, and *Tabanus sulcifrons* Macquart, in dry soil in gullies (Schomberg 1952, Schomburg 1955).

Bovine Production Losses

Research studies have implicated tabanid flies in various types of production losses. Losses stem from reduced weight gains, reduced milk production, weight loss and even anemia and death (Steelman 1976, Hollander and Wright 1980b, Perich et al. 1986). Direct blood loss and time spent in defensive grouping or in shelter away from forage sites, leads to less foraging and can quickly impact livestock performance. Behavior dynamics and social standing of herd animals can be altered due to fly pressure (Duncan and Vigne 1979, Mooring et al. 2007). Behaviors in herd animals have evolved in order to provide protection for young, avoidance of predators and shared grooming. Social standing plays a role in herd size. Equids will often separate in smaller groups based on stallion competition for mares and forage location. In months with high tabanid activity, it has been observed that domesticated and feral herds will form larger groups regardless of forage availability (Duncan and Vigne 1979, Rutberg 1987, Christensen et al. 2002). The estimated loss in production due to tabanid attacks and control costs in the United States, circa late 1970s was $40 million. Of that, $30 million was contributed to a loss in weight gain. (Geden and Hogsette 1994). Perich et al. (1986) demonstrated in Oklahoma that when heifers were exposed to tabanid attacks it resulted in a $10.08 loss over an 84-day exposure period. The exposed heifers gained 6.7 kilograms less than those protected from tabanid attack and would need 1.32 kilograms more feed a day in order to achieve the same rate of gain as the protected heifers (Perich et al. 1986). In dairy production, milk production can be impacted up to 100% over a three week sustained period of attack (Howard 1916, Zumft 1949, Decker 1955) . Feral and domesticated equid and bovid herds have been shown to alter feeding and social behaviors due to tabanid pressure (Duncan and Vigne 1979, Duncan and Cowtan 1980, Hughes et al. 1981, Collins and Urness 1982, Keipler and Berger 1982, Rutberg 1987). Physical production losses are a quick way to see the monetary impact of tabanids. The more interesting impact may not be the tabanids themselves but the organisms they transfer.

Disease Transmission

The family Tabanidae is considered an efficient disease vector of many veterinary important pathogens. Tabanids are considered telmophages and cause excessive bleeding at the feeding site. They use their large knife-like mouthparts to open large cuts in the skin, allowing the blood to pool and run free. The flies will feed until interrupted, often returning to the same host or a host nearby (Foil 1983, Barros and Foil 2007). Tabanids have been documented to fly kilometers between herds in order to finish feeding (Thornhill and Hays 1972, Sheppard and Wilson 1976, Cooksey and Wright 1987). This type of persistent feeding, creates large wounds in the skin, providing a site for secondary infections and routes for other flies to feed, such as house and flesh flies, which can also transmit pathogens into these feeding sites. Transmission of pathogens between animals can occur quickly and efficiently. Tabanids are linked to two different types of transmission: mechanical and biological.

In general, mechanical transmission of certain pathogens is common for tabanids. Yet, for mechanical transmission to be effective, a few parameters must be met which include available hosts, pathogen maintenance within the environment and infectious tabanid vectors. Tabanid mechanical transmission has been linked to bacteria, viruses, helminths, and protozoa. Several viruses have been shown to be transmitted by flies of the genus *Tabanus*. Foil et al. 1988 used a known carrier cow of Bovine Leukemia (BL Virus Genus: *Deltaretrovirus*) to demonstrate transmission using *Tabanus fucicostatus* Hine. Bovine Leukemia Virus (BLV) was transferred from the carrier cow to groups of sheep and goats by disrupting the feeding of the flies on the carrier and transferring to naïve animals (Foil et a. 1988). Again in 1997 Foil et al. studied the amount of blood left on the mouth parts of a tabanid during an Equine Infectious Anemia Virus (EIA) study. They found that there was adequate blood left on the mouthparts of *T. fusciostatus* to effectively transmit EIA. The flies were allowed to feed to engorgement on carrier equids (*Equis caballus* Linnaeus). The flies were then taken back into the lab and their heads removed at five different time intervals. The quantifiable blood meal on the mouthparts immediately after feeding was calculated to be 10nl. This was an adequate amount as shown by previous studies (Foil et al. 1997, Hawkins et al. 1976). Bovine Anaplasmosis, *Anaplasma marginale* Theiler, is primarily vectored by ticks, but can be spread by blood-feeding flies and fomites (Dikmans 1950). Most flies in the genus *Tabanus* have been implicated or confirmed as a competent vector (Sanborn et al. 1932, Morris et al. 1936, Howell et al. 1941, Lotze and Yiengst 1941, Dikmans 1950). Bovine Anaplasmosis is very quick to debilitate animals and herds. Often cattlemen are not aware of infected cattle, until they find them deceased. This is concerning considering the number of wildlife reservoirs and the intense movement of cattle (*Bos tarus* and *B. indicus* L.) in North

America (Darlington 1926, Howe and Hepworth 1965, Zaugg et al. 1996, Taylor et al. 1997, Kuttler 2013). Other mechanically transmitted diseases include but are not limited to: Potomac Horse Fever (*Neorickettsia (Ehrlichia) risticii* Holland)(Levine et al. 1992) , Classical Swine Fever (Classical Swine Fever Virus Genus: *Pestivirus*)(Tidwell et al. 1972), Tularemia (*Francisella tularensis* McCoy) (Petersen et al. 2009), California (California encephalitis virus Genus: *Orthobunyavirus*) and Western Equine Encephalitis (Western Equine encephalitis virus Genus: *Alphavirus*) (DeFoliart et al. 1969, Miller et al. 1983), Anthrax (*Bacillus anthracis* Cohn) (Morris 1918), Lyme (*Borrelia burgdorferi* Burgdofer) (Magnarelli et al. 1986), and Brucellosis (*Brucella* sp. Meyer and Shaw)(Wellman 1951, O'Brien et al. 2017) have been isolated and confirmed from tabanids. An outbreak of tularemia in Utah in 2007, was linked to deer flies (*Chrysops* genus) after five people became ill after being bitten by the flies. A search of the area found the flies, as well as other infected hosts, hares and rabbits (Peterson et al 2008a). Both of the Encephalitis viruses have been isolated from tabanids in multiple studies, however, no research has documented the transmission (DeFoliart et al. 1969, Miller et al. 1983).

Biological transmission is slightly different than mechanical. The tabanid will obtain the pathogen by feeding but then the pathogen agent utilizes the tabanid vector to complete its lifecycle or replicate inside the fly. This allows a greater chance of transmission even if the initial viral or bacterial load was low, or if enough time had passed to desiccate the agent. This behavior has been implicated in the spread and reemergence of deadly livestock, wildlife and even human diseases. Tabanids can carry multicellular pathogens with ease. Many of these are not only transmitted by tabanids but actually require the tabanid in order to complete their lifecycle. Helminths and trypanosomes use tabanids as intermediate hosts, cycling through different life stages before becoming infective for their hosts. Even the smaller deer flies have been confirmed biological vectors of the arterial worm, *Eleaophora schneideri* Dikmans (Grunenwald et al. 2018). The arterial worm is found from coast to coast in moose (*Alces alces* Linnaeus), elk

(*Cervas elaphus* Linnaeus), white-tail deer (*Odocoileus virginianus* Zimmermann), black-tailed deer (*Odocoileus hemionus* Rafinesque) and other ungulates (Clark and Hibler 1973, Weinmann et al. 2013, Grunenwald et al. 2018). In its natural hosts, the arterial worm causes little clinical symptoms(Clark and Hibler 1973). Yet the worm can be transmitted to sheep (*Ovis aries* Linnaeus), goats (*Capra hircus* Linnaeus) and equids, causing serious neurological damage in the abnormal hosts (Couvillion et al. 1985). Trypanosomes are found worldwide, and each species has an associated tabanid vector species. In North America, tabanids can carry *Trypanosoma theileri* Laveran, and *T. evansi* Steel (Krinsky 1976, Foil 1989). *T. theileri* utilizes many large flies in the genus Tabanus. The trypanosome has been confirmed in elk, moose, and white-tailed deer (Davies and G 1974, Stuht 1975, Böse et al. 1987).

Regardless of the disease type, species associated, or the type of host, tabanids are still considered a diverse group of flies that should still be given an increased amount of consideration in the area of livestock production. An area of focus related to tabanid vector ecology that has not been well defined is the concept of landscape epidemiology. Landscape epidemiology can determine how the tabanid vector interacts with the temporal presence of the host in relation to pathogen load and how transmission can either be enhanced or impeded by the vegetation landscape (Reisen 2010, Baldacchino et al. 2013). This is further explained when the concept of nidality of disease in which pathogens are associated with specific landscapes are linked to certain vegetation types (Pavlosky 1966, Reisen 2010). Pathogen transmission by tabanids can be potentially linked to landscapes which include Eastern Red Cedar especially for pathogens that are mechanically transmitted to naïve animals.

As the ERC expands its range in the plains, it could be providing an expanded habitat for arthropod vectors like tabanids and the pathogens they can carry. By examining adult tabanid habitat use of ERC, we can better understand the relationship between tabanids and their environment and if manipulation of the habitat by removing ERC is a viable control option for tabanids. By eliminating red berry juniper in pastures in Texas, adult tabanid populations were significantly lowered and stayed low, even with the reintroduction of cattle. Differences between the mechanical removal of junipers and control areas was examined for tabanid larval survival, with both mechanical methods showing significant decrease in larval populations in the leaf litter (Wiedenmann et al. 2005).

CHAPTER II

Introduction

Beef cattle producers are faced with many challenges that include providing a safe food source by ensuring they raise healthy animals while keeping in mind the public demand for limited antibiotic use by eliminating drug residue that could lead to antibiotic resistance. In Oklahoma, beef producers are faced with limiting disease as well as reducing potential routes for infection. Managing herd health can be as minimal as ensuring shelter during weather events to quarantine of new animals or simple biosecurity measures such as maintaining fences. However, beef producers have been dealing with parasites that not only cause production losses but also serve as vectors for important pathogens.

External parasites of cattle can cause mild irritation to the animal as well as decrease efficiency. However, they also serve as important vectors for viruses, bacteria, protozoa and internal parasites. Biting flies and ticks can cause significant production losses and serve as important vectors of pathogens. Certain pathogens that can cause diseases such as bovine anaplasmosis, infectious equine anemia and classical swine fever have a quick onset and often cause high mortality rates. Other important pathogens that cause diseases such as tularemia, anthrax, and Lyme disease are zoonotic and are linked to an arthropod vector. One of the best methods to limit of vector-borne disease within livestock is prevention. This is best achieved by altering the environment. Chemical control measures, pasture rotation, and vegetation manipulation are used in conjunction with one another to limit important arthropod vectors.

Highly mobile pests like biting flies are often difficult to control in pasture systems due to their feeding and behavior. Most biting flies have a strict preference for adult and larval habitat as well as food sources and feeding sites. Pests like horn flies, stable flies and mosquitos are efficiently managed based on their adult behavior and larval development requirements. Unfortunately, Oklahoma producers must also deal with another flying pest, the horse fly.

Horse flies (tabanids) are in the order Diptera and the family Tabanidae. These tabanids are blood feeding flies with a painful bite. They are persistent feeders that will visit multiple hosts or fly miles in between herds if feeding is disrupted. Hosts are found using their large complex eyes that have developed to pick large animals out of the landscape. They are considered very difficult to control due to their behavior and mobility. Many adult behaviors are not documented properly due to the complexity of the tabanidae family as well as the difficulty of mark and recapture. Adult tabanids have an equitable association with wooded habitat but little has been done to prove or breakdown this association.

In Oklahoma, a phenomenon related to woody plant encroachment is known as the green glacier which has altered much of the central plains landscape. One particular woody plant species is the Eastern Red Cedar (ERC) which is a quick growing juniper species with a high fecundity and growth rate. The dense foliage leads to competition and shading out of native plants. The canopy retains humidity and serves as a temperature moderator. These characteristics have made it useful to humans and wildlife alike, serving as wind breaks and refuge for various species.

Ticks and mosquitos have shown significant use for ERC. As well, similar juniper species have been proven to have an association with tabanids in the rolling plains of Texas. Previous work has not clearly defined whether certain tabanid flies are associated with ERC or even varying degrees of ERC density within a pasture utilized for cattle grazing. The overall goal was to determine the relationship of ERC within cattle pastures to different tabanid populations. The objectives of this study were to 1) determine habitat use by tabanidae in relation to ERC and 2) determine the abiotic factors that are associated with tabanidae use of ERC habitat.

CHAPTER III

MATERIALS AND METHODS

This study was conducted at the Oklahoma State University Range Research Station approximately 13 km west of Stillwater, Oklahoma. The Range Research Station is located in the western part of the Cross Timbers ecosystem which spans from southeastern Kansas to northcentral Texas. The Cross Timbers ecosystem is a mosaic of upland deciduous forest, savanna, and Tallgrass prairie that typifies the broad region between the eastern deciduous forest and the grasslands of the southern Great Plains. Multiple locations on the Range Research Station were scouted and selected based on Eastern Red Cedar densities. Habitat classifications were grouped into four distinct groups:

1. Open Canopy Cedar Habitat – native grass pasture that has significant ERC development, but the tree canopy was not completely closed due to ERC, and was over 60% mature ERC (tree height over 8 m with a canopy width of at least 2m) within a 50 m radius, representing the majority of tree species within that location
2. Closed Canopy Cedar Habitat – a closed canopy system, with more than 60% mature ERC (tree height over 8 m with a canopy width of at least 2m) within a 50 m radius, representing the majority of tree species within that location
3. Oak Habitat – a closed canopy system, with less than 40% cedar of any age present and the majority of tree species within a 50 m radius identified as post oak (*Quercus stellate*) and blackjack oak (*Q. marilandica*)

4. Pasture Habitat – open grassland with no cedars over 2 m in height within a 50 m radius, or mature wooded canopies within 300m, representing native tallgrass species within the Southern Great Plains ecotone

The Open Canopy and Closed Canopy systems were distinct with the Open Canopy habitat containing open native grass areas interspersed among the ERC. The Closed Canopy habitat was consistently defined as areas of dense ERC habitat with the plant canopy completely closed no open native grass areas with little to no oak trees present. The Pasture habitat was distinct with no ERC or other woody plant canopies within a minimum of 300 m distance of trap locations. Each habitat classification was sampled weekly for Tabanidae for a 20-week period in 2017 and again in 2018. Abiotic weather variables were collected from the Oklahoma Mesonet (Oklahoma Mesonet, 2017 & 2018) environmental monitoring system using the Marena Mesonet Site. The Marena Site is 0.74 kilometers from the closet trap location and 5.47 kilometers from the furthest. Weekly data was downloaded and utilized for the trapping periods in 2017 and 2018.

Any site that did not fall strictly into a category was not used. A brief description of each location was taken, including nearby water, fencing and presence of livestock. Pictures of each trap location was taken to ensure correct habitat classification and a GPS coordinate taken. Cattle were not permanently present in the pastures used, but traps placed were always within 400 meters of adjoining permanently pastured cattle.

Each habitat category received a minimum of five bucket traps as well as two HorsePal® (Newman Enterprises, Omro, WI) traps, each individually numbered. Variation in the number of buckets was due to the lack of wooded areas without Eastern Red Cedar encroachment. Care was taken not to disturb the habitat vegetation structure, but grass and weeds were managed in the immediate area of the trap placement to conserve trap integrity. One trapping location was

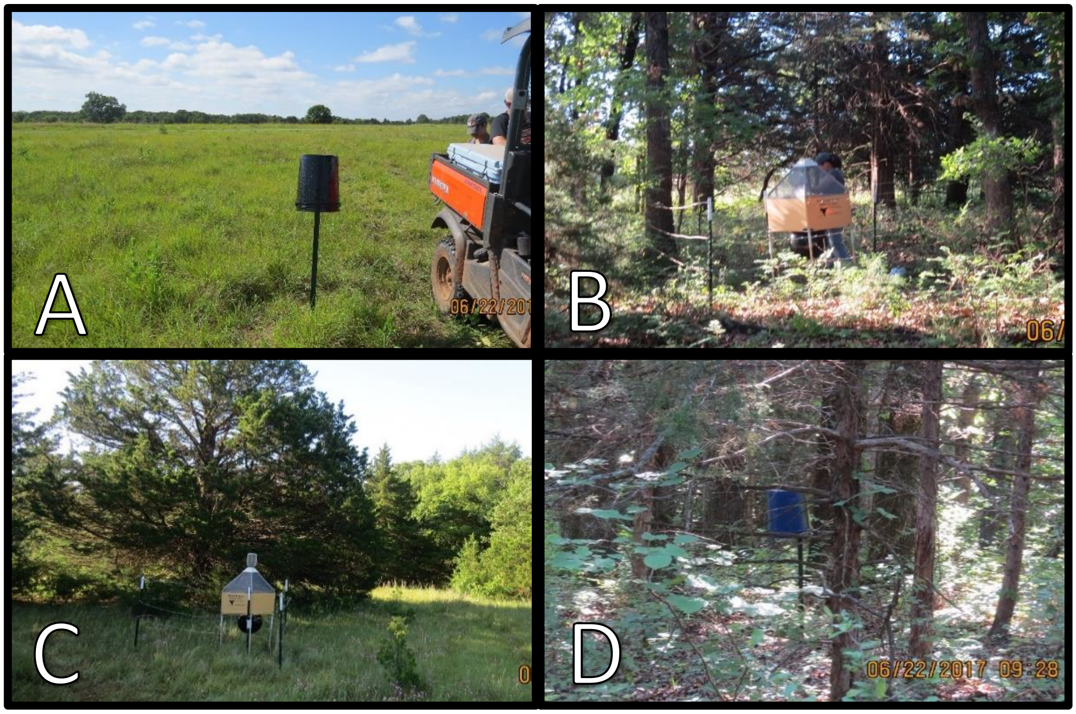

Figure 1 - Habitat Classification: *Pasture Habitat with no cedars over 2 meters in view (A);Oak Habitat in a closed canopy system, with less than 40% cedar of any age present (B); Open Canopy Cedar Habitat, pasture that had been taken over, and was over 60% mature ERC (C); Closed Canopy Cedar Habitat in a closed canopy system, with more than 60% mature ERC (D).*

removed in 2018, due to accessibility issues. To ensure that trap sites were located inside the specified habitat categories, we confirmed each trap location through GPS and then georeferenced from Google Earth Pro with a collection date of 2018 (Figure 2).

