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SURVEILLANCE OF SOUTH DAKOTA MOSQUITO ABUNDANCE, INFECTION RATE, AND INSECTICIDE SUSCEPTIBILITY

BY

GEOFFREY P. VINCENT

A dissertation proposal in fulfillment of the requirements for the

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2018

SURVEILLANCE OF SOUTH DAKOTA MOSQUITO ABUNDANCE, INFECTION RATE, AND INSECTICIDE SUSCEPTIBILITY

GEOFFREY P. VINCENT

This dissertation is approved as a creditable and independent investigation by a candidate for the Doctor of Philosophy in Biological Sciences degree and is acceptable for meeting the dissertation requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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This dissertation is dedicated to my son, Greyson P. Vincent, who will soon be born into this world.

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ABSTRACT

SURVEILLANCE OF SOUTH DAKOTA MOSQUITO ABUNDANCE, INFECTION RATE, AND INSECTICIDE SUSCEPTIBILITY

GEOFFREY P. VINCENT

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The aim of this dissertation was to survey and evaluate the abundance, infection rate, and insecticide susceptibility of mosquito fauna present in South Dakota. Mosquito surveillance has been conducted across South Dakota to record and track potential West Nile virus (WNV) vectors from 2004 to 2017. The nuisance mosquito *Aedes vexans* was found to be the most abundant species overall in the state and most abundant in many of the regions. The WNV vector, *Culex tarsalis*, was found to be the second most abundant mosquito and the most abundant vector mosquito across the state. However, geospatial variation did exist between both the vector and nuisance, as well as between different WNV vectors latitudinally across the state. A total of 22 mosquito species were identified, and 6 were found each year. Positive relationships were found between average *Cx. tarsalis* weekly abundance and average weekly human cases of WNV at a two to three-week lag. Weaker relationships existed between *Ae. vexans* and human cases of WNV and between the ratio of *Cx. tarsalis* to total mosquito abundance and human cases of WNV. We were not able to identify any relationship between yearly vector abundance and human cases. Logistic regression modeling using mean daily temperatures and total daily precipitation determined the best weekly collection period for collections to have the highest proportion of *Cx. tarsalis*. Infection rates of potential mosquito vectors were calculated using the minimum infection rate method. Though *Cx. tarsalis* infection rate was lower than other present vector species, due to its large relative abundance to other highly susceptible species it is still considered to be the primary method for human WNV infection. In testing susceptibility to the insecticide permethrin, we determined the diagnostic dose for multiple time periods and ranged from 27.0 µg/ml at 60 min to 38.4 µg/ml at 30 min. There was no significant difference detected in mortality rates between *Cx. tarsalis* and *Ae. vexans* for any diagnostic time and dose. For practical purposes, mosquitoes in 2017 were tested at 38 µg/ml for 30 min; expected mortality rates were 93.38% for *Cx. tarsalis* and 94.93% for *Ae. vexans.* Actual 2017 mortality rates were 92.68% for *Cx. tarsalis* and 96.12% for *Ae. vexans*, validating the usefulness of this baseline at an additional location and year.

These findings suggest that mosquito control efforts are not selectively diminishing nuisance mosquitoes that may contribute to human avoidance behaviors that may limit exposure to WNV. These base line time and diagnostic values can allow for future studies to monitor these two important South Dakota mosquito species for resistance to insecticides.

Chapter 1: Introduction

Mosquito-borne diseases have had a significant impact in human health. In the United States, mosquitoes are responsible for transmission of various pathogens such as West Nile virus (WNV), Chikungunya, St. Louis encephalitis, Western and Eastern equine encephalitis, La Crosse virus, and most recently Zika virus (ZIKV). Out of the many mosquito species populating any region, only a few typically vector diseases endemic to that region. As the arbodiseases change in a region, the relative importance of each species as vectors may also change. Species that are not vectoring regionally endemic diseases serve only as a nuisance to the people in that region. Yet, there is growing recognition that even nuisance mosquito species may be important in limiting disease transmission by discouraging human behaviors associated with transmission by vector species (Gujral et al. 2007). Therefore, it is not only important to understand population dynamics for current vectors in a given region, it is also important to understand these dynamics for the predominant species that are currently functioning only as a nuisance.

Prior to 2002, *Culex. tarsalis* Coquillett was only a vector for Saint Louis encephalitis (SLE) and Western equine encephalitis (WEE) in the Northern Great Plains (Janousek and Kramer 1998); however, the last major outbreak for either of these diseases in SD occurred in 1975 (Easton, Coker, and Ballinger 1986). The first human case of WNV was reported in SD in 2002 (Kightlinger 2017). *Culex tarsalis* has been considered the primary WNV vector throughout the northern Great Plains, including SD

(Bell et al. 2006), and is considered to be the second most abundant mosquito in the state (Gerhardt 1966; Easton 1987b). *Aedes vexans* (Meigen) has been recorded as the most abundant species in the state (Easton 1987b), and is considered a major nuisance mosquito. Recently, *Ae. vexans* has been documented as a possible vector for ZIKV (O'Donnell et al. 2017), but as local transmission of ZIKV in SD has not occurred, this mosquito is still considered a nuisance mosquito for this region. As a major nuisance mosquito in SD, its high abundance may be significant in diminishing human behaviors associated with WNV transmission risk (Gujral et al. 2007).

Few mosquito surveys have been performed in the past 50 years for South Dakota. Gerhardt (1966) published a general survey that listed 40 species found in South Dakota based upon mosquito collection surveys and previous studies. In 1984 and 1985, a survey on mosquitos from select Native American reservations across South Dakota documented 10 species of mosquito (Easton 1987b). Both surveys noted that *Ae. vexans* (Meigen) was the most abundant species collected followed by *Cx. tarsalis*.

In addition to *Cx. tarsalis,* South Dakota has three additional *Culex* species that can vector WNV, including: *Culex pipiens* L, *Culex restuans* Theobald, and *Culex salinarius* Coquillett. Though these species have not been considered as important vectors for WNV in South Dakota, *Cx. pipiens* is an important vector throughout eastern U.S.A. (Molaei et al. 2006). Mosquito populations in the neighboring states of Minnesota and Iowa recorded *Cx. pipiens* as more abundant than *Cx. tarsalis* (Dunphy, Rowley, and Bartholomay 2014; Kinsley et al. 2016). Studies in both Nebraska and Colorado found *Cx. tarsalis* to be the most abundant vector for WNV (Barker et al. 2009; Janousek and

Kramer 1999) while *Cx. pipiens* was one of the least abundant captured in both states. In general, these studies show an apparent east to west spatial change in vector abundance from *Cx. pipiens* to *Cx. tarsalis.*

Minnesota, Iowa, Nebraska, and Colorado all list their most abundant nuisance mosquito species as *Ae. vexans* (Barker et al. 2009; Barr 1958; Kinsley et al. 2016; Dunphy, Rowley, and Bartholomay 2014) After this, the number of mosquito species identified and their abundances vary between the states and even within different sites. *Aedes albopictus* (Skuse) has become a mosquito of concern for its potential to transmit the ZIKA virus. This mosquito has been found in the midwestern states of Minnesota, Iowa, Nebraska, Kansas, and Missouri, over the past ten years (Moore and Mitchell 1997). Some of these areas that *Ae. albopictus* have been found in these neighboring states are within some of the same ecoregions found in South Dakota, raising concerns that this invasive mosquito could also become established in this region (Bailey et al. 1994).

In addition to surveillance of mosquito species and abundance, monitoring infectivity of potential vector mosquitoes is necessary for a more complete picture on predicting human risk of disease transmission. Locally, data sets are often created and maintained in order monitor these risks. In South Dakota, mosquito species composition and abundance, West Nile virus (WNV) infection rate, and the number of human cases reported have been created and used since 2002 to help assess risk potential for human infection of WNV. These different data sets have been used to create a variety of methods for assessing risk.

A common method for monitoring human risk is through the minimum infection rate (MIR) which is the ratio of positive pools of mosquitoes to the total number of mosquitoes tested. Mosquito pool testing is useful in assessing the prevalence of WNV virus in the bridge vector mosquitoes (i.e. mosquitoes that feed on the bird reservoir and humans); however, the assumption in this technique is that only one mosquito in a pool size is infected which can severely underestimate the true infection rate if the virus is abundant in the population (Gu, Lampman, and Novak 2003). Additionally, this technique does not account for the varying levels of vector competency of multiple vectors within a geographical region. The vector index was designed to create a more complete picture using abundance, the species tendency to feed on mammals, infection prevalence in mosquitoes and an index for the vectors competence (Kilpatrick et al. 2005). However, in diseases such as West Nile virus (WNV), the reservoir hosts are not mammals, and so higher ratios of mammal feeding will not necessarily increase human risk.

Other effective tools include datasets that utilize meteorological and landscape ecology and have been used to developed models which have assessed human WNV risk based upon multiple factors, such as temperature and rainfall in various times of the year (Parham and Michael 2010; Wimberly et al. 2014), and land cover as an influence (Chuang, Hockett, et al. 2012).

Recently, a combination of both infection rates and environmental effects have been combined to create prediction models for human WNV risk that has been successful in South Dakota (Davis et al. 2017). This technique calculates mosquito infection growth rate (MIGR) and found that human risk was at its highest when the environmental conditions were met, and early season infection data was available (Davies in press). However, the process of collecting, sorting, transporting mosquitoes to testing facilities, infection testing, and ultimately reporting the results can take multiple weeks to complete. This lag in testing shortens the window of predictive capabilities available to the model.

Understanding the various impacts of these factors may help public health officials to predict and respond to threats of mosquito transmitted pathogens. While some success has been had in predicting the severity and timing of WNV outbreaks in South Dakota, the relationship of early season mosquito abundance to virus prevalence has not been thoroughly studied. Should early season mosquito abundance be able give insights into WNV amplification, the need for testing mosquito infection rate could decrease as the season progresses.

From 2002 until 2004, all species collected in South Dakota were tested for infection of WNV. After this time, the South Dakota Department of Health focused on the vector mosquito *Culex tarsalis* (Kightlinger 2017) and has focused its efforts on monitoring the MIR of this species. As mentioned in chapter 2, increases in *Cx. tarsalis* abundance are correlated with increases in human WNV cases; however, the overall abundance of the *Cx. tarsalis* does not relate to the overall incidence of human cases (Nielsen et al. 2008). From a public health perspective, identifying all sources of potential transmission as well as predicting the level of amplification of virus within those vectors could further the predictive ability of current models.

Multiple species that have been confirmed as potential vectors are present in the state. While *Cx. tarsalis* was the most abundant vector species in the state, *Culex pipiens* was also present in the state, predominantly on the eastern side. Both species are known to switch from feeding on birds in the spring to mammals later in the summer (Goddard et al. 2002; Tempelis 1975). The species *Culex salinarius* is known to feed on both birds and mammals and may also contribute to the amplification of the virus (Molaei et al. 2006). *Culiseta inornata* is one of the first species to appear in traps, and while it does primarily feed on mammals, it is known to take avian blood meals (Tempelis 1975; Anderson and Gallaway 1987). Having some avian host preference combined with a moderate ability to transmit WNV makes it an excellent candidate for early season amplification (Goddard et al. 2002). Both *Aedes vexans* and *Aedes dorsalis* have experimentally been shown to transmit WNV; however, both of these species prefer feeding on mammals (Kramer, Reisen, and Chiles 1998; Molaei and Andreadis 2006); however, *Ae. vexans* was the most abundant mosquito in many areas of the state, especially those with high numbers of human cases.

In this study we aimed to determine if mosquito abundance of these experimentally determined vectors present in South Dakota influence infection rates derived either from MIR or through MIGR. Additionally, we investigated precipitation and temperature effects on the abundance of two important species in the state.

Surveillance of mosquito resistance to insecticides is also needed to assist in the prevention of mosquito transmitted pathogens. Insecticides play a major role in controlling vector-borne diseases. One of the first major movements to eradicate a vector-borne disease was in the 1950's when the World Health Organization attempted to use DDT to eradicate malaria carrying mosquitoes. This, however, ended in failure as resistance to the insecticide ultimately ceased the effectiveness of DDT. Today, we still use new insecticides to help control populations of insects that can transmit human diseases; however, a focus on monitoring, measuring, and understanding the impacts of insecticide resistance has come to the forefront.

The two major classes of insecticides used today are organophosphates and pyrethroids. Organophosphates work by inactivating acetylcholinesterase which is necessary for proper nerve function in many animals, including insects. Concerns over its impact on human health have been raised and has led to a ban by the EPA for residential use since 2001. Pyrethroids also attack the nervous system by preventing the closure of voltage-gated sodium channels in the axonal membranes. This prevents nerves from repolarizing, leaving them in a permanent depolarized state causing paralysis. Pyrethroids have recently risen in usage for both residential and agricultural usage as they are considered generally safer than organophosphates (USGS).

Insect resistance to pyrethroids has been documented in many disease vectoring insects including the common house fly, black flies, Tsetse fly, fleas, and mosquitoes (Naqqash et al. 2016). Resistance has also been found in crop pests such as the diamondback moth, tobacco budworm, and cotton leafworm (Elghar et al. 2005). The use of pyrethrin, in both agricultural use and urban mosquito control, could increase resistance to pyrethrin in disease vectoring mosquitoes in certain areas of the world.

Mosquito transmission of arboviruses to humans depends on multiple factors (Kilpatrick et al. 2005; Kilpatrick and Pape 2013), including human behavior [3]. Gujral et. al. (Gujral et al. 2007) suggest that human behavioral risk factors, such as the use of personal protectants, can be influenced by the “biting pressure” created by local mosquito populations. These populations include potential vector species and non-vector nuisance mosquitoes, and the presence of many nuisance mosquitoes could increase the use of personal protectants or avoidant behavior, thereby reducing the chance of potential viral transmission by vector mosquitoes. Conversely, the lack of abundant nuisance mosquitoes may have the opposite effect. Therefore, it is important to consider nuisance as well as vector mosquitoes when developing comprehensive strategies for mosquito reduction and disease control.

Community adulticiding efforts can limit both disease and nuisance issues caused by mosquitoes, but the common use of insecticides has prompted concerns over growing resistance to insecticides in mosquito populations. Permethrin, a broad-spectrum insecticide in the pyrethroid family, is the primary adulticide used in the United States (EPA) and is used for agriculture to reduce crop and livestock pests (Catangui and Berg 2002; Campbell, Boxler, and Davis 2001), as well as in residential areas to control nuisance and vector mosquito populations. Long-term usage of this class of insecticide has been shown to cause increased resistance in mosquito populations (Naqqash et al. 2016). Because of its broad use and the documented cases of resistance, monitoring of permethrin resistance is important to mosquito control efforts (Brogdon and McAllister 1998b; Strong et al. 2008).

Most studies on insecticide resistance have focused primarily on vector mosquito species, and few have included common nuisance mosquitoes within arbovirus-endemic areas. Richards et al. (2017) evaluated the susceptibilities of a potential Zika virus (ZIKV) vector (*Aedes albopictus* (Skuse)) and 2 West Nile virus (WNV) vectors (*Culex pipiens* L. and *Culex quinquefasciatus* (Say)) to 6 different common insecticides in a study that included 26 mosquito populations from four different U.S.A. regions. They also included the tree-hole mosquito, *Aedes triseriatus* (Say), in this study, though this species is not a significant vector for Zika or WNV and is generally only a minor species in most regions. They found that the *Aedes* species tested were less likely to exhibit resistance when compared with *Culex* species, particularly for etofenprox and malathion, and found that all *Aedes* spp. populations tested were either susceptible or possibly resistant to permethrin while most *Culex* spp. populations were resistant. Given the potential role of nuisance mosquitoes in encouraging the use of personal protectants, the susceptibility of non-vector mosquitoes to insecticides should be evaluated especially in arbovirus-endemic regions where nuisance species are far more abundant than the vector species.

In the U.S.A. Northern Great Plains, *Culex tarsalis* Coquillett is the primary vector for WNV, and *Aedes vexans* (Meigen) is generally the most predominant nuisance mosquito (Barr 1958; Easton, Coker, and Ballinger 1986; Bell et al. 2006; Barker et al. 2009). Recently, *Ae. vexans* has been reported as a potential vector for ZIKV (Gendernalik et al. 2017; O'Donnell et al. 2017); however, there have been no reported cases of local transmission of ZIKV in the Northern Great Plains. In eastern South

Dakota, *Ae. vexans* populations generally swell to very large numbers in the spring and remain high during the WNV transmission season (Chuang et al. 2011). The aggressive biting of this nuisance mosquito can motivate people to use personal protection (Gujral et al. 2007). Despite its potential public health significance, we have found no studies directly comparing permethrin susceptibility for *Cx. tarsalis* and *Ae. vexans*.

Brookings County, located in east-central South Dakota, is the fifth-most populated county and contains the fourth largest city in South Dakota, though it mostly consists of farmland. Both species of interest are abundant within this county. The city of Brookings has utilized a mosquito control program involving permethrin for over 20 years, and the small cities in the county have had similar programs for over 10 years. The purpose of the present study is to compare susceptibilities of *Ae. vexans* and *Cx. tarsalis* to reagent-grade permethrin in a CDC bottle bioassay protocol involving multiple concentrations and time periods. For this comparison, we used adult mosquitoes freshly captured in Brookings County using CO_2 baited light traps. Use of wild-caught adult mosquitoes in this type of bioassay is considered acceptable from both the CDC bottle bioassay protocol and the WHO test procedures for insecticide resistance, and wild-caught adult mosquitoes have been used in previous studies where mosquito aquatic stages were not consistently available (CDC 2013; Rakotoson et al. 2017; WHO 2016; Marcombe et al. 2017). Because both species prefer to lay their eggs throughout natural habits, harvesting *Cx. tarsalis* and *Ae. vexans* eggs and then growing adults for the assay was not practical, and results can be inconsistent when rearing mosquitoes from eggs in a lab (Strong et al. 2008). In our area, the consistent collection of *Cx. tarsalis* larvae in large enough numbers to adequately compare its susceptibility to *Ae. vexans* was also not practical. To minimize concerns about potential high variability for data collected from wild-caught adults, the susceptibility comparisons involved a large number of mosquitoes evaluated in multiple assays conducted throughout the mosquito season. The use of field collected mosquitoes for this comparison also allowed for testing both species together in the same bottles and testing them in the various natural physiological conditions representative of the wild populations' age distribution at any given time (WHO 2016).

