

THE ROLE OF TEMPERATURE, WIND SPEED, AND PRECIPITATION ON THE ABUNDANCE OF *CULEX* SPECIES AND WEST NILE VIRUS INFECTION RATE IN RURAL WEST-CENTRAL ILLINOIS

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ABSTRACT. While most research on West Nile virus (WNV) and its main vector, the *Culex* mosquito, has been conducted in laboratory or urban settings, studies with field-caught mosquitoes in rural areas, such as west-central Illinois, are lacking. The objective of this research was to investigate key abiotic factors using macroclimate data, including temperature, precipitation, and wind speed, to determine their influence on field-caught mosquito abundance in 4 rural counties in Illinois from 2014 to 2016. Additionally, the relationship between minimum infection rate (MIR) and thermal time was examined. Using gravid traps at 15 sites, *Culex* mosquitoes were collected twice a week. A total of 5,255 adult female *Culex* mosquitoes (*Cx. pipiens*, *Cx. quinquefasciatus*, and *Cx. restuans*) were collected in 2014; 9,138 in 2015; and 5,702 in 2016. Regression models were developed based on outcomes of relationships between field-caught mosquitoes and abiotic factors. Precipitation and thermal time had the most significant relationship with mosquito abundance ($r^2 = 0.993$ and $r^2 = 0.993$, respectively), while wind speed was less ($r^2 = 0.714$). The greatest number of *Culex* and the highest annual MIR were observed in 2015, which was also the driest of the 3 sampling seasons. Mosquito abundance was observed to increase with warmer degree days and MIR was found to increase with abundance in mosquitoes. These models can be used for other mosquito surveillance and monitoring studies in various climate types and environments.

KEY WORDS abiotic factors, *Culex*, mosquito abundance, regression model, West Nile virus

INTRODUCTION

West Nile virus (WNV) was introduced to the United States in 1999 and to Illinois in 2001 (IDPH 2023). The *Culex* genus of mosquitoes is widely known as the main vector for WNV, which is maintained in an enzootic transmission cycle using avian and sometimes accidental mammalian hosts to replicate. This includes its primary reservoir host, the bird, specifically corvid species (Vogels et al. 2016, CDC 2023); these hosts are also known as amplifier hosts due to the virus's ability to reach high viral copy number in birds. The mosquito and WNV life cycle and distribution can be influenced by many factors such as location (urban versus rural) and various abiotic factors including temperature, precipitation, and wind speed (Di Pol et al. 2022). Successful development of the mosquito life cycle depends on the correct abiotic factors (Yoo et al. 2016). While wind can alter adult mosquito behavior and flight patterns, sufficient precipitation is needed for the larval stage to thrive and for some female mosquitoes, including *Culex* species, to oviposit their eggs on water surfaces (Rosa et al. 2014).

The distribution and intensity of vector-borne pathogen transmission is strongly influenced by the

interaction of temperature, vectors, and hosts (Kilpatrick et al. 2008). Temperature has the potential to increase growth rates of vector populations (Ruiz et al. 2010, Dale et al. 2023) by enhancing mosquito reproduction rates and extending breeding seasons, creating a longer contact time period between vector and host. Additionally, as increased temperature lengthens suitable environmental conditions for vector breeding, viral replication is increased within the mosquito itself (Paz and Albersheim 2008, Moser et al. 2023). The replication cycle of WNV accelerates with warmer temperatures, therefore increasing the concentration within a host as well (Kilpatrick et al. 2008, Paz and Albersheim 2008).

After overwintering, adult mosquitoes take an initial blood meal in the spring; this is a temperature dependent event that varies with species (Kunkel et al. 2006). For example, the lower development threshold temperature for *Cx. pipiens* L. has been observed at 5.5°C (42°F) (Loetti et al. 2011). Thus, changes in temperature have been observed to be associated with the intensity of pathogen transmission, causing changes in the extrinsic incubation period, longevity, and feeding rate of vectors (Kunkel et al. 2006, Kilpatrick et al. 2008, Paz and Albersheim 2008, Di Pol et al. 2022). Kunkel et al. (2006) observed increasing temperature to alter the time, or crossover dates, in which two *Culex* spp. (*Cx. pipiens* and *Cx. restuans* Theobald) most likely to vector WNV are equal, encouraging earlier appearances of the virus and resulting in a greater likelihood of transmission.

During a WNV outbreak in Chicago, IL (2004–2008), years that reported elevated numbers of WNV cases corresponded with warmer than average temperatures (Ruiz et al. 2010). Warmer spring months

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were observed to encourage early emergence of adults from hibernation, leading to a greater abundance of mosquitoes in later summer months (Walsh et al. 2008). At much warmer temperatures, the mosquito biting rate increased; the frequency between blood meals was observed to increase linearly with temperature (Ruybal et al. 2016).