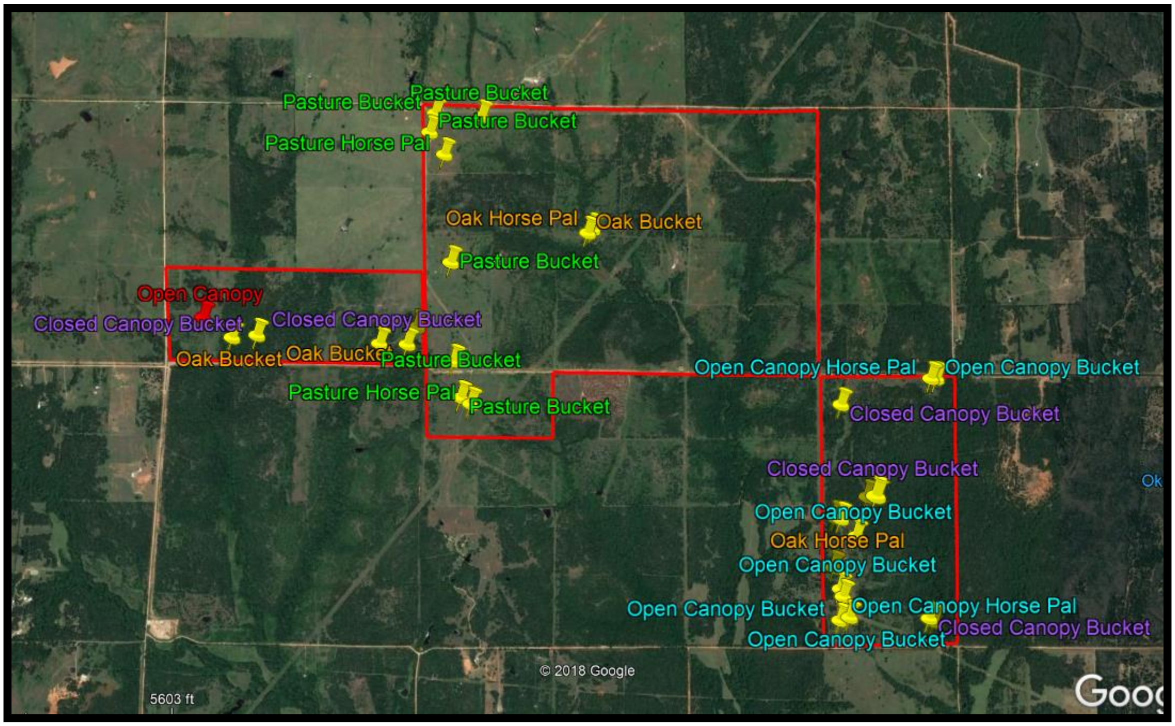

Figure 2 - Trap Placement on OSU Research Range Station, Stillwater Oklahoma: Open Canopy Cedar Traps are in blue, Closed Canopy Cedar in purple, Oak in orange, and Pasture in green. The trap that was removed for the 2018 season is denoted in red.

Trap Selection

The two traps utilized in this study relied on the host seeking behavior exhibited by Tabanidae that use visual cues to find suitable hosts. No lures such as carbon-dioxide or octanol were used to ensure tabanids trapped in each habitat type were not lured from an adjacent habitat by an attractant. The modified bucket traps found in Moore et al. (1996) were selected based on the reactivity and reliability the traps had in various habitat types. The HorsePal® traps were a commercial selection that mimics a Manitoba trap. Both trap designs differed enough visually to appeal to multiple tabanid species. Examples of the traps with trapped tabanid flies are shown in Figure 3.

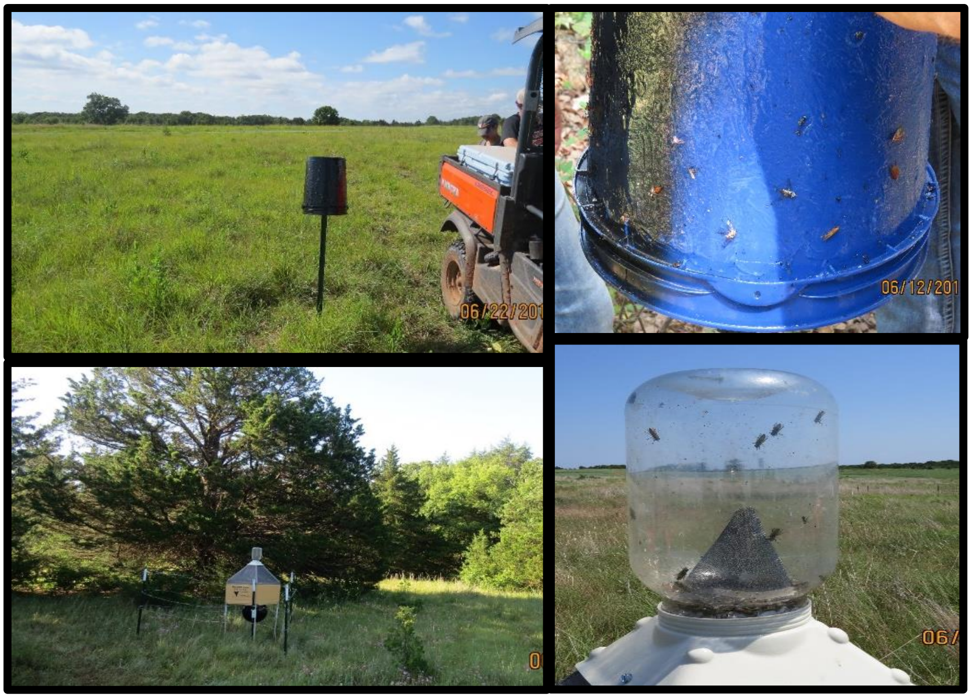

Figure 3 - Trap Styles: A view of what the two trap types, bucket trap (top) and HorsePal (bottom), would appear from a distance and a closer view of tabanids stuck to the adhesive and in the collection jar.

The modified buckets traps were made using a blue 5-gallon bucket with a 0.31-meter length of 7.62-centimeter PVC screwed to the inside. The bucket was then inverted over a t-post with the post inserted into the opening of the PVC for stability. The buckets were then spray painted a glossy black over one-half of the outside, numbered and covered in a layer of adhesive TAD® All-Weather™ (Tangle-trap) (Trece Adhesive Division, Great Lakes IPM). Colors were selected based on the recommendation of multiple studies in the literature (Tashiro and Schwardt 1953, Bracken et al. 1962, Hanec and Bracken 1962, Moore et al. 1996, Sasaki 2003, Horváth et al. 2010). The adhesive was applied in the lab to ensure even coat coverage and consistency. To transport to the field sites, a trash bag would be used to protect the adhesive and the transport vehicles. The traps were deployed for one – two weeks depending on weather conditions and wear of the adhesive. When collected, the previously used bucket was placed in a plastic bag, labeled with the trap number, and then transported to the lab where the Tabanids were sorted. After the tabanids were removed then the buckets were scraped, and adhesive was reapplied after any required maintenance.

Within each habitat two HorsePal® traps were deployed. These traps were put in close range, but not within view of a bucket trap in the Open Canopy Cedar, Closed Canopy Cedar and Oak Habitats. In the Pasture Habitats, the paired HorsePal® traps were a minimum of 60 meters from the bucket trap. Each week the collection jar would be emptied and replaced in the field, as well as any other trap maintenance.

Counting and Identification

Tabanidae is a very diverse family. For this study the genera of *Tabanus, Chrysops* and *Hybomitra* were counted. Total tabanidae and genera counts were taken immediately after field collection. Tabanidae were identified using morphological characters on the head thorax and the wing venation pattern. Separation into genera required the inspection of the head for ocelli, the antennal segments and the presence of hind tibial spurs. During genera counts, if the tabanidae had lost its head, it was not included in the genera counts, because the location of an ocelli on the ventral side of the head is used to separate the *Tabanus* and *Hybomitra* generas. Tabanids were cleaned with Histo-Clear II® (Great Lakes IPM), then placed in water and ethanol baths. The flies were stored in 80% ethanol, in dated, numbered vials corresponding to the trap location and week. Identification of selected species with the aid of a dissection microscope was made after field work was completed.

Selected species identified included: *Tabanus abactor* Philip, *T. atratus* Fabricus, *T. equalis* Hine, *T. molestus* Say, *T. sulcifrons* Macquart, *T. subsimilis* Bellardi, *T. styguis* Say, *T.*

trimaculatus Palisot De Beauvios, *Chrysops callidus* Osten-Sacken, *C. flavidus* Wiedmann, and *Hybomitra (Tabanus) lasiophthalma* Macquart (Table 1).

Table 1 – Previously described tabanid species important to livestock.

Tabanidae Species of Interest	Previously Described in Oklahoma	Important Veterinary Pathogen / Disease Association
Tabanus abactor Philip	(Hollander and Wright 1980a, 1980b, Wright et al. 1984, 1986)	Bovine Anaplasmosis (Howell et al. 1941)
T. atratus Fabricus	(Hollander and Wright 1980a, 1980b, Wright et al. 1984, 1986)	*Bacillus anthracis* (Morris 1918), *Trypanosoma theileri* (Packchanian 1957, Mohler and Thompson 1911)
*T. equalis** Hine	(Hollander and Wright 1980a, 1980b, Wright et al. 1984, 1986)	Bovine Anaplasmosis (Sanborn et al. 1932, Howell et al. 1941)
*T. molestus** Say	(Wright et al. 1984, 1986)	
T. styguis Say	(Wright et al. 1984, 1986)	
*T. subsimilis** Bellardi	(Hollander and Wright 1980a, 1980b, Wright et al. 1984, 1986)	Vesicular Stomatitis Virus** (Ferris et al.)
*T. sulcifrons** Macquart	(Hollander and Wright 1980a, 1980b, Wright et al. 1984, 1986)	Equine Infectious Anemia Virus (Stein et al.1942), Classical Swine Fever Virus (Tidwell et al. 1972), Bovine Anaplasmosis (Sanborn et al. 1932, Howell et al. 1941)
T. trimaculatus Palisot DeBeauvios	(Hollander and Wright 1980a, 1980b, Wright et al. 1984, 1986)	
Hybomitra (Tabanus) lasiophthalma Macquart	(Hollander and Wright 1980a, 1980b, Wright et al. 1984, 1986)	Equine Infectious Anemia Virus (Stein et al.1942), California Encephalitis Virus (DeFoilart et al. 1969), Vesicular Stomatitis Virus (Ferris et al.), Lyme Disease (Magnarelli et al. 1986)
Chrysops callidus Osten-Sacken	(Hollander and Wright 1980a, Wright et al. 1984, 1986)	Lyme Disease (Magnarelli et al. 1986)
C. flavidus Wiedmann	(Wright et al. 1984, 1986)	

**Each species marked are considered the main species of interest in a complex*

***Pathogens marked was cited using a previous synonym or a species included as part of a complex*

Each species was selected based on disease transmission capability as well as abundance in earlier tabanidae studies of the region (Sanborn et al. 1932, Krinsky 1976, Hollander and Wright

1980a, 1980b, Foil 1989). Other genera and species were collected and noted but not used in the study analysis due to low population numbers.

Identification of species was made using multiple keys and the published descriptions if available. The Catalog of Tabanidae (Diptera) of North America North of Mexico (Burger 1995) was used to match species with previous synonyms and ranges. Keys and descriptions included: Prodrome of a Monograph of the Tabanidae of the United States (Osten-Sacken 1875), Tabanidae of Ohio with a Catalogue and Bibliography of the Species from America North of Mexico (Hine 1903), Tabanidae of Ohio with a Catalogue and Bibliography of the Species from America North of Mexico (Hine 1903), Horseflies of Arkansas (Schwardt 1936), The Horseflies of the Sub Family Tabaninae of the Neartic Region (Stone 1938), New North American Tabanidae (Diptera) Part II Tabanidae (Philip 1950), New North American Tabanidae(Diptera) III. Notes on *Tabanus molestus* and Related Horseflies with a prominent Single Row of Triangles on the Abdomen (Philip 1950), New North American Tabanidae (Diptera). VI Descriptions of Tabanidae and New Distributional Data (Philip 1954), The Tabanidae (Diptera) of Louisiana (Tidwell 1973), The Horse and Deer Flies (Diptera: Tabanidae) of Texas (Goodwin and Drees 1996), The Diptera, or True flies, of Illinois I. Tabanidae (Pechuman et al. 1983), and The Tabanidae of Tennessee (Goodwin et al. 1985). Taxonomic descriptions were compiled for each species from and added to a previously unpublished taxonomic key for Tabanidae of Oklahoma (Wright, unpublished). Due to the close relationship of species and multiple intermediate morphological forms, there are four species complexes. These closely aligned complexes of species were documented previously in literature and depending on the region (Table 2). If identification of a sample was indeterminate between the main species and another, it was counted in the complex total. If the sample was able to be identified as one of the aligned species but did not show characteristics of the main species of interest, the sample did not count towards the complex total. Table 2 lists the four complexes as well as the species considered in the complex and the discussion in the literature.

Table 2 - Description of the four species complexes used in identification

Main Species of Interest	Aligned Species	Discussion in Literature
*Tabanus equalis**	*T. turbidus* Wiedemann	Tidwell 1973
*Tabanus molestus***	*T. mixis* Say**	Stone 1938, Philip 1950c, Tidwell 1973, Goodwin et al. 1985, Wright et al. 1986,
*Tabanus sulcifrons**	*T. abdominalis* Fabricius, *T. gladiator* Stone, *T. limbatinevris* Macquart	Osten-Sacken 1876, Hine 1903, Stone 1938, Philip 1950c , Tidwell 1973, Pechuman et al. 1983, Goodwin et al. 1985, Wright et al. 1986,
Tabanus subsimilis	*T. similis* Macquart , *T. lineola* Fabricius	Osten-Sacken 1876, Stone 1938, Tidwell 1973, Goodwin et al. 1985, Wright et al. 1986,

**Tabanus equalis* and *sulcifrons* are superficially similar in size and coloration. Care was taken to look at coloration of antennae, and forelegs to make the distinction. Even though *T. equalis* populations peak in early summer and *T. sulcifrons* peak in late summer, overlap in Oklahoma does occur.

***Tabanus molestus*, *mixis*, and *trimaculatus* are superficially similar in size and coloration. Care was taken to look at coloration of markings and wing venation to make the distinction.

Statistical Analysis

Tabanidae abundance and weather data were summarized for each habitat, trap type and trap location using Microsoft Excel. Comparisons were further analyzed by analyzed by analysis of variance (ANOVA) using the PROC GLM procedure with means separated by a LSMEANS test (SAS 9.4, SAS Institute), for habitat use by total abundance of tabanidae and genera, as well as trap type preference by genera with an α of 0.05.

Ordination Statistics Methods

We used multivariate statistical techniques to understand complex taxonomic and ecological associations of the Tabanidae species composition data, specifically unconstrained and constrained ordination (ter Braak 1997). For all ordination analyses, we pooled traps together by site within each sampling week. We only used data confirmed to the species level for 10 species (*C. callidus*, *C. flavidus*, *T. abactor*, *T. atratus*, *T. equalis*, *T. molestus*, *T. subsimilis*, *T. sulcifrons*, *T. styguis*, and *T. trimaculatus*), however *Hybomitra* which were pooled at the genus level although this genus was dominated by *H. lasiophthalma*. All ordination analyses were run separately by year. We first conducted unconstrained ordination analyses using Principal Components Analysis (PCA) using only the Tabanidae species composition data. We applied a log transformation to the species data and centered by species. Sample diversity was expressed using the Shannon-Wiener diversity index. The percent of variation explained along the first and second PCA axis were calculated and presented. We then conducted constrained ordination analyses using Redundancy Analysis (RDA) using the Tabanidae species composition data constrained to habitat classification (Open Canopy Cedar, Closed Canopy Cedar, Oak, Pasture) and weather variables including wind speed, solar radiation, humidity (minimum, average, and maximum), and air temperature (minimum, average, and maximum) (Lin et al. 2016; Pérez-Marcos et al. 2018). Day of sampling was also used as a constraining variable in order to better understand seasonality of Tabanidae species distributions (Baldacchino et al. 2014). We applied a log transformation to the species data, centered by species, and applied a Hellinger standardization to samples (Legendre and Gallagher 2001). To test if all ordination axes were significant, we used a permutation test with 1,000 iterations and a random number generator to seed the test. Sample diversity was expressed using the Shannon-Wiener diversity index. The percent of variation explained along the first and second RDA axis were calculated and presented along with a pseudo-F statistic and p-value for all axes and the total variation explained. Finally, we developed species response attribute plots by year for a sub-set of Tabanidae species based on the strongest environmental or seasonality gradients explaining Tabanidae species composition (Potocký et al. 2018) identified in the RDA that included solar radiation, day of sampling, solar, and air temperature (average). These plots display species response curves used a generalized linear model with a quasi-Poisson distribution and log link function. Summary statistics for each species response curve graph include F statistic, p-value, optimum value, and tolerance (width of the curve). All ordination analyses were conducted in CANOCO v 5.0 (Šmilauer and Lepš 2014).

Multivariate Analysis

A multivariate analysis of variance (MANOVA) was conducted using the PROC MEANS and PROC GLM procedures (SAS 9.4, SAS Institute) with total Tabanidae abundance, total abundance within each genera (*Tabanus, Chrysops,* and *Hybomitra*), *Tabanus abactor* abundance, *T. equalis* complex abundance, *T. molestus* complex abundance, *T. styguis* abundance, *T. sulcifrons* complex abundance, *T. trimaculatus* and *Chrysops callidus* abundance were considered dependent variables. Independent variables were Julian date, weekly average maximum temperature, weekly average minimum temperature, weekly average temperature, weekly average maximum humidity, weekly average minimum humidity, weekly average humidity, weekly average precipitation, weekly average wind speed, and weekly average solar radiation as well as categorical variables of habitat type and trap type. Any independent variable considered significant in contributing to abundance of the different Tabanidae was considered significant at α of <0.05.

CHAPTER IV

RESULTS

Total Tabanidae Abundance

In 2017 there was a total of 12,955 tabanid flies trapped. *Tabanus* (n=10,020), *Hybomitra* (n=3), and *Chrysops* (n=978) accounted for the genera collected (Figure 4). There was a significant difference overall in use of habitat by tabanid adults. Open-Canopy Cedar habitat had significantly higher populations of tabanids than any other habitat (F = 8.99, P<.0001) (Figure 5).