The data from this study were used to calculate a base-line diagnostic dose and time for permethrin susceptibility for both species that can be used in future studies for evaluating changes in permethrin resistance from this region. We have found no studies identifying values for diagnostic dose and time on permethrin susceptibility for *Cx. tarsalis* and *Ae. vexans*, and none have been reported to the Arthropod Pesticide Resistance Database (Database] 2014). According to the Centers for Disease Control and Prevention (CDC) diagnostic dose and times should be determined for each type of insecticide used, and for each species of concern within a region (CDC 2013), and studies often emphasize the lack of reported diagnostic dose and times reported for *Culex* species, and more so with *Cx. tarsalis* (Richards et al. 2017). CDC guidelines recommend creating the diagnostic dose and time from susceptible populations; however, permethrin is used throughout the state for both agricultural and mosquito control purposes. The Center for Disease Control guidelines also recommend establishing these baselines from mosquito populations in areas where treatments are applied as a reference point for future comparisons.

Chapter 2: Population Dynamics of Mosquitoes in South Dakota: A 14 Year Study

Abstract

Mosquito surveillance has been conducted across South Dakota (SD) to record and track potential West Nile virus (WNV) vectors since 2004. During this time, communities from 29 counties collected nearly 5.5 million mosquitoes providing data from over 60,000 unique trapping nights. The nuisance mosquito, *Aedes vexans*, was the most abundant species in the state (39.9%), and most abundant in a majority of the regions. The WNV vector, *Culex tarsalis*, was the second most abundant species (20.5%), and 26 times more abundant than the other *Culex* species that also transmit WNV. However, geospatial variation did exist between WNV vectors species, as well as relative abundance of vector and nuisance mosquitoes. The majority of *Ae. vexans* samples were caught between weeks 27 and 28 in the eastern portion of SD, whereas the majority of this species were captured by week 25 in the southwestern portion of the state. No relationship was found between yearly human cases of WNV and yearly abundance or relative abundance of *Cx. tarsalis* and *Ae. vexans.* Positive relationships were found between average *Cx. tarsalis* weekly abundance and average weekly human cases of WNV at a two to three week lag. Weaker relationships existed between *Ae. vexans* and human cases of WNV and between the ratio of *Cx. tarsalis* to total mosquito abundance and human cases of WNV. Logistic regression modeling using mean daily temperatures and total daily precipitation determined the best weekly collection period for collections to have the highest proportion of *Cx. tarsalis*. This study addressed the

need for an updated summary of mosquito species present in South Dakota and impacts of vector and nuisance mosquito on human transmission of WNV.

Introduction

Mosquito-borne diseases have had a significant impact in human health. In the United States, mosquitoes are responsible for transmission of various pathogens such as West Nile virus (WNV), Chikungunya, St. Louis encephalitis (SLE), Western and Eastern equine encephalitis, La Crosse virus, and most recently Zika virus (ZIKV). Out of the many mosquito species populating in any region, only a few typically vector diseases endemic to that region. As the arbodiseases change in a region, the relative importance of each species as vectors may also change. Species that are not vectoring regionally endemic diseases, including both species that are not competent for disease transmission and species that are competent yet not infected, serve only as a nuisance to the people in that region. Yet, there are some studies that recognize that human behaviors may be responsible for varying levels of risk to mosquito transmitted pathogens (Oidtman et al. 2016; Zielinski-Gutierrez and Hayden 2006). There is also recognition that even nuisance mosquito species may be important in limiting disease transmission by discouraging human behaviors associated with transmission by vector species (Gujral et al. 2007). Therefore, it is not only important to understand population dynamics for current vectors in a given region, it is also important to understand these dynamics for the predominant species that are currently functioning only as a nuisance.

Prior to 2002, *Culex tarsalis* Coquillett was only a vector for SLE and Western equine encephalitis (WEE) in the Northern Great Plains (NGP) (Janousek and Kramer

1998); however, the last major outbreak for either of these diseases in South Dakota (SD) occurred in 1975 (Easton, Coker, and Ballinger 1986). The first human case of WNV was reported in SD in 2002, and since then, it has become widespread throughout the (NGP) causing the highest incidence of WNV neuroinvasive disease of any region in the nation (Kightlinger 2017). States within the NGP generally include: North and South Dakota, Montana, Wyoming and Nebraska. Species in the genus *Culex* are the most important vectors for WNV (Turell et al. 2005), and *Cx. tarsalis* is considered to be the primary WNV vector in throughout the NGP (Bell et al. 2006), and the second most abundant mosquito in the SD (Easton 1987a; Gerhardt 1966). *Aedes vexans* (Meigen) has been recorded as the most abundant species in the state (Easton 1987a), and is considered a major nuisance mosquito in the region. Recently, *Ae. vexans* has been documented as a possible vector for ZIKV (O'Donnell et al. 2017), but as local transmission of ZIKV in SD has not occurred, this mosquito is still considered a nuisance mosquito for this region. As a major nuisance mosquito in SD, its high abundance may be significant in diminishing human behaviors associated with WNV transmission risk (Gujral et al. 2007).

Few mosquito surveys have been performed in SD. Gerhardt (1966) published a review and qualitative survey that listed 43 mosquito species found in SD based upon larval and adult mosquito collections from 38 different communities (in 37 counties) and also based on previous reports. Among the communities surveyed, *Ae. vexans*, *Cx. tarsalis*, *Aedes dorsalis* (Meigen), *Aedes nigromaculis* (Ludlow), *Aedes triseriatus* (Say), and *Aedes spencerii* (Theobald) were listed as the most prevalent species. In addition to

Cx. tarsalis, Gerhardt (1966) lists five additional *Culex* species from SD, including: *Cx. pipiens* L, *Cx. restuans* Theobald, *Cx. salinarius* Coquillett, *Cx. territans* (Walker), and *Cx. erraticus* (Dyar and Knab). More recent surveys from 15 Native American tribal sites in SD conducted in 1983 (Easton et al, 1986) and 1984-1985 documented 19 species of mosquito. Most of the mosquitoes collected in these two studies were trapped in New Jersey traps, but encephalitis virus surveillance traps producing carbon dioxide and CDC miniature light traps baited with dry ice were also used at a few sites. Among all but the western-most SD site, *Ae. vexans* was the most abundant species collected, comprising 77.9% of the trapped mosquitoes. Overall, 13.7% of the total mosquitoes were *Cx. tarsalis*, but this ranged from 6.9% from an eastern SD site to 35.0% from the western-most site. Among the remaining 8.4% of mosquitoes, *Ae. dorsalis*, *Coquillettidia perturbans* (Walker, 1856) and *Ae. nigromaculis* were the only species above 1%. *Culex salinarius* was the only other *Culex* species found in these two surveys, and it only comprised 0.5% of the population in SD.

A two-year study involving 11 Nebraska communities identified more than 1.8 million mosquitoes involving 27 species (Janousek et al, 1999). They found 6 species of *Culex*; 85.6% of the genera were *Cx. tarsalis*, 6.7% were *Cx. salinarius*, 6.6% *Cx. restuans*, 0.9% *Cx. erraticus*, and only 0.2% *Cx. pipiens*. Only one specimen of *Cx. territans* was found. Among all mosquitoes identified, *Cx. tarsalis* provided 11.0% while *Ae. vexans* provided 74.1%; of the remaining species, 5.4% were *Aedes trivittatus* (Coquillett), 1.5% *Aedes melanimon* (Dyar), 1.0% *Ae. Triseriatus*, and the remaining 7.0% were spread out among the other species at level less than 1% per species. Among sites located along the eastern edge of Nebraska, the percentage of *Ae. vexans* was relatively high, ranging from 78.2% to 91.0%, but decreased to 56.4% on sites located in the western region of the state. An opposite east-to-west population trend occurred with *Cx*. *tarsalis*.

North Dakota studies conducted from 2002 to 2004 found the most abundant mosquito species to be *Ae. vexans, Ae. dorsalis,* and *Cx. tarsalis* (Bell et al. 2006)*. Aedes vexans* was not the most dominant species in all years in this study as *Ae. dorsalis* was the most dominant species in 2004. The WNV vector, *Cx. tarsalis,* decreased in both over all abundance and relative abundance in each consecutive year. The authors of this study indicated that the reduction in vector populations was one of the causes for reduced human cases of WNV in 2004 (Bell, Mickelson, and Vaughan 2005)

In neighboring states east of SD, the prevalence of *Cx. pipiens* increases significantly as *Cx. tarsalis* decreased. In an oviposition study conducted in central Iowa, 25.4% of the *Culex* eggs were *Cx. pipiens*, 55.0% were *Cx. restuans*, 12.2% *Cx. salinarius*, and only 0.14% were *Cx*. *tarsalis* (Lee and Rowley 2000). While they demonstrated that ovitraps underestimate the presence of *Cx*. *tarsalis*, New Jersey traps from that area showed that its prevalence was less than 4.6%. DeGroote et al. (2008) documented the changing relative abundance of *Cx. tarsalis* and *Cx. pipiens* throughout Iowa where the *Cx. pipiens*/*Cx. restuans* complex was more common WNV vector statewide, but that *Cx. tarsalis* increased significantly in the western portion. Statewide Iowa data collected over 35 years showed that 71% of the collected mosquitoes were *Ae. vexans*, 16% were the *Cx. pipiens*/*Cx. restuans* complex, 6% were *Ae. trivittatus*, 3% *Cx. tarsalis*, and 2% were *Anopheles punctipennis* Say; all other species accounted for less than 1%. The only recent mosquito survey Minnesota involved a one-year study from the northern region where less than 2.5% of the mosquitoes were members of the genus *Culex*, and *Cq. perturbans* was the most abundant species 62.1% (Kinsley et al. 2016). Older data suggest that mosquito populations in the southern regions are more similar to those in Iowa (Barr, 1958).

A previous study in Wyoming found the most abundant mosquito species to be *Cx. tarsalis,* followed by *Ae. vexans* and *Ae. dorsalis.* This study also determined that peak *Cx. tarsalis* abundance occurred between the second week of June and the first week of July (Doherty, M.K. 2007 Thesis). A similar study conducted in Montana during 2005 and 2006 had the same three species listed as the most abundant species but recorded that *Ae. dorsalis* was the most abundant followed by *Ae. vexans* and *Cx. tarsalis* which were relative close in overall abundance (Friesen and Johnson 2014). Seasonal host-seeking for *Ae. vexans* in Montana occurred between the middle of June through the middle of July peaking in captures during the last week of June. The abundance of *Ae. vexans* nearly disappear during the last week of July but reappear between mid to late August. *Culex tarsalis* was predominantly captured between the last week of June and the last week of July with a peak at July 15th. *Culex pipiens* was only recorded in the Wyoming study where it consisted of less than 1% of all species captured.

Aedes albopictus (Skuse) has become a mosquito of concern for its potential to transmit the ZIKA virus. This mosquito has been found in the midwestern states of Minnesota, Iowa, Nebraska, Kansas, and Missouri, over the past ten years (Moore and

Mitchell 1997). Some of these areas that *Ae. albopictus* have been found are within some of the same ecoregions found in SD, raising concerns that this invasive mosquito could also become established in this region (Bailey et al. 1994).

Our study aims to provide a current inventory of the mosquito species trapped in carbon dioxide-baited light traps located throughout SD between 2004 and 2017. Sites included in the study were in communities participating in a surveillance of mosquitoes for WNV, and therefore, the distribution, intensity and longevity of trapping varied considerably based upon the perceived risk of WNV and on community resources that were available. From 2002 to 2016, 71.4% of SD WNV human cases came from residents of counties east of the Missouri River (Kightlinger, 2017). For this reason, the vast majority of mosquitoes collected in communities east of the Missouri River. Population dynamics are also reported from these traps for the major WNV vector and nuisance mosquito species.

Methods

Mosquito collections and identification:

Mosquito surveillance was conducted in almost half of the counties across SD using $C0_2$-baited CDC miniature light traps with air-activated gates (John W. Hock Model 1012-CO_2, set to deliver 0.5 L CO_2/min) from 2004 to 2017. Traps were suspended approximately1.5 meters from the ground and located in areas with moderate-heavy tree cover and vegetation. Tapping occurred overnight and were activated using light sensors.

Over the 14 years of this study, communities from 30 of the 66 counties contributed mosquitoes for varying numbers of years (Table 1). Trap locations were most commonly found in populated communities. Over half of the trap-nights occurred in the Sioux Falls area (Minnehaha and Lincoln counties), and almost 30% occurred in Brookings, Brown and Coddington counties. Collections in the southwest portion of the state occurred near the Black Hills area included Fall River, Custer, Pennington, and Meade counties. Because of their proximity to a unique ecoregion of the state, these counties were combined during regional analysis and labeled as southwest SD.

Thoughout the study, 5,486,692 mosquitoes were captured creating 60,317 unique samples from trapping nights. The number of data points collected in counties east of the Missouri River accounted for 96.4% of the data points, and these eastern sites trapped 97.7% of all mosquitoes included in this study. Trapping began as early as April and continued as late as the end of October, but most collections were made from June 1 through August 15. All sites identified *Cx. tarsalis*, but the specificity of mosquito identification for the other species varied depending on trap location and year. As designated in Table 1, some sites (Brookings, Minnehaha/Lincoln, Hughes, Butte/Harding) identified mosquitoes to the species level based upon morphological characteristics (Darsie 2005). A category called "non-*Culex tarsalis*" was used for mosquitoes that were not identified to species. In some areas, such as Brown County and counties located in the southwestern portion of SD, the non-*Culex tarsalis* category became inflated with highly abundant mosquitoes, especially *Ae. vexans*. Differentiation of *Cx. restuans* and *Cx. pipiens* can prove difficult morphologically as features used are not reliable (Harrington and Poulson 2008). Distinguishing between *Cx. restuans* and *pippiens* was based upon the presence or absence of white dots located on the scutum. Because of issues in reliably identifing these two species, we lumped the totals of these two species together for any statistical studies. When evaluating the regional population dynamics for *Ae. vexans* and *Cx. tarsalis*, both the weekly *Ae. vexans* and non-*Cx. tarsalis* were calculated because some regions did not identify most of their non-*Cx. tarsalis* mosquitoes to the species level.

Human cases:

Human case counts were obtained from the South Dakota Department of Health (SDDOH) on a weekly, statewide basis for all weeks in 2004-2017. Cases were assigned to the week they began showing symptoms. These data were collected as part of a pre-existing surveillance system with a formal data-sharing agreement between the SDDOH and South Dakota State University. These data were considered exempt from IRB because the human case data are collected as a normal part of the SDDOH's surveillance process and do not contain any personally identifying information.

Statistical model of human cases:

Weekly number of human cases in the state were related to various measures of mosquito populations collected some time before the week in question. For every week in 2004-2017 we defined three covariates: average *Cx. tarsalis* collected per trap per week, average *Ae. vexans* collected, and the ratio of *Cx. tarsalis* to total mosquitoes collected per week. These were related individually to the number of human cases in the state in every week, when lagged either 0, 1, 2, or 3 weeks. The Spearman rank correlation coefficient (Spearman's ρ) was calculated to determine the strength of any correlation.

Table 1: Counties participating in mosquito Surveillance

County	Region	# of Years Collected	# mosquitoes	# of data points
Beadle	East Central (ER)	12	170,965	591
Brookings*	East Central (ER)	14	1,207,741	7,060
Brown	Northeastern (ER)	14	2,031,810	8,641
Butte/Harding*	Northwestern (WR)	3	14,255	554
Clay	Southeastern (ER)	1	1,073	67
Coddington	East Central (ER)	13	111,677	2,020
Custer	Southwestern (WR)	4	4,485	104
Davison	Southeastern (ER)	12	64,773	316
Dewey	North Central (WR)	2	6,175	72
Edmunds	North Central (ER)	4	15,943	155
Fall River	Southwestern (WR)	4	3,725	297
Grant	Northeastern (ER)	4	21,607	85
Hand	Central (ER)	4	9,692	68
Hughes*	Central (ER)	14	159,732	959
Lake	East Central (ER)	11	48,088	968
Lincoln/Minnehaha*	Southeastern (ER)	14	1,470,899	36,464
Marshal	Northeastern (ER)	3	5,652	52
Meade	West Central (WR)	10	24,969	764
Moody	East Central (ER)	6	28,731	519
Pennington	West Central (WR)	9	68,001	352
Perkins	Northwestern (WR)	2	419	20
Sanborn	East Central (ER)	1	53	7
Spink	Northeastern (ER)	2	177	25
Turner	Southeastern (ER)	1	28	7
Union	Southeastern (ER)	3	12,637	95
Yankton	Southeastern (ER)	2	1,119	43
Ziebach	Northwestern (WR)	1	2,266	12

*Denotes region that identified most mosquitoes to species. (ER) Denotes counties located east of Missouri River (WR) Denotes counties located West of Missouri River

Results

State-wide mosquito survey:

Twenty-two species were identified during the 14-year study period and eight species were present in every year surveyed (Table 2). Using data from counties identifying mosquitoes to species level for all years, *Ae. vexans* and *Cx. tarsalis* were the two most abundant species, accounting for 67.8% and 21.0% respectively. *Aedes vexans* populations ranged from 31.67% to 72.10% in areas in which personnel identified mosquitoes to the species level every year, whereas, *Cx. tarsalis* ranged from 15.95% to 62.27%. Brown county reported *Ae. vexans* as only 2.78 % of their total catch; however, this county only sporadically identified non-Culex tarsalis mosquitoes sporadically, and yet *Ae. vexans* almost certainly contributed to a bulk of the species reported in the non-identified category. The six other species present in each year include: *Aedes trivittatus, Ae. dorsalis, Culiseta inornata* (Williston)*, Culex restuans, Cx. salinarius,* and *Anopheles punctipennis*. These six species only accounted for 5.9% of the total mosquitoes in counties where all mosquitoes were identified to species. With the exception of *Cx. salinarius* in Beadle county, these mosquitoes were recorded in all the regions, but not all sites within some counties. *Culex. restuans* and *Cx. salinarius* are minor vectors for WNV, accounting for less than 1% of all mosquitoes collected. Though these species are far fewer in abundance that *Cx. tarsalis,* Culex*. restuans* was more abundant and most frequently recorded in Brookings county while *Cx. salinarius* was most abundant in Brookings and Hughes counties (Table 2).