Additionally, mosquito populations depend on the distribution of precipitation with daily amounts being an important determining factor of mosquito abundance (Valdez et al. 2017). With equally distributed rainfall over several days, mosquito populations will increase, yet when rainfall ceases, oviposition sites and larval habitats are adversely impacted, which causes populations to shrink (Gardner et al. 2014, Valdez et al. 2017). On the other hand, too much rainfall can also have an adverse effect on larvae by flushing breeding sites and affecting the bacterial contents of larval habitats (Ruiz et al. 2010, Valdez et al. 2017, Shutt et al. 2022).

While many studies of abiotic factors and their respective roles on vector competence have been conducted in the laboratory, this study collected data from a field setting, including macroclimate data and an assembly of real organisms over multiple years. Cass, Fulton, McDonough, and Schuyler counties, located in west-central Illinois, are rural. Here, rural is defined as the individual areas not delineated as urban (USCB 2010). In west-central Illinois counties, Cass is composed of approximately 52% rural land, Fulton is composed of 60% rural land, McDonough is composed of 29.5% rural land, and Schuyler is composed of 57.6% rural land. On the other hand, Cook and DuPage counties in the greater Chicago metropolitan area each have approximately 0.05% rural land (USCB 2010). Rural counties assessed in this study are at an increased risk of WNV infection due to growing elderly populations, lack of awareness of vector-borne diseases and their vectors, and larger numbers of individuals employed in agriculture operations, as all of these factors increase the risk for WNV infection and vector-host contact (Ruiz et al. 2004).

The objective of the present study was to develop models based on field collected data of a real assemblage of organisms over multiple seasons and locations demonstrating a relationship between them and abiotic factors (temperature, wind speed, and precipitation) and their association with WNV. More specifically, the goal was to establish which abiotic factor(s) are most important within west-central Illinois that also influence the abundance of WNV-positive mosquitoes. This is a field-based study over a spatial and temporal extent using macroclimate data to determine or verify relationships between mosquito abundance and key abiotic factors. The findings of this study can lead to an understanding of what abiotic factors drive mosquito abundance and WNV infection in a rural Midwestern area that can be applied to other locations.

MATERIALS AND METHODS

Mosquito traps and collections

Mosquito surveillance was conducted in west-central Illinois throughout Cass, Schuyler, Fulton, and McDonough counties from June through September 2014, June through October 2015, and May through October 2016. Traps were deployed at 15 sites (Fig. 1a and Fig. 1b) across the four counties. Sample locations contained suitable habitats for *Culex* mosquitoes: populated/residential, agricultural/farmland, and marshy wetlands. Mosquitoes were collected using CDC Gravid Traps (Model 1712; John W. Hock Company®, Gainesville, FL). The natural water sources favored by *Culex* mosquitoes were mimicked in the traps to attract female mosquitoes to oviposit. This included approximately 30 gal (114 liters) of water and 0.5 kg of dissolved organic materials. The organic materials used to mimic the natural habitat included rabbit chow infusions and animal feces. This created the foul, dirty water that *Culex* spp. prefers.

Adult mosquito collections took place twice weekly from June 19 through September 30, 2014, June 19 through October 2, 2015, and May 25 through October 11, 2016. Note that in 2014, traps were only set in Cass, Fulton, and McDonough counties; sites in Schuyler County were added in 2015. After collection, female *Culex* mosquitoes were sorted, counted, and stored for further analysis at 20°C in labeled, sterile Eppendorf tubes grouped according to trap site and date. Mosquito identification was completed using existing morphological keys, including the Illinois Natural History Survey's synopsis of Illinois mosquitoes (Ross and Horsfall 1965) and Darsie and Ward's (2005) *Identification and Geographical Distribution of the Mosquitoes of North America, North of Mexico*.

Abiotic factor data

During 2014–2016, local weather data were acquired from the Macomb Municipal Airport (MQB) in Macomb, IL, to the west, and the General Wayne A. Downing Peoria International Airport (PIA) in Peoria, IL, to the east (Fig. 1a). Any missing atmospheric data were collected from the Midwestern Regional Climate Center (MRCC) and Weather Underground. MQB and PIA are separated by 87 km (54 mi) and have topographic relief and surrounding land use and land cover that are largely representative of conditions across the study sites. Atmospheric data were obtained from Automated Surface Observation System (ASOS) stations at MQB and PIA (NOAA 2017) and averaged on a daily or weekly basis as needed prior to being used in subsequent data analyses.