In 2018 there was a total of 12,209 tabanid flies caught. *Tabanus* (n=8,561), *Hybomitra* (n=496), and *Chrysops* (n=2,387) accounted for the genera collected (Figure 4). Due to earlier emergence than in previous studies (Wright et al. 1986) in the initial year of this study (2017) sentinel traps were deployed beginning in March of 2018. The first tabanids were recorded on 30^{th} of April 2018. Once sentinel traps averaged five flies per trap, all traps were deployed on the 4^{th} of May 2018. Average tabanid caught per habitat by year showed that there were significantly higher numbers of tabanids caught in Open Canopy Cedar habitat (Figure 5). Open-Canopy Cedar habitat had significantly higher populations of tabanids than any other habitat (F = 38.01, P<.001).

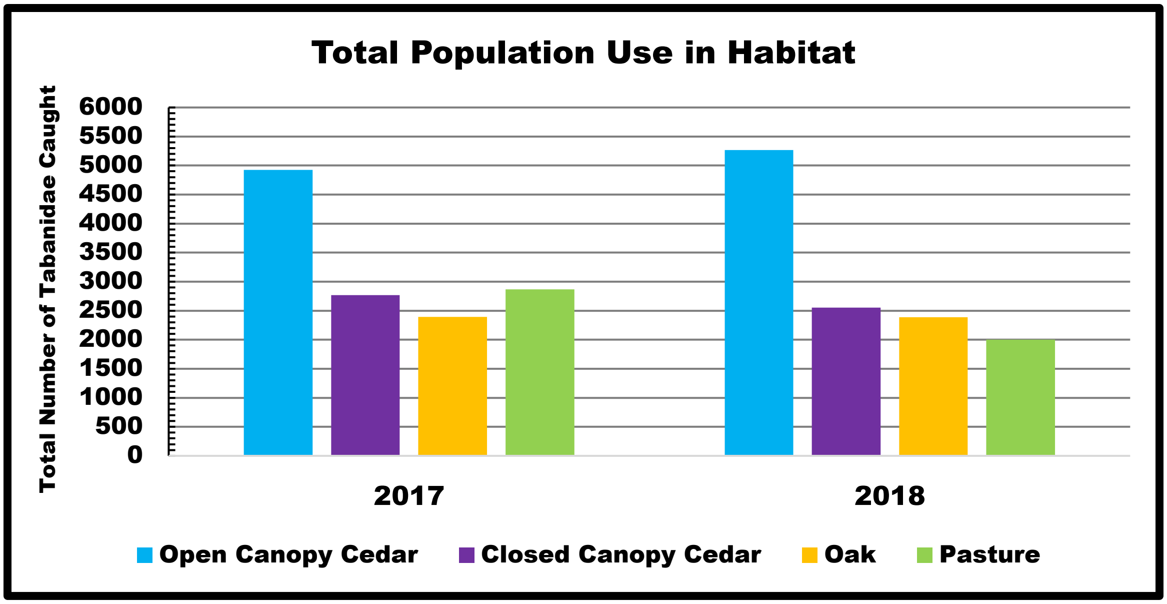

Figure 4 - Total population use of habitat by year

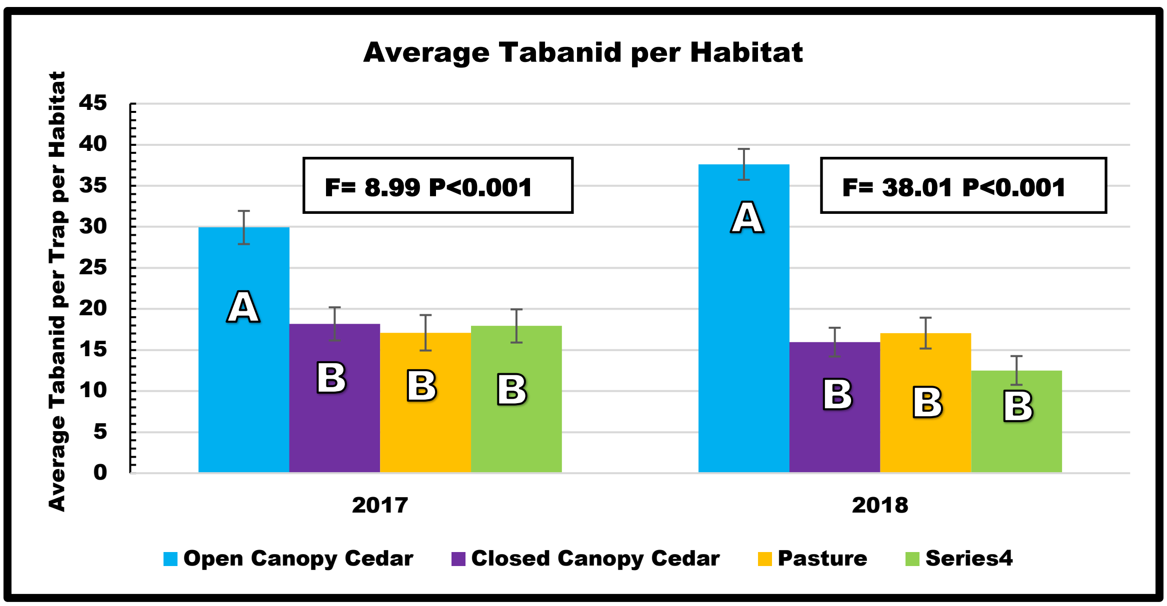

Figure 5 – Average tabanid caught per trap per habitat type by year. Bars with different letters indicate detectable differences (P<0.05) between habitat use within genera. Values are least squared means.

When comparing the total tabanid populations the 2017 seasons showed two distinctive peaks in population, the first in week 5 (June 15th, 1328 tabanids) and the second in week 13

(August 10th, 1247 tabanids)(Figure 6). The 2018 seasonal peaks were shifted slightly and were not as well defined as in 2017 (Figure 6). The first peak occurred on week 3 (May 25th, 1035 tabanids), a second smaller peak at week 7 (June 22nd, 897 tabanids) and a third large peak week 17 (August 31st, 1063 tabanids). Sentinel traps placed to capture the initial emergence, went from an average of 8.25 tabanids per trap to 23.82 tabanids in the seven-day period between the last sentinel and the first full trapping week in 2018. Each of the peaks were driven by *Tabanus* populations in 2017 (Figure 7) and by *Chrysops* and *Tabanus* populations in 2018 (Figure 8).

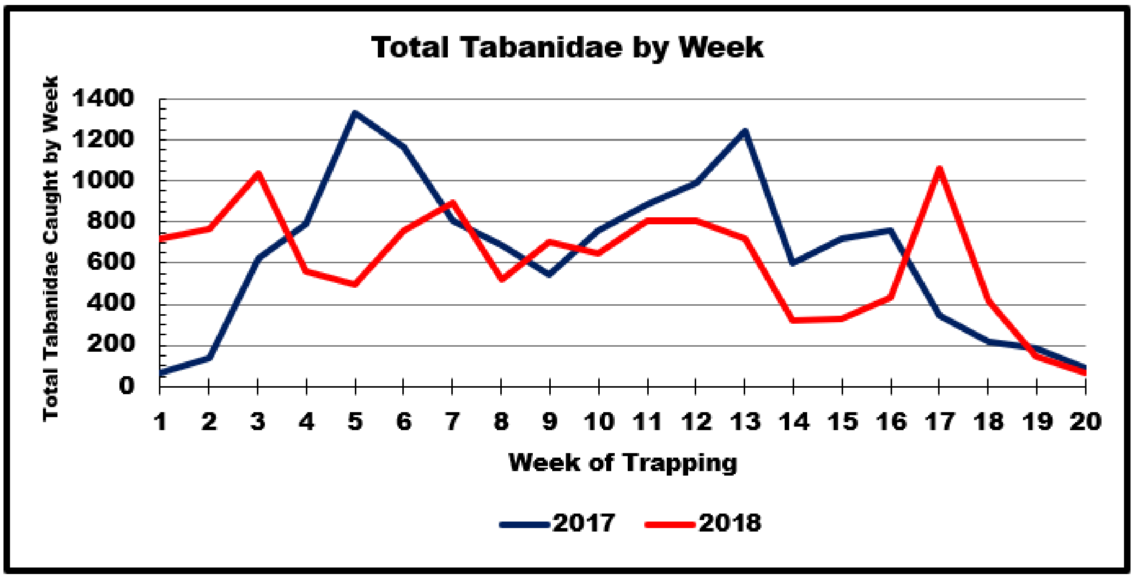

Figure 6 - Comparison of Total Tabanidae for 2017 and 2018

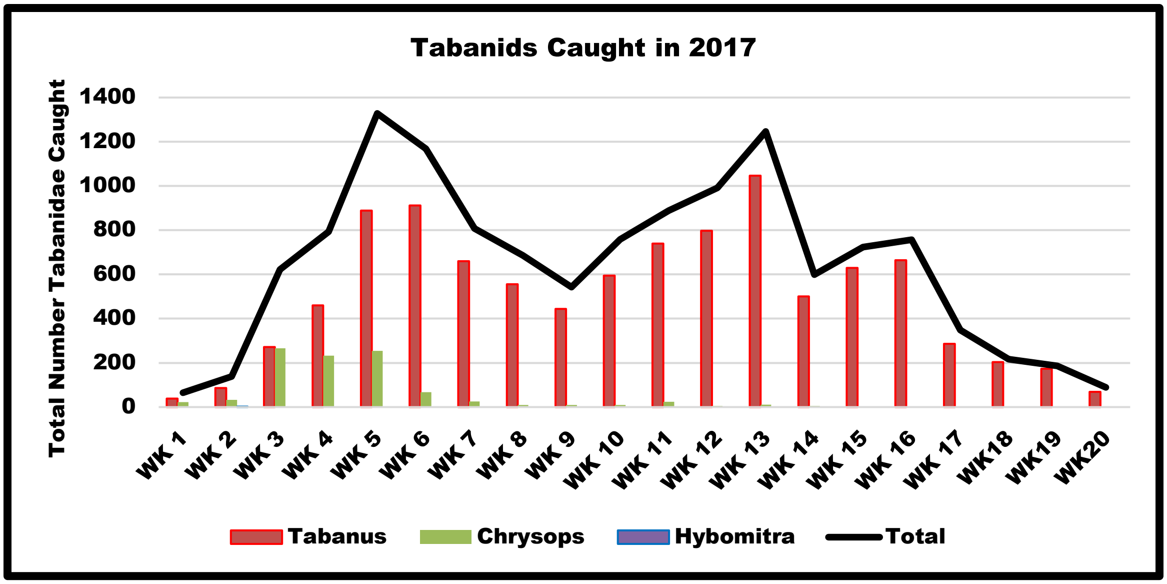

Figure 7 - Comparison of different tabanidae genera totals by week for 2017

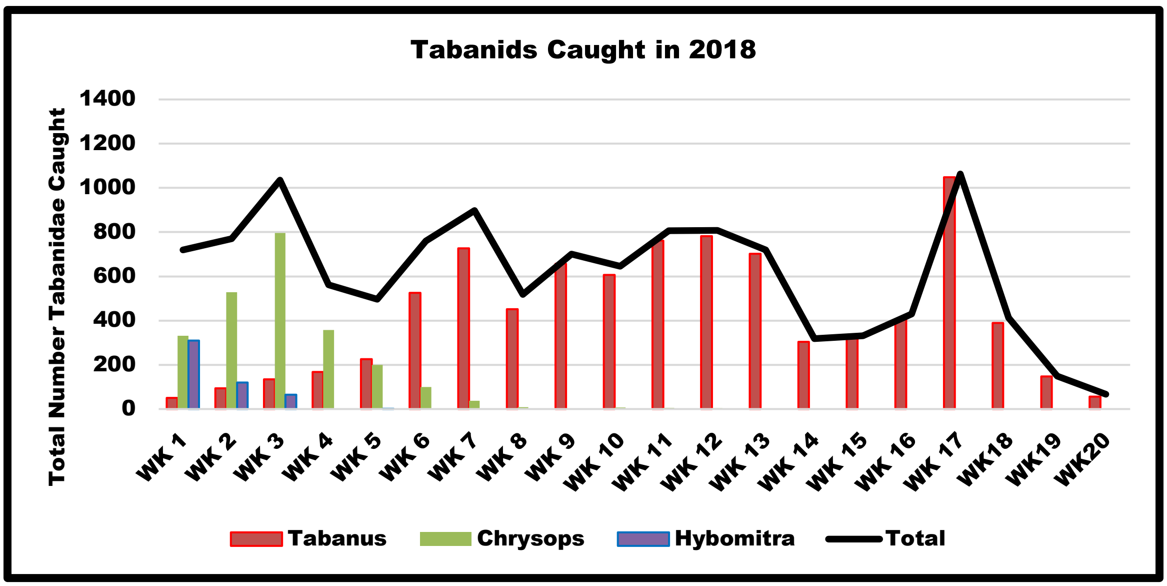

Figure 8 - Comparison of different tabanidae genera totals by week for 2018

Habitat Use by Week

Habitat use by week was analyzed and out of the 40 weeks trapped in this study, 30 weeks were significantly different in population use between habitats. Weeks that showed significant differences between habitat types, demonstrated that Open Canopy Cedar was significantly higher than at least one other habitat classification.

In 2017, 14 of the 20 weeks sampled were significantly different for tabanid use of habitat. During the first population peak in week 5 (June 15th), tabanid use of Open Canopy Cedar, Pasture and Oak was significantly higher than Closed Canopy Cedar (F=3.72, P=0.029). During the second peak in week 13 (August 10th), tabanid use of Open Canopy Cedar was significantly higher than any other habitat (F=6.09, P=0.004). (Figure 9)

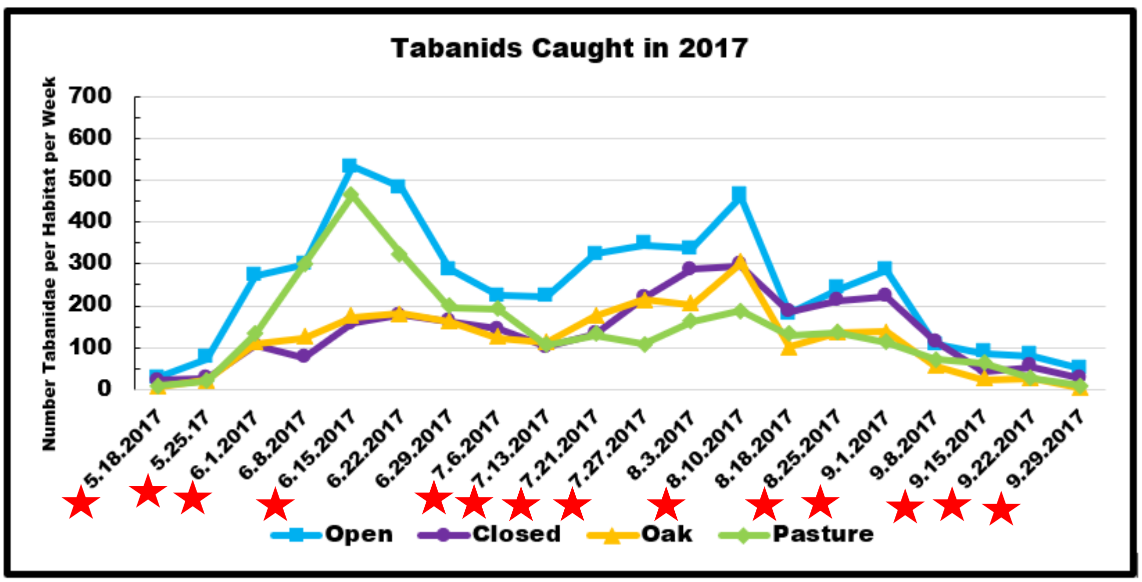

Figure 9 - Tabanidae habitat use by week in 2017. The dates marked with a star showed a significant difference in habitat use at Alpha 0.05.

In 2018, 16 of the 20 weeks sampled were significantly different between tabanid use of habitat. During all three populations peaks, tabanid use of Open Canopy Cedar was significantly higher than any other habitat (week 3 population peak F=17.48, P<0.001; week 7 population peak F=7.06, P=0.003; week 17 population peak F=18.71, P<0.001). (Figure 10)

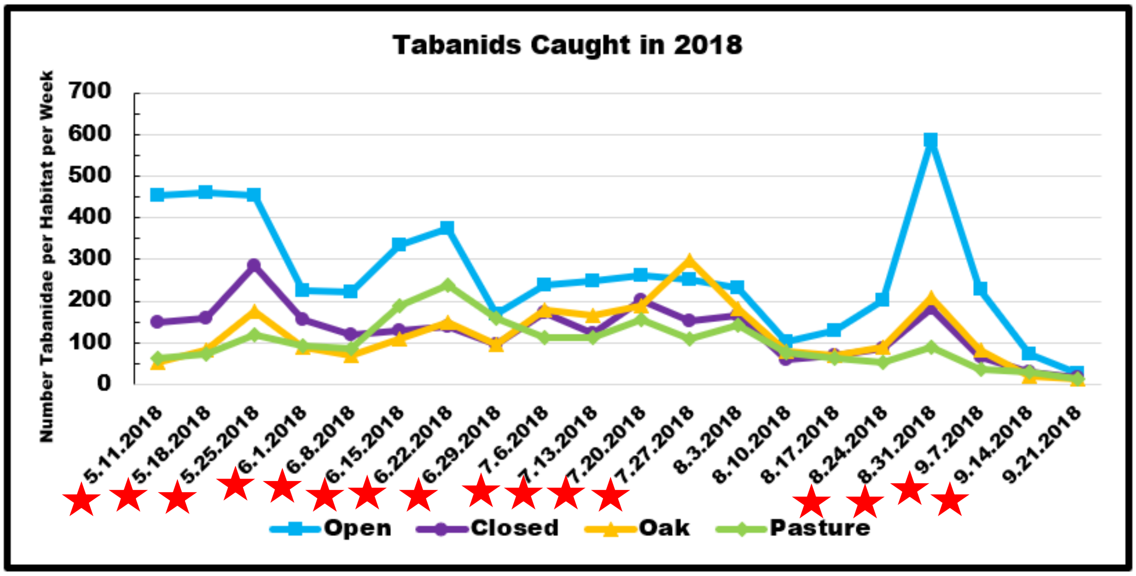

Figure 10 - Tabanidae habitat use by week in 2018. The dates marked with a star showed a significant difference in habitat use at Alpha 0.05.