Table 2: Mosquitoes present annually within Representative South Dakota Areas from 2004 to 2017.

	Lincoln/ Minnehaha	Brookings	Brown	Beadle	Hughes	Fall River/Custer/ Pennington/Meade
Species	% total	% total	% total	% total	% total	% total
Other	1.49%	6.19%	80.56%*	4.24%	0.63%	43.44%
Ae. vexans	72.10%	67.23%	2.78%*	70.01%	31.67%	9.43%
Cx. tarsalis	18.29%	18.84%	15.95%	22.40%	62.27%	43.42%
Ae. trivittatus	3.05%	0.89%	0.06%*	0.69%	0.62%	2.13%
Ae. dorsalis	1.93%	0.85%	0.08%*	1.10%	2.45%	0.58%
Cs. Inornata	1.40%	0.55%	0.07%*	0.45%	1.16%	0.37%
Cx. restuans	0.25%	1.23%	0.06%*	0.88%	0.60%	0.23%
Cx. salinarius	0.04%	0.29%	0.12%*	0.00%	0.31%	0.02%
An. punctipennis	0.17%	0.02%	0.01%*	0.14%	0.04%	0.04%

*Did not identify non-*Cx. tarsalis* mosquitoes except during 1 year

Other species were not collected every year and tended to be present in only certain areas of the state, or in certain habitat under specific conditions, and could occasionally become a dominant species within a specific area (Table 3). These species included: *Cq. perturbans*, *Ae. triseriatus, Aedes fitchii, Psoraphora cyanescens, Ae. sollicitans, Anopheles walkeri, Uranotaenia sapphrina, Culex territans, Anopheles quadrimaculatus, Aedes japonicas, Ae. canadensis, Ae. cinereus, and Cx. erraticus.* Though these minor species only accounted for 1.25% of the mosquitoes identified statewide and during all years, occasionally their abundance played an important role in certain locations. For example, while Cx. pipiens does not play a major role in vectoring WMV throughout South Dakota, it could become a significant vector in Lincoln and Minnehaha counties during some years (Table 3 and figure 3). Certain of the other species could serve as vectors for other diseases not currently endemic to this the

Northern Plains, currently, they would only function as nuisance mosquitoes in certain locations. For example, *Cq. perturbans* served as an important nuisance in some sites within Brookings County (Table 3). *Aedes japonicus* has been sporadically detected within South Dakota. So far, nine specimens have been captured, four in 2009 and five in 2016, all in Lincoln and Minnehaha counties located on the eastern edge of the state.

Table 3: Mosquitoes present in South Dakota, but not every year.

	Lincoln/ Minnehaha	Brookings	Brown*	Beadle	Hughes	Southwest SD
Species	% total	% total	% total	% total	% total	% total
Cx. pipiens	0.63%	0.15%	0.02%	0.00%	0.06%	0.00%
Cq. perturbans	0.00%	3.67%	0.04%	0.00%	0.06%	0.00%
Ae. triseriatus	0.23%	0.05%	0.01%	0.01%	0.04%	0.07%
Ae. fitchii	0.13%	0.01%	0.01%	0.00%	0.01%	0.24%
Ps. cyanescens	0.00%	0.00%	0.09%	0.00%	0.00%	0.00%
Ae. sollicitans	0.00%	0.00%	0.04%	0.00%	0.00%	0.01%
An. walkeri	0.01%	0.01%	0.01%	0.06%	0.07%	0.00%
U. sapphrina	0.00%	0.01%	0.00%	0.00%	0.00%	0.00%
Cx. territans	0.01%	0.02%	0.00%	0.00%	0.00%	0.00%
An. quadrimaculatus	0.01%	0.00%	0.00%	0.00%	0.01%	0.00%
Ae. japonicus	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Ae. canadensis	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Ae. cinereus	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Cx. erraticus	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%

*Did not identify non-*Cx. tarsalis* mosquitoes except during 1 year

Year-to-year statewide variations:

On a statewide basis, *Ae. vexans* was the most abundant species during every year except 2007 and 2013, when *Cx. tarsalis* became slightly more abundant (Figure 1). The mean total number of mosquitoes per trap night for both species varied considerably on a yearly basis. During 2006 and 2012, the total population was particularly low, and particularly high during 2010. The ratios of *Ae. vexans* to *Cx. tarsalis* were high during most of the other years.

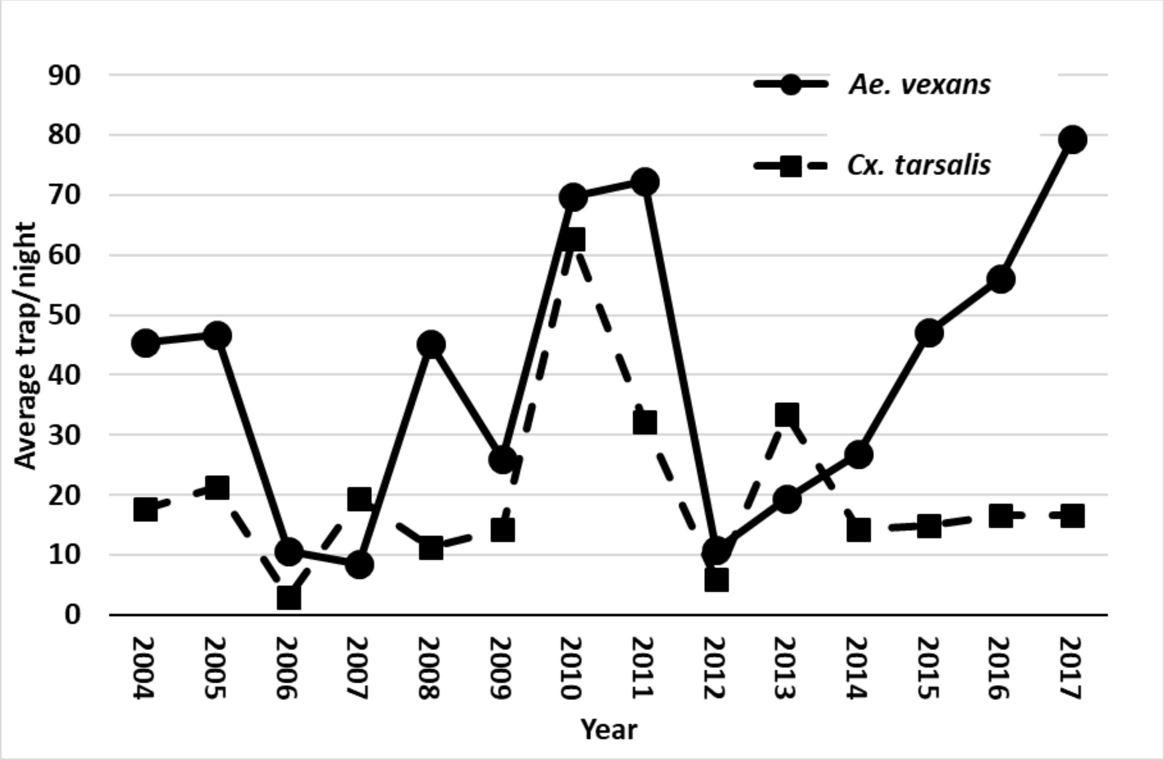

Figure 1: Yearly, season-long mean mosquito numbers trap per night for the two most abundant mosquitoes in South Dakota.

The yearly proportion for the three most common minor species each reached or exceeded 5% in at least one of the 14 years. For *Ae. trivittatus*, populations spikes occurred in 2004 and 2006. For *Cs. inornata* and *Cq. perturbans,* the spikes occurred in 2012 and 2017 respectively. These spikes were not only created through an increase in the abundance of these three species, but also affected by the low abundance of the two major species. This enable these species to become more important as nuisance mosquitoes during this years. The proportions for these less common species never exceeded 2.5%. The statewide proportion of *Cx. pipiens* remainded extremely low throughout the study, but did exceeded 1% during 2016 and 2017. Proportions for *Cx. restuans* exceeded 1% during 2006, 2007, 2009 and 2013; during this later year, it exceeded 2% of the mosquitoes collected. In regions where these 2 potential WNV vectors are more common, they could contribute significantly to viral amplification during years when *Cx. tarsalis* populations are low. The proportion of *Aedes* dorsalis exceeded 1% during three years: 2006, 2010 and 2014.

Seasonal population dynamics of *Cx. tarsalis* and *Ae. vexans*:

Within each mosquito season (Figure 2), the statewide mean 14-year population values for *Aedes vexans* tended to increase rapidly at around week 20, peaking at 180 mosquitoes per trap night at week 27. In contrast, *Cx. tarsalis* populations tended to not begin increasing until week 22 and reached its peak of 50 mosquito/night at week 28. The population decrease for *Cx. tarsalis* was fairly linear from week 28 to the population disappearance at week 37 (Figure 2). The Ae*. vexans* population decreased rapidly until week 33, and then increased again to week 35 until decreasing again down to zero at week 42. It should be noted that before the sharp increase of human incidence of week 29 (Figure 5), *Ae. vexans* abundance drops by a third (Figure 2). Collections for half the annual *Cx. tarsalis* samples occurred between weeks 28 and 30 in all locations whereas half the annual state wide collections for *Ae. vexans* occurs between weeks 27 and 28.

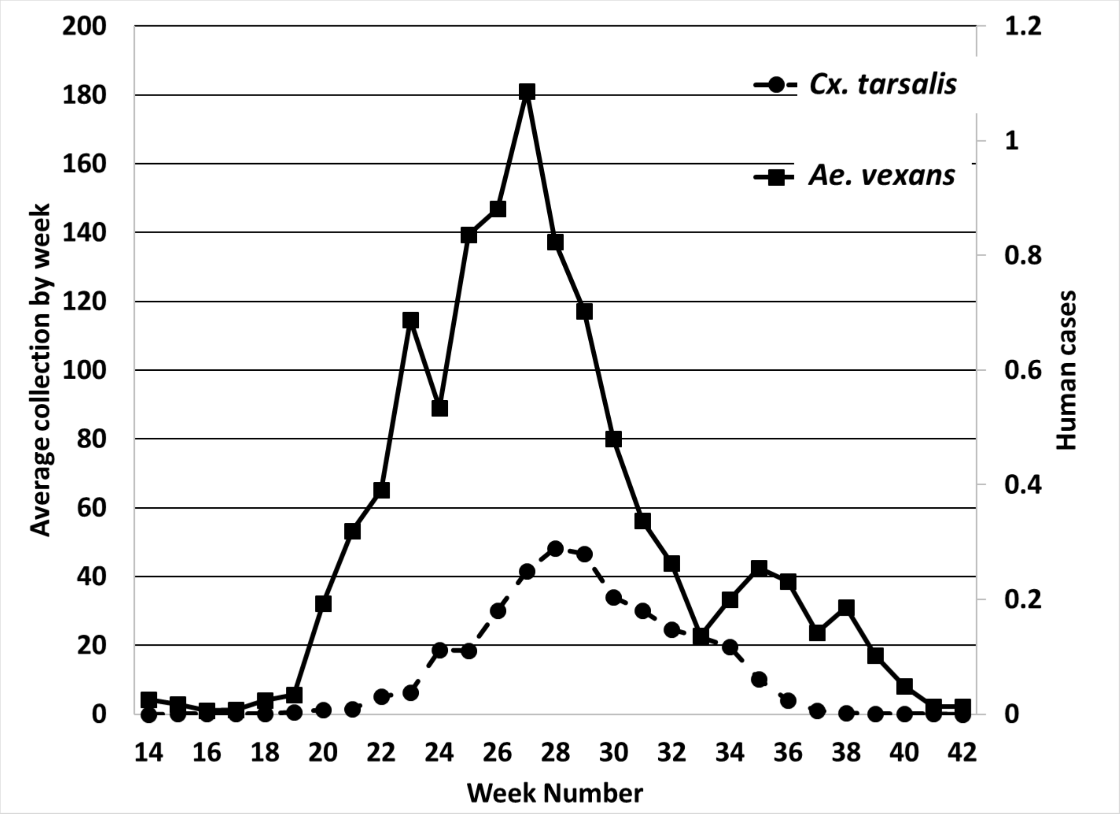

Figure 2: Statewide 14-year mean for the number of *Ae. vexans* and *Cx. tarsalis* females captured per trap per week.

Regional variations in *Cx. tarsalis* and *Ae. vexans* populations:

For each of the six SD regions shown in figure 3, the weekly population dynamics for *Cx. tarsalis* and *Ae. vexans* are expressed as then mean of all 14 years. The number of trap sites for each region is reported in Table 1. In this figure, the non-*Cx. tarsalis* values were also added to the graphs because in some regions (e.g. Brown County), the number of *Ae. vexans* were artificial low because most of the mosquitoes were not identified to the species level. Therefore, in regions such as Minnehaha/Lincoln, Brookings, Beadle, and Hughes counties, non-*Cx. tarsalis* and *Ae. vexans* are very similar; whereas in Brown county and Fall River/Custer/Pennington/ Mead counties, *Ae. vexans* are artificially below their actual values, and the non-*Cx. tarsalis* values would more closely represent the *Ae. vexans* population for that specific region.

With the exception of Lincoln/Minnehaha counties, all of the eastern SD regions (i.e. Brookings, Beadle and Brown counties) showed relatively high numbers of *Ae. vexans* (or non-*Cx. tarsalis* species) compared to the western regions (i.e. Hughes County and Fall River/Custer/Pennington/Meade counties). These eastern counties also showed evidence of a later resurgence of *Ae. vexans* populations after week 33. *Culex tarsalis* populations were quite low in Lincoln/Minnehaha counties, and in Fall River/Custer/Pennington/Meade counties. They were higher in the other counties, and highest in Hughes county, located in the center of the state. Therefore, overall average abundance for *Cx. tarsalis* is lower than the non-*Cx. tarsalis* and *Ae. vexans* population for all regions except Hughes county.

Over 10% of the *Culex tarsalis* captured for the season occurred by week 26 in Lincoln/Minnehaha counties. This occurred slightly earlier for Brookings county at week 23, for Hughes county week 23, and Beadle in week 22 . *Culex tarsalis* reached this mark in week 27 in Brown county annd in southwest South Dakota week 26. In weeks 28 to 30 we consistently saw over half the annual average mosquito collections occur. Over 95% of the annual collections occurred between weeks 34 and 36 in all regions studied.

Over 10% of the *Aedes vexans* (or non-*Cx. tarsalis* mosquitoes) annual collections were captured by 23 to 25 for all regions studied, and 50% were consistently by weeks 27 to 28, with the exception of the southwest region in which this occurred in week 25. Collections of 95% of the average annual collections varied between areas studied. In Lincoln, Minnehaha, and Brookings county this occurred the latests between weeks 37 and 39. In the Brown, Hughes, and Beadle couties this had occurred by weeks 34 to 35. In the southwest region of South Dakota 95% of the average annual collections occurred in week 31. Abundance of *Ae. vexans* (or non-*Cx. tarsalis* mosquitoes) in the central and western portions of the state reached zero at or before week 40, while in the eastern portion of the state the presence of these mosquitoes could remain until as late as week 42.

From the peak of both *Ae. vexans* and *Cx. tarsalis*, both species decline in average abundance until around week 40; however, the rate at which they decline is slower for *Cx. tarsalis.* This caused periods in which the average *Cx. tarsalis* abundance to meet or exceed *Ae. vexans* average abundance for certain weeks. This occures betweeen weeks 32 and 34 in Lincoln, Minnehaha, and Brookings counties, week 34 in Brown county, weeks 24, 25, 30 and 31 in Beadle county, and nearly all weeks for Hughes and southwest South Dakota.

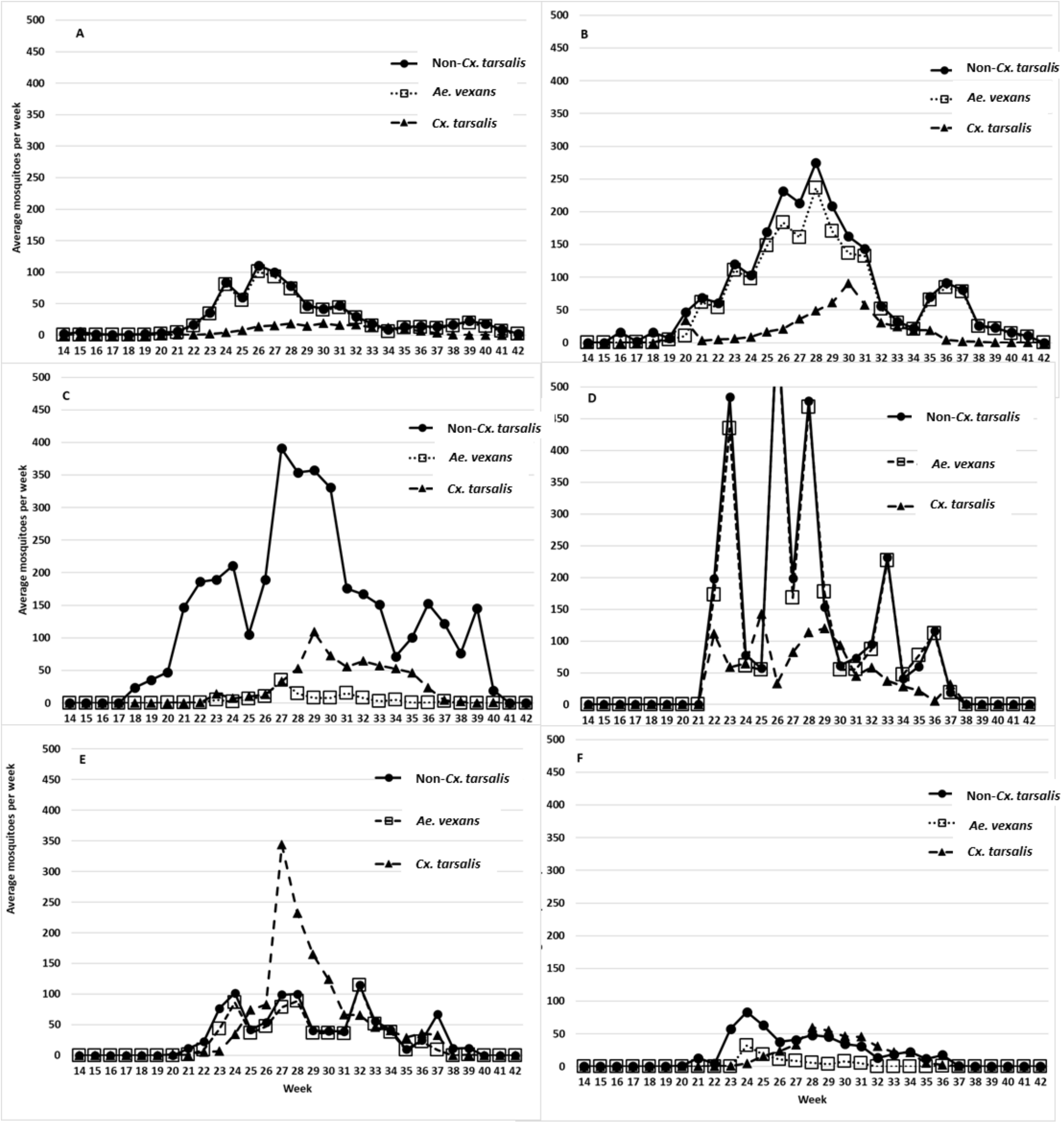

Figure 3: Average mosquito collections per trap over all years. A) Lincoln/Minnehaha counties B) Brookings county C) Brown county D) Beadle County E) Hughes County F) Fall River/Custer/Pennington/Meade counties.