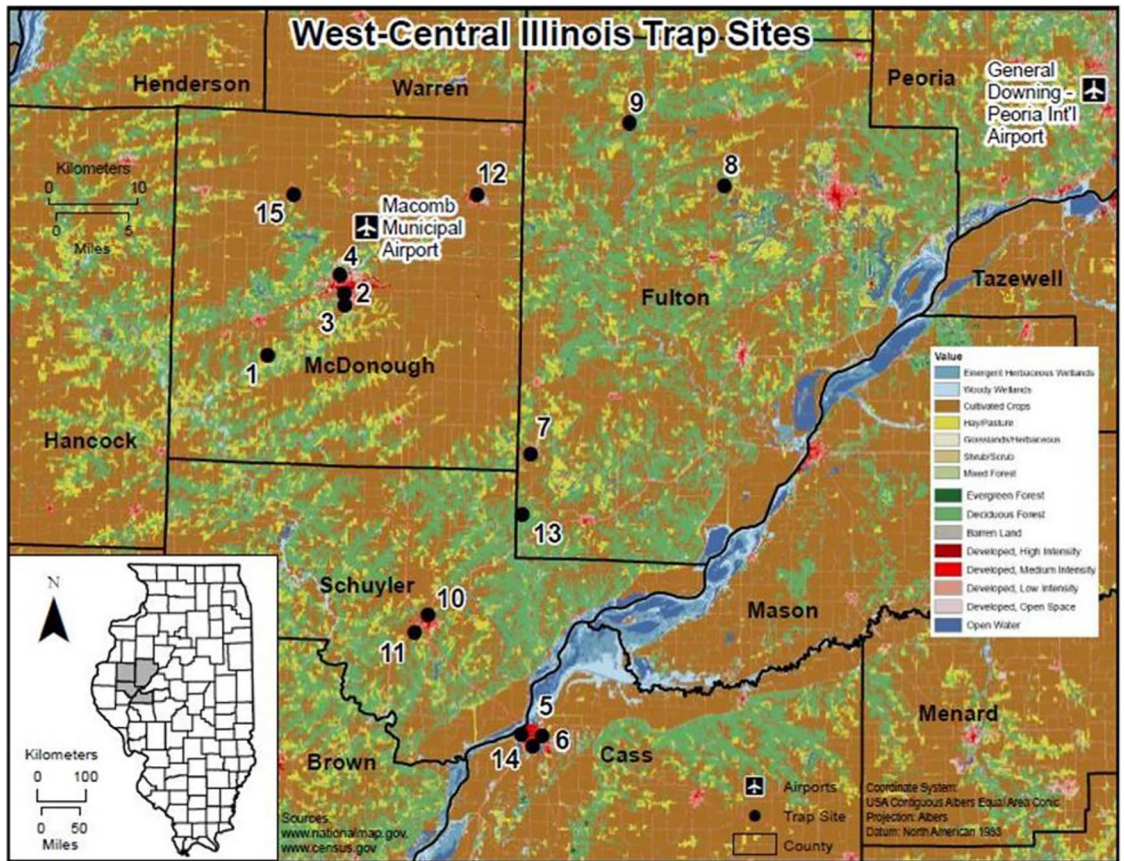


Fig. 1a. West-central IL sites displaying the land use land cover of where traps were deployed across four counties. Local weather data were collected from the Macomb Municipal Airport and General Wayne A. Downing Peoria International Airport in McDonough County and Peoria County, respectively. There were 15 mosquito surveillance sites; numbers in this figure represent the locations of these sites.

WNV assays

Testing for WNV was performed using two techniques: VecTest® WNV antigen assay (Medical Analysis Systems, Camarillo, CA) and the rapid analyte measurement platform (RAMP®) WNV test (Response

Biomedical Corporation, Vancouver, BC, Canada). The VecTest and RAMP sample methods for the separate 3 field seasons are displayed in Table 1.

Infection rates were calculated from those which tested positive for WNV (Table 2). The infection rates are estimates of the number of mosquitoes that

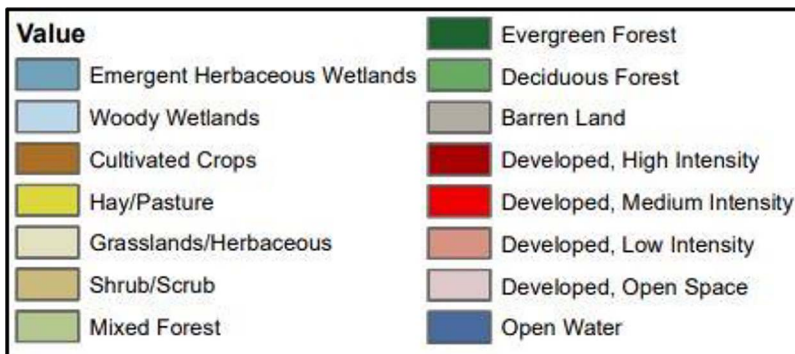


Fig. 1b. Land use land cover (LULC) legend for the map in Fig. 1a enlarged.

Table 1. Summary of sampling duration, location, and West Nile virus test method used in field studies.

| Sampling season | Counties sampled | WNV assay method | |
|-----------------|-----------------------------------|--------------------|--------------------------------|
| | | VecTest Site # | RAMP test, ¹ Site # |
| 2014 | McDonough, Fulton | 1, 2, 3, 4, 12, 15 | 7, 8, 9, 13 |
| 2015 | McDonough, Fulton, Schuyler, Cass | - | 1-15 |
| 2016 | McDonough, Fulton, Schuyler, Cass | - | 1-15 |

¹ RAMP® ≥ 100 units for positive WNV finding in the state of Illinois.

could possibly be carrying the virus per 1,000 mosquitoes. Infection rates were determined using the CDC’s “PooledInfRate V.4.0” program with a 95% confidence rate (Biggerstaff 2009).