Tabanid Genera Sampled by Trap Type and Habitat

Tabanus, *Chrysops*, and *Hybomitra* were analyzed by trap type. Tabanus both years were trapped significantly more in the HorsePal® trap (F=110.64, P<0.001; F=147.29, P<0.001). *Chrysops* populations were trapped in significantly higher numbers for the bucket traps in 2017 (F=15.90, P<0.001) and higher numerical numbers in 2018 but showed no significance in 2018 (F=3.73, P=0.054). The three *Hybomitra* flies in 2017 were found exclusively on bucket traps, however the low population was non-significant (P=0.429). *Hybomitra* populations in 2018 were more robust and were caught in significantly higher numbers on bucket traps (F=23.77, P<0.001).

Table 3 - Specific tabanid genera trapping comparison between the bucket trap and the HorsePal® trap.

	2017				2018			
	N	dF	F	P	N	dF	F	P
Tabanus	620	1	110.64	<0.001	600	1	147.29	<0.001
Chrysops	620	1	15.9	<0.001	600	1	3.73	0.054
Hybomitra	620	1	0.63	0.429	600	1	23.77	<0.001

The total tabanid sampled by genera within each habitat in 2017 was significantly different for *Tabanus* and *Chrysops*. *Tabanus* and *Chrysops* both had significantly higher populations in Open Canopy Cedar habitat than any other habitat ($F=7.16$, $P<0.001$; $F=10.15$, $P<0.001$)(Figure 12). *Hybomitra* populations showed no significant difference between habitats ($P=0.535$) and were active during a short period in 2017. Since the flies were already active during the first week of trapping, (May 11th-18th), the *Hybomitra* had already peaked and were in a decline.

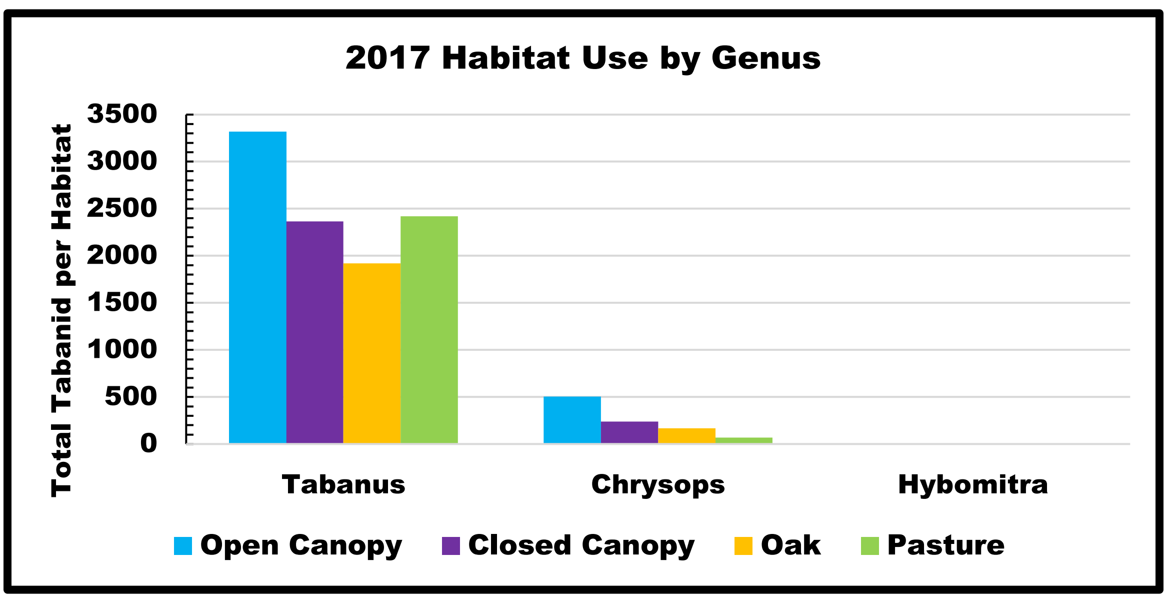

Figure 11 – Total tabanidae abundance by genus within habitat in 2017

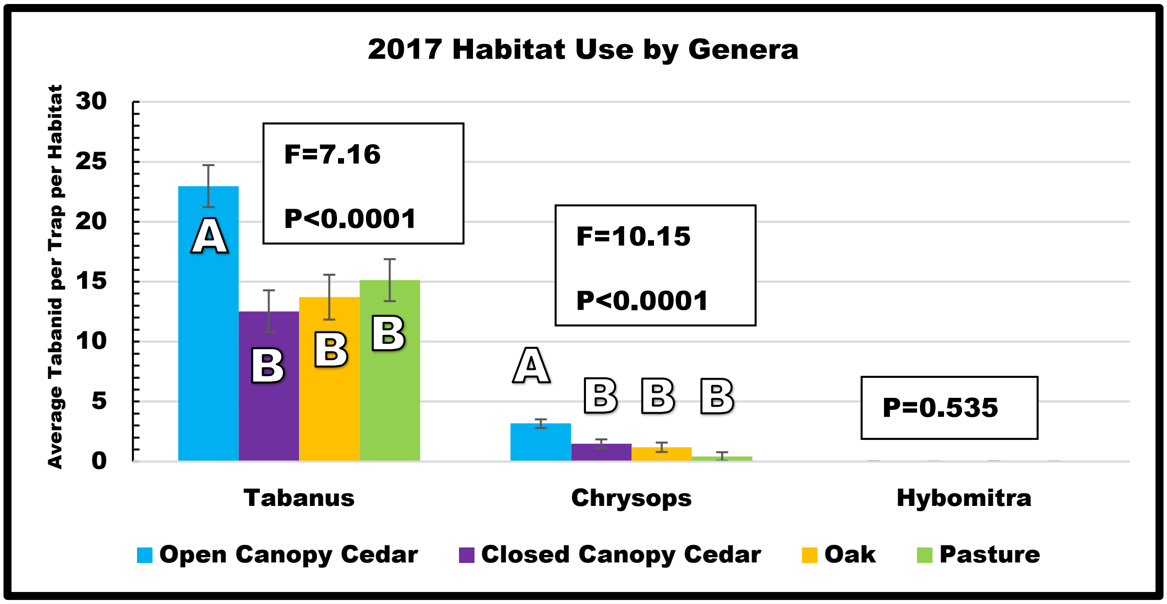

Figure 12 - Average Tabanid Caught per Trap per Habitat by Genus 2017. Bars with different letters indicate detectable differences (P<0.05) between habitat use within genera. Values are least squared means.

In 2018, total tabanid sampled by genera within each habitat was significantly different for *Tabanus* and *Hybomitra*. *Tabanus* and *Hybomitra* both had significantly higher populations in Open Canopy Cedar habitat than any other habitat (F=15.47, P<0.001; F=11.04, P<0.001)(Figure 9). *Chrysops* showed significant difference between habitats (F=3.33, P=0.019). Populations in Open Canopy Cedar habitat were not significantly different from Pasture habitat but were significantly higher than Closed Canopy Cedar and Oak habitats (Figure 14). Pasture, Closed Canopy Cedar and Oak were not significantly different.

To further determine if the number of tabanid genera in habitat type were influenced by trap type, additional analysis was conducted. In 2017, the average tabanid caught per bucket trap within each habitat type was significantly different.

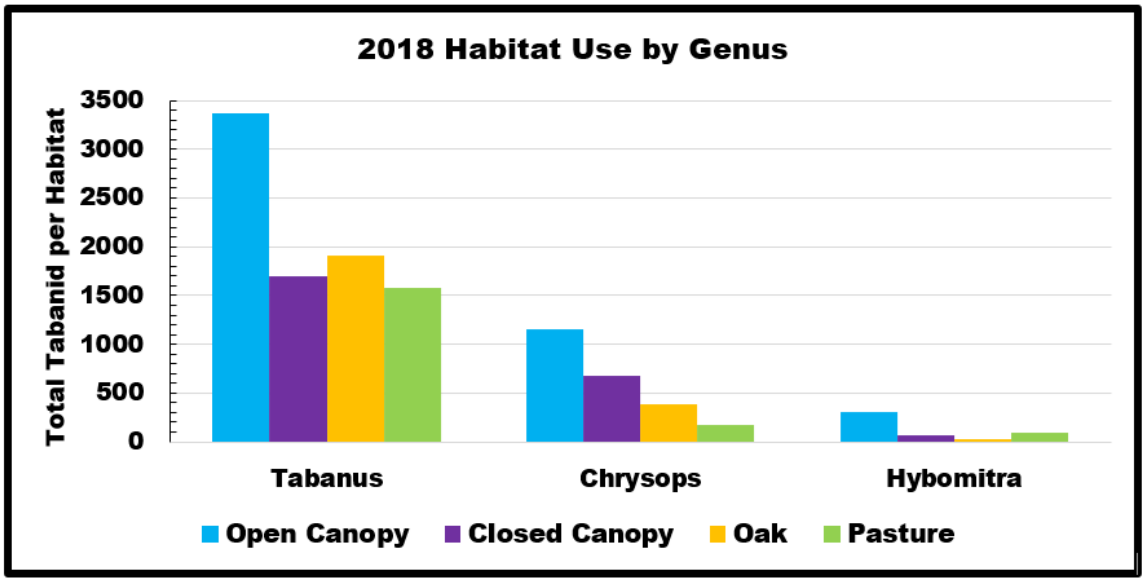

Figure 13 - Total tabanidae abundance by genus within habitat in 2018

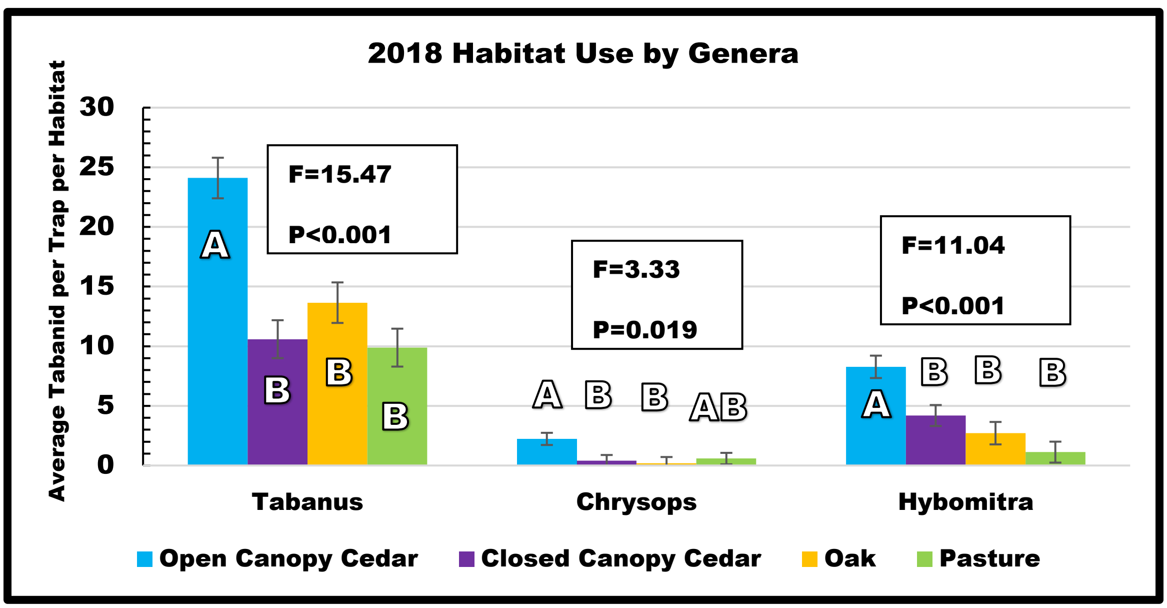

Figure 14 - Average tabanid caught per trap per habitat by genus 2018. Bars with different letters indicate detectable differences (P<0.05) between habitat use within genera. Values are least squared means.

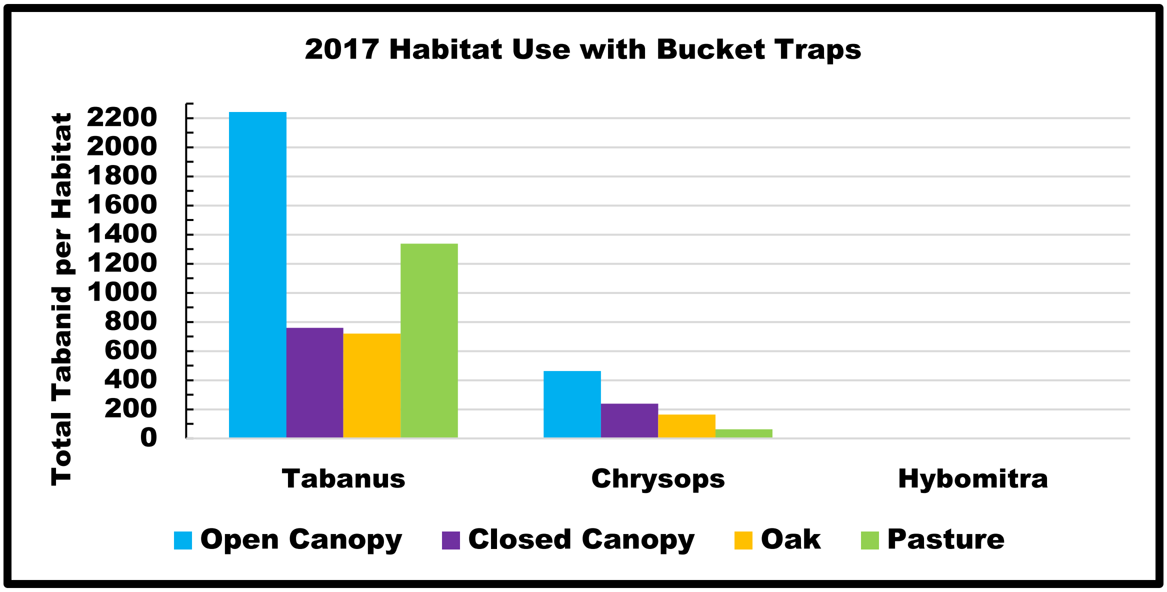

Figure 15 – Total tabanidae abundance by genera within each habitat type associated with bucket traps in 2017

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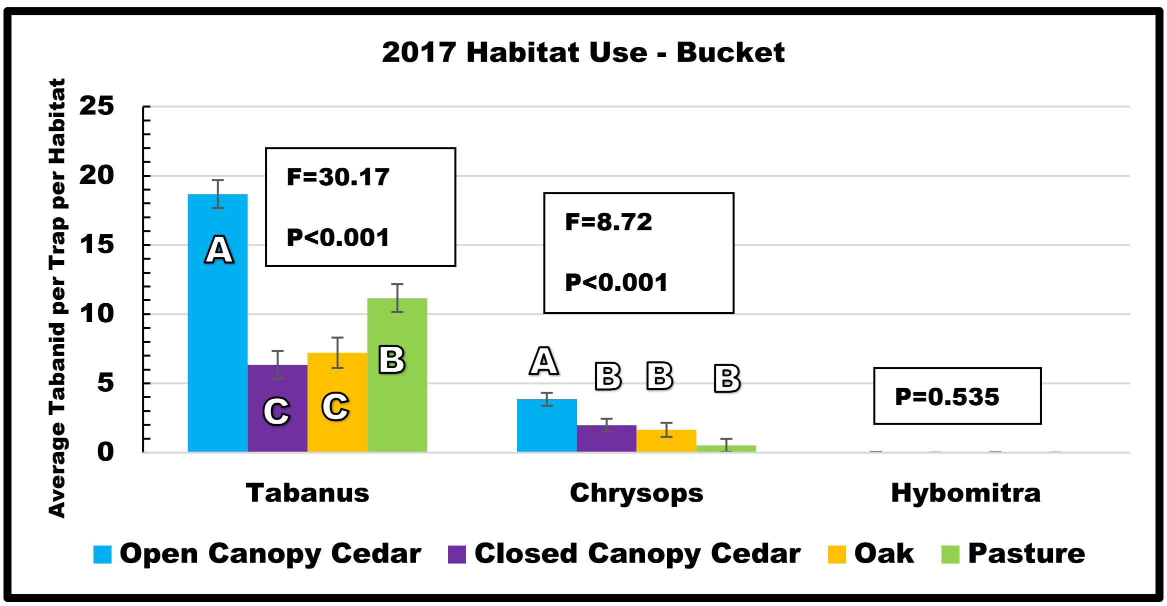

Figure 16 – Average tabanidae caught by genera within each habitat type associated with bucket traps in 2017. Bars with different letters indicate detectable differences (P<0.05) between habitat use within genera. Values are least squared means.

Tabanus had significantly higher populations in Open Canopy Cedar habitat than any other habitat, and Pasture was significantly higher than Closed Canopy Cedar or Oak (F=30.17, P<0.001)(Figure 16). For *Chrysops* Open Canopy Cedar habitat was significantly higher than any other habitat (F=8.72, P<0.001*)*(Figure 16) *Hybomitra* were only captured on bucket traps in Open Canopy Cedar and Oak habitats but showed no significant difference between habitats (P=0.535) due to low numbers.

In regard to HorsePal® traps for 2017, the number of *Tabanus* were not significantly different between habitats (P=0.736)(Figure 18). *Chrysops* was significantly higher in Open Canopy Cedar habitat than Closed Canopy Cedar and Oak, but not Pasture (F=3.52, P=0.016)(Figure 18). *Hybomitra* were only captured on bucket traps in Open Canopy Cedar and Oak habitats, so no data was produced for this trap.

