MODELING THE IMPACT OF VARIABLE CLIMATIC FACTORS ON THE CROSSOVER OF CULEX RESTUANS AND CULEX PIPiens (DIPTERA: CULICIDAE), VECTORS OF WEST NILE VIRUS IN ILLINOIS

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Abstract. The aim of this study was to model the impact of temperature on the timing of the seasonal shift in relative proportion of Culex restuans Theobald and Culex pipiens L. in Illinois. The temporal pattern of West Nile virus (WNV) and St. Louis encephalitis virus transmission in the midwest exhibits a late summer to early fall peak in activity, which parallels the temporal increase in the abundance of Cx. pipiens. The daily number of egg rafts oviposited by each species has been monitored at multiple surveillance sites in Urbana-Champaign in central Illinois for more than 13 years. The time when the two Culex species are in equal abundance (crossover) varies considerably from year to year. Our investigation of several thermal measures indicated that this variation was related in large part to climatic conditions with warmer (cooler) temperatures correlated to earlier (later) crossover dates. Models based on degree days and the number of days in which the daily maximum temperature exceeded an upper temperature threshold explained more than 60% of the variance in crossover dates. In contrast, models based on the number of days in which the daily minimum temperature exceeded a lower temperature threshold explained no more than 52% of the variance. An evaluation of these models demonstrated that they provide relatively simple and accurate estimates of crossover date from daily temperature data, a necessary component for developing an overall climatic index for the risk of WNV transmission in Illinois.

INTRODUCTION

West Nile Virus (WNV) is a mosquito-borne flavivirus typically transmitted between birds and mosquitoes, and endemic to Africa, Europe, the Middle East, west and central Asia, and Oceania. The virus was first identified in the United States in the New York metropolitan area in the fall of 1999, and since then has emerged as a threat to public, equine, and wildlife health in North America. In 2002, WNV emerged as a full-scale epidemic in the United States, being reported in 2,531 counties in 44 states, compared with 359 counties in 27 states in 2001. In addition, humans were more heavily implicated in 2002 than in previous years with a total of 4,156 human cases and 284 related deaths reported. In contrast, 149 cases and 18 deaths were detected during the entire period between 1999 and 2001 (http://www.cdc.gov/ncidod/dvbd/westnile/qa/cases.htm). Illinois reported the highest numbers of human cases (884) and deaths (66) in 2002 with 100 counties reporting WNV-positive birds, mosquitoes, humans, or horses (http://www.idph.state.il.us/envhealth/wnv.htm). A similar high level of transmission activity occurred in 2003 in the Great Plains and WNV was reported from 2,289 counties in 46 states, but epidemic transmission in Illinois was markedly lower in that year, with 54 cases and one death and transmission activity reported from 77 counties.

Culex species appear to be the predominant vectors in the enzootic and epizootic bird-mosquito transmission cycles of WNV and three large urban outbreaks in the 1990s in the eastern hemisphere (Romania and southern Russia) and western hemisphere (New York City) implicated Culex pipiens L for the first time as the primary vector. An important component for developing vector and disease management programs is an understanding of the population dynamics of the mosquito vectors. Since its introduction into North America, the vectors of WNV have proven to vary regionally, homologous to that observed for the closely related flavivirus St. Louis encephalitis virus (SLEV). Although other species are vector competent under laboratory conditions, the predominant WNV-positive species from field collections in the midwest and parts of the northeastern United States have been Cx. pipiens and Cx. restuans Theobald.