Relationship between mosquito abundance and human cases:

We ran Spearman's rank correlations between human cases of WNV and all four measures of mosquito abundance (*Cx. tarsalis* and *Ae. vexans* trap per night totals and relative abundance) by year, and in no case was a relationship found ($p > 0.05$) The highest abundance of *Cx. tarsalis* occurred in both 2010 and 2011, yet the number of human cases for WNV were among the lowest (Fig 4). Conversely, years with some of the highest numbers of WNV cases were years where the abundance of *Cx. tarsalis* were particularly low such as in 2006, 2007, and 2012. There were also years in which both human cases of WNV and abundance of *Cx. tarsalis* were both low (2008 and 2009), and where human WNV cases and *Cx. tarsalis* abundance were high (2013) (Figure 4A). When comparing the proportion of *Cx. tarsalis* and *Ae. vexans* relative to the total number of mosquitoes collected, we did see years of high human WNV incidence in which the proportion of *Cx. tarsalis* to *Ae. vexans* was higher such as in 2007 and 2013 (Figure 4B). However, this trend was not seen in all years such as 2005 and 2012 which has large numbers of human cases and proportionally more *Ae. vexans.*

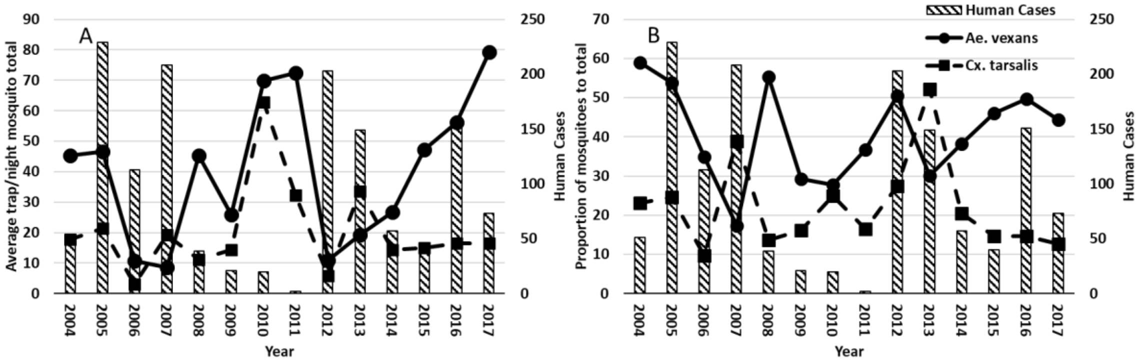

Figure 4: (A) Average abundance per trapping night of *Ae. vexans* and *Cx. tarsalis* using statewide data from 2004 to 2017 (Line left axis) along with human cases of WNV (Bar right axis). (B) Proportion of *Ae. vexans* and *Cx. tarsalis* to total mosquitoes captured.

Analysis of weekly mosquito averages and mean human cases of WNV show some positive relationships at a three week lag. Human WNV cases began increasing at week 24, and continued to increase long after the the *Cx. tarsalis* population peaked (Figure 5). Human cases continue about three weeks after the disappearance of the *Cx. tarsalis* population. The population decrease for *Cx. tarsalis* was fairly linear from week 28 to the population disappearance at week 37. The mean population for *Ae. vexans* decreased rapidly until week 33, and then increased again to week 35 until decreasing again down to zero at week 42. It should be noted that before the sharp increase of human incidence of week 29, *Ae. vexans* abundance drops by a third.

The results of a Spearman rank correlation coefficient analysis to evaluate potential relationships between the abundance of *Cx. tarsalis* and *Ae. vexans* versus weekly human WNV cases statewide are shown in Table 3 at four different lags periods. In tests of significance, all coefficients differed from 0 significantly (p < 0.0001 in all cases). Increases in both the average number of *Cx. tarsalis* and its ratio to the total population have a positive relationship with human cases of WNV, most noteably at a three week lag.

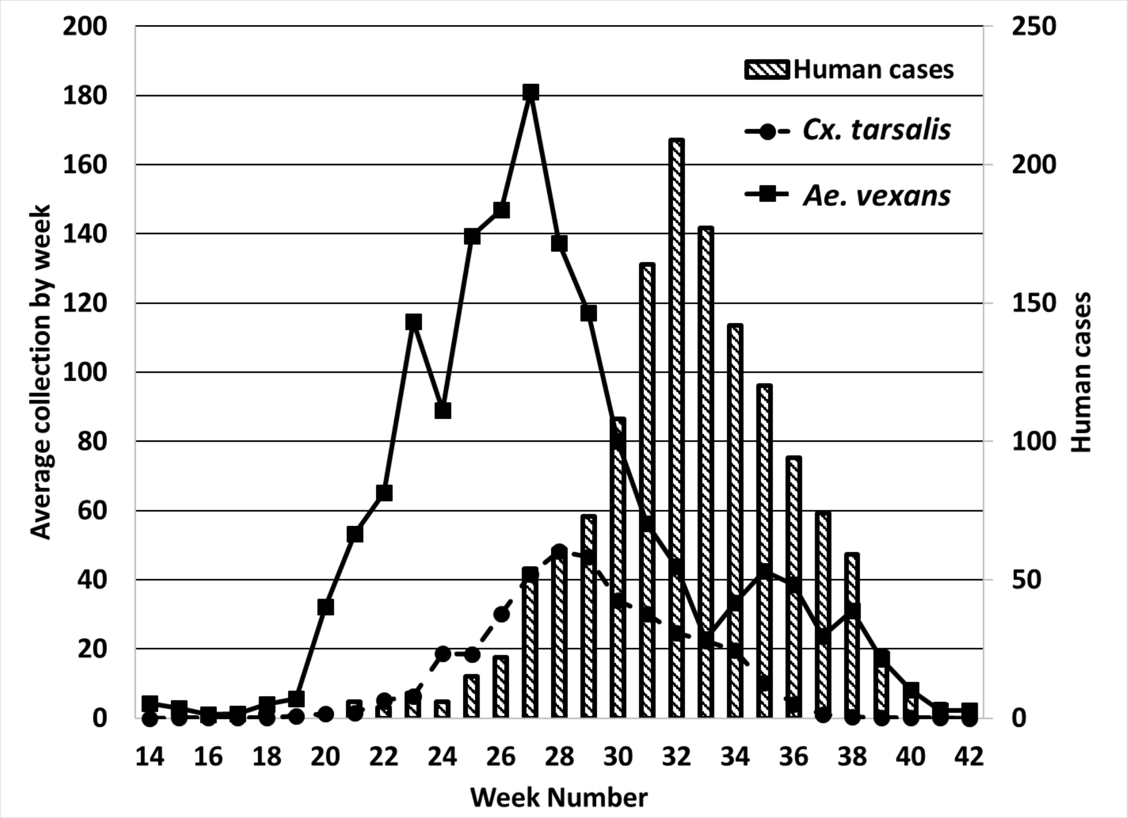

Figure 5: Statewide average of the mosquitoes *Ae. vexans* and *Cx. tarsalis* captured per trap per week for all years against human cases denoted by the bar graph for years 2004-2016.

Table 3: Spearman rank correlation coefficients for weekly human cases statewide vs. average *Cx. tarsalis*, *Ae. vexans* and the ratio of *Cx. tarsalis* to total mosquitoes.

	lag 0	lag 1	lag 2	lag 3
Average *Cx. tarsalis* collected	0.607	0.74	0.827	0.865
Average *Ae. vexans* collected	0.265	0.362	0.444	0.506
Ratio of *Cx. tarsalis* to total	0.433	0.498	0.519	0.503

Discussion

This study updates and expands on previous studies of mosquito populations in South Dakota and compares patterns of population dynamics for *Cx. tarsalis* and *Ae. vexans* that might influence WNV transmission to humans throughout the NGP. The few previous studies conducted from this region suggest that *Cx. tarsalis* and *Ae. vexans* are the two most common species present (Easton 1987a; Easton, Coker, and Ballinger 1986; Gerhardt 1966), and this is supported by the present study where both species accounted for 88.8% of all species identified. Our study has demonstrated that *Ae. vexans* is the primary nuisance mosquito for this region, and that *Cx. tarsalis* is the primary vector for WNV. All of the other 20 species reported in this study have been identified in other studies from SD or neighboring states (Bell, Mickelson, and Vaughan 2005; DeGroote et al. 2007; Easton, Coker, and Ballinger 1986; Friesen and Johnson 2014; Janousek and Kramer 1999). Small numbers of *Aedes japonicus* has been found in Iowa and Minnesota, but had not been identified in states within the NGP (Kaufman and Fonseca, 2014), and our report of a few specimens captured on two separate years in the Sioux Falls area constitutes a new record for SD. Recently, the potential ZIKA virus vectors, *Aedes albopictus* and *Aedes aegypti,* have been specifically targeted for surveillance within eastern South Dakota, and though neither species has been detected, Kaufman and Forseca (2014) point out that *Ae. japonicus* share similar habitat preferences to *Ae. albopictus*, which indicates that the Sioux Falls area is a logical location for continued surveillance for these ZIKA vectors.

Because 97.7% of the mosquitoes collected in this study were from counties east of the Missouri River, the population intensities described more closely resembled distributions in Minnesota, Iowa and eastern Nebraska, than those found in neighboring states to the west. Further studies involving additional locations from western SD would be useful in determining predominant species in those locations as well confirming the presence of species previously recorded in South Dakota that did not appear in this study, such as *Aedes nigromaculis.*

In addition to *Cx. tarsalis,* SD has three additional *Culex* species that can vector WNV, including: *Culex pipiens* L, *Culex restuans* Theobald, and *Culex salinarius.* In spite of the growing recognition that *Cx. tarsalis* is the primary vector for WNV throughout the NGP, no studies have evaluated the year-to-year population fluctuations occurring in this vector species, or the weekly population changes that occur throughout an average mosquito season within this region.

The purposes of Gerhardt (1966) and older studies in South Dakota were to determine the mosquito species present, utilizing multiple trapping techniques throughout most regions of the state, and therefore, identified more species than was found in the present study utilizing only the carbon dioxide-baited trap. This may be due to some mosquitoes having geographical limitations and do not appear in eastern South Dakota, such as *Culiseta impatiens* (Walker) that inhabits coniferous forests in the western region of South Dakota and *Aedes nigromaculis* (Ludlow) that was caught in areas where our current study has limited data west of the Missouri River (Gerhardt 1966). His study and ours do agree that after almost 50 years, *Ae. vexans* and *Cx. tarsalis* are still the most abundant mosquito species. However, our data shows that the peak abundance for *Cx. tarsalis* to be in July compared to Gerhardt's observation of their peak a couple weeks before fall (Gerhardt 1966). Another study located in north central South Dakota also showed that *Ae. vexans* and *Cx. tarsalis* to be the most abundant species during their survey in 1984 and 1985. This study was able to concur with our study that *Cx. tarsalis* is at its most abundant during July (Bolling et al. 2009; Fauver et al. 2016).

A short coming of these previous studies was a lack of geospatial comparisons between the raw abundance and specific proportional differences, as well as limited information on the temporal abundance of important mosquito species. These previous studies showed *Cx. tarsalis* to be the most abundant ins Lincoln/Minnehaha counties, but our study showed *Ae. vexans* to be the most abundant. In Hughes County, Gerhardt reported *Ae. vexans* to be the most abundant mosquito species while this current study not only shows that *Cx. tarsalis* was the most abundant, but that *Ae. vexans* abundance was the lowest of the regions that identified this species. Our study showed that the abundance of an important nuisance mosquito, *Ae. vexans,* to be the most abundant species in South Dakota; however, this is only at the state level. Regionally, *Ae. vexans* varies between predominant and secondary in abundance and this drop in relative abundance occurs from east to west through the state. This east to west trend in reduced relative abundance of *Ae. vexans* is supported through studies neighboring SD. Iowa shows this species to be their most abundant mosquito statewide whereas studies in Montana showed relative abundance of *Ae. vexans* and *Cx. tarsalis* to be more similar (DeGroote et al. 2007; Friesen and Johnson 2014). These changes in relative abundance between vectors and non-vectors could impact human avoidance behaviors if important nuisance mosquitoes are not as abundant. Reduction in biting pressure generated by non-vector mosquitoes could reduce avoidance behaviors such as applying repellent or seeking shelter. Previous studies have shown that the public is more inclined to take action to prevent bites when they are aware of mosquito presence (Zielinski-Gutierrez and Hayden 2006).

In addition to changes between vector and non-vector abundance, we saw a shift in abundance between two major WNV vectors. The increase in *Cx. tarsalis* abundance from the eastern portion of the state to the central portion has similar patterns in other surrounding midwestern states. *Culex tarsalis* is far less abundant in Iowa while having higher numbers of *Cx. pipiens.* In Nebraska, mosquito surveys showed the abundance of *Cx. tarsalis* increased from east to west while the abundance of non-*Cx. tarsalis* mosquitoes decreased in overall abundance towards the central portions of the state. Another important WNV vector, *Cx. pipiens,* falls in abundance as we move westward in South Dakota. Iowa reported this mosquito as their second most abundant mosquito, while in Nebraska, both its abundance and relative abundance drops progressively towards the west. Studies conducted in states located to the west of South Dakota report that *Cx. pipiens* is either not present or less than 1% of total captures. South Dakota appears to be a part of a large longitudinal boundary in the upper great plains for a transitional shift between two prominent WNV vectors, *Cx. tarsalis* and *Cx. pipiens.* These geospatial shifts between mosquito vectors and between vectors and non-vectors abundance may be important in monitoring human risk of mosquito transmitted pathogens.

Our study was not able to find any significant relationship between the abundance, or relative abundance between *Cx. tarsalis* and *Ae. vexans* and human WNV on a yearly basis. However, our statistical models were able to find a relationship between the weekly abundance of *Cx. tarsalis* and human infection of WNV particularly at the two and three week lags, which is expected as diagnosis of WNV in humans can take weeks after the infectious bite, though this may also be a result of the seasonality of both the vector and human cases of WNV. Vector abundance has been used in attempts to predict potential human risk to WNV and has been shown in other studies to positively correlate with a few week lag in human cases (Bolling et al. 2009; Kilpatrick and Pape 2013). However, infection rates of mosquitoes increased while the average abundance of the vector sharply decreased. This could indicate that the proportion of infected mosquitoes is more important than the abundance of the potential vector. In this situation, non-infected vectors act nuisance mosquitoes that could apply biting pressure to encourage avoidance behaviors in humans. Therefore, an increase in the number of infected mosquitoes with a decrease in overall numbers could be the driving factor in increased human cases. In another study, two cities where infection rates were similar, compared vector index and human cases and found a negative association

(Gujral et al. 2007). They concluded that while the infection rates were the same, the increase in vector index was due to increased mosquito abundance that resulted in avoidance behaviors.

Recently, South Dakota has used a new model which has shown success over the past few years (Davis et al. 2017). This new technique uses mosquito infection data along with climate variables to predict human risk of WNV, however, the model does not use any mosquito abundance data. Understanding the roles and impacts of the various mosquitoes have on infection rates, human behaviors, and ultimately human infection of WNV, could enhance models currently used to predict human risk of WNV.

Chapter 3: Comparisons of Vector Abundance to Minimum Infection Rate and Mosquito Infection Growth Rate

Abstract

Predicting human risk for West Nile virus (WNV) in North America has been a topic of study since its inception in 1999. Many factors have been used to predict human risk including mosquito abundance and infection rate. In this paper we intend to study the relationship between early season mosquito abundance and two different methods of estimating infection rate in six mosquito species found in the Northern Great plains. Mosquito abundance was calculated as the average collected per trapping night. Minimum infection rate (MIR) was calculated for all species that have tested positive for WNV in South Dakota to determine which species could potentially drive infection prevalence. Minimum infection rate and the mosquito infection growth rate (MIGR) were then compared, using the Pearson statistic, to mosquito abundance collected before July 15th from four counties in South Dakota. Infection rate for all species that have tested positive for WNV in order of highest infection rate to lowest are: *Aedes fitchii, Culex pipiens/restuans, Aedes cinereus, Culex salinarius, Culex tarsalis, Aedes dorsalis, Culiseta inornata,* and *Aedes vexans.* Of the six species, *Aedes vexans, Aedes dorsalis,* and *Culex tarsalis* abundance all had a negative relationship. *Culex pipiens/restuans* and *Culex salinarius* abundance had negative correlations with MIR but had positive correlations with MIGR. *Culiseta inornata* abundance maintained a positive correlation compared to both MIR and MIGR. Effects of temperature and precipitations were evaluated on the abundance of *Ae. vexans* and *Cx. tarsalis* as they are the most

abundant species found across the state. Short term effects on *Cx. tarsalis* were positively correlated for both temperature and precipitation; however, these effects were relatively small compared to *Ae. vexans.* Precipitation had a large effect on *Ae. vexans* abundance at the two to three week lag period. Temperature had a positive, but declining, relationship with *Ae. vexans* abundance during the zero to two week lag period, turning to a negative relationship past the three week lag. Understanding the relationship of mosquito abundance to mosquito infection rates within a system can be important in understanding the dynamic and complex system of virus amplification. Though most species abundance was negatively associated with infection rates, this could indicate that environmental conditions may have a greater driving force in viral amplification than just vector abundance alone. The positive relationship seen with *Cs. inornata* could indicate that this early emerging mosquito may, in conjunction with environmental factors, have an important impact in early season amplification.