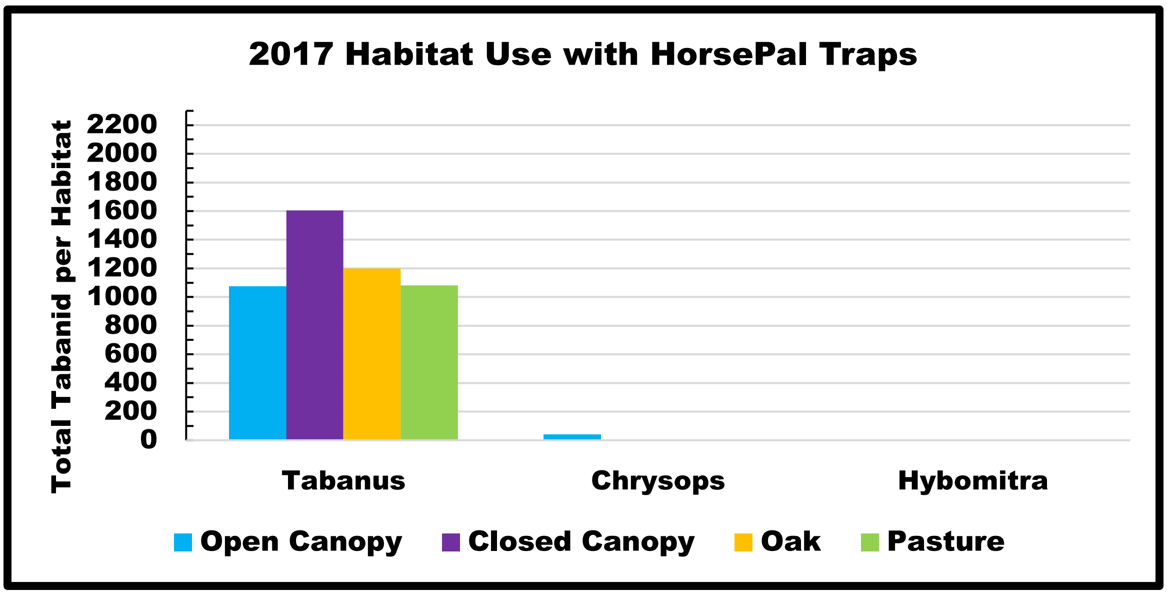

Figure 17- Total tabanidae abundance by genera within each habitat type associated with HorsePal® traps in 2017

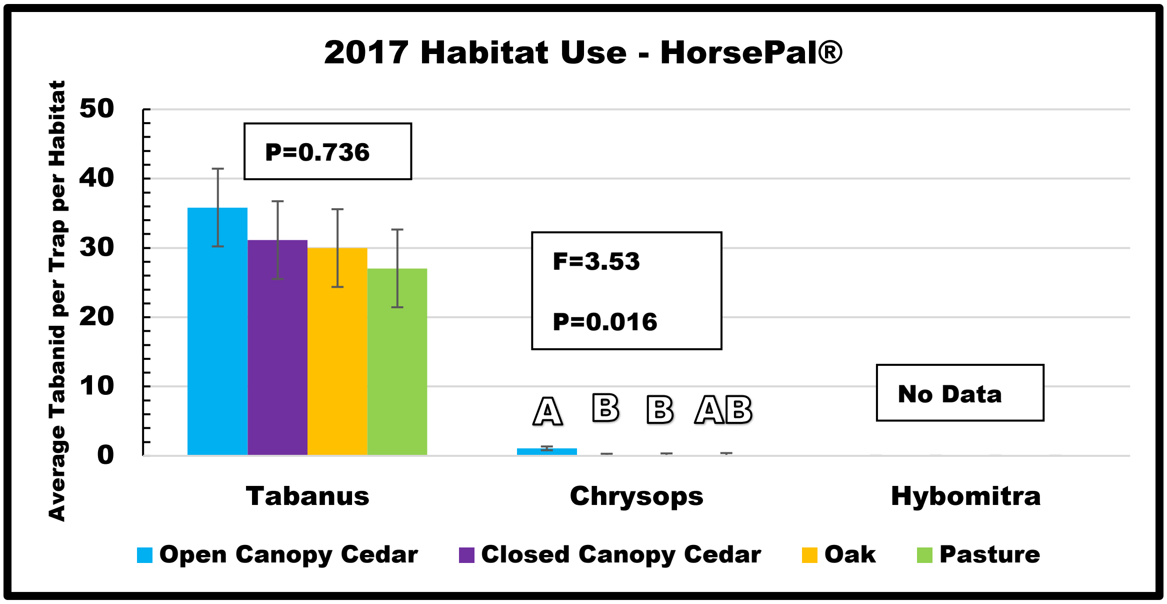

Figure 18- Average tabanidae caught by genera within each habitat type associated with HorsePal® traps in 2017. Bars with different letters indicate detectable differences (P<0.05) between habitat use within genera. Values are least squared means.

In 2018, the average number of *Tabanus* caught per bucket trap within each habitat type was significantly different. (F=45.38, P<0.001)(Figure 20). *Chrysops* use of Open Canopy Cedar habitat was significantly higher than Oak and Pasture habitats but not Closed Canopy Cedar (F=3.57, P=0.014)(Figure 20). *Hybomitra* had significantly higher populations in Open Canopy Cedar habitat than any other habitat (F=11.72, P<0.001)(Figure 20).

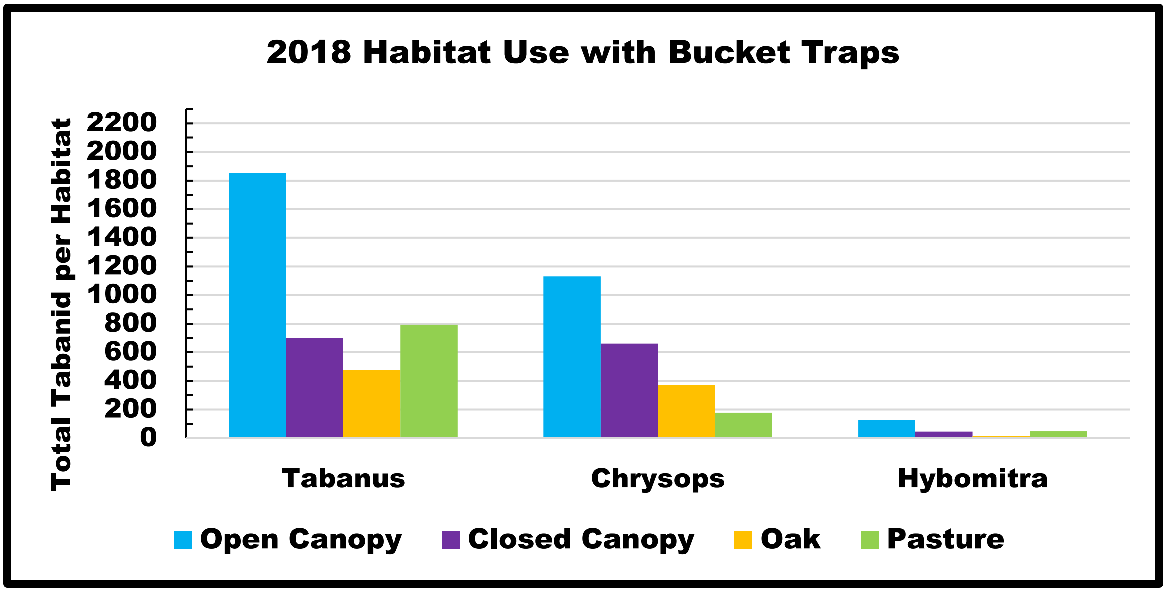

Figure 19- Total tabanidae abundance by genera within each habitat type associated with bucket traps in 2018

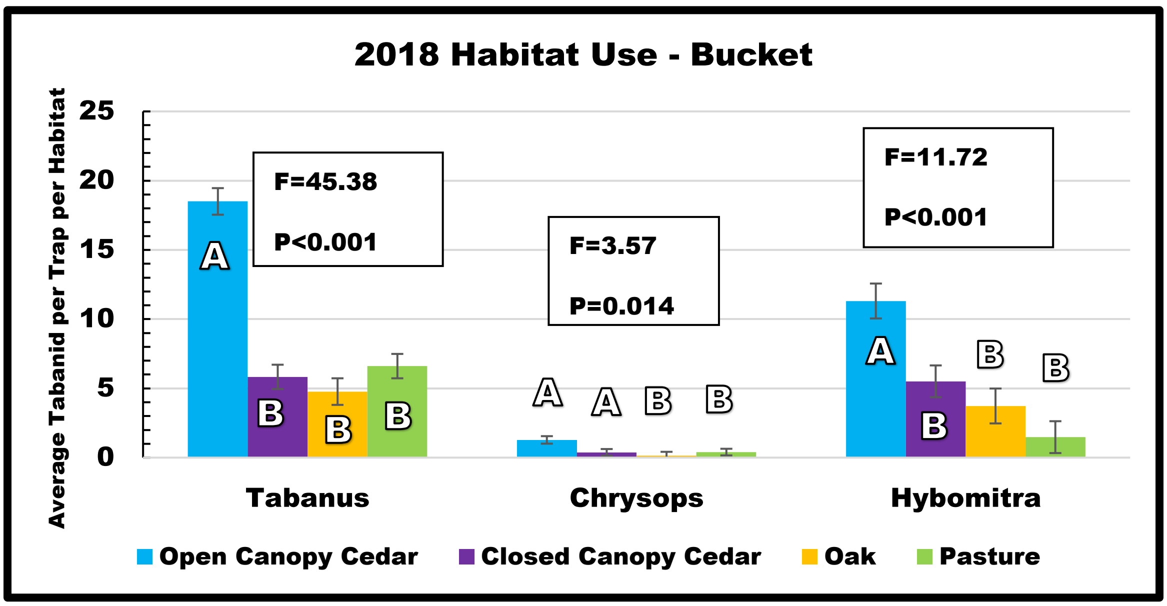

Figure 20- Average tabanidae caught by genera within each habitat type associated with bucket traps in 2018. Bars with different letters indicate detectable differences (P<0.05) between habitat use within genera. Values are least squared means.

In regard to HorsePal® traps for 2018, *Tabanus* populations were significantly different between habitats. *Tabanus* populations were significantly higher in Open Canopy Cedar than Pasture but not Closed Canopy Cedar or Oak, however *Tabanus* populations sampled in the Pasture habitat were not significantly different from those sampled in the Closed Canopy Cedar or Oak (F=3.29, P=0.022)(Figure 22). *Chrysops* populations sampled by the HorsePal® trap within habitats were not significantly different (P=0.259)(Figure 22). *Hybomitra* populations sampled by the HorsePal® trap were significantly higher in Open Canopy Cedar than Closed Canopy Cedar or Oak but had no significant difference with those sampled within the Pasture habitat (F=5.05, P=0.002)(Figure 22).

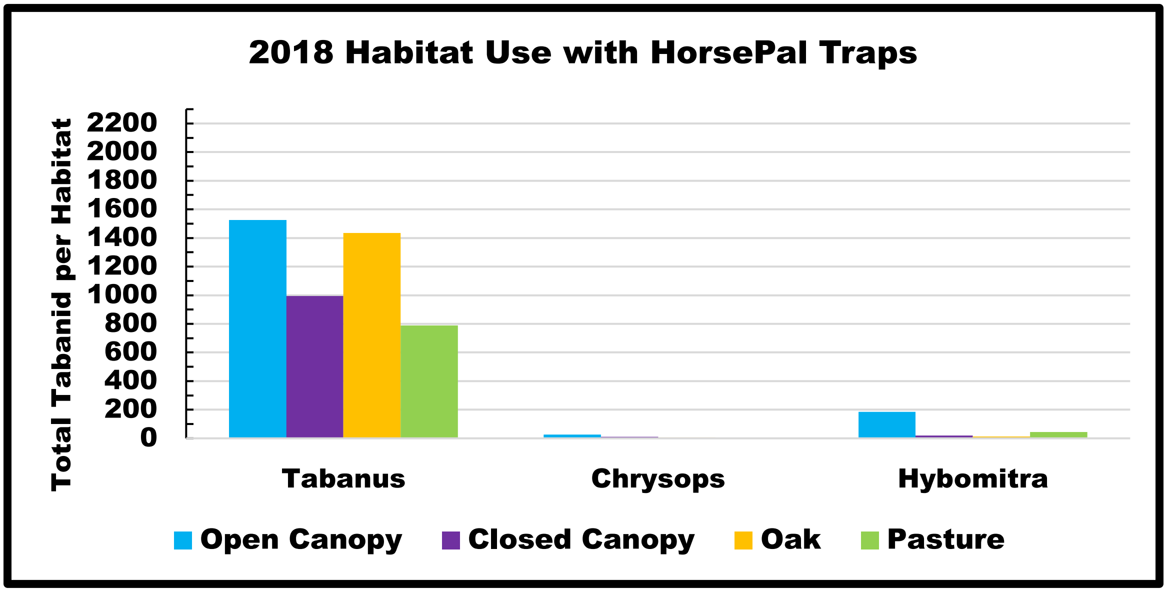

Figure 21 - Total tabanidae abundance by genera within each habitat type associated with HorsePal® traps in 2018

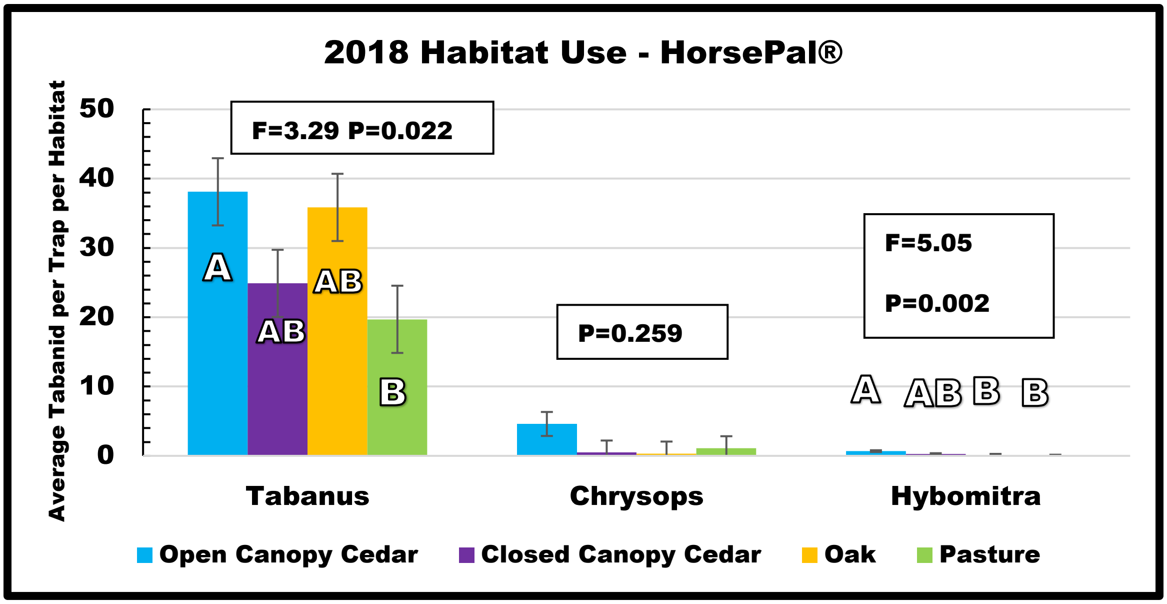

Figure 22 - Average tabanidae caught by genera within each habitat type associated with HorsePal® traps in 2018. Bars with different letters indicate detectable differences (P<0.05) between habitat use within genera. Values are least squared means.

Tabanid Community and Abiotic Factors

To determine the community associations between selected tabanid species, the population data of important tabanid species known to be vectors of certain pathogens (Table 1) were submitted to unconstrained ordination, principle components analysis (PCA). In 2017, the first PCA axis explained 40.73% of variation, the second PCA axis explained 37.14% for a total of 77.87% of the variation explained by the model submitted. The unconstrained PCA (Figure 23) for 2017 showed correlation of multiple species distributed across the first axis. *T. abactor* and *T. sulcifrons* complex separated themselves from the other species along the left side of the first axis. Four of the *Tabanus* species (*T. equalis, T. molestus, T. styguis* and *T. trimaculatus*) showed a close association and *Hybomitra* and *C. callidus* grouped together along the right side of the first axis.

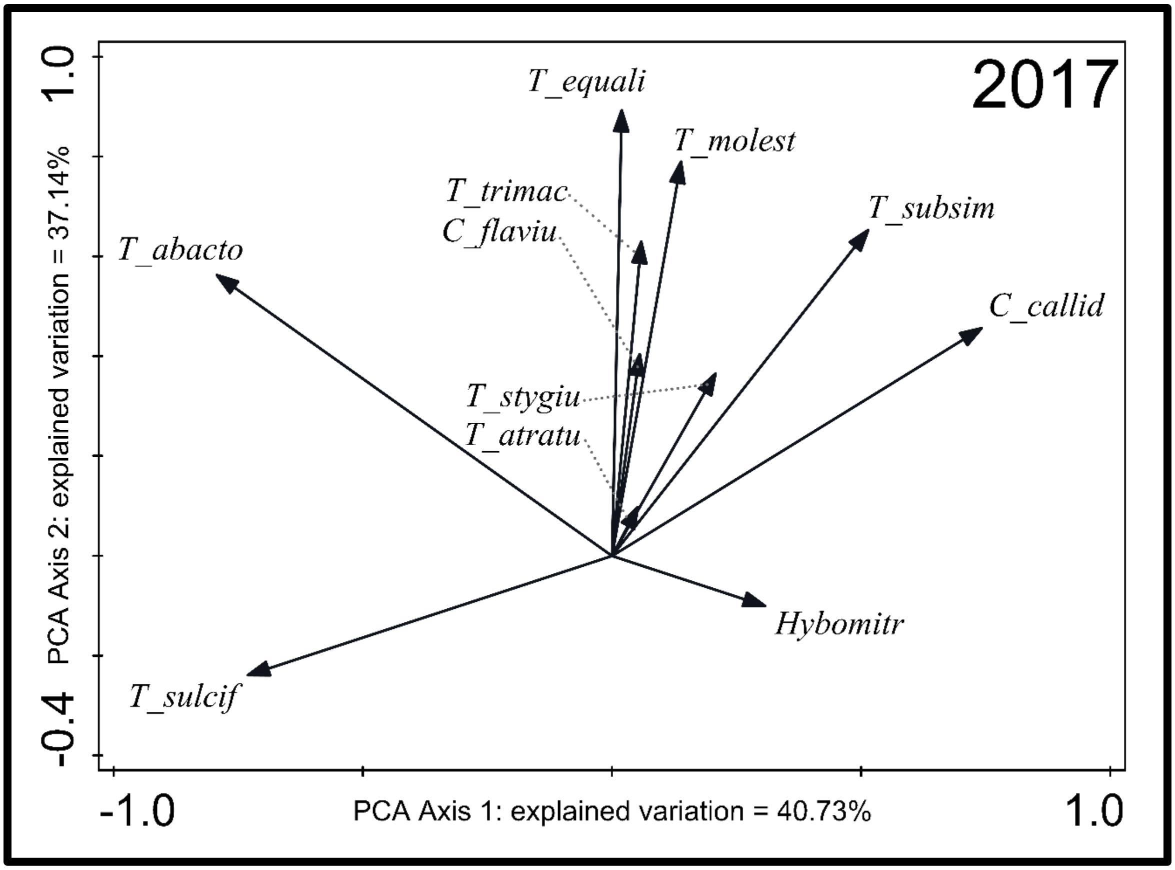

Figure 23 – Unconstrained Principle Component Analysis (PCA) for selected tabanid species in 2017

To further understand both the community of selected tabanid species and how those are influenced by certain abiotic factors a constrained Redundancy analysis (RDA)was utilized for 2017 which included weather and habitat variables (Figure 24). Species that had separated or grouped with the PCA, were preserved. *T. abactor* positively correlated with the temperature variables, suggesting the population is driven by temperature more than other abiotic variables. The direct opposite was noted for *C. callidus* populations. This *Chrysops* species positively correlated with wind and negatively with the temperature variables. The *Tabanus* species of *T. equalis*, *T. molestus*, *T. styguis* and *T. trimaculatus* showed a positive correlation with Solar. The first axis explained 75.81% of the variation, while the second axis explained 13.78% for a total of 89.59% of the variation explained by the submitted model. Open-Canopy and Closed-Canopy Cedar habitats disassociated from the Oak and Pasture Habitats.