The majority of overwintering Cx. pipiens and Cx. restuans are mated, nonblood-fed females. Diapause termination in Cx. pipiens is dependent upon juvenile hormone biosynthesis; however, in most temperate areas, the initiation of blood feeding in the spring is dependent upon temperature. The time from egg raft oviposition to adult emergence generally takes from 8 to 12 days in east-central Illinois and varies depending mainly on water temperature, nutrient quantity and quality, and larval crowding. Culex restuans egg rafts are often first detected between mid-April and May in central states such as Illinois, Iowa, and Indiana, and it rapidly becomes the dominant Culex species until June or early July. In contrast, Cx. pipiens is typically a rare species in the midwest from April to June and oviposition peaks between August and early September. Crossover of the two Culex species is defined as the time during this transition when the relative proportions of the two species are equal. The shift in abundance from an early season dominance of Cx. restuans to a late season dominance of Cx. pipiens has led to the suggestion that the former species may initiate enzootic cycles, whereas the latter species may amplify the number of infected avian hosts. In addition to amplifying WNV among birds, Cx. pipiens is also considered the main epidemic vector in the midwest of WNV and SLEV. This is based on a demonstrated vector competency in the laboratory and a seasonal pattern of infection and abundance that both precedes and parallels the temporal pattern of human cases for the two flaviviruses.

Although the general pattern of crossover is similar over a broad geographic area, the exact timing of when the two species are in equal abundance is quite variable (ranging between July and September in central Illinois) and probably repre-
sents an interaction of meteorologic, ecologic, and density-dependent factors. A previous study indicated that annual shifts in crossover were not related to any obvious differences in environmental conditions between years. The aim of our study was to discover whether meteorologic and climatic factors play a role in the relative abundance of Cx. pipiens. This report addresses this issue and whether crossover can be predicted based on known meteorologic conditions. Our long-term goal is to develop a climatic index that accurately reflects the temporal abundance of this potential amplification vector of WNV and SLEV to be used in flavivirus risk models. Unfortunately, there is no known data set for the absolute abundance of Cx. restuans and Cx. pipiens in an area of WNV transmission; however, the relative proportion of the two species has been recorded in the Urbana-Champaign, Illinois area for more than a decade using oviposition traps.

MATERIALS AND METHODS

Since 1988, Culex oviposition has been monitored in the Urbana-Champaign area (40°06′N, 88°15′W) of east-central Illinois by the Illinois Natural History Survey. Relative abundance of Cx. pipiens and Cx. restuans were estimated by collecting egg rafts daily throughout the two cities in oviposition buckets baited with a rabbit chow infusion. The emerging larvae were identified to species in the third or fourth instar. Proportions of the two species were computed and plotted to determine the date of crossover, which is the date when the number of egg rafts from the two species are equal in pooled data from the various collection sites.

Observations of daily maximum temperature (T_{max}), daily minimum temperature (T_{min}), and daily precipitation were collected by the Illinois State Water Survey at its headquarters located in Champaign on the University of Illinois campus. This is an official cooperative observer site of the National Weather Service (site name: Urbana, site identification number: 118740). Temperature data were not available for each mosquito collection site. The Urbana site was the nearest climate station within the city limits (within 1–7 km of the study sites). The climate station is located on the southern edge of Champaign-Urbana (population approximately 100,000) in a grassy area surrounded by a mixture of buildings, trees, roads, and agricultural fields. All of the mosquito collection sites were in residential neighborhoods with land cover of similar character to the climate station site. Because of the lack of topographic variability in this area, the close proximity to study sites, and the similarity in land cover between the climate station and the collection sites, we concluded that the meteorologic observations from the climate station provided reasonable estimates for all the collection sites.

A cursory comparison of temperature data with the crossover dates indicated that warmer (cooler) summers were characterized by earlier (later) crossover dates. Many studies have found that one measure of the thermal environment, degree days (DDs), is related to insect development. Degree days are calculated as follows:

DD = \begin{cases} T_{\text{mean}} - T_{\text{base}} & \text{if } T_{\text{mean}} > T_{\text{base}} \\ 0 & \text{if } T_{\text{mean}} \leq T_{\text{base}} \end{cases}

where DD = number of degree days for a particular day, T_{\text{mean}} = mean temperature of the day, and T_{\text{base}} = base temperature. The most appropriate value of T_{\text{base}} varies by species. In the present study, DDs with different T_{\text{base}} were investigated to determine whether these were related to the crossover date. In addition, two other measures of the thermal environment were investigated: number of days that T_{\text{max}} was greater than an upper threshold temperature and number of days T_{\text{min}} was below a threshold temperature. The deviation of T_{\text{max}} and T_{\text{min}} above or below maximum and minimum thresholds, respectively, are termed exceedances in this report. As with DDs, a series of upper and lower thresholds were correlated to crossover dates. Although these latter two temperature threshold exceedances are not commonly used to reflect insect development, our preliminary inspection of the data suggested a possible relationship. In addition, the analysis was performed for two periods: 1) last spring freeze to crossover, and 2) January 1 to crossover.

A simple linear function of the following form was assumed to express the dependence of the time of crossover on the thermal measures:

\[ D_{\text{cross}} = S \Delta T + I \]  

where D_{\text{cross}} = day of year of crossover, \( \Delta T = \text{thermal measure} \) (degree day accumulation or number of days with threshold exceedances) accumulated from the beginning day to D_{\text{cross}} and expressed as a deviation from the climatic average, S = slope, and I = intercept. The thermal measure, \( \Delta T \), is calculated as follows:

\[ \Delta T = \sum_{i=1}^{N} (t_i - a_i) \]  

where t_i = value of thermal measure on day i, a_i = average value of the thermal measure on day i, n = beginning day for accumulation of the thermal measure (either 1 for January 1 or the day of year of the first spring freeze), and N = day of year of crossover. \( \Delta T \)s were calculated with various values specified for DD base temperature or for the exceedance threshold temperatures. The average value of a_i for the thermal measure was calculated for the period 1971–2000 using historical data from the same climate station. In this function (equation 2), the intercept physically represents the estimated crossover date for average temperature conditions and the slope represents the sensitivity of the crossover date to the thermal measure; an example is given in the next section. Hereafter, the term model will refer to equation 2 with one of the thermal measures specified for \( \Delta T \).

The three models were tested using a jackknife regression. For each year with historically observed crossover dates, a regression was run, using observed values of D_{\text{cross}} and \( \Delta T \), excluding the year to be modeled, to obtain values of I and S to be applied to the year excluded from the regression. Since the intent of the model is as an operational/forecasting tool, it was tested in a manner similar to the way it would be applied. That is, let j = current day of year. For each day, an estimated crossover day (E_{\text{cross}}) is calculated from equations 2 and 3:

\[ E_{\text{cross}} = I + S \sum_{i=j}^{N} (t_i - a_i) \]  

If E_{\text{cross}} > j, then we assume that the crossover date has not yet occurred. On the first day that E_{\text{cross}} ≤ j, crossover is
assumed to occur and this day is denoted as $N_{\text{cross}}$. Correlation coefficients between $N_{\text{cross}}$ and observed values of $D_{\text{cross}}$ for all the years with observations were calculated for each combination of thermal measure, base value (DD base or temperature threshold), and starting date. These correlations coefficients were used to identify the best predictive model.

**RESULTS**

Table 1 lists the dates when the first egg rafts of *Cx. pipiens* were detected and when crossover occurred (equal number of egg rafts for both *Culex* species). The mean number of days to crossover from January 1, based on 13 years of data for 1988–2003 was 219 days ($SD = \pm 22$ days). The crossover day of year varied from 191 to 255, a range of approximately 9 weeks.

A scattergram of crossover day of year versus the number of days with $T_{\text{max}}$ exceeding 27°C from January 1 to the day of crossover (Figure 1) illustrates the inverse relationship between the thermal environment and the day of crossover. The relationship appears to be linear, a characteristic common to all of the following combinations of model type and base/threshold temperature. The slope of the linear fit is $-1.4$ days/day and the intercept is 219 days. Thus, for an average number of days with $T_{\text{max}}$ exceeding 27°C, the crossover day of year is expected to be 219 (August 7). For every additional (compared with the average) day above 27°C, the crossover day of year on average is earlier by 1.4 days.