Introduction

Human transmission risks associated with mosquito-borne diseases has become an area of recent study in disease modeling across the world (Reiner et al. 2013). Locally, data sets are often created and maintained in order to monitor these risks (Sucaet et al. 2008). In South Dakota, mosquito species composition and abundance, West Nile virus (WNV) infection rate, and the number of human cases reported have been created and used since 2002 to help assess risk potential for human infection of WNV. These different data sets have been used to create a variety of methods for assessing risk (Chuang, Hockett, et al. 2012; Davis et al. 2017; Fauver et al. 2016;

Janousek and Kramer 1999; Jian et al. 2016; Karki et al. 2018; Kilpatrick and Pape 2013; Wimberly et al. 2014).

A common method for assessing human risk to WNV is through monitoring the minimum infection rate (MIR) for vector mosquitoes, which is the ratio of virus positive pools of mosquitoes (usually tested in groups of less than 50 mosquitoes) to the total number of mosquitoes tested for that time period. Mosquito pool testing is useful in assessing the prevalence of WNV virus in the bridge vector mosquitoes (i.e. mosquitoes that feed on the bird reservoir and humans); however, in doing so, it is assumed in this technique that only one mosquito in a pool is infected, which can lead to an underestimation of the true infection rate if the virus is abundant in the population (Gu, Lampman, and Novak 2003). Additionally, this technique does not account for the varying levels of vector competency of multiple vectors within a geographical region. The vector index was designed to create a more complete picture by including the abundance of each vector, the species tendency to feed on mammals, infection prevalence in mosquitoes and an index for the vectors competence (Kilpatrick et al. 2005). However, in diseases such as West Nile virus (WNV), the reservoir hosts are not mammals, and so higher ratios of mammal feeding will not necessarily increase human risk.

Other effective tools in monitoring human risks to WNV include datasets that utilize meteorological and landscape ecology combined with developed models which than then assess human WNV risk based upon multiple factors, such as temperature and rainfall in various times of the year (Parham and Michael 2010; Wimberly et al. 2014), and land cover as an influence (Chuang, Hockett, et al. 2012).

Recently, a combination of both infection rates and environmental effects have been combined to create prediction models for human WNV risk that have been successful in South Dakota (Davis et al. 2017). This technique calculates mosquito infection growth rate (MIGR) and found that human risk was at its highest when the environmental conditions were met, and early season infection data showed that the virus was being amplified (Davis in press). However, the process of collecting, sorting, transporting mosquitoes to testing facilities, infection testing, and ultimately reporting the results can take multiple weeks to complete. This lag in testing shortens the window of predictive capabilities available to the model.

Understanding the various impacts of these factors may help public health officials to predict and respond to threats of mosquito transmitted pathogens. While some success has been had in predicting the severity and timing of WNV outbreaks in South Dakota, the relationship of early season mosquito abundance to virus prevalence has not been thoroughly studied. Mosquito control programs can often operate with limited resources (Kilpatrick and Pape 2013), so finding means for determining predictive factors that are both cost effective and available sooner should be a priority.

From 2002 until 2004, all species collected in South Dakota were tested for infection of WNV. After this time, the South Dakota Department of Health focused on the vector mosquito *Culex tarsalis* (Kightlinger 2017) and has focused its efforts to monitor the MIR of this species. As mentioned in chapter 2, increases in *Cx. tarsalis* abundance are correlated with increases in human WNV cases within a season; however, the overall year-to-year abundance of the *Cx. tarsalis* does not relate to the overall incidence of human cases (Nielsen et al. 2008). From a public health perspective, identifying all sources of potential transmission as well as predicting the level of amplification of virus within those vectors could further the predictive ability of current models.

Multiple species that have been confirmed as potential vectors are present in the state. While *Cx. tarsalis* was the most abundant vector species in the state, *Culex pipiens* was also present in the state, predominantly on the eastern side. Both species are known to switch from feeding on birds in the spring to mammals later in the summer (Goddard et al. 2002; Tempelis 1975). *Culex salinarius* is known to feed on both birds and mammals, and may also contribute to the amplification of the virus (Molaei et al. 2006). *Culiseta inornata* is one of the first species to appear in traps, and while it does primarily feed on mammals, it is known to take avian blood meals (Tempelis 1975; Anderson and Gallaway 1987). Having some avian host preference combined with a moderate ability to transmit WNV makes it an excellent candidate for early season amplification (Goddard et al. 2002). *Aedes vexans* was the most abundant mosquito in many areas of the state, especially those with high numbers of human cases, and both *Aedes vexans* and *Aedes dorsalis* have experimentally been shown to transmit WNV; however, both of these species also prefer feeding on mammals (Kramer, Reisen, and Chiles 1998; Molaei and Andreadis 2006), In this study we aimed to determine if mosquito abundance of these experimentally determined vectors present in South Dakota influence infection rates derived either from MIR or through MIGR. Additionally, we investigated precipitation and temperature effects on the change in abundance of two important species in the state.

Materials and methods

Mosquito collections:

Mosquito surveillance was conducted in approximately half the counties in SD using $C0_2$-baited CDC miniature light traps with air-activated gates (John W. Hock Model 1012-CO_2, set to deliver 0.5 L CO_2/min) from 2004 to 2017. Traps were suspended approximately 1.5 meters from the ground and located in areas with moderate-heavy tree cover and vegetation. Tapping occurred overnight and were activated using light sensors. Mosquito species were identified species level based upon morphological characteristics (Darsie 2005). Due to the difficulty in consistently identifying *Cx. pipiens* and *Cx. restuans* correctly based upon morphological features alone, these two species have been combined for analysis.

Infection rate:

Most mosquito testing was performed by the SDDOH using standardized testing (Lanciotti et al. 2000). Testing in Lincoln, Minnehaha, and Brown counties were conducted using the RAMP test (ADAPCO) according to the manufacturer's recommendation.

The minimum infection rate (MIR) was calculated as the total number of infected pools divided by the total number of mosquitoes tested, and then multiplied by 10,000; this is a standard summary statistic and assumes that any positive pool contained only one infected mosquito. Data from any point in the year were used. The MIGR was defined as the rate at which positive pools appeared in the early season of a given year; that is, how quickly the virus replicated in the mosquito population. Specifically, the probability of a positive pool anywhere in the state before July 15th was modeled by a generalized linear mixed model linear in the day of the year, with random effects on intercept and slope by year (glmer function, lme4 library). The MIGR was then defined as the estimated slope of the fit. This is conceptualized below in Figure 6.

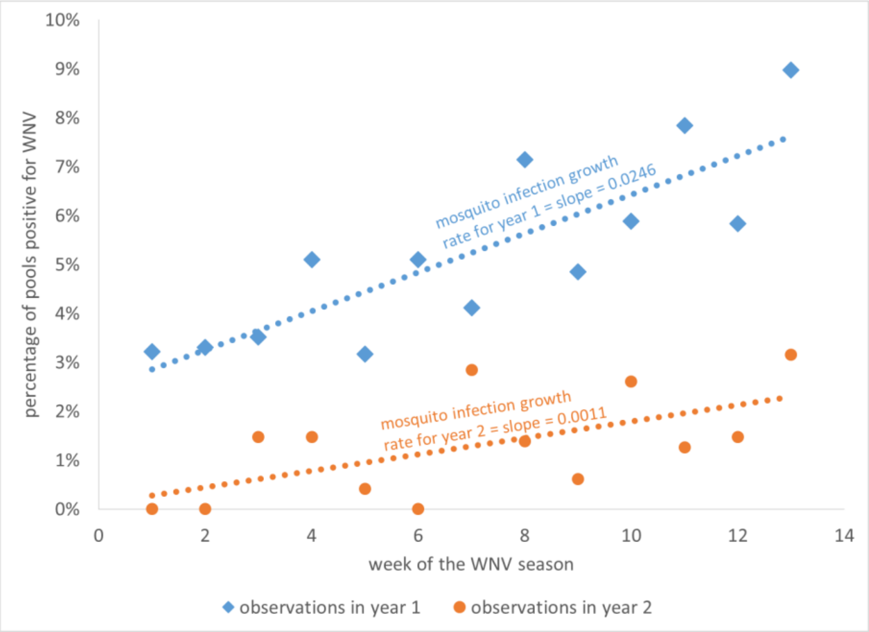

Figure 6: Conceptualization of the mosquito infection growth rate (MIGR) in two years. Observations are represented by points, and the mixed effects model produces the fit line with a slope.

Relationship of mosquito abundance to MIR and MIGR:

Female mosquito collections from Lincoln, Minnehaha, Brookings, and Hughes counties before July 15th of any year were log-transformed and averaged, to yield an early-season yearly abundance for every species and year for those species that have been known to be infected with WNV and are present every year from 2004 to 2017. These abundances were compared to the MIR and MIGR calculated above, and relationships between the two were sought with Pearson correlation coefficients (ρ).

Statistical model of mosquito abundance:

Our goal was to predict the average weekly collections of *Culex tarsalis* and *Aedes vexans* as a function of temperature and precipitation. Collections of each species were averaged by week and county.

Raw hourly mean temperature (deg C) and total precipitation (mm) data were obtained for all dates beginning Jan. 1, 2003 from the North American Land Data Assimilation System (NLDAS) atmospheric forcing data. Temperature and precipitation were obtained for every combination of county and hour by sampling the appropriate NLDAS layer at each county centroid. These values were then aggregated to compute daily mean temperature and total daily precipitation for every combination of county and day.

Each combination of week and county was then associated with 362 lagged meteorological variables, including temperature and precipitation on the day the trap was set (lag 0) up to 180 days or roughly six months prior (lag 180), giving each county and week a six-month meteorological history.

Lagged model:

The average mosquito collection of each species for each week within each of the four counties sampled was log-transformed and then modeled by a linear model, with indicator for county to capture spatial variation, and an Almon-type distributed (a sixth-degree polynomial) to summarize the relationship between abundance and two environmental covariates, precipitation and temperature (Almon 1965). The model was fit on all historical data from the counties in the lm function (stats package, R 3.4.3).

Results

From 2002 to 2004, 18 different species of mosquitoes were tested for the presence of WNV; however, only 9 of these species resulted in a positive WNV detection. After 2004, *Cx. tarsalis* was primary species tested for WNV, but *Cx. pipiens/restuans* was also tested in areas where they were commonly present (Table 4). Overall, *Cx. tarsalis* had the most number of mosquitoes (n = 790,046) and pools tested (n = 23,681), as well as the most positive pools (n = 1,205) which resulted in an MIR of 15.25. *Culex pipiens/restuans* being the second most tested mosquito in terms of number (n = 24,154) and pools (n = 2,238), had only 57 positive pool, yet has a higher overall MIR value (23.60) than *Cx. tarsalis. Culex salinarius* also has a MIR value higher than *Cx. tarsalis* at 18.50, but with far fewer mosquitoes (n = 1,081) pools tested (n = 230) so that the potential variability is also higher. Of the *Aedes* species, *Aedes fitchii* had the highest infection rate (MIR = 84.03) but was the least tested mosquito with only 238 mosquitoes tested. *Aedes dorsalis* MIR (7.47) was lower than the *Culex* species but was still higher than *Ae. vexans* (MIR = 0.44) and *Cs. inornata* (MIR = 7.27).

To compare infection rates within similar time periods, the values for *Cx. tarsalis* and *Cx. pipiens/restuans* were calculated using only data collected from 2002 – 2004. Even in this reduced sample, *Cx. tarsalis* still had the most number of mosquitoes (n = 29,941), pools tested (n = 976), and the MIR increased to 18.70. *Culex pipiens/restuans* MIR value calculated at 37.42, the highest for that time.

Table 4: Results of statewide WNV mosquito testing 2002 to 2017.

Species	# of pools	# of mosquitoes tested	# of positive pools	MIR
Ae. cinereus	67	525	1	19.05
Ae. dorsalis	254	2676	2	7.47
Ae. fitchii	37	238	2	84.03
Ae. vexans	580	22743	1	0.44
Cs. inornata	210	1376	1	7.27
Cx. pipiens/restuans	2238	24154	57	23.60
Cx. pipiens/restuans (2002-2004)	312	1069	4	37.42
Cx. salinarius	230	1081	2	18.50
Cx. tarsalis	23681	790046	1205	15.25
Cx. tarsalis (2002-2004)	976	29941	56	18.70

The effects of mean abundance for each mosquito species tested on the cumulative yearly MIR in South Dakota varied among the species, but all had some negative effect (Figure 7). The nuisance mosquito *Ae. dorsalis* and the vector species *Cx. pipiens* and *Cx. salinarius* showed little relationship to predicting that years MIR; however, the Pearson correlation statistic for all three of these species did show that there is some potential negative relationship at -0.28, -0.18 and -0.06 respectively. Both the nuisance mosquito *Ae. vexans* and the vector *Cx. tarsalis* showed a negative relationship to yearly cumulative MIR with the Pearson correlation coefficient at -0.61 and -0.60 respectively. *Culiseta inornata* was the only species to show a positive relationship with yearly MIR with a Pearson correlation coefficient at 0.63.

The effect of mean abundance for mosquitoes tested on the MIGR statistic in South Dakota also varied among species (Figure 8). The nuisance mosquitoes *Ae. dorsalis* and *Ae. vexans*, as well as the primary vector mosquito *Cx. tarsalis* all showed a negative relationship to the MIGR. The strongest relationship was with *Ae. vexans* with a Pearson coefficient of -0.82, followed by *Ae. dorsalis* at -0.50. The vector, *Cx. tarsalis* had the negative relationship at -0.35. The remaining vector mosquitoes *Cx. pipiens, Cx. salinarius,* and *Cs. Inornata* all had positive, yet relatively weak, associations with MIGR with Pearson correlation coefficients at 0.27, 0.20, 0.13 respectively.

Comparing the results of the two techniques of calculating infection rate (Figures 7 and 8), *Ae. vexans* maintains a strong negative relationship to infectivity of mosquitoes with a much stronger relationship shown to the MIGR. While *Ae. dorsalis, Cx. tarsalis* and *Cs. inornata* all maintain the direction of their influence on infection rates, they both had less influence in the MIGR model. Both *Cx. pipiens* and *Cx. salinarius* had weak associations with measures of infection rate; however, the direction of influence switched with *Cx. pipiens* and *Cx. salinarius,* both becoming positively associated with MIGR.

The relationship between the number of *Cx. tarsalis* mosquitoes collected and the mean level of precipitation and temperature was very week in that increases in precipitation and temperature increased abundance by only a small amount between 0 and 3 weeks (Figure 9C and D). However, there was a strong positive association between precipitation and *Ae. vexans* population levels, particularly between two to three weeks after a rain event (Figure 9A). Temperature had a very short- term, positive relationship to *Ae. vexans* numbers; however, as temperatures increase we saw a negative effect on *Ae. vexans* abundance around the three week lag (9B).

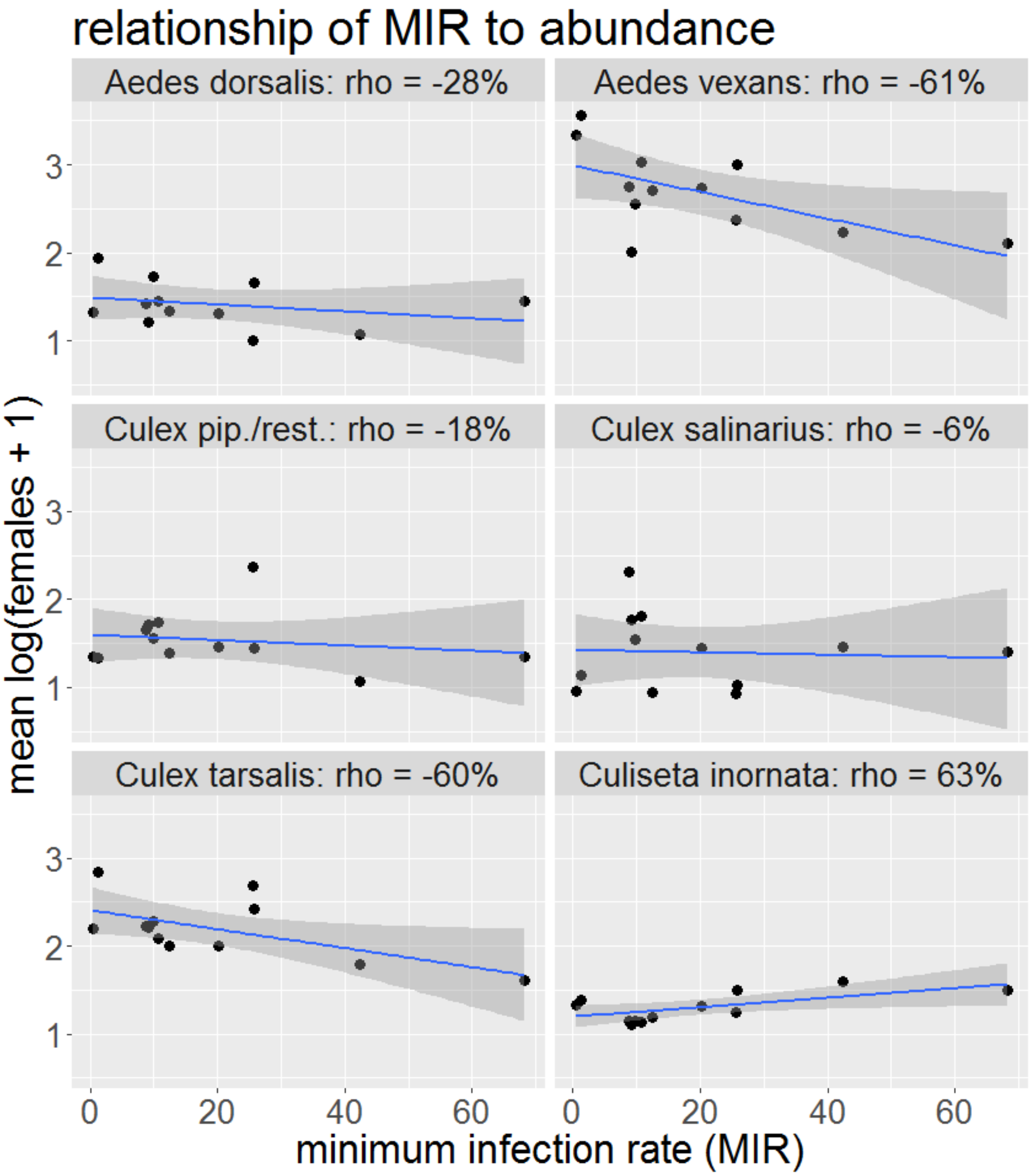

Figure 7: Cumulative MIR of WNV in *Culex tarsalis* mosquitoes in a year, against the log mean mosquito collections per species before week 26 of the same year.