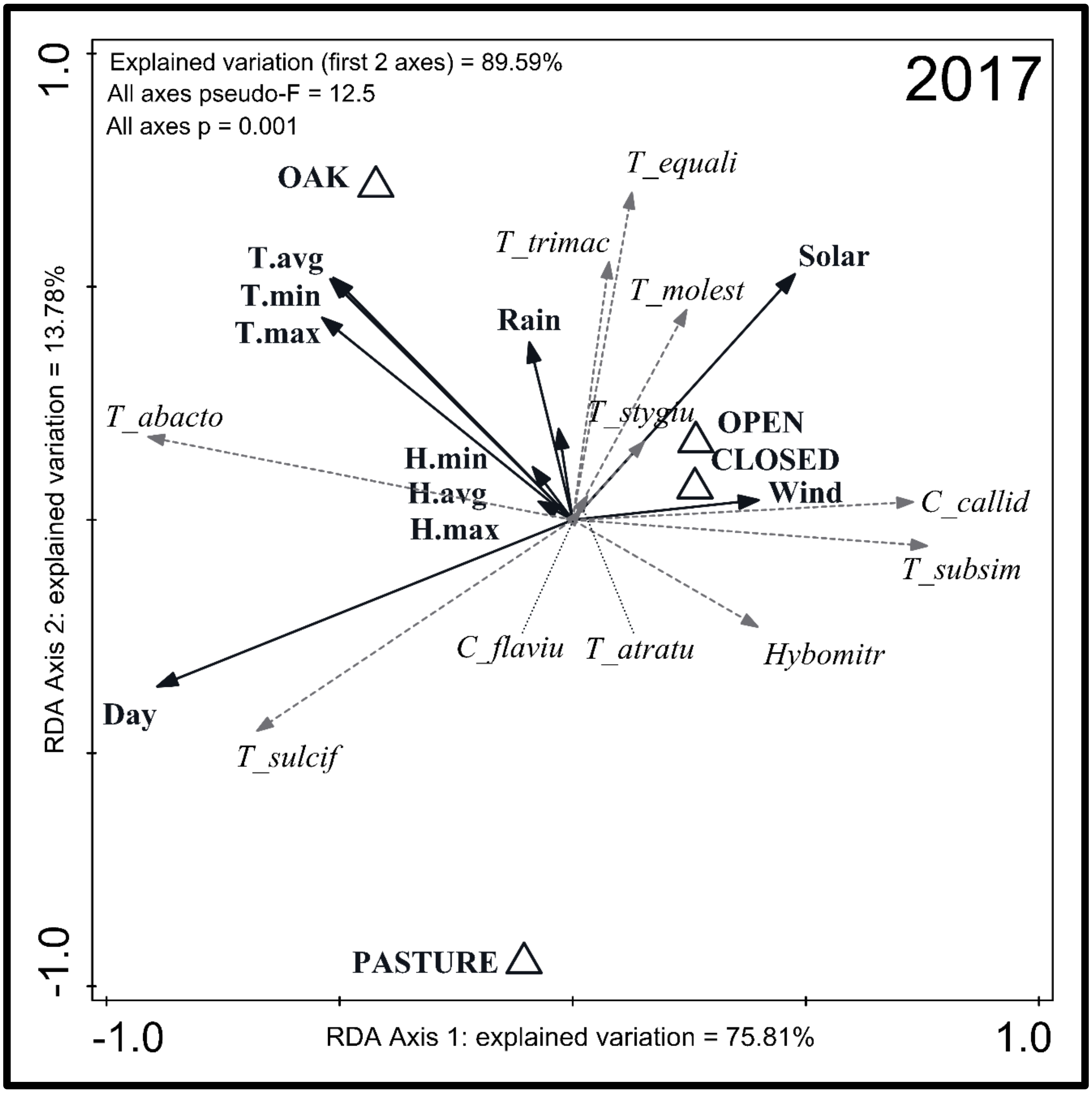

Figure 24 – Constrained Redundancy Analysis RDA of selected tabanidae species along with abiotic and habitat factors in 2017

The unconstrained PCA for 2018 (Figure 25) showed the same distinction of species from 2017. The first PCA axis explained 58.18% of variation, the second PCA axis explained 23.95% for a total of 82.13% of the variation explained by the model submitted.

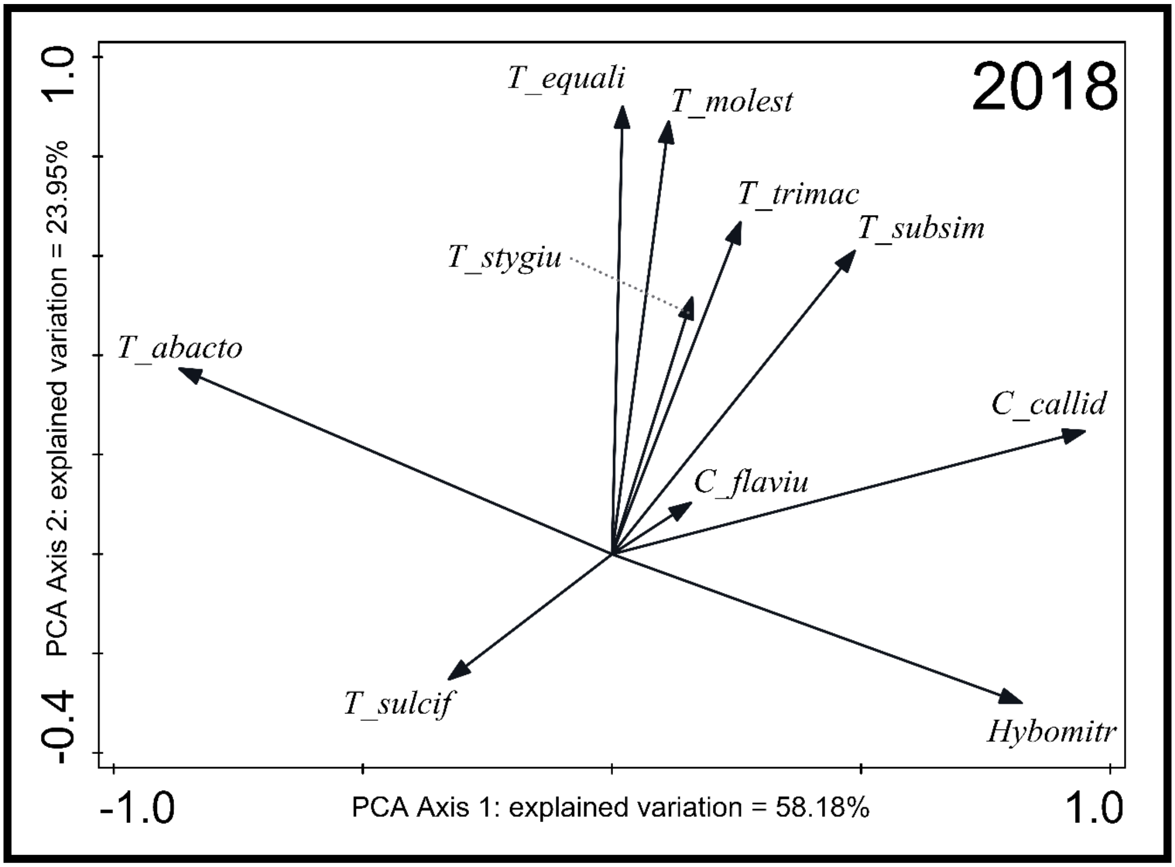

Figure 25 - Unconstrained Principle Component Analysis (PCA) for selected tabanid species in 2018

The constrained RDA analysis (Figure 26) was similar to 2017. The first axis explained 77.34% of the variation, while the second axis explained 13.12% for a total of 90.46% of the variation explained by the submitted model. The same close positive correlation between the temperature variables and *T. abactor* was duplicated. The *Hybomitra* and *C. callidus* populations had a positive correlation with wind and negative for the temperature values. The *Tabanus* species of *T. equalis*, *T. molestus*, *T. styguis* and *T. trimaculatus* still showed a positive correlation with Solar. Habitat disassociation held true from 2017. Open-Canopy and Closed-Canopy Cedar habitats were modeled close together and were disassociated from the Oak and Pasture Habitats.

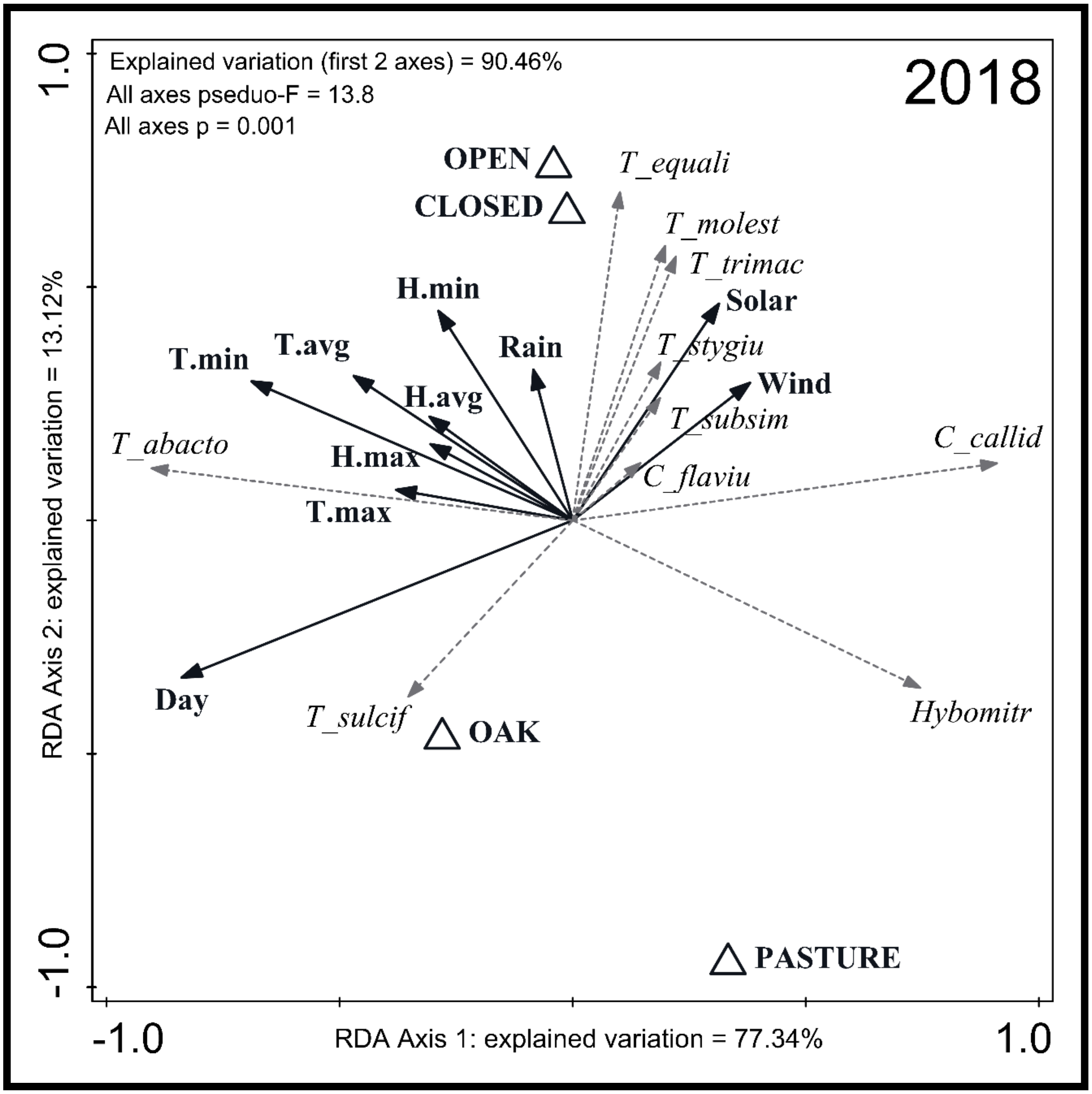

Figure 26 - Constrained Redundancy Analysis RDA of selected tabanidae species along with abiotic and habitat factors in 2018

Important Tabanid Vector Species Response Curves

Based on the RDA for 2017 and 2018, significant species response curves for the values of Julian date, average temperature and solar were generated. *T. abactor T. sulcifrons*, *Hybomitra* and *C. callidus* species showed significance for observations associated with Julian dates, as well as average temperature.

Table 4 – Tabanidae species with relationship to Julian Date for their peak population

	Day (Julian Date)							
	2017				*2018*			
	F	P	**Opt**	Tolerance	**F**	P	**Opt**	Tolerance
Tabanus abactor	**53.9**	<0.001	**201**	27	**109.8**	<0.001	**197**	24
Tabanus sulcifrons	**43**	<0.001	**228**	26	**24.2**	<0.001	**237**	26
Hybomitra	**31.8**	<0.001	**138**	2	**78.7**	<0.001	**117**	10
Chrysops callidus	**5.3**	<0.001	**153**	17	**NS**	NS	**-**	-

Julian date was analyzed for *T. abactor T. sulcifrons*, *Hybomitra* and *C. callidus*. The Julian date was used to find the optimum day of year for total abundance for each species as well as their tolerance or deviation from the optimum dates. *Tabanus abactor* for both 2017 and 2018 was four days apart for the optimum day for the populations to peak and gave similar tolerance ranges (F=53.9, P<0.001; F=109.8 P<0.001) (Figure 27). *T. abactor* populations should peak in late July but could peak 24 – 27 days before or after.

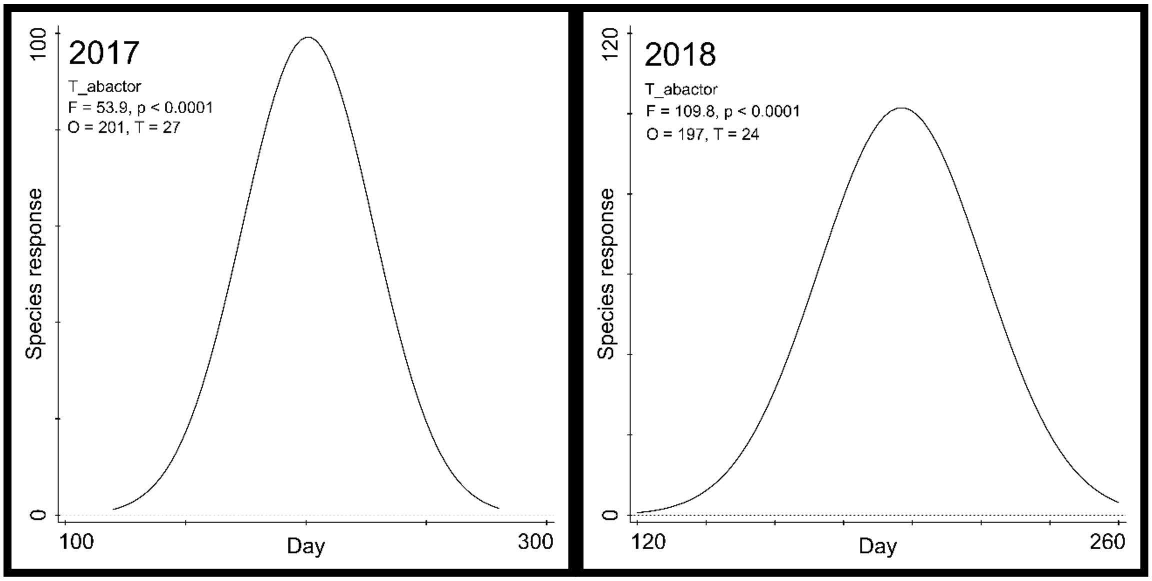

Figure 27 – Species response curves for *Tabanus abactor* in relation to Julian Date for population peak

Tabanus sulcifrons for both 2017 and 2018 was within 10 days apart for optimum day for the populations to peak (228, 237) and gave similar tolerance ranges (F=43.0, P<0.001; F=24.2 P<0.001)(Figure 28). *T. sulcifrons* populations should peak in August but could peak 26 days before or after.