Correlation coefficients were calculated for the model using DDs as the thermal measure for base temperatures from 5°C to 25°C (Figure 2). For the case of January 1 as the starting date for accumulation (solid curve), the $r^2$ values are between 0.6 and 0.7 for base temperatures between 5°C to 22°C, with the maximum value of 0.68 occurring at 17°C. For the case of the last freeze as the starting date for accumulation (dashed curve), the shape of the correlation curve is similar to that for a January 1 starting date. The magnitudes of the correlations are lower, with the highest $r^2$ value being 0.54 for a base temperature of 11°C.

Correlation coefficients for the number of $T_{\text{max}}$ exceedances as a function of a threshold (Figure 3) show a pronounced dependence on temperature. For the case of January 1 as the starting date (solid curve), there is a peak $r^2$ value of 0.52 at 20°C. For the case of the last freeze as the starting date (dashed curve), the maximum $r^2$ value is only 0.27 at a threshold of 20°C. Thus the best correlation coefficients for devia-

![Figure 1](image1.png)

**Figure 1.** Deviation from the long-term average of the number of days with a daily maximum (max) temperature approximately 27°C versus the day of year of crossover from *Culex restuans* to *Cx. pipiens*. The number of days is accumulated from January 1.

![Figure 2](image2.png)

**Figure 2.** Square of the correlation coefficient between crossover date and accumulated degree days as a function of degree day base for data from the period 1988–2003 (except for 1989, 1993, and 2000). Degree days are accumulated from January 1 (solid line) or the date of the last spring freeze (Frz) (dashed line).

<table>
<thead>
<tr>
<th>Year</th>
<th>Last spring freeze</th>
<th>First <em>Cx. pipiens</em> in trap</th>
<th>Crossover date (day of year)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1988</td>
<td>April 20</td>
<td>June 27</td>
<td>July 10 (192)</td>
</tr>
<tr>
<td>1990</td>
<td>April 18</td>
<td>June 19</td>
<td>August 7 (219)</td>
</tr>
<tr>
<td>1991</td>
<td>April 11</td>
<td>June 12</td>
<td>July 10 (191)</td>
</tr>
<tr>
<td>1992</td>
<td>April 28</td>
<td>July 10</td>
<td>September 11 (255)</td>
</tr>
<tr>
<td>1994</td>
<td>April 8</td>
<td>June 15</td>
<td>July 25 (206)</td>
</tr>
<tr>
<td>1995</td>
<td>April 5</td>
<td>June 14</td>
<td>July 29 (210)</td>
</tr>
<tr>
<td>1996</td>
<td>April 27</td>
<td>July 1</td>
<td>September 9 (253)</td>
</tr>
<tr>
<td>1997</td>
<td>April 18</td>
<td>June 24</td>
<td>August 31 (243)</td>
</tr>
<tr>
<td>1998</td>
<td>March 24</td>
<td>June 10</td>
<td>July 20 (201)</td>
</tr>
<tr>
<td>1999</td>
<td>March 28</td>
<td>June 13</td>
<td>July 29 (210)</td>
</tr>
<tr>
<td>2001</td>
<td>April 18</td>
<td>June 17</td>
<td>August 22 (234)</td>
</tr>
<tr>
<td>2002</td>
<td>April 7</td>
<td>June 11</td>
<td>July 28 (209)</td>
</tr>
<tr>
<td>2003</td>
<td>April 13</td>
<td>June 14</td>
<td>August 23 (235)</td>
</tr>
</tbody>
</table>
DISCUSSION

West Nile virus by its very nature of having visible signs of transmission (dead birds), recurrent outbreaks, and high infection rates in vectors provides a unique opportunity to investigate the intricacies of the transmission cycle and the impact of meteorologic and ecologic variables on it. In our study, we focused on modeling the impact of temperature on the timing of the Cx. pipiens increase in abundance, which coincides with the amplification phase of WNV transmission in Illinois over the past three years (Novak RJ, Lampman RL, Gu W, unpublished data). The DD and T_{max} exceedance models explain more than 65% of the observed variance in crossover date for some values of the base/threshold temperature, whereas the best T_{min} exceedance model explains up to 52% of the variance. Also, the use of January 1 as the starting date for the accumulation of the thermal measure generally results in higher values of the explained variance compared with the use of the last freeze as the starting date.