Thermal time

To standardize the calculation of thermal time over multiple sampling seasons, thermal time was determined January 1 of each year. Since traps were emptied approximately twice a week, a weekly timeframe was used in subsequent analyses as opposed to the more common daily time interval used in phenological studies. The average weekly thermal time and accumulated thermal time were determined according to Equations 1 and 2, respectively.

$$\Delta t = t_w - t_{lo} \begin{cases} \Delta t \leq 0, & t_t = 0 \\ \Delta t > 0, & t_t = \Delta t \end{cases} \quad (1)$$

where t_w = average weekly temperature, t_{lo} = lower thermal developmental threshold for female *Cx. pipiens* mosquitoes (5.5°C [42°F] per Loetti et al. (2011) t_t = average weekly thermal developmental time

$$T_{t_n} = \sum_{i=1}^n t_{t_i} \quad (2)$$

Where n = week of accumulation and i = individual weekly thermal time (i = 1, 2, . . . , 52).

Table 2. Summary of *Culex* collected, WNV positive pools, annual MIR, and sampling duration and summary of degree week and thermal time in 2014, 2015, and 2016.

| | 2014 | 2015 | 2016 |
|--|-----------------|-----------------|-----------------|
| Total <i>Culex</i> collected | 5,255 | 9,138 | 5,702 |
| Number WNV positive pools ¹ | 3 | 31 | 11 |
| Annual minimum infection rate (%) ² | 0.57 | 3.4 | 1.9 |
| Sampling duration (weeks) | 14 | 19 | 19 |
| First annual accumulation of thermal time (week number) | 2.0°C·wk (14) | 2.4°C·wk (11) | 5.4°C·wk (11) |
| Thermal time accumulated at start of annual sampling (week number) | 133.0°C·wk (25) | 94.6°C·wk (22) | 83.9°C·wk (22) |
| Thermal time accumulated at peak <i>Culex</i> collection (week number) | 281.6°C·wk (34) | 305.5°C·wk (34) | 173.7°C·wk (27) |
| Thermal time accumulated over annual sampling period | 314.2°C·wk | 295.6°C·wk | 317.8°C·wk |
| Annual thermal time accumulated | 389.1°C·wk | 446.2°C·wk | 458.5°C·wk |

¹ 25 mosquitoes per pool; a “positive” pool can contain between 1 and 25 “positive” mosquitoes.

² Determined using PooledInfRate, V.4.0 (Biggerstaff 2009).

Abiotic factor collection

Precipitation, wind speed, and temperature were collected daily for each sampling period throughout the sampling field seasons. HOBO® data loggers (Onset, Bourne, MA) were attached at each mosquito trap to collect hourly air temperature. Precipitation and wind speed were collected from the Macomb Municipal Airport in Macomb, IL, and the General Wayne A. Downing Peoria International Airport in Peoria, IL, using an ASOS; this sensor group collects weather observations hourly. Temperature was used to calculate thermal time during the year that sampling occurred. Precipitation was calculated by average daily cumulative amounts (cm) between collection dates, and wind speed was calculated using the average wind speed (m/s) between collection dates. Precipitation amounts of 0–0.5 cm were also investigated for a more detailed look in comparison to *Culex* collected.

RESULTS

The 2014–2015 and 2015–2016 sampling seasons yielded 5,255, 9,138, and 5,702 field-caught *Culex* mosquitoes, respectively. The total number of *Culex* and corresponding WNV minimum infection rates (MIR) are presented in Table 2; the assay method used at each site was presented in Table 1. Cass County had no WNV positive samples detected using VecTest. In McDonough County, one pool of

mosquitoes tested positive for WNV from a September 14, 2014 collection using VecTest. A sample from Fulton County was positive from a collection date of September 4, 2014. This sample yielded a high reading of 443.8 units using the RAMP system yet failed to produce a positive result on the VecTest.

When examining the relationship between MIR and the number of *Culex* collected, a linear relationship was observed ($r^2 = 0.980$). Equation 3, where C is the number of *Culex* collected in a season, was used to calculate *Culex* collected and MIR from the combined seasons 2014–2016.

$$MIR = (7.0 \times 10^{-4})C - 2.49 \tag{3}$$

where MIR = annual minimum infection rate and C = the total number of *Culex* collected in a sampling season.

Regression model

Calculating thermal time was previously described; the general linear model used for *Culex* collected and thermal time was

$$C = mT_{t-s} + b$$

Where C = annual *Culex* collected and T_{t-s} = thermal time accumulated over an annual sampling period, ($^{\circ}C \cdot wk$).