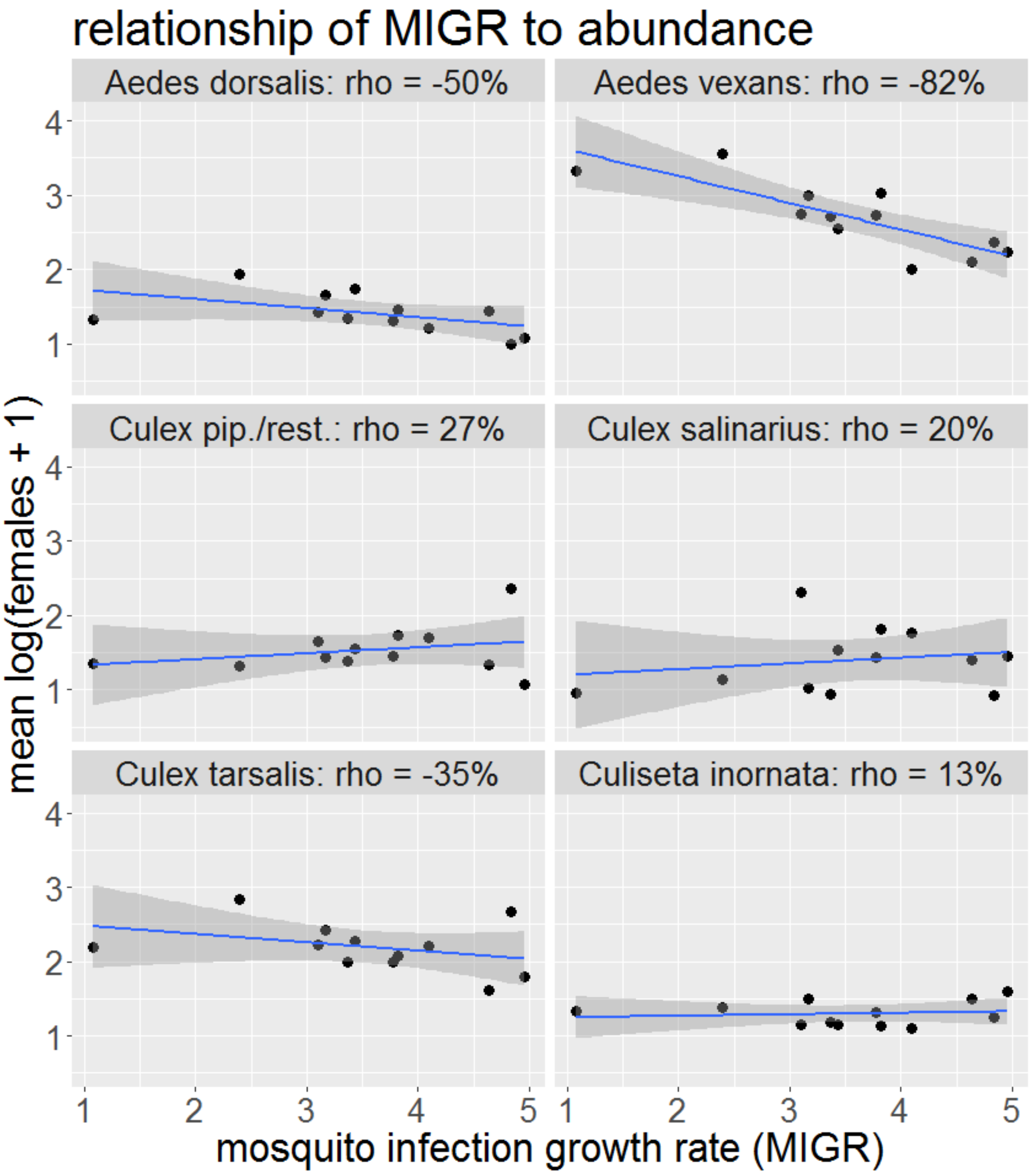

Figure 8: Estimated mosquito infection growth rate (MIGR) of WNV in *Culex tarsalis* mosquitoes in a year, against the log mean mosquito collections per species before week 26 of the same year.

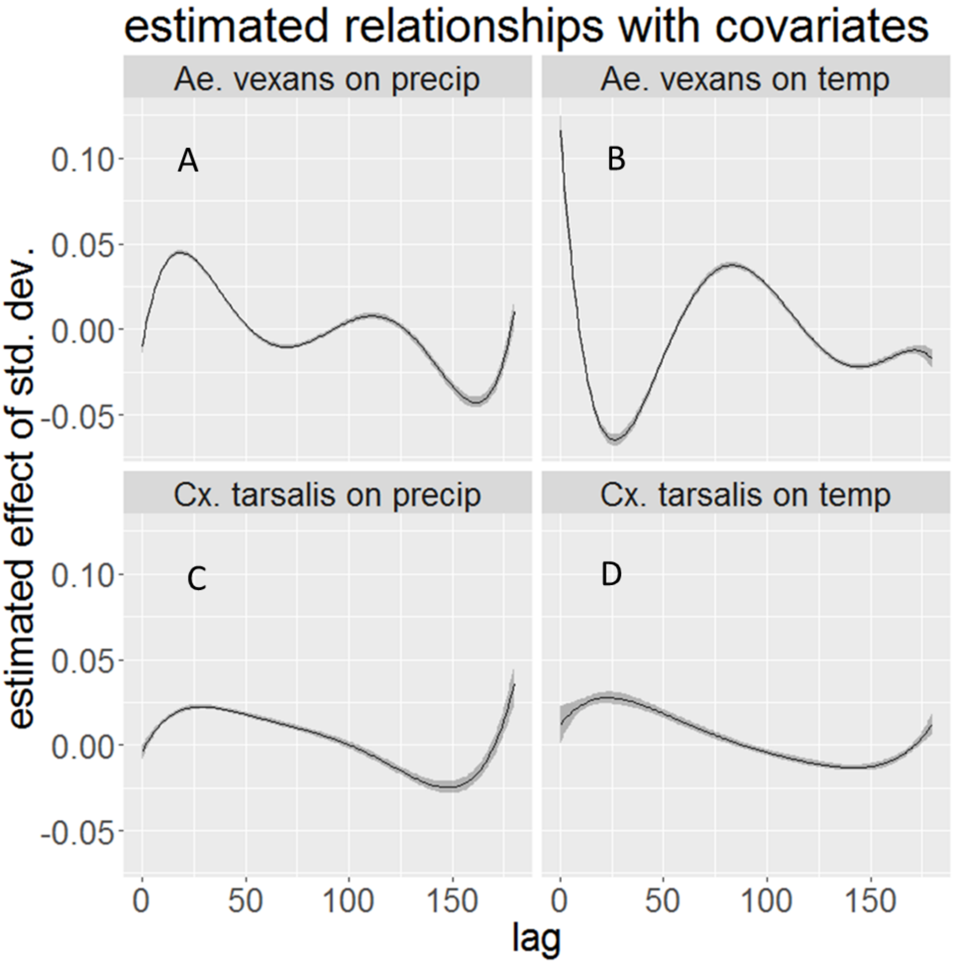

Figure 9: Effects of precipitation and temperature on the abundance of *Ae. vexans* (A and B) and *Cx. tarsalis* (C and D). Relationships have been standardized so that one standard deviation increase in either environmental covariate is directly comparable.

Discussion

Our goal was to determine if the abundances of mosquitoes that are considered competent for transmission of West Nile virus and found infected with WNV in South Dakota influence the overall infection rates among all mosquitoes in the counties tested. We first looked at the mosquitoes that have been tested in South Dakota to determine those species that have been found positive for WNV and the rate in which they were.

Aedes fitchii had the highest MIR of all the WNV positive mosquitoes tested in South Dakota but was also the least tested mosquito and is not often collected in traps. This particular species has been tested elsewhere and found to be infected with WNV, but is considered to primarily feed on mammals and not thought to be a major contributor to amplification or human transmission (Phippen, Bio, and Phippen 2014) *Aedes cinereus* had the third highest MIR; however, this mosquito is also rarely found in South Dakota traps. Both of these species may be limited in collection due to being univoltine species that generally emerge between April and June (Ross 1947), thus making them unlikely candidates for amplification of the virus, and thus removed from comparisons to MIR and MIGR comparisons.

Culex tarsalis and *Culex pipiens/restuans* are generally considered to be the most important vectors for human WNV infection throughout the USA and they both have higher MIR values in South Dakota. The *Cx. pipiens/restuans* species had a higher average cumulative MIR than *Cx. tarsalis* when considering all years and the subset between 2002 and 2004. However, the infection rate of these mosquitoes for this time period was less than half of infection rates found in North Dakota (Bell et al. 2006; Bell, Mickelson, and Vaughan 2005). This also contrasts other studies where *Cx. tarsalis* is considered the primary vector, as in Colorado reported the infection rate of *Cx. tarsalis* to be equal or higher than *Cx. pipiens* (Fauver et al. 2016) Though infection rates are higher in *Cx. pipiens/restuans* in SD, the abundance of *Cx. tarsalis* is far greater than *Cx. pipiens/restuans* in all regions of the state (see Chapter 2), making *Cx. tarsalis* a much more important vector for WNV in South Dakota. However, *Cx. pipiens/restuans* should be considered as a possible virus amplifier in the wild, especially in eastern South Dakota where it is more abundant. Additionally, *Cx. salinarius* also had a similar MIR value to *Cx. tarsalis* between 2002 and 2004. This mosquito has been collected less than both *Cx. tarsalis* and *Cx. pipiens/restuans*; however, *Cx. salinarius* is known to feed on both birds and mammals and is considered to be a bridge vector, especially in years of high virus prevalence (Bernard et al. 2001; Vaidyanathan et al. 1997; Crans 1964). The mosquito species *Cs. inornata* and *Ae. dorsalis* had MIR values that were less than half of the *Culex* species, and *Ae. vexans* was the lowest reported value despite being the most abundant mosquito in many areas of the state. Though they may have been found to be competent vectors, infection rates may be lower due to their preference for feeding on mammals. Both *Cs. inornata* and *Ae dorsalis* had similar MIR values; however, *Cx. inornata* is generally an early spring and *Ae. dorsalis* abundance can fluctuate greatly depending on meteorological conditions, which makes them potential candidates for impacting early season amplification of WNV.

It is not entirely surprising that these relationships between the abundance of most of the vectors and infection rate are not positively correlated due to the complexity of the WNV transmission cycle. However, there are some patterns within our results that do seem to offer some insights on how some pieces fit into the larger puzzle.

The primary vectors for WNV in South Dakota, *Cx. tarsalis* and *Cx. pipiens* showed very little relationship to rate of infection within themselves. This indicates that while these species have a role in transmitting WNV humans, the abundance of these mosquitoes may not be directly related to the amplification of the virus, or at the least, is not the limiting factor in the environment. The only species to show any positive correlation when comparing species abundance to the traditional calculation for infection rate (i.e. MIR) was *Cs. inornata.* This mosquito is the first to be found in mosquito traps in South Dakota, and is often noted as an early season mosquito in other areas of the United States (Reisen, Meyer, and Milby 1989). Correlations have been found in previous studies that warmer winters and springs increase risk in human WNV infections (Wimberly et al. 2014). Warmer winters and springs could allow for *Cs. inornata* to emerge earlier and allow for early amplification of WNV. Both *Ae. vexans* and *Ae. dorsalis* showed a negative association with both measurements of infection rate described within this paper. It may not be that these two species directly impacting infection rates of *Cx. tarsalis*, and may instead indicate that environmental factors favoring to these two species are unfavorable to the amplification of WNV and so an investigation into environmental factors that drive these two species was done.

The effects of precipitation on *Ae. vexans* abundance was considerably higher than its effects on *Cx. tarsalis,* especially between the two and three week lags. This is somewhat expected as the strategies for reproduction differs between these two species. Whereas, Ae. *vexans* lays its eggs on a dry substrate and waits to be submerged with water following large rain events to trigger the beginning of their life cycle, *Cx. tarsalis* lays its eggs directly on standing water which can't exist without a precipitation event. The positive association with precipitation in *Ae. vexans* is contradictory to another in our region. In Iowa, precipitation was found to have a negative association between the 2 and 3 week lag period (DeGroote et al. 2007). However, they claimed low collections of mosquitoes in early samples may have strongly influenced their calculations and this study was based upon samples for one year. A second Iowa study using eight years of data found the impacts of precipitation on *Cx. tarsalis* and *Ae. vexans* to be similar to ours showing that large rainfall events left *Cx. tarsalis* abundance relative unchanged while *Ae. vexans* abundance increased significantly (Rowley 1995).

The effect of increased temperatures on *Cx. tarsalis* populations were very similar to that of precipitation in that increased temperatures showed only a small increase in abundance. Short term lags in temperature have a positive effect on *Ae. vexans* which move towards a negative effect as it nears a three week lag, which is similar past studies done in South Dakota (Chuang, Henebry, et al. 2012). Again, this contradicts other local studies that showed increased temperatures positively related to *Ae. vexans* abundance (DeGroote et al. 2007). This negative effect may explain the general bimodal abundance pattern we see statewide in *Ae. vexans* population dynamics across the state. Peak abundance for *Ae. vexans* generally occurs during week 27 (Chapter 2 Figure 2) which is when temperatures begin to reach their highest across the state. High temperatures could then be driving abundance downward until temperatures cool enough to allow for *Ae. vexans* populations to rebound. While higher temperatures do decrease time for mosquito larvae to mature, temperatures above 30^{o} have been shown to decrease survival rate (Brady et al. 2013; Shelton 1973). Temperature has also been strongly associated with higher risk of WNV transmission (Davies in press), which could be the result of lower *Ae. vexans* abundance that could alter human behavior that would make them vulnerable to vector mosquitoes (Gujral et al. 2007). The weekly average abundance of *Cx. tarsalis* and *Ae. vexans* has previously been shown to have some relationship to weekly average human cases of WNV (Chapter 2).

These approaches do have some limitations in that it is taking a linear approach to what is possibly a non-linear system. Non-environmental factors, such as immunity levels in reservoir hosts, could dramatically impact amplification of virus in vectors (Allan et al. 2009). Additionally, the effects of mosquito abatement programs could reduce amplification by reducing vector abundance. However, if these mosquito control steps should only impact infectivity after they are implemented, and often they are only implemented when nuisance mosquito populations are high, or virus has been detected in vectors of human concern. These measures are unlikely to impact very early season amplification which could be accomplished by *Cs. inornata.* Further studies should be done to investigate the infection rate and roles of early season mosquitoes to the amplification of WNV in the wild. Knowledge of this could lead to factors that are available sooner and with greater cost effectiveness that could increase the lead time for prediction human risk of WNV.

Chapter 4: Permethrin Susceptibility for the Vector *Culex tarsalis* and a Nuisance Mosquito *Aedes vexans* in an Area Endemic for West Nile Virus

Abstract

In 2016, we compared susceptibility to the insecticide, permethrin, between the West Nile virus vector, *Culex tarsalis* Coquillett, and a major nuisance mosquito, *Aedes vexans* (Meigen) using baseline diagnostic dose and time values determined using the CDC bottle bioassay protocol. Mosquitoes were collected in the wild in Brookings County, South Dakota, situated in the Northern Great Plains of the USA. The determined diagnostic dose and time were then used in 2017 to validate these measurements for the same 2 mosquito species, collected at a second location within Brookings County. The diagnostic dose was determined for multiple time periods and ranged from 27.0 µg/ml at 60 min to 38.4 µg/ml at 30 min. There was no significant difference detected in mortality rates between *Cx. tarsalis* and *Ae. vexans* for any diagnostic time and dose. For practical purposes, mosquitoes in 2017 were tested at 38 µg/ml for 30 min; expected mortality rates were 93.38% for *Cx. tarsalis* and 94.93% for *Ae. vexans.* Actual 2017 mortality rates were 92.68% for *Cx. tarsalis* and 96.12% for *Ae. vexans*, validating the usefulness of this baseline at an additional location and year.

Introduction

Mosquito transmission of arboviruses to humans depends on multiple factors (Kilpatrick et al. 2005; Kilpatrick and Pape 2013), including human behavior [3]. Gujral et. al. (Gujral et al. 2007) suggest that human behavioral risk factors, such as the use of personal protectants, can be influenced by the “biting pressure” created by local mosquito populations. These populations include potential vector species and non-vector nuisance mosquitoes, and the presence of many nuisance mosquitoes could increase the use of personal protectants or avoidant behavior, thereby reducing the chance of potential viral transmission by vector mosquitoes. Conversely, the lack of abundant nuisance mosquitoes may have the opposite effect. Therefore, it is important to consider nuisance as well as vector mosquitoes when developing comprehensive strategies for mosquito reduction and disease control.