Using as examples those cases with the highest correlations, the DD base of 17°C and the T_{max} threshold of 27°C were chosen to illustrate the behavior of two operational models applied to the historical data. The model estimates of the crossover date obtained from the jackknife regression (Figure 5) show that the large interannual variations in crossover date are predicted rather well. There is little difference between the two models. In one year (1996), the model estimates are 3–4 weeks early. In half of the years, the model estimates are within one week of observed crossover. In other years, the model estimates are within 1–2 weeks of observed. Thus, the DD and T_{max} exceedance models perform with approximately equal accuracy when using optimum values of the base/threshold temperatures. Surprisingly, the accuracy of the DD model was relatively insensitive to base temperature, whereas the T_{max} exceedance model was quite sensitive to the temperature threshold chosen.

Since the use of a fixed calendar date (January 1) produces higher correlations than the use of last freeze date (which varies from year to year), these models are very straightforward to apply. A remaining question in terms of a climate index is its applicability to other regions of the country.

The initial evidence from field studies suggests that WNV is similar to SLEV in that the main enzootic and epizootic vectors may vary regionally in the United States, but primarily involve Culex species. Although considerable emphasis has been placed on the potential role of many mammal-feeding mosquito species as bridge vectors, particularly Stegomyia albopicta and Ochlerotatus japonicus, there is little field evidence that supports these non-Culex species as important epidemic vectors in the east-central United States. In Illinois, S. albopicta is common throughout the southern

![Figure 3](image-url) Figure 3. Square of the correlation coefficient between crossover date and accumulated number of maximum temperature (T_{max}) exceedances as a function of exceedance threshold for data from the period 1988–2003 (except for 1989, 1993, and 2000). Threshold exceedances are accumulated from January 1 (solid line) or the date of the last spring freeze (Frz) (dashed line).

![Figure 4](image-url) Figure 4. Square of the correlation coefficient between crossover date and accumulated number of minimum temperature (T_{min}) exceedances as a function of exceedance threshold for data from the period 1988–2003 (except for 1989, 1993, and 2000). Threshold exceedances are accumulated from January 1 (solid line) or the date of the last spring freeze (Frz) (dashed line).

![Figure 5](image-url) Figure 5. Time series of the day of year of crossover from Culex restuans to Cx. pipiens for 1988–2003 for observed (Obs) data (thick solid line), estimates from a degree day model (DD Mod) using base temperature of 17°C (dashed line), and estimates from a maximum temperature (T_{max}) exceedances model (T_{max} Mod) using a threshold of 27°C (thin solid line).
quarter of the state, but is only sparsely distributed in the central and northern regions. Neither of these species were present in Champaign-Urbana. The only species with a significant number of positive pools from dry ice-baited traps and gravid traps were Culex species. The most common non-Culex species in which WNV RNA was detected in central Illinois was Aedes vexans, although the number of pools and infection rates were well below 1 per 1,000 mosquitoes (Novak RJ, Lampman RL, Gu W, unpublished data).

Major outbreaks of SLEV and WNV tend to occur when temperatures are above average and rainfall is average to below average, although outbreaks may be preceded by above average rainfall. High temperatures may favor flavivirus transmission by increasing the development rate of the vector, decreasing the interval between blood meals, and increasing the virus replication rate and magnitude of infection in mosquitoes, thus considerably shortening the extrinsic incubation period. For SLEV, temperature exceedances and DD accumulations above specific temperature limits have been reported to correlate with the development of flaviviruses or with transmission rates. Our study suggests that temperature plays an additional role in transmission of flaviviruses by altering the crossover date of Cx. restuans and Cx. pipiens