The general model used for *Culex* collected and wind speed was

$$f(x) = e^{-(a^2(x-b)^2 - c)}$$

where a , b , and c are constants

If “ a ” is a constant, then a^2 is also a constant. We can call the new constant “ d ”; now we can rewrite the model as

$$f(x) = e^{-(d(x-b)^2 - c)}$$

What the constants do and what they correspond to:

- d = controls the width of the *Culex* peak. Higher “ d ” results in a narrower peak.
- b = sets the center of the *Culex* peak; higher “ b ” moves the center of the peak to the right and lower “ b ” moves the center of the peak to the left.
- c = sets the amplitude of the *Culex* peak. Higher “ c ” results in a higher peak amplitude; lower “ c ” reduces the peak amplitude.

The general model used for *Culex* collected and precipitation was

$$f(x) = ke^{-\frac{x}{n}}$$

Where k and n are constants. We can further write the equation as

$$f(x) = ke^{-x*p}$$

p = precipitation

Thermal time

Since mosquito development is known to be a temperature driven process, the relationship between *Culex* collected and thermal time was examined. A linear relationship was observed between annual *Culex* collected and thermal time accumulated (see Equation 4, $r^2 = 0.993$).

$$C = 32.25T_{t-s} - 3005 \tag{4}$$

Where T_{t-s} = thermal time accumulated over annual sampling period, ($^{\circ}C \cdot wk$).

These data were representative of general trends observed between *Culex* collected and thermal time in 2015 and 2016. Data collected over thermal time is presented for the 2014 sampling season in Fig. 2. A summary of thermal time accumulated at various points in each sampling season was presented in Table 2.

Based on the linear relationships determined between *Culex* collected and MIR (Equation 3) and accumulated thermal time-weighted temperature, a further linear relationship ($r^2 = 0.996$) was determined for MIR and *Culex* collected (Equation 5).

$$MIR = 0.026T_{t-s} - 4.764 \tag{5}$$

Where T_{t-s} = thermal time accumulated over annual sampling period, ($^{\circ}C \cdot wk$).

Precipitation

The relationship between *Culex* collected and average daily precipitation, p , for data collected in 2014, 2015, and 2016 is presented and in a detailed view of *Culex* collected over the range $0 \leq p \leq 0.5$ cm in Figs. 3a and 3b. In the raw meteorological data, instances of “trace” precipitation were noted, though no numerical value was provided. In these cases, a value of 2.54×10^{-3} cm (0.001 in.) was used for subsequent data reduction. Based on a visual inspection, a first order relationship between *Culex* collected and average cumulative daily precipitation was examined. Based on nonlinear regression analysis, the specific form of the model is presented in Equation 6 where $r^2 = 0.993$.

$$C = 13965e^{-401p} \tag{6}$$

Where p = average daily precipitation (cm).

Wind speed

The relationship between *Culex* collected and average daily wind speed for data collected in 2014, 2015, and 2016 in west-central Illinois

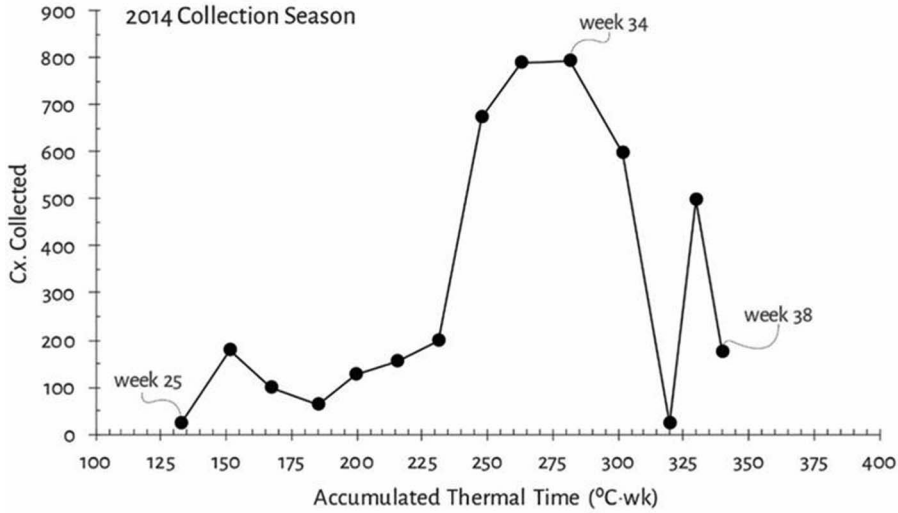


Fig. 2. This is an example of thermal time accumulating over a sampling period of *Culex* mosquitoes collected and thermal time during 2014. Accumulation of thermal time over various time points over the multiple sampling seasons was shown previously in Table 2.

(Fig. 4) was described by a model (Equation 7) of the following general form:

$$f(x) = e^{-(a^2(x-b)^2-c)} \tag{7}$$

Where a, b, and c are constants.

Based on nonlinear regression analysis, the specific form of the model (Equation 8) (Fig. 4) is $r^2 = 0.714$:

$$C = e^{-((0.61)^2(x-2.8)^2-7.9)} \tag{8}$$

Where C = *Culex* collected and w = average daily wind speed (m/s).