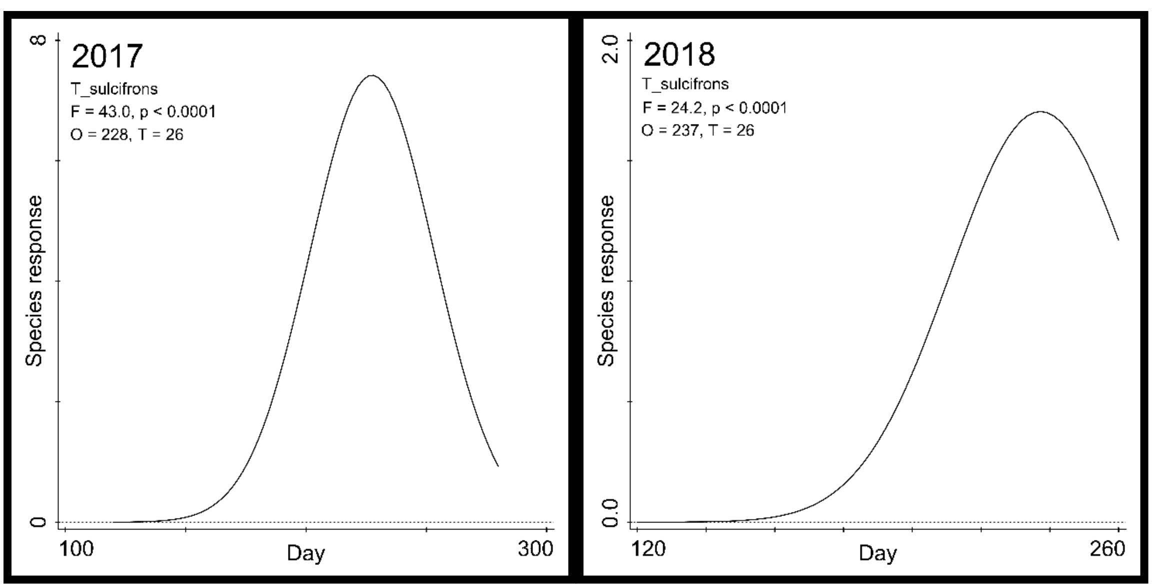

Figure 28 – Species response curves for *Tabanus sulcifrons* in relation to Julian Date for population peak

Hybomitra for both 2017 and 2018 was less similar than the *Tabanus* species for optimum day for populations to peak (138, 117) and tolerance ranges of 2 and 10 (F=31.8, P<0.001; F=78.7 P<0.001). (Figure 29). *Hybomitra* populations should peak in late spring which is earlier than other tabanid species.

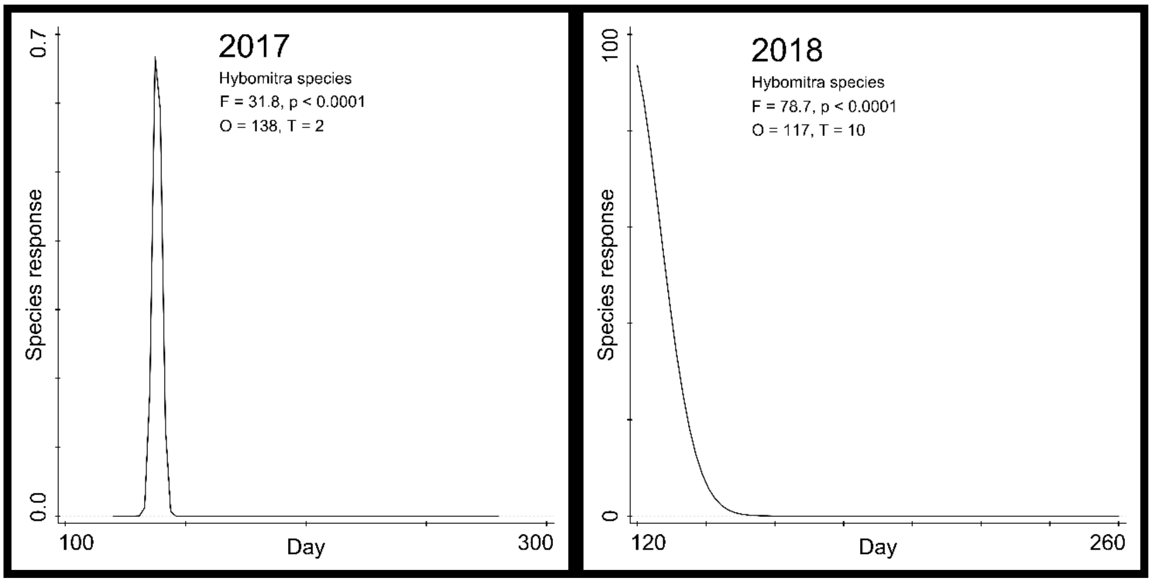

Figure 29 - Species response curves for *Hybomitra* in relation to Julian Date for population peak

Chrysops callidus was only significant for 2017, optimum day for populations to peak was calculated to be 153 and a tolerance of 17 days (F=5.3, P<0.01). *C. callidus* populations should peak in June however this data is inconclusive in determining when peak *C. callidus* populations could occur in Oklahoma.

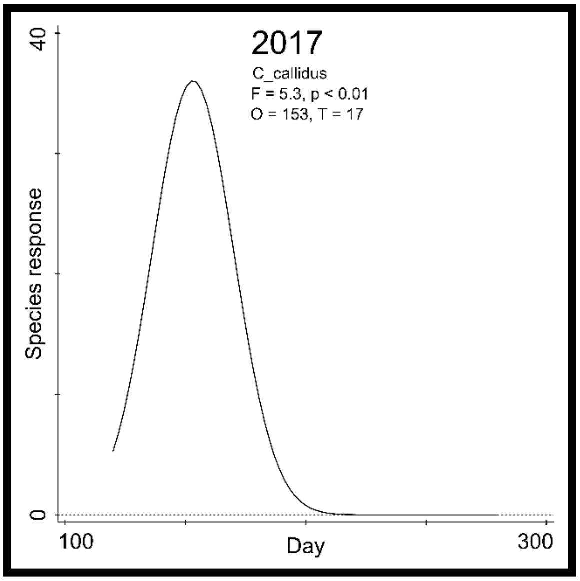

Figure 30 - Species response curves for *Chrysops callidus* in relation to Julian Date for population peak

Weekly average temperature was analyzed for *Tabanus abactor*, *Hybomitra*, and *Chrysops callidus*. The average temperature during sample week was used to find the optimum average temperature for each population peak as well as their tolerance. *Tabanus abactor* for optimum weekly average temperature was 28°C in 2017 and 50°C degrees in 2018. Both were highly significant and are reasonable with the late summer optimum Julian date predicted population peak however the 2018 optimum temperature of 50°C is likely due to never reaching an upper temperature that could predict the peak population of this species (F=35.5, P<0.001; F=22.3 P<0.001). Weekly average temperature for *Hybomitra* was significant for both years in determining the presence of this genera but the exact relationship is unknown to no optimum weekly average temperatures being associated with this tabanid group (F=187.0, P<0.001; F=9.5, P<0.001). Weekly average temperature was significant for *Chrysops callidus* in 2018 only, optimum weekly average temperature was 23°C (F=20.7, P<0.001).

The *Tabanus* species of *T. equalis*, *T. molestus*, *T. styguis* and *T. trimaculatus* were used to analyze the effect of solar, which is a measure of solar exposure in the form of total solar energy for a day and measured in megajoules per square meter (MJ/m^2). Optimum solar comparisons varied by species and by year. Solar was significant in both years for *T. equalis*, optimum solar for populations to peak were reported at 27 MJ/m^2 and 32 MJ/m^2 with narrow tolerances of 3 MJ/m^2 and 4 MJ/m^2 ($F=13$, $P<0.001$; $F=12.3$ $P<0.001$)(Figure 31). Solar radiation was significant for *T. molestus* populations and had highly variable optimum solar radiation ranges between years ($F=27.4$, $P<0.001$; $F=6.8$ $P=0.002$)(Figure 31). When considering the effects of solar radiation on *T. styguis* it was only influencing this species significantly for 2018 ($P=0.09$; $F=5.6$ $P<0.01$). The impact of solar radiation on *T. trimaculatus* was highly significant in both years with similar optimum solar measurements of 28 MJ/m^2 and 25 MJ/m^2 ($F=19.2$, $P<0.01$; $F=11.3$ $P<0.001$).

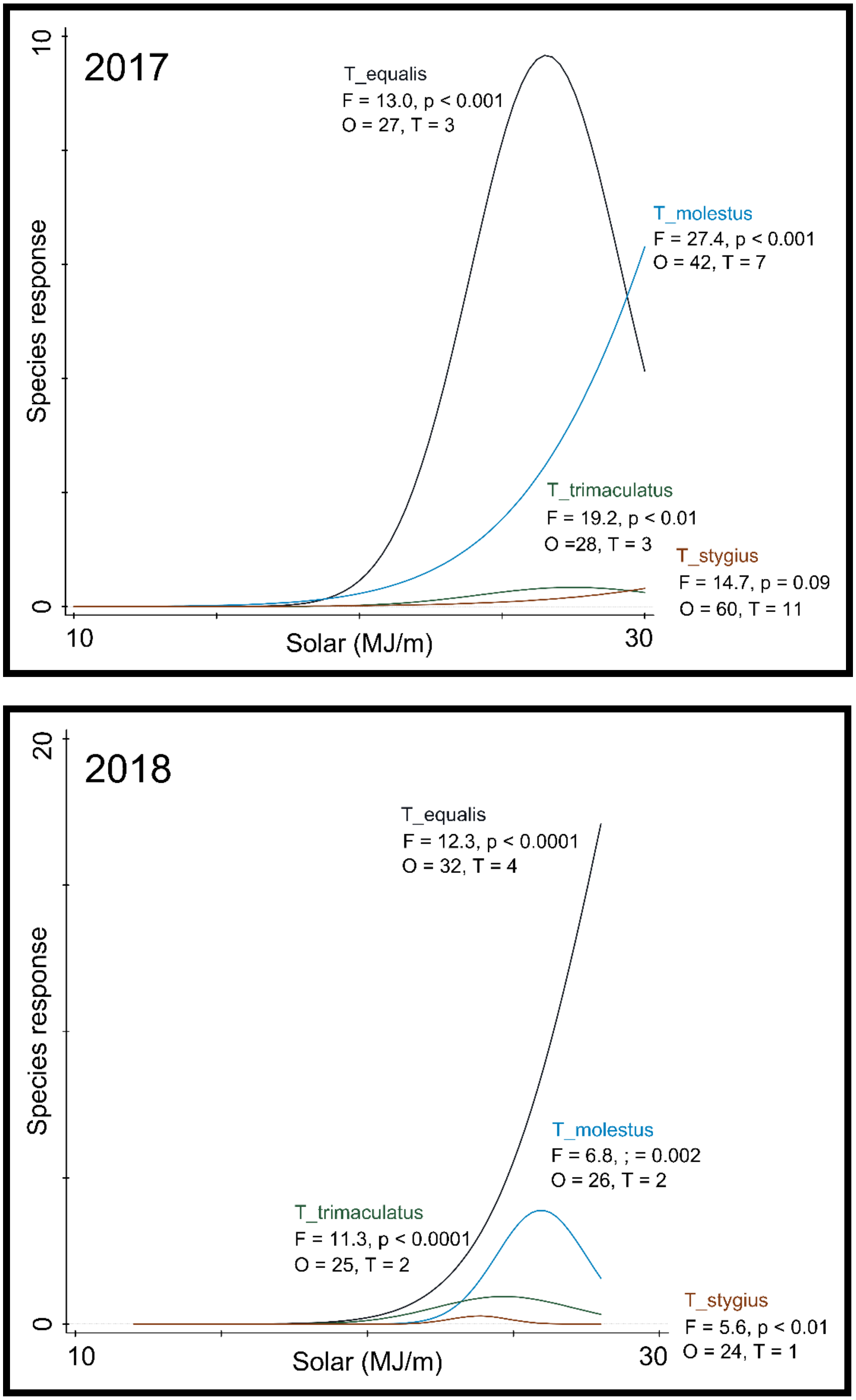

Figure 31 – Species response curves for *Tabanus equalis, T. molestus, T. styguis* and *t. trimaculatus* in relation to weekly average solar radiation for population peaks

The abiotic factors submitted to the RDA were also analyzed using a multivariate analysis of variance (MANOVA) to better understand the abiotic drivers behind the populations of *Tabanus abactor*, *T. equalis*, *T. molestus*, *T. sulcifrons T. styguis*, *T. trimaculatus*, *Hybomitra* and *Chrysops callidus*. The MANOVA output below also lists the associated F statistic and P value for each species and is considered significant in the different tabanid grouping of species at an α≤0.05 for each year.

Table 5 Multivariate Analysis of Variance for 2017 and 2018. Each column lists the number of observations (N), the associated mean and standard deviation (Mean ±SD), Minimum number caught in a single trap (MIN), the maximum number caught in a single trap (MAX), and the calculated F statistic and P-value.

	2017						2018					
	N	**Mean ±SD**	**MIN**	**MAX**	**F**	**P**	**N**	**Mean ±SD**	**MIN**	**MAX**	**F**	**P**
Total Tabanidae	619	20.93 (26.01)	0	181	21.24	<0.001	600	20.35 (24.16)	0	181	24.35	<0.001
Total *Tabanus*	619	16.18 (22.48)	0	171	16.74	<0.001	600	14.27 (20.86)	0	181	30.44	<0.001
Total *Hybomitra*	619	0.005 (0.09)	0	2	1.61	0.072	600	0.83 (6.12)	0	131	7.67	<0.001
Total *Chrysops*	619	1.59 (4.70)	0	56	11.33	<0.001	600	3.98 (11.43)	0	77	31.88	<0.001
Tabanus abactor	619	6.09 (9.54)	0	73	19.45	<0.001	600	5.78 (10.41)	0	102	28.46	<0.001
***T. equalis* complex**	619	0.55 (1.81)	0	16	10.91	<0.001	600	0.46 (1.82)	0	23	11.72	<0.001
***T. molestus* complex**	619	0.15 (0.99)	0	20	4.49	<0.001	600	0.20 (1.19)	0	23	6.67	<0.001
***T. sulcifrons* complex**	619	0.42 (1.25)	0	14	11.44	<0.001	600	0.09 (0.34)	0	3	4.04	<0.001
T. styguis	619	0.01 (0.10)	0	1	10.38	<0.001	600	0.01 (0.11)	0	1	3.5	<0.001
T. trimaculatus	619	0.02 (0.14)	0	1	4.43	<0.001	600	0.07 (0.31)	0	3	4.57	<0.001
Chrysops callidus	619	1.45 (5.49)	0	83	38.03	<0.001	600	3.52 (10.81)	0	75	30.57	<0.001

To further analyze the effect of abiotic factors, the fixed independent effects of habitat type and trap type were utilized to determine if these were still significant factors in tabanid abundance. In 2017, the only factors in the MANOVA that were contributing to significantly to the overall model for *T. abactor* were Habitat and Trap Type (F=19.58 P<0.001; F=82.14 P<0.001)(Table 6). When looking at factors contributing to *T. equalis* abundance there were multiple abiotic factors as well as habitat and trap type that were significant. The abiotic factors of importance to *T. equalis* abundance were: weekly average minimum temperature (°C) (F=13.39 P=0.0003), weekly average humidity (% relative humidity RH) (F=13.82 P=0.0002), weekly average rain accumulation (millimeters mm)(F=39.23 P<0.001), average wind speed (meters per second m/s)(F=31.18 P<0.001) and weekly solar radiation (F=12.72 P=0.0004), one unit increase of each factor was associated with *T. equalis* increased populations. Factors important to *T. molestus* abundance were weekly average humidity (%RH) (F=4.03 P=0.045), weekly average rain accumulation (mm) (F=9.14 P=0.003), and trap type (F=29.63 P<0.001). As both weekly average humidity (%RH) and weekly average rain accumulation (mm) increased by one unit, there was an increase in *T. molestus* population. The factors important to *T. sulcifrons* abundance were habitat (F=6.84, P=0.0002) and trap type (F=86.43, P<0.001), as well as multiple abiotic factors. The abiotic factors important to *T. sulcifrons* abundance were Julian date (F=10.21, P=0.002) and weekly average maximum temperature (°C) (F=3.88, P=0.049), which demonstrated an increase of *T. sulcifrons* with one unit increase of factor. However, a one unit increase in weekly average humidity (%RH) (F=6.23, P=0.013), and weekly average rain accumulation (mm) (F=7.98, P=0.005), were associated with a decrease in *T. sulcifrons* abundance. Several factors were important in determining *T. trimaculatus* abundance which were habitat (F=3.05, P=0.028) and trap type (F=7.71, P=0.006), as well as multiple abiotic factors. Weekly average humidity (%RH) (F=6.09, P=0.014), weekly average rain accumulation (mm) (F=18.81, P<0.001), average wind speed (m/s) (F=6.32, P=0.012), and weekly solar radiation (F=8.88, P=0.003), demonstrated an increase of *T. trimaculatus* with one unit increase of these factors. However, a one unit increase in weekly average temperature (°C) (F=9.68, P =0.002) was associated with a decrease in population of this species. There were no factors within this study that could reliable influence *Hybomitra* abundance (P=0.072). Many factors contributed to *Chrysops callidus* abundance including habitat (F=10.42, P<0.001) and trap type (F=17.14, P<0.001), as well as multiple abiotic factors which were Julian date (F=4.94, P=0.027), weekly average maximum temperature (°C) (F=5.35, P=0.021), and average wind speed (m/s) (F=6.56, P=0.011) that demonstrated a decrease of the number of *C. callidus* with one unit increase of these factors.