Community adulticiding efforts can limit both disease and nuisance issues caused by mosquitoes, but the common use of insecticides has prompted concerns over growing resistance to insecticides in mosquito populations. Permethrin, a broad-spectrum insecticide in the pyrethroid family, is the primary adulticide used in the United States (EPA) and is used for agriculture to reduce crop and livestock pests (Catangui and Berg 2002; Campbell, Boxler, and Davis 2001), as well as in residential areas to control nuisance and vector mosquito populations. Long-term usage of this class of insecticide has been shown to cause increased resistance in mosquito populations (Naqqash et al. 2016). Because of its broad use and the documented cases of resistance, monitoring of permethrin resistance is important to mosquito control efforts (Brogdon and McAllister 1998b; Strong et al. 2008).

Most studies on insecticide resistance have focused primarily on vector mosquito species, and few have included common nuisance mosquitoes within arbovirus-endemic areas. Richards et al. (2017) evaluated the susceptibilities of a potential Zika virus (ZIKV) vector (*Aedes albopictus* (Skuse)) and 2 West Nile virus (WNV) vectors (*Culex pipiens* L. and *Culex quinquefasciatus* (Say)) to 6 different common insecticides in a study that included 26 mosquito populations from four different U.S.A. regions. They also included the tree-hole mosquito, *Aedes triseriatus* (Say), in this study, though this species is not a significant vector for Zika or WNV and is generally only a minor species in most regions. They found that the *Aedes* species tested were less likely to exhibit resistance when compared with *Culex* species, particularly for etofenprox and malathion, and found that all *Aedes* spp. populations tested were either susceptible or possibly resistant to permethrin while most *Culex* spp. populations were resistant. Given the potential role of nuisance mosquitoes in encouraging the use of personal protectants, the susceptibility of non-vector mosquitoes to insecticides should be evaluated especially in arbovirus-endemic regions where nuisance species are far more abundant than the vector species.

In the U.S.A. Northern Great Plains, *Culex tarsalis* Coquillett is the primary vector for WNV, and *Aedes vexans* (Meigen) is generally the most predominant nuisance mosquito (Barr 1958; Easton, Coker, and Ballinger 1986; Bell et al. 2006; Barker et al. 2009). Recently, *Ae. vexans* has been reported as a potential vector for ZIKV (Gendernalik et al. 2017; O'Donnell et al. 2017); however, there have been no reported cases of local transmission of ZIKV in the Northern Great Plains. In eastern South Dakota, *Ae. vexans* populations generally swell to very large numbers in the spring and remain high during the WNV transmission season (Chuang et al. 2011). The aggressive biting of this nuisance mosquito can motivate people to use personal protection (Gujral et al. 2007). Despite its potential public health significance, we have found no studies directly comparing permethrin susceptibility for *Cx. tarsalis* and *Ae. vexans*.

Brookings County, located in east-central South Dakota, is the fifth-most populated county and contains the fourth largest city in South Dakota, though it mostly consists of farmland. Both species of interest are abundant within this county. The city of Brookings has utilized a mosquito control program involving permethrin for over 20 years, and the small cities in the county have had similar programs for over 10 years. The purpose of the present study is to compare susceptibilities of *Ae. vexans* and *Cx. tarsalis* to reagent-grade permethrin in a CDC bottle bioassay protocol involving multiple concentrations and time periods. For this comparison, we used adult mosquitoes freshly captured in Brookings County using CO_2 baited light traps. Use of wild-caught adult mosquitoes in this type of bioassay is considered acceptable from both the CDC bottle bioassay protocol and the WHO test procedures for insecticide resistance, and wild-caught adult mosquitoes have been used in previous studies where mosquito aquatic stages were not consistently available (CDC 2013; Rakotoson et al. 2017; WHO 2016; Marcombe et al. 2017). Because both species prefer to lay their eggs throughout natural habits, harvesting *Cx. tarsalis* and *Ae. vexans* eggs and then growing adults for the assay was not practical, and results can be inconsistent when rearing mosquitoes from eggs in a lab (Strong et al. 2008). In our area, the consistent collection of *Cx. tarsalis* larvae in large enough numbers to adequately compare its susceptibility to *Ae. vexans* was also not practical. To minimize concerns about potential high variability for data collected from wild-caught adults, the susceptibility comparisons involved a large number of mosquitoes evaluated in multiple assays conducted throughout the mosquito season. The use of field collected mosquitoes for this comparison also allowed for testing both species together in the same bottles and testing them in the various natural physiological conditions representative of the wild populations' age distribution at any given time (WHO 2016).

The data from this study were used to calculate a base-line diagnostic dose and time for permethrin susceptibility for both species that can be used in future studies for evaluating changes in permethrin resistance from this region. We have found no studies identifying values for diagnostic dose and time on permethrin susceptibility for *Cx. tarsalis* and *Ae. vexans*, and none have been reported to the Arthropod Pesticide Resistance Database (Database] 2014). According to the Centers for Disease Control and Prevention (CDC) diagnostic dose and times should be determined for each type of insecticide used, and for each species of concern within a region (CDC 2013), and studies often emphasize the lack of reported diagnostic dose and times reported for *Culex* species, and more so with *Cx. tarsalis* (Richards et al. 2017). CDC guidelines recommend creating the diagnostic dose and time from susceptible populations; however, permethrin is used throughout the state for both agricultural and mosquito control purposes. The Center for Disease Control guidelines also recommend establishing these baselines from mosquito populations in areas where treatments are applied as a reference point for future comparisons.

Methods

Mosquito collections:

From June through August of 2016, mosquitoes were captured using CDC Miniature Light Trap Model 512 equipped with photoswitches and air-actuated gate system (John W. Hock Company, Gainesville, Florida) and baited with CO_2 at a farm located 1.8 km from the southwestern city limits of Brookings, South Dakota (44.25^o, -96.81^o). This site was selected as populations of *Cx. tarsalis* and *Ae. vexans* are present in large enough abundances to test mortality rates for multiple permethrin concentrations. This location is also on the outskirts of a city where insecticide fogging applications targeting mosquitoes have occurred sporadically for 25 years. In June of 2017, mosquitoes were again collected using the same method but from Oakwood State Park (44.45^o, -96.98^o) located 25 km northwest. In neither year were targeted mosquito control applications of insecticide administered near either site.

CDC bottle bioassay:

The CDC bottle bioassay was chosen for its effectiveness in testing field-collected mosquitoes (McAllister, Godsey, and Scott 2012; Sun et al. 2014). Calibration of the CDC bottle bioassay was performed per the CDC Bottle Bioassay protocol (CDC 2013). These guidelines recommend that a baseline diagnostic value should be determined for use in comparisons for future resistance testing on specific mosquito species and geographical regions. In determining the diagnostic dose and times, we identified a concentration that would result in 100% mortality between 30 and 60 min, and then estimated the LC98, or the lowest concentration which was lethal for 98% of mosquitoes at those time spans. For the 2016 bioassay calibration, tested concentrations were 1, 10, 20, and 40 µg/ml of laboratory grade permethrin (Sigma-Aldrich, St. Louis, Mo.) suspended in acetone. Due to varying physiological states of wild-caught mosquitoes, a large sample size was used for each replicate. Additionally, any mosquitoes that died or had physical injuries at the start of each trial, such as broken or missing wings or legs, were excluded. Replicates of each concentration were run until at least 100 mosquitoes of each species were tested. Each test bottle was cleaned with detergent, rinsed, and then dried in an oven ensure removal of residual contaminants. Each treatment consisted of clear 250 ml bottles coated with 1 ml of a specific concentration containing permethrin with 1 control bottle coated with 1 ml of acetone only. For the 2017 validation bioassays, permethrin was prepared at a concentration of 38 µg/ml.

Prior to testing, captured mosquitoes were maintained in holding cages in the laboratory for 3 to 4 hours to acclimate to their environment. Mosquitoes were transferred to the experimental bottles via a mechanical aspirator to avoid introducing condensation to the treatment bottles. The CDC bottle bioassay protocol recommended determining diagnostic times between 30 and 60 minutes so mosquito mortality was measured every 5 min for 60 min. Mosquitoes that would no longer right themselves after slowly rolling the bottle were considered dead. Mosquitoes that survived past 60 min were removed from the bottle and euthanized separately. During the 2017 bioassays, mosquitoes were only evaluated for 30 min. Afterwards, all mosquitoes were identified to species. Only data from *Cx. tarsalis* and *Ae. vexans* were used in this study.

Corrected mortality was not needed as no mortality occurred in any of the control bottles.

Statistical analyses:

Using mosquitoes collected in 2016, a probit model (glm function, stats package, R x64 version 3.2.2) was used to model the relationship between proportion mortality (dead per total in bottle, binomial family) as a linear function of time, with slope and intercept depending on species as a factor and concentration linearly. The estimated diagnostic dose or LC98 was defined as the lowest dose for which the expected mortality exceeded the susceptibility threshold defined by the CDC (98% mortality) for each species and concentration at 30, 45, and 60 min (CDC 2013; WHO 2013).

A likelihood ratio test (ANOVA function, stats package) was performed against a simpler model in which mortality depended on concentration and time but not species, to determine whether the 2 species differed by mortality in any systematic way during the experiment. For analysis of the 2017 mosquito data, 95% exact confidence intervals were calculated for proportion mortality at the chosen dose and time (binconf function, Hmisc package) to compare with estimates obtained in 2016.

Results

Culex tarsalis (n = 421) and *Ae. vexans* (n = 1084) were tested in 2016 at various concentrations of permethrin. An additional 159 *Cx. tarsalis* and 207 *Ae. vexans* were used in control bottles containing acetone only. No mortality in the control bottles occurred during the experimental period. Observed mortality rates as functions of time, species, and permethrin concentration are displayed in Figure 10. Mortality rates increased as permethrin concentration increased and were nearly linear in time for each concentration of permethrin except for 40 µg/ml, which reached near 100% mortality at approximately 30 min for both species. At 20 µg/ml, mortality reached 50% for both target species at 60 min.

The estimated LC98 for *Ae. vexans* was 38.4 µg/ml at 30 min and 27.0 for 60 min. For *Cx. tarsalis* it was 38.3 at 30 min and 27.5 µg/ml at 60 min (Table 5). This showed that increased permethrin concentrations achieved the same mortality in less time for both species. For a given time, mortality rates are similar between the 2 species. We saw some variation at longer times using 1 ug/ml, but this may have been due to one trial rapidly spiking to 70% mortality near 55 minutes (Fig.10 and 11A). Mortality rates at 10 ug/ml were consistent for both species at most times with one trial reaching near 100% mortality at 60 minutes (Fig. 10 and 11B). *Aedes vexans* showed a slightly higher mortality rate at 20 ug/ml for between 30 and 60 minute time intervals (Fig.10) with 1 trial reaching 100% mortality at 40 minutes and a second at 60 minutes (Fig. 11C). Mortality rates at 40 ug/ml for *Cx. tarsalis* were slightly higher than *Ae. vexans* at earlier times; however, both species reached 100% mortality at approximately the same time interval (Fig. 10). Mortality rates reached 100% in all trials within the bioassay time limit. Using a diagnostic time longer than 30 min is likely inadvisable, as both species showed 100% mortality soon after that for the 40 µg/ml concentration (Fig 10).

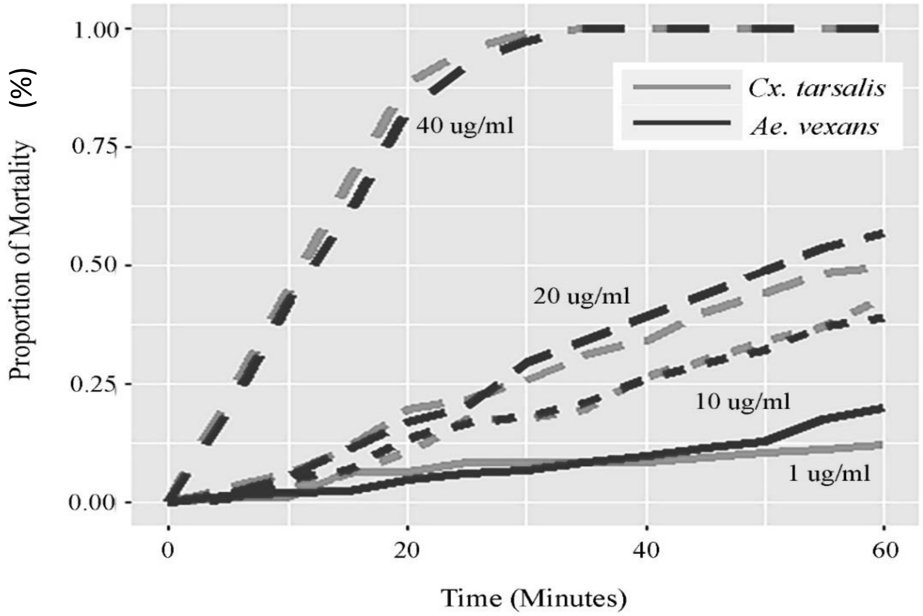

Figure 10: Observed mortality rates for *Cx. tarsalis* and *Ae. vexans* as a function of time for various concentrations of permethrin in 2016.

Table 5. LC98 Diagnostic doses and times for *Cx. tarsalis* and *Ae. vexans* with lower (LCL) and upper (UCL) confidence limits.

Species	Time (min)	Diagnostic dose (ug/ml)	LCL (ug/ml)	UCL (ug/ml)
Cx. tarsalis	30	38.3	36.9	40.0
Ae. vexans	30	38.4	37.0	40.0
Cx. tarsalis	45	32.1	30.8	33.6
Ae. vexans	45	31.8	30.5	33.3
Cx. tarsalis	60	27.5	26.3	28.8
Ae. vexans	60	27.0	25.9	28.3

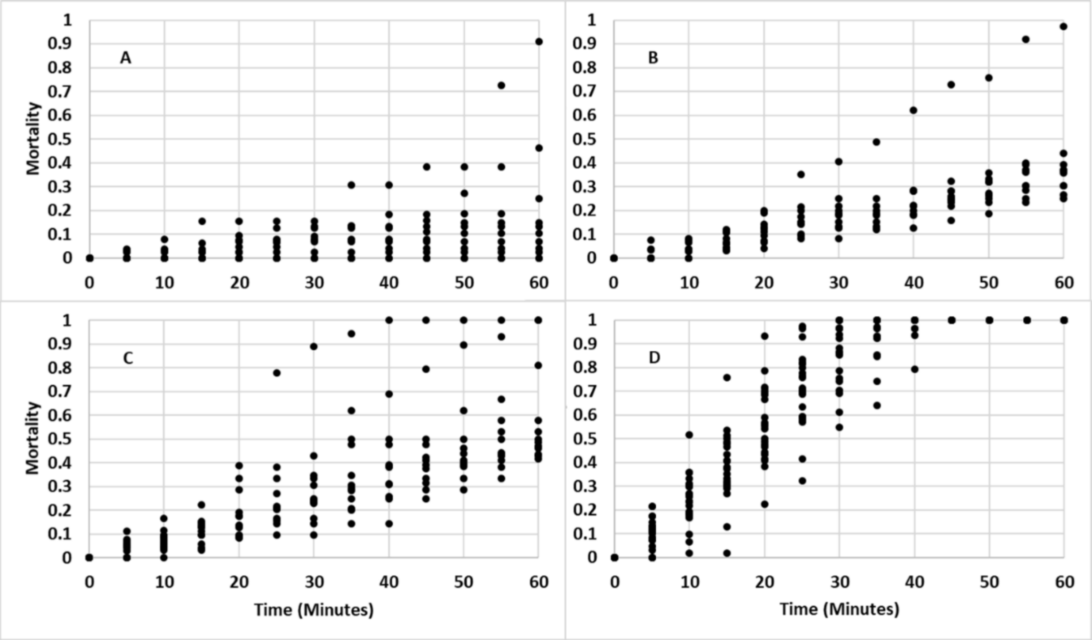

Figure 11: Observed mortality rates for each bottle bioassay replicate at time intervals for 1 ug/ml (A), 10 ug/ml (B), 20 ug/ml (C), and 40 ug/ml (D) of permethrin.

A likelihood ratio test between the main model, used to calculate LC98s, and a simplified model, in which species was no longer a predictor, indicated that the mortality rates of the 2 species had statistically indistinguishable rates of mortality over concentration and time (p = 0.7803), and any function of model estimates (such as the diagnostic dose for a given time) is unlikely to differ between the 2 species.

During the 2017 study, the estimated diagnostic doses determined in 2016 were rounded to 38 µg/ml for both species at 30 minutes. This dose was chosen rather than the estimated LC98s both for convenience and also so that at least some (approximately 1 in 20) mosquitoes should be alive at 30 min. The mortality rates based on our model's diagnostic dose estimated mortality at 30 minutes to be 94.93% for *Ae. vexans* and

93.38% for *Cx. tarsalis*. In early 2017, 380 *Ae. vexans* and 123 *Cx. tarsalis* adult females were used to validate these calculations. Observed mortality rates for mosquitoes at the diagnostic time for *Ae. vexans* populations collected in 2017 at Oakwood State Park were 297/309 = 96.12% with a 95% exact confidence interval of 93.31 to 97.98%. Mortality rates for *Cx. tarsalis* collected at Oakwood State Park at the diagnostic time were 114/123 = 92.68% with 95% exact CI (86.56, 96.60%). In all cases, the 95% CI for observed mortality in 2017 contained the expected mortality rate estimated in 2016. For both species, estimated and observed mortality rates as functions of time are displayed in Figure 15.