Note that there is no reason to assume zero *Culex* collected when the average wind speed is zero.

We have evaluated the ways *Culex* mosquito abundance is influenced by three discrete abiotic factors (thermal time, wind speed, and precipitation). There are no functional relationships between thermal time, wind speed, and/or precipitation; that is,

$$\text{thermal time} \neq f(\text{wind speed}) \neq f(\text{precipitation})$$

$$\text{wind speed} \neq f(\text{precipitation}) \neq f(\text{thermal time})$$

$$\text{precipitation} \neq f(\text{thermal time}) \neq f(\text{wind speed})$$

Consequently, the model of *Culex* abundance as a function of thermal time exists independently of the model for *Culex* abundance as a function of wind speed

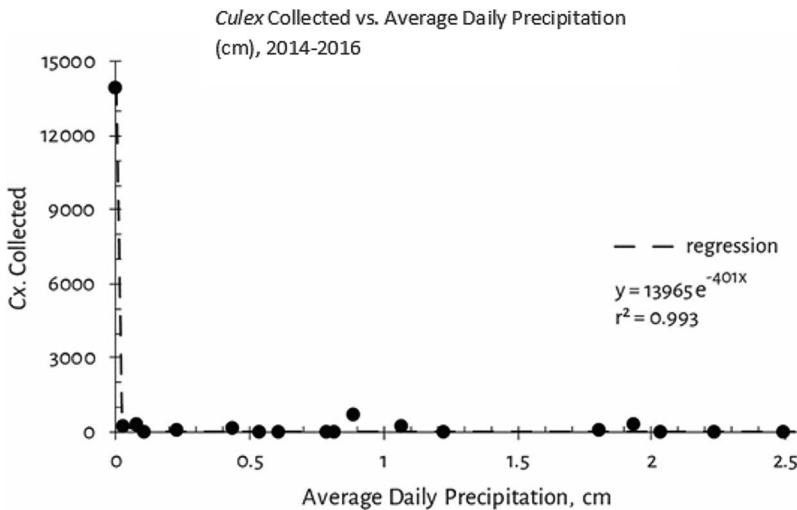


Fig. 3a. Number of *Culex* collected and average cumulative daily precipitation with instances of trace precipitation up to 0.5 cm. *Culex* collected vs. average precipitation remained constant at an average of 401 *Culex* from 0.5 to 8.0 cm.

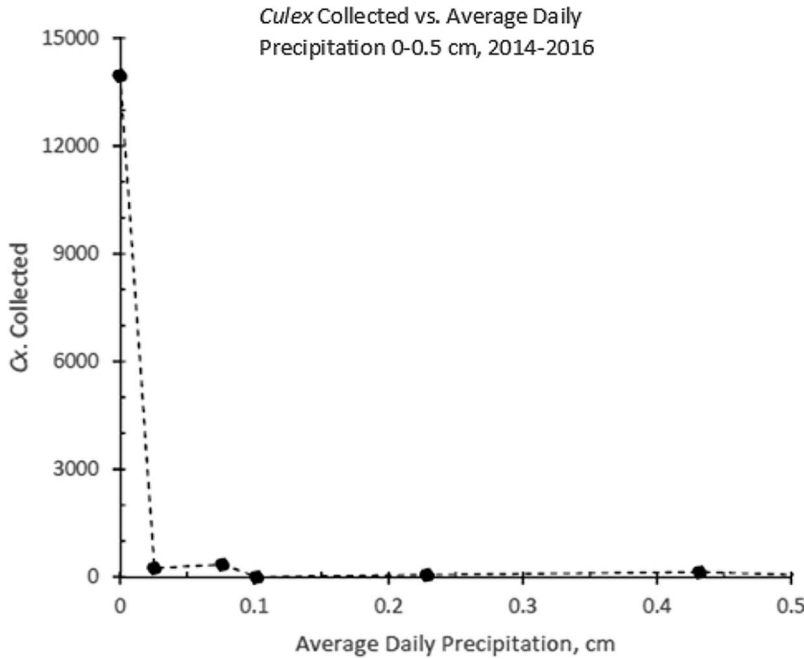


Fig. 3b. Number of *Culex* collected and average cumulative daily precipitation up to 0.5 cm, demonstrating the initial declining trend in detail. Precipitation was collected from local airports Macomb Municipal Airport and General Wayne A. Downing Peoria International Airport; any missing data were collected from the Midwestern Regional Climate Center and Weather Underground.

or precipitation. Therefore, as a result, $Culex \neq f(\text{thermal time, wind speed, precipitation})$; rather, $Culex = f(\text{thermal time})$ and $f(\text{wind speed})$ and $f(\text{precipitation})$.