Table 6 – Important abiotic as well as habitat and trap factors that influence tabanidae species in 2017

2017	Total Tabanidae	**Total *Tabanus***	Total *Hybomitra*	**Total *Chrysops***	*Tabanus abactor*	***T. equalis* complex**	*T. molestus* complex	***T. sulcifrons* complex**	*T. styguis*	***T. trimaculatus***	*Chrysops callidus*
Julian Day	*NS*	***NS***	*NS*	**18.17 (<0.001)**	*NS*	***NS***	*NS*	**10.21 (0.002)**	*NS*	***NS***	4.94 (0.027)
Maximum Temperature	*NS*	***NS***	*NS*	***NS***	*NS*	***NS***	*NS*	**3.88 (0.049)**	*NS*	**5.75 (0.0168)**	5.35 (0.021)
Minimum Temperature	3.87 (0.0497)	***NS***	*NS*	***NS***	*NS*	**13.39 (0.0003)**	*NS*	***NS***	*NS*	**8.26 (0.004)**	*NS*
Average Temperature	*NS*	***NS***	*NS*	***NS***	*NS*	***NS***	*NS*	***NS***	*NS*	**9.68 (0.002)**	3.93 (0.048)
Maximum Humidity	*NS*	***NS***	*NS*	***NS***	*NS*	**13.82 (0.0002)**	*NS*	**6.14 (0.014)**	*NS*	**7.66 (0.006)**	*NS*
Minimum Humidity	*NS*	***NS***	*NS*	***NS***	*NS*	**39.23 (<.0001)**	*NS*	**6.36 (0.012)**	*NS*	**4.07 (0.044)**	*NS*
Average Humidity	*NS*	***NS***	*NS*	***NS***	*NS*	**31.13 (<.0001)**	4.03 (0.045)	**6.23 (0.013)**	*NS*	**6.09 (0.014)**	*NS*
Rain	*NS*	***NS***	*NS*	***NS***	*NS*	**78.23 (<.0001)**	9.14 (0.002)	**7.98 (0.005)**	*NS*	**18.81 (<.0001)**	*NS*
Wind Speed	*NS*	***NS***	*NS*	***NS***	*NS*	**31.18 (<.0001)**	*NS*	**2.88 (0.090)**	*NS*	**6.32 (0.012)**	6.56 (0.011)
Solar	*NS*	***NS***	*NS*	***NS***	*NS*	**12.72 (0.0004)**	*NS*	***NS***	*NS*	**8.88 (0.003)**	*NS*
Habitat	13.23 (<0.001)	**9.77 (<0.001)**	*NS*	**11.84 (<0.001)**	19.58 (<.0001)	**2.8 (0.039)**	*NS*	**6.84 (0.0002)**	*NS*	**3.05 (0.028)**	10.42 (<.0001)
Trap Type	162.58 (<0.001)	**129.87 (<0.001)**	*NS*	**18.48 (<0.001)**	82.14 (<.0001)	**16.43 (<.0001)**	29.63 (<.0001)	**86.43 (<.0001)**	*NS*	**7.71 (0.006)**	17.41 (<.0001)

*This table lists the interactions between the species and the abiotic factors. The F statistic and associate P value are listed for each variable and species. P values are in parenthesis. Non-significant interactions are labeled with **NS**.*

In 2018, important factors that contributed to *T abactor* abundance were habitat and trap type (F=11.3, P<0.001; F=168.79 P<0.001), as well as multiple abiotic variables. For weekly average minimum temperature (°C)(F=20.3, P<0.001), weekly average rain accumulation (mm)(F=7.51, P=0.006), and weekly average solar radiation (F=6.9, P=0.009), one unit increase of each of these factors, the *T. abactor* populations increased. However, for the factors of weekly maximum temperature (°C)(F=8.79, P=0.003), and weekly average wind speed (m/s)(F=25.07, P<0.001), one unit increase in these factors were associated with a decrease in the *T. abactor* population. Factors contributing to *T. equalis* abundance were habitat and trap type (F=4.16, P=0.006, F=6.69 P=0.01) and for the factors of weekly average minimum temperature (°C) (F=11.26 P=0.0008), and weekly average solar radiation (F=36.62 P<0.001), for every one unit increase of each factor, *T. equalis* populations increased. For the factor of weekly average rain accumulation (mm)(F=8.89 P=0.003) a one unit increase was associated with a decrease in *T. equalis* abundance. Factors contributing to *T. molestus* abundance were habitat (F=6.88 P=0.001) and trap type (F=13.31 P=0.0003). Weekly minimum humidity (%RH)(F=7.57 P=0.006), and weekly average solar radiation (F=8.77 P=0.003) were associated with an increase of *T. molestus* with one unit increase in these factors. Weekly average rain accumulation (mm)(F=10.63 P=0.001) was associated with a decrease in *T. molestus* population. Important factors influencing *T. sulcifrons* abundance were habitat (F=4.31, P=0.005) and Julian date (F=7, P=0.008). Julian date was associated in an increase of *T. sulcifrons* with a one unit increase. Julian date demonstrated an effect on this species. Factors important to *T. styguis* abundance were trap type (F=20.69, P<0.001) and weekly average temperature (°C)(F=5.03, P=0.025). Weekly average temperature (°C) increase was positively associated with *T. styguis* increase. Factors contributing to *T. trimaculatus* abundance were habitat (F=4.13, P=0.007) as well as weekly average rain accumulation (mm)(F=4.55, P=0.033) and weekly average solar radiation (F=11.62, P=0.0007). When weekly solar radiation demonstrated an increase then *T. trimaculatus* would also increase. However, a one unit increase in weekly rain accumulation was associated with a decrease in *T. trimaculatus* population. Factors influencing *Hybomitra* abundance were habitat (F=3.72, P=0.011), trap type (F=4.04, P=0.045), and positively influenced by Julian date (F=9.79, P=0.002). A one unit increase in Julian date was associated with a decrease in *Hybomitra* abundance, meaning that more *Hybomitra* were caught earlier in the active fly season. Factors influencing *C. callidus* abundance were habitat (F=18.16, P<0.001) and trap type (F=38.38, P<0.001), as well as multiple abiotic factors. For example, an increase in weekly average wind speed (m/s)(F=42.87, P<0.001) was associated with an increase in *C. callidus* abundance. However, Julian date (F=48.71, P<0.001), weekly average temperature (°C)(F=9.96, P=0.002), average minimum humidity (%RH)(F=7.24, P=0.007), weekly average rain accumulation (mm)(F=32.14, P<0.001) demonstrated a decrease of the number of *C. callidus* with an increase of these factors.

Table 7 - Important abiotic as well as habitat and trap factors that influence tabanidae species in 2018

2018	Total Tabanidae	**Total Tabanus**	Total Hybomitra	**Total Chrysops**	Tabanus abactor	**T. equalis complex**	T. molestus complex	**T. sulcifrons complex**	T. styguis	**T. trimaculatus**	Chrysops callidus
Julian Day	6.12 (0.014)	**5.12 (0.024)**	9.79 (0.002)	**52.68 (<0.001)**	NS	**NS**	NS	**7 (0.008)**	NS	**NS**	48.71 (<.0001)
Maximum Temperature	NS	**4.22 (0.040)**	NS	**12.24 (0.0005)**	8.79 (0.003)	**NS**	NS	**NS**	NS	**NS**	11.6 (0.0007)
Minimum Temperature	12.35 (0.0005)	**15.47 (<0.001)**	NS	**NS**	20.3 (<.0001)	**11.26 (0.0008)**	NS	**NS**	8.62 (0.003)	**NS**	NS
Average Temperature	NS	**NS**	NS	**8.09 (0.005)**	NS	**NS**	NS	**NS**	5.03 (0.025)	**NS**	9.96 (0.002)
Maximum Humidity	NS	**NS**	NS	**26.52 (<0.001)**	NS	**NS**	NS	**NS**	NS	**NS**	27.24 (<.0001)
Minimum Humidity	4.36 (0.037)	**NS**	NS	**4.85 (0.028)**	NS	**NS**	7.57 (0.006)	**NS**	NS	**NS**	7.24 (0.007)
Average Humidity	NS	**NS**	NS	**NS**	NS	**NS**	NS	**NS**	NS	**NS**	NS
Rain	NS	**NS**	NS	**30.48 (<0.001)**	7.51 (0.006)	**8.89 (0.003)**	10.63 (0.001)	**NS**	NS	**4.55 (0.033)**	32.14 (<.001)
Wind Speed	NS	**NS**	NS	**42.26 (<0.001)**	25.07 (<.001)	**NS**	NS	**NS**	NS	**NS**	42.87 (<.001)
Solar	5.37 (0.021)	**11.86 (0.0006)**	NS	**NS**	6.9 (0.009)	**36.62 (<.001)**	8.77 (0.003)	**NS**	NS	**11.62 (0.0007)**	NS
Habitat	47.27 (<0.001)	**21.98 (<0.001)**	3.72 (0.011)	**18.81 (<0.001)**	11.3 (<.001)	**4.16 (0.006)**	6.88 (0.001)	**4.31 (0.005)**	NS	**4.13 (0.006)**	18.16 (<.001)
Trap Type	110.92 (<0.001)	**192.73 (<0.001)**	4.04 (0.045)	**41.53 (<0.001)**	168.79 (<.001)	**6.69 (0.01)**	13.31 (0.0003)	**NS**	20.69 (<.001)	**NS**	38.31 (<.001)

*This table lists the interactions between the species and the abiotic factors. The F statistic and associate P value are listed for each variable and species. P values are in parenthesis. Non-significant interactions are labeled with **NS**.*

CHAPTER V

DISCUSSION

This study has demonstrated that Tabanidae populations use Open Canopy Cedar habitat more than Closed Canopy Cedar, Oak or Pasture habitats. Regardless of genus or trap type, tabanids preferred Open Canopy Cedar habitat. Tabanid behavior is based on finding hosts, mates and refuge for themselves and their offspring. Baldacchino et al. 2017 found that land use changes and fragmentation of the landscape in several countries in Europe, led to a decline in the population of smaller tabanids but increase in population of medium and large tabanids (Baldacchino et al. 2017). This inverse relationship between the European species was also linked to the increase in intensively grazed cattle pastures (higher numbers of hosts) and incidences of diseases from pathogens transmitted by tabanids. O'Brien and Reiskind (2013) found similar results with vector mosquitoes in areas of fragmentation caused by Eastern Red Cedar encroachment in Oklahoma. Mosquitoes were found in higher numbers based on woody vegetation density, specifically more often in areas of ERC (O'Brien and Reiskind 2013). Tick populations in Oklahoma have been previously linked to ERC habitats by (Noden and Dubie 2017, and Mitcham et al. 2018). Established populations of ticks have been found in the more arid, dry biomes of Oklahoma in relation to ERC (Noden and Dubie 2017, Mitcham et al. 2018). The ERC not only provided refuge for the tick but hosts that are known to harbor both immature and mature tick stages.

Tabanids depend on visual cues more than odor cues used by mosquitoes and ticks (Allan et al. 1987). Tabanids have been shown to use polarized light to distinguish hosts from the environment. Darker, solid colored hosts are easier to pick out than lighter, spotted or striped hosts (Tashiro and Schwardt 1953, Horváth et al. 2010, Blaho et al. 2012a, Egri et al. 2012, Horváth et al. 2017). Difference in successfully distinguishing a host in the open versus in a shaded area has been confirmed by modeling the degree of polarization reflected from a range of host colors and habitats (Horváth et al. 2010, Blaho et al. 2012b, Egri et al. 2012). By searching for hosts in habitats with open areas, instead of shaded canopies, tabanids ensure their success of finding a suitable host. In areas with mature ERC encroachment, channels of open area between the cedar are still utilized by grazing cattle. These open areas can help concentrate the hosts into the open and can also serve as flight corridors for the tabanid flies. Thick vegetation structure, like the canopy of ERC, is used as a natural barrier to flying insects. The dense canopy obstructs vison and flight ability but also can serve as refuge and resting sites for tabanids which was demonstrated in Kingston et al. 1986. Host seeking behavior within the habitats was verified by capturing higher numbers of tabanids in Open Canopy Cedar than Closed Canopy Cedar habitat (Figure 4).

Visual based traps were selected over traps baited with carbon dioxide or octenol to ensure the captured flies were host searching in the selected habitats and not drawn away from different adjoining habitats. By ensuring the tabanids were actively using the area around the traps, the effect of trap placement was minimized, even for large tabanids with a longer flight range. Differences in trap use can be explained by feeding behavior of the species. The HorsePal® trap required the flies to land under the main trap body, before ascending to the collection jar. Field observation noted many tabanids landing and resting on the upper part of the trap. Based on feeding site selection studies, *Chrysops, Tabanus atratus,* and *T. sulcifrons* tend to land and feed from the head, back and upper rib of animals (Hollander and Wright 1980a). *Tabanus abactor* and *T. subsimilis* feed in sites such as the legs and belly of animals (Hollander and Wright 1980a). This behavior in feeding site selection drove the effectiveness of the traps on a per species basis.

Both trapping years presented the same snapshot of relationships between species and abiotic weather factors (Figure 15 and Figure 17). Further detailed analysis from important factors associated with certain tabanids showed more unique associations between the abiotic variables and the selected species.

Tabanus is a very diverse genera and the data collected confirms this. Differences in emergence (linked to Julian Date) and abiotic factors such as weekly temperature, humidity, and rain had different effects on species but not on the genera in total. *Tabanus* was driven by the *T. abactor* abundance. *T. abactor* were the most abundant species both years of the study (2017 n= 3,771), (2018 n = 3,470). In 2017, *T. abactor* populations were associated with the Julian Date and weekly average temperature variables with the constrained ordination statistics but were only significant for the categorical variables of Habitat and Trap type. In 2018 however, *T. abactor* populations were associated again with the Julian Date and weekly average temperature variables with the constrained ordination statistics and were significant for the categorical variables of Habitat and Trap type along with weekly minimum temperature, weekly rain accumulation, average weekly wind speed and weekly solar radiation. With the differences in the two years it is hard to say which abiotic factors have larger impact on *T. abactor* abundance. Extensive work done on *T. abactor* in the Rolling Plains as well as previous studies in the region, frame the biology and behavior quite well (Davis and Sanders 1981, Wright et al. 1984, Kingston et al. 1986, Cooksey and Wright 1987, Slosser et al. 2000). This study further demonstrated that *T. abactor* emergence is predicted for late July and higher weekly temperatures are favored by this species. *T. abactor* is a known vector of *Anaplasma marginale,* which causes bovine anaplasmosis, flourishes in the late summer when overall tabanid pressure and temperature is highest. This study along with the extensive work done in the Rolling Plains, lends to *Tabanus abactor* serving as a model for tabanid activity and behavior in relation to ERC habitats (Davis and Sanders 1981, Wright et al. 1984, Kingston et al. 1986, Cooksey and Wright 1987, Slosser et al. 2000).

Hybomitra were driven primarily by Julian Date. This is expected since previous literature states that this genus has a very abrupt emergence and decline. In Northern regions, *Hybomitra lasiophthalma* is a major pathogen vector and one of the most abundant species throughout the tabanid season. However, in Oklahoma, populations are the first to emerge and are short lived but can drive the amount of tabanid pressure in the late spring and early summer (Figure 7 and Figure 8). *Hybomitra* were modeled to have a very narrow tolerance for their optimum Julian Date for their population to peak both years. By using *Hybomitra* as a model to predict when tabanids will begin emerging, we can predict when tabanids can begin to cause production losses in cattle.

Chrysops were driven by Julian Date and the categorical variables in 2017 but was significant for all variables submitted in 2018 except for weekly average minimum temperature and weekly solar radiation. *Chrysops* was driven by *C. callidus* abundance. *C. callidus* was the second most abundant species in both years (2017 n = 818) (2018 n = 2,114). In 2017, *C. callidus* populations were associated with the Julian Date and weekly average temperature, and weekly average wind speed variables but were significant for the categorical variables of Habitat and Trap type as well. In 2018, *C. callidus* populations were associated again with the Julian Date and weekly average temperature, and weekly average wind speed variables and were significant for the categorical variables of Habitat and Trap type. Additionally, weekly average humidity and weekly rain accumulation were significant in driving the population. This *Chrysops* species peaked in June both years, and decline before temperatures begin to rise, falling in line with the model submitted for optimum Julian Date population peak. However, this species was present in low numbers for the entire tabanid trapping season both years.

With the habitat types separating in terms of tabanid abundance, we can make inferences that the habitats are not utilized equally with more tabanids being caught in the Open Canopy Cedar habitats. However, by grouping of the Open Canopy Cedar and Closed Canopy Cedar habitats with constrained ordination and disassociated from the Oak and Pasture habitats, the two ERC habitats may be used more similarly by tabanids. This study shows that pastures that have ERC, both established (Closed Canopy Cedar) and encroaching into native grassland (Open Canopy Cedar) could potentially see increased tabanid pressure.

CHAPTER VI

CONCLUSIONS AND CONSIDERATIONS

Tabanids are a major livestock pest and have the potential to impact animal production as well as health. Many of the specific behaviors and vectorial capacity of tabanid species are not known, especially in Oklahoma. The first objective of the study was to determine habitat use of tabanids in relation to Eastern Red Cedar. This study has shown tabanid use of Eastern Red Cedar habitat is significantly higher than that of other habitats. By establishing this higher use, livestock producers could manage ERC to limit the tabanid feeding pressure on their cattle as well as lower the chance for diseases such as bovine anaplasmosis. In Oklahoma, beef producers do not have many options for tabanid control. Cultural control requires producers to remove cattle from areas with high tabanid pressure. Many producers do not have the land available to rotate cattle away from problem pastures. Insecticidal control of adult tabanids is limited to use of pyrethroids. It is not considered economical and using pyrethroids can lead to resistance issues in other important beef pests such as horn flies (*Haematobia irritans*)(Foil and Hogsette 1994). Mechanical removal of ERC could prove to be a viable strategy in an integrated pest management program for tabanids associated with cattle production.

The second objective of the study was to determine the abiotic factors that drive tabanid use of ERC habitat. Understanding the reasons why tabanids use ERC habitats, especially the vector species, can aide in the understanding the epidemiology of vector-borne pathogens transmitted by tabanids. This study demonstrated that an important vector of bovine anaplasmosis, *Tabanus abactor*, could serve as a model species to monitor tabanid populations and predict outbreaks of vector-borne pathogens transmitted by tabanids. *T. abactor* demonstrated a close relationship with an increase in weekly average temperature and their peak populations in relation to day of year was consistent for both years. Species such as *T. equalis* and *T. sulcifrons* could help further expand the requirements for predicting tabanid emergence. Both objectives were completed and correlated with known tabanid behaviors and biology. Tabanids have been associated with wooded habitat, but the use of the differences in wooded habitat were unknown. By better understanding the tabanid use of wooded habitat, especially that of ERC encroachment, producers can better prevent production losses and pathogen transmission of this difficult pest group.

Limitations to this study was the lack of knowledge on biology of certian tabanid species. Many of the species were described from specimens caught in the eastern United States and were debated when described. Descriptions were often made on single or incomplete specimens from very small regions and few specimens were examined from the central and western United States. Most tabanid species were not considered to be present in Oklahoma. Tabanids are difficult to observe in their natural environment and with the added complexity of not being able to rear them in colony, then identification and biology becomes complex. Many tabanid species lack physical descriptions for the males, larvae, or even larval habitat. There were constant debates over synonyms of previous species or a subspecies, but the original specimens were never compared between scientists at the time. The tabanid species documented in this study were previously observed yet the earliest tabanid studies in Oklahoma were demonstrating vectorial capability and did not focus on their ecology. There needs to be a better understanding of the basic biology and ecology of important tabanid species before we can implement successful control straitigies.

Further studies should include exploring the physical area needed, practicality and economic viability of removing ERC to control tabanid populations and how long the effect on tabanid populations can be maintained. Other studies should consider exploration of larval habitat in pastures with ERC and conduct more detailed microhabitat measurements of abiotic factors such as temperature and relative humidity at a finer scale to determine where exactly tabanids utilize ERC for their success. The three species, *T. abactor*, *T. equalis* and *T. sulcifrons*, have all been previously found in non-aquatic habitats. By quantifying larval habitat and adult resting sites associated with ERC, we can better understand how vector populations of tabanids are maintained in relation to habitat fragmentation of open grassland.

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