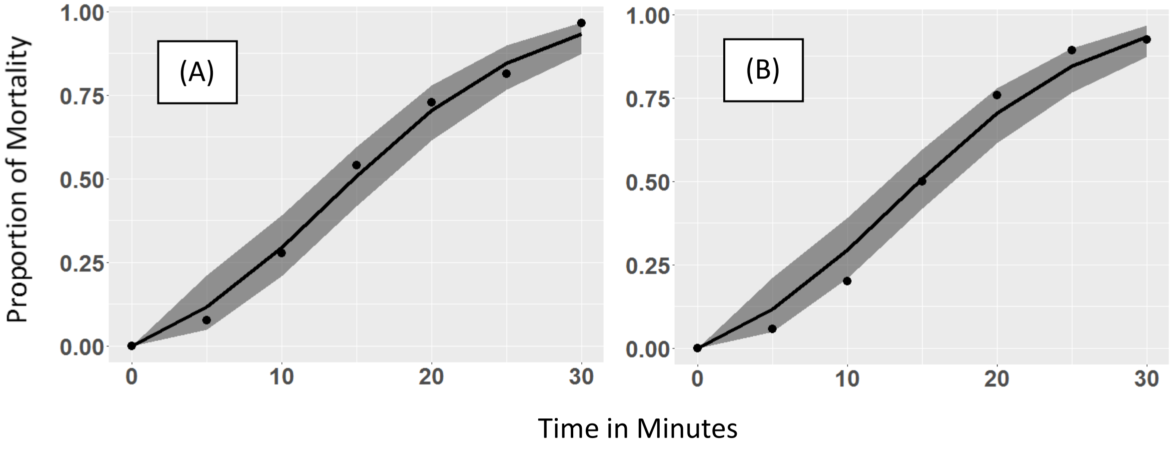

Figure 12: Bioassay mortality rates for *Ae. vexans* (A) and *Cx. tarsalis* (B) collected in 2017 (solid circles) using 38 µg/ml of permethrin, against estimated calibration curve (line) with 95% confidence interval (grey shaded band) generated from data collected in

Discussion

This study is the first direct comparison of the permethrin susceptibilities of a major vector for WNV, *Cx. tarsalis*, to a major nuisance mosquito, *Ae. vexans*, where WNV is endemic to the region, and was conducted using collected adult mosquito from eastern South Dakota exposed to multiple concentrations of permethrin in a CDC bottle bioassay. Scatter-plots of the data showed that while there were some variations in individual bottles, there was good consistency in bottles for each permethrin concentration (Fig.11). The overall results showed similar mortality rates at all times for all concentrations between *Cx. tarsalis* and *Ae. vexans,* and we found no statistical difference between the calculated mortality rates of these 2 species. Comparisons were made based upon calculated diagnostic dose and times as recommended by CDC when testing for insecticide resistance (CDC 2013), and the similarities between each species were also reflected in the calculated diagnostic doses and times. The susceptibilities of *Ae. vexans* and *Cx. tarsalis* remained constant between 2016 and 2017, between 2 study sites, and within and between both species. A diagnostic dose and time has not been previously reported for either of these species. Our calculated value for both species was 38 µg/ml in 30 min, which is more than twice the recommended diagnostic dose and time of 15 µg/ml at 30 min listed for *Aedes spp.* in the CDC bioassay protocol (CDC 2013). A California study used 30 µg/ml of permethrin and was unable to knockdown 50% of their wild-caught *Cx. pipiens* (McAbee et al. 2004). Richards et. al. (Richards et al. 2017) found 2 *Aedes* spp. collected from 13 different locations within the United States that were either susceptible or possibly resistant with mortality rates ranging from 91%

to 100% using 15 µg/ml doses at 30 min. This same study tested 26 *Cx. pipiens* collected from St. Paul, Minnesota and achieved 96% mortality using 15 µg/ml permethrin at 30 min. A similar study to ours tested 49 *Cx. tarsalis* adults reared from field caught larva and determined a median lethal dose of 50 µg/ml; however, they reported that this same concentration caused 100% mortality the following year and had to reduce their median lethal dose to 10 µg/ml (Strong et al. 2008).

Our trials were able to attain consistent results from mosquitoes with varying physiological attributes between years and geographical locations, and the results should encapsulate the variation found in the wild. This is essential in disease control and mosquito abatement programs whose primary concern is to reduce vector and/or nuisance populations (Brogdon and McAllister 1998a) We determined multiple options for base-line diagnostic doses and times that can be used in the Northern Great Plains. By using this method, we have a basis for comparing various species levels of susceptibility and allows for future testing and comparisons of resistance in and between *Cx. tarsalis* and *Ae. vexans* using the CDC bottle bioassay.

Both the vector and nuisance mosquitoes are heavily targeted within the state of South Dakota due to the former's ability to transmit WNV and the latter's large abundance creating a nuisance for residents. Since nuisance mosquitoes may help motivate humans to seek shelter or use personal protection (Gujral et al. 2007), higher susceptibility to insecticides in nuisance mosquitoes compared to vector mosquitoes may cause mosquito control efforts to actually increase human risk for WNV. Our findings showed that the susceptibility to permethrin between the WNV vector, *Cx.*

tarsalis, and the nuisance mosquito, *Ae. vexans,* were the similar in this region and that treatment will be equally effective, thus it will not diminish the nuisance mosquito more than the vector. There is a great need for future studies to understand the level of insecticide resistance developing in vector mosquitoes throughout the United States; however, these studies should also include predominant nuisance mosquitoes in areas where both groups are abundant to ensure that susceptibility of the vectors are similar or higher so that attempts to control vector species will not have a significantly greater effect on nuisance mosquitoes, and thus lowering the avoidance behaviors in humans.

Final Discussion

This body of work, as a whole, was designed to use multiple methods of surveillance to enhance the body of knowledge surrounding the risk mosquitoes pose in in three areas: 1) Assess the current mosquito fauna presence and abundance in South Dakota, 2) evaluate the vector risk for these mosquito species and compare their abundance to virus amplification, and 3) evaluate the effectiveness of a commonly use insecticide on the primary mosquitoes of South Dakota.

An older study in South Dakota determined the mosquito fauna through the use of collection techniques as well as data from other authors (Gerhardt 1966). The author from the study acknowledged that their data set was limited; however, this list compiled from Gerhardt contained more species than what we found in the course of our study. This may be due to some mosquitoes having geographical limitations and do not appear in eastern South Dakota, such as *Culiseta impatiens* (Walker) that inhabits coniferous forests in the western region of South Dakota (Gerhardt 1966). His study and ours do agree that after almost 50 years, *Ae. vexans* and *Cx. tarsalis* are still the most abundant mosquito species. However, our data shows that the peak abundance for *Cx. tarsalis* to be in July compared to Gerhardt's observation of their peak a couple weeks before fall (Gerhardt 1966). Another study located in north central South Dakota also showed that *Ae. vexans* and *Cx. tarsalis* to be the most abundant species during their survey in 1984 and 1985 (Easton 1987b). This study was able to concur with our study that *Cx. tarsalis* is at its most abundant during July.

A short coming of these previous studies was a lack of geospatial comparisons. These previous studies showed *Cx. tarsalis* to be the most abundant ins Lincoln/Minnehaha counties, but our study showed *Ae. vexans* to be the most abundant. In Hughes county, Gerhardt reported *Ae. vexans* to be the most abundant mosquito species while this current study not only shows that *Cx. tarsalis* was the most abundant, but that *Ae. vexans* abundance was the lowest of the regions that identified this species.

The increase in *Cx. tarsalis* abundance from the eastern portion of the state to the central portion has similar patterns in other surrounding midwestern states. *Culex. tarsalis* is far less abundant in Iowa while having higher numbers of *Cx. pipiens.* In Nebraska, mosquito surveys showed the abundance of *Cx. tarsalis* increased from east to west while the abundance of non-*Cx. tarsalis* mosquitoes decreased in overall abundance towards the central portions of the state. Another Important WNV vector, *Cx. pipiens,* falls in abundance as we move westward in South Dakota. Iowa reported this mosquito as their second most abundant mosquito, while in Nebraska, both its abundance and relative abundance drops progressively towards the west. This geospatial shift in mosquito species abundance may be important in monitoring human risk of mosquito transmitted pathogens. Differences in relative abundance between vectors and non-vectors can impact amplification of viruses and change human avoidance behaviors if important nuisance mosquitoes are not present.

Recently, South Dakota has used a new model which has shown success over the past few years (Davis et al. 2017). This new technique uses mosquito infection data along with climate variables to predict human risk of WNV, however, the model does not use any mosquito abundance data. Our statistical models were able to find a relationship between the weekly abundance of *Cx. tarsalis* and human infection of WNV particularly at the two and three week lags, which is expected as diagnosis of WNV in humans can take weeks after the infectious bite, though this may also be a result of the seasonality of both the vector and human cases of WNV. Abundance is often used as a part of an equation to calculate a vector index when predicting WNV risk to humans (Kilpatrick and Pape 2013). We also investigated if a relationship of the proportion of the vector mosquito to the total population, we found this relationship to be not as strong than *Cx. tarsalis* alone*.* While we did find a statistically significant and strong association between *Cx. tarsalis* and human infection of WNV, seasonality was still a better predicter. Even though the strength of the correlation when including other non-vector mosquitoes was weaker, the impact of non-vector mosquitoes may still be an important factor in WNV transmission through its biting pressure it applies to humans.

Understanding of the population dynamics of the state's vector mosquitoes is an important resource to state health departments and mosquito abatement programs. Understanding the ebb and flow of the vector abundance can help direct resources to specific times within a season. The use of historical mosquito data and recent meteorological events can allow for efficient vector collections for pathogen testing. This also allows for mosquito abatement programs to proactively treat for prevalent nuisance mosquito populations before they can impact the public. Though our study did not find a strong relationship between vector and nuisance mosquito populations on human WNV transmission at a state wide level, additionally studies at local levels where human WNV transmission is common may yield further insight to how population dynamics of the mosquitoes present may influence human infection of WNV.

Our goal was to determine if the abundance of mosquitoes considered competent for transmission of West Nile virus found in South Dakota influence overall infection rates found in South Dakota. We first looked at the mosquitoes that have been tested in South Dakota to determine those species that have been found positive for WNV and the rate in which they were.

Aedes fitchii had the highest MIR of all the WNV positive mosquitoes tested in South Dakota but was also the least tested mosquito and is not often collected in traps. *Aedes cinereus* had the third highest MIR; however, this mosquito is also rarely found in South Dakota traps. Both of these species may be limited in collection due to being univoltine species that generally emerge between April and June (Ross 1947), thus making them unlikely candidates for amplification of the virus, and thus removed from comparisons to MIR and MIGR comparisons.

The two species generally considered to be the vectors for human WNV infection remain higher end of the MIR scale in South Dakota. The *Cx. pipiens/restuans* species had a higher MIR across all years studied and when evaluation infection rate between 2002 and 2004 only when compared to *Cx. tarsalis.* Though infection rates are higher, the abundance of *Cx. tarsalis* is far greater than *Cx. pipiens/restuans* in all regions of the state, making *Cx. tarsalis* the more likely primary vector for WNV in South Dakota.

However, *Cx. pipiens/restuans* should be considered as a possible virus amplifier in the wild, especially in eastern South Dakota where it is more abundant. Additionally, *Cx. salinarius* also had a similar MIR value for the years it was tested. This mosquito has been collected less than both *Cx. tarsalis* and *Cx. pipiens/restuans*; however, *Cx. salinarius* is known to feed on both birds and mammals and so should also be considered an important vector, especially in years of high virus prevalence.

The remaining species, *Cs. inornata, Ae. dorsalis,* and *Ae. vexans* were on the lower end of the infection rate scale. Though they may have been found to be competent vectors, infection rates may be lower due to their preference for feeding on mammals. *Aedes vexans* is the most abundant mosquito found in the state, and a large number were tested during the first few epidemic years of WNV in the state, yet only one pool tested positive. Both *Cs. inornata* and *Ae dorsalis* had similar MIR values; however, *Cx. inornata* is generally an early spring and *Ae. dorsalis* abundance can fluctuate greatly depending on meteorological conditions, which made them potential candidates for impacting early season amplification of WNV.

It is not entirely surprising that these relationships between the abundance of most of the vectors and infection rate are not positively correlated due to the complexity of the WNV transmission cycle. Modeling attempts using meteorological data, mosquito abundance, mosquito infection rates, vector competency, avian population susceptibilities, host-seeking patterns, host-feeding shifts, and host behaviors have not been perfect at predicting WNV transmission. However, there are patterns within these results that do seem to offer some insights on how some pieces fit into the larger puzzle.

The primary vectors for WNV in South Dakota, *Cx. tarsalis* and *Cx. pipiens* showed very little relationship to rate of infection within themselves. This indicates that while these species have a role in transmitting WNV humans, it may not be directly related to the amplification of the virus, or at the least, is not the limiting factor in the wild. The only species to show any positive correlation when comparing species abundance to traditional calculation of infection rate was *Cs. inornata.* This mosquito is the first to be found in mosquito traps in South Dakota, and is often noted as an early season mosquito in other areas of the United States (Reisen, Meyer, and Milby 1989). Both *Ae. vexans* and *Ae. dorsalis* both showed a negative association with both measurements of infection rate described within this paper. It may not be that these two species directly impact infection rate of *Cx. tarsalis* and may instead indicate that environmental factors that are favorable to these two species is unfavorable to the amplification of WNV.

The effects of temperature on and precipitation between these two species varied in some degrees. The effects of precipitation on *Ae. vexans* abundance was considerably higher than its effects on *Cx. tarsalis,* especially between the two and three week lags. This is somewhat expected as the strategies for reproduction differs between these two species. Where Ae. *vexans* lays its eggs on a dry substrate and waits to be submerged for water, large rain events will trigger the beginning of their life cycle, *Cx. tarsalis* lays its eggs directly on standing water which can exist without a precipitation event. Increased temperatures effects on *Cx. tarsalis* were very similar to the effects of precipitation, yet we saw that increased temperatures have a strong negative effect on *Ae. vexans*. This negative effect may explain the general bimodal abundance pattern we see statewide in *Ae. vexans* across the state. Peak abundance for *Ae. vexans* generally occurs during week 27 (Chapter 2 Figure 4) which is when temperatures begin to reach their highest across the state. High temperatures could then be driving abundance downward until temperatures cool enough to allow for *Ae. vexans* populations to rebound. Temperature has also been strongly associated with higher risk of WNV transmission (Davies in press), which could be the result of lower *Ae. vexans* abundance that could alter human behavior that would make them vulnerable to vector mosquitoes. Precipitation was shown to reduce the overall proportion of the vector to nuisance, this is most likely caused by a dramatic increase in *Ae. vexans* abundance. The weekly average abundance of *Cx. tarsalis* and *Ae. vexans* has previously show to have some relationship to weekly average human cases of WNV. Because of this, predicting increases in abundance of these, and other mosquitoes, is a concern to state health department and mosquito abatement programs.

However, this approach does have some limitations in that it is taking a linear approach to what is possibly a non-linear system. Non-environmental factors, such as immunity levels in reservoir hosts, could dramatically impact amplification of virus in vectors. Additionally, the effects of mosquito abatement programs could reduce amplification by reducing vector abundance. However, if these mosquito control steps should only impact infectivity after they are implemented, and often they are only implemented when nuisance mosquito populations are high, or virus has been detected in vectors of human concern. These measures are unlikely to impact very early season amplification which could be accomplished by *Cs. inornata.* Further studies should be done to investigate the infection rate and roles of early season mosquitoes to the amplification of WNV in the wild. Knowledge of this could lead to factors that are available sooner and with greater cost effectiveness that could increase the lead time for prediction human risk of WNV.

The final study on susceptibility to insecticides is the first direct comparison of the permethrin susceptibilities of a major vector for WNV, *Cx. tarsalis*, to a major nuisance mosquito, *Ae. vexans*, where WNV is endemic to the region, and was conducted using collected adult mosquito from eastern South Dakota exposed to multiple concentrations of permethrin in a CDC bottle bioassay. Scatter-plots of the data showed that while there were some variations in individual bottles, there was good consistency in bottles for each permethrin concentration (Fig. 15). The overall results showed similar mortality rates at all times for all concentrations between *Cx. tarsalis* and *Ae. vexans,* and we found no statistical difference between the calculated mortality rates of these 2 species. Comparisons were made based upon calculated diagnostic dose and times as recommended by CDC when testing for insecticide resistance [17], and the similarities between each species were also reflected in the calculated diagnostic doses and times. The susceptibilities of *Ae. vexans* and *Cx. tarsalis* remained constant between 2016 and 2017, between 2 study sites, and within and between both species. A diagnostic dose and time has not been previously reported for either of these species.

Our calculated value for both species was 38 µg/ml in 30 min, which is more than twice the recommended diagnostic dose and time of 15 µg/ml at 30 min listed for *Aedes spp.* in the CDC bioassay protocol (CDC 2013). A California study used 30 µg/ml of permethrin and was unable to knockdown 50% of their wild-caught *Cx. pipiens* (McAbee et al. 2004). Richards et. al. (Richards et al. 2017) found 2 *Aedes* spp. collected from 13 different locations within the United States that were either susceptible or possibly resistant with mortality rates ranging from 91% to 100% using 15 µg/ml doses at 30 min. This same study tested 26 *Cx. pipiens* collected from St. Paul, Minnesota and achieved 96% mortality using 15 µg/ml permethrin at 30 min. A similar study to ours tested 49 *Cx. tarsalis* adults reared from field caught larva and determined a median lethal dose of 50 µg/ml; however, they reported that this same concentration caused 100% mortality the following year and had to reduce their median lethal dose to 10 µg/ml (Strong et al. 2008).

Our trials were able to attain consistent results from mosquitoes with varying physiological attributes between years and geographical locations, and the results should encapsulate the variation found in the wild. This is essential in disease control and mosquito abatement programs whose primary concern is to reduce vector and/or nuisance populations (Brogdon and McAllister 1998a) We determined multiple options for base-line diagnostic doses and times that can be used in the Northern Great Plains. By using this method, we have a basis for comparing various species levels of susceptibility and allows for future testing and comparisons of resistance in and between *Cx. tarsalis* and *Ae. vexans* using the CDC bottle bioassay.

Both the vector and nuisance mosquitoes are heavily targeted within the state of South Dakota due to the former's ability to transmit WNV and the latter's large abundance creating a nuisance for residents. Since nuisance mosquitoes may help motivate humans to seek shelter or use personal protection (Gujral et al. 2007), higher susceptibility to insecticides in nuisance mosquitoes compared to vector mosquitoes may cause mosquito control efforts to actually increase human risk for WNV. Our findings showed that the susceptibility to permethrin between the WNV vector, *Cx. tarsalis,* and the nuisance mosquito, *Ae. vexans,* were the similar in this region and that treatment will be equally effective, thus it will not diminish the nuisance mosquito more than the vector. There is a great need for future studies to understand the level of insecticide resistance developing in vector mosquitoes throughout the United States; however, these studies should also include predominant nuisance mosquitoes in areas where both groups are abundant to ensure that susceptibility of the vectors are similar or higher so that attempts to control vector species will not have a significantly greater effect on nuisance mosquitoes, and thus lowering the avoidance behaviors in humans.

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