DISCUSSION

Throughout the USA, other state mosquito surveillance programs have found correlations between

mosquito abundance and environmental variables, including the abiotic factors included here. In Colorado, Louisiana, Pennsylvania, and Nebraska there have been recorded positive correlations with WNV infection, high temperatures, and agricultural land use, while Texas has showed a strong relationship between rural and agricultural land with mosquito abundance (Deichmeister and Telang 2010). Overall,

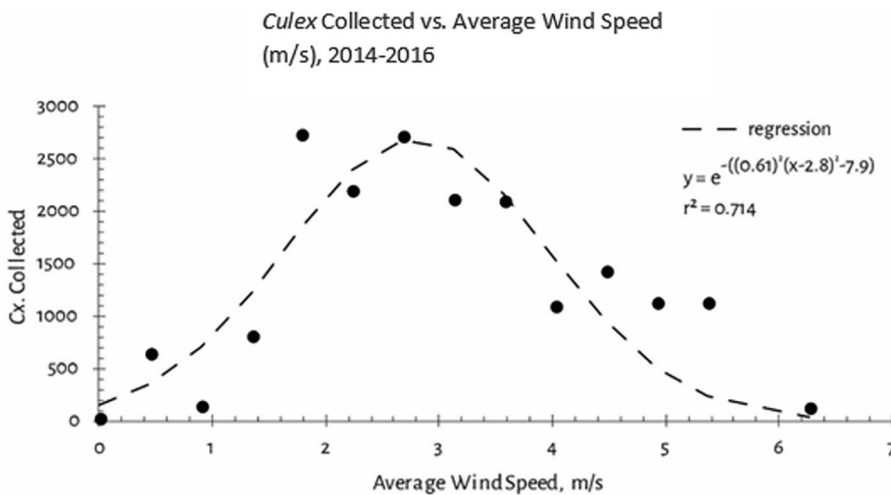


Fig. 4. Number of *Culex* collected and average wind speed during 2014, 2015, and 2016. Wind speed data were collected from local airports Macomb Municipal Airport and General Wayne A. Downing Peoria International Airport; any missing data were collected from the Midwestern Regional Climate Center and Weather Underground.

in the three sampling seasons, the number of mosquito positive pools have increased with the number of mosquitoes collected for testing. The total number of *Culex* mosquitoes captured has increased from 2014 to 2015, as presented in Table 2. In 2014 there were three WNV positive mosquito pools, in 2015 there were 31 WNV positive mosquito pools, and in 2016 there were 11 WNV positive mosquito pools, all ≥ 100 units (Table 2).

The RAMP WNV test is stated to be approximately 100-fold more sensitive than the VecTest WNV antigen assay (Burkhalter et al. 2006). The RAMP manual states a sample that is ≥ 30 units and above is considered positive; however, in the state of Illinois only samples ≥ 100 units are considered positive (Response Biomedical Corp. 2005). Other states have different standard cutoffs compared to the state of Illinois, ranging from ≥ 80 to ≥ 200 units as being considered positive and due to these inconsistencies, nicknamed the gray zones, states have varying RAMP standard cutoffs (Kesavaraju et al. 2012). There is a possibility for RAMP to result in false positives, which hinders its ability to provide reliable results. In order to accommodate the gray zones, it has been suggested from Response Biomedical Corp. for all users to increase cut off units to > 80 to avoid false positives.

There were many samples in this study that fell between 30 and 100 RAMP units, specifically in the 2015 sampling season. The 2015 field season yielded 31 positives above the RAMP unit cutoff in the state of Illinois, from a total of 9,138 *Culex* mosquitoes, yet there were still 20 suspected positives. In 2016, 11 WNV positives and 2 suspected positives falling within the 30–100 RAMP unit category were recorded from a total of 5,702 *Culex* collected. This creates an issue with how to manage any suspected RAMP positives. With so many samples falling in the suspected positive category, this could mean WNV is still present in the sample and was replicating inside the mosquito, but not high enough to warrant a report of virus. This leads to speculation on how many mosquitoes in that suspected sample were carrying the virus but not recorded due to the units reading below 100. Reevaluation of state levels for WNV positivity should be considered, as well as creating a standardized cutoff level nationwide. This information is useful and relevant to mosquito and vector control organizations that have less funding and lack more modern capabilities and/or higher sensitivity of testing for mosquito-borne viruses, such as a polymerase chain reaction (PCR) machine.

Over the study period, 2015 had the highest number of *Culex* collected and the highest annual MIR. The driest sampling season was 2015 and drought across the west-central Illinois region during that year could be a possible explanation for the increase in *Culex* collected and the increase in MIR. The large populations of mosquitoes likely increased host-vector contact and WNV infection, the latter of which was increased during the 2015 sampling season in comparison with the 2014 and 2016 seasons, respectively. These findings

are similar to other studies which show lower than average precipitation seasons positively correlating with higher infection rates (Ruiz et al. 2010). Mosquito population abundance also correlates with higher infection rates as presented in Table 2 and is similar to data collected from other mosquito monitoring studies (Deichmeister and Telang 2010, Ruiz et al. 2010). The infection rate from mosquito surveillance overall can give important indicators of transmission activities and peak human infection risk (CDC 2023). The MIR data presented from this study allow some insight into what environmental factors may be associated with elevated risk of human WNV infection.

In 2014 and 2015, peak *Culex* collection occurred at 281.6 and 305.5°C·wk, respectively. In both cases, peak *Culex* collection occurred during week 34 (the third week of August) which is consistent with the findings of others who reported oviposition peaks for *Culex pipiens* from August through early September (Lampman and Novak 1996, Lee and Rowley 2000, Kunkel et al. 2006). In contrast, peak *Culex* collection in 2016 occurred at 173.7°C·wk (week 27). Among other data in Table 2, there were no significant trends between *Culex* collected and 1) thermal time at the start of sampling, 2) thermal time accumulated at peak *Culex* collection, or 3) annual thermal time accumulated. However, a linear relationship was observed between annual *Culex* collected and the thermal time accumulated over the sampling period indicating a strong relationship between the two variables. Mosquito abundance has also been positively correlated with temperature (Deichmeister and Telang 2010, Arora et al. 2022, Di Pol et al. 2022, Moser et al. 2023). While there were no significant trends between the 3 relationships previously described and presented in Table 2, other variables could be affecting the number of *Culex* collected throughout the sampling periods, such as precipitation.

The general model developed to describe precipitation and mosquito abundance demonstrated a strong relationship between these, suggesting precipitation in west-central Illinois does influence their abundance. *Culex* mosquitoes have been observed to prefer climate which allows formation of habitats for oviposition and larvae survival, such as wet periods followed by warm, dry time frames (Soverow et al. 2009, Wang et al. 2010, Messina et al. 2011, Rosa et al. 2014). The precipitation allows puddles to form in small cavities, drains, and artificial containers where water temporarily pools, thus constructing habitats for oviposition and larval development. However, too much heavy precipitation can potentially disrupt and destroy those necessary habitats, only until the next warm-dry period occurs, letting water settle and pool. These observations match other studies that have noted the same patterns (Soverow et al. 2009, Wang et al. 2010, Rosa et al. 2014).

Based on results of the analysis, the proposed model did not fully describe the relationship between mosquito abundance and wind speed. At west-central IL

sites, regression analysis of discrete annual data yielded very low r^2 values (≤ 0.173). When west-central IL data were combined for 2014, 2015, and 2016, the goodness of fit improved to 0.714. This is likely due to the greater number and larger range of mosquito abundance and wind speed in the data set.

As previously discussed, MIR was noted to increase with mosquito abundance especially during observed dry conditions; increased incidence of WNV infection was associated with warmer maximum weekly temperatures (Soverow et al. 2009, Wang et al. 2010). During a surveillance study in VA, high mosquito abundance was observed and positively correlated with a lower than average precipitation season in 2005 (Deichmeister and Telang 2010) similar to the 2015 data presented in Figs. 3a and 3b, where a first order relationship was determined between *Culex* collected and average daily precipitation. In contrast, 2014 and 2016 did not experience as low precipitation levels as 2015 did during the sampling season. Both years had nearly equal numbers of *Culex* collected and lower accumulated thermal time at peak *Culex* collection and the sampling period compared to data from 2015 (Table 2). When WNV appeared in the USA in 1999 and the years following, bursts in WNV infection and mosquito abundance were also contributed to dry conditions and warmer than usual temperatures (Roehrig 2013).

Overall, the present study determined relationships between field-caught mosquito abundance and key abiotic factors (temperature, precipitation, and wind speed) that are measured regularly across multiple locations in west-central IL. Additionally, the model of *Culex* abundance as a function of thermal time exists independently of the model for *Culex* abundance as a function of wind speed or precipitation. Through observations using these macroclimate data and a study that was as controlled as it can be in a field setting, general models were developed to determine relationships over a spatial and temporal extent. As a result, $Culex \neq f(\text{thermal time, wind speed, precipitation})$; rather, $Culex = f(\text{thermal time})$ and $f(\text{wind speed})$ and $f(\text{precipitation})$. It should be further noted that none of the cause and effect of relationships from this study happened inside the laboratory, all of it occurred in an outside environment and with individual generations of field-caught mosquitoes.

Rural areas face many obstacles compared to urban and metropolitan regions, including lack of and poor access to health care facilities and services, a growing elderly population, and more individuals working in outdoor occupations, such as farming and agriculture, all leading to a higher risk of contact with mosquitoes and WNV infection (Morken et al. 2017, James et al. 2018). Overall, the elderly population is increasing each year; by 2030 approximately 12% of the global population will be older adults (He et al. 2016). The demographic population of rural communities is also changing and growing, with more racial and ethnic disparities becoming apparent

in the community (James et al. 2018). Individuals working outdoors are exposed to mosquito biting risks daily, and some may not take or be aware of protective measures to prevent contact with mosquitoes carrying harmful diseases (Swai et al. 2016). The models presented here may aid in our understanding of the complex relationships between the abiotic factors that help drive mosquito population size and infection rate for pathogens such as West Nile virus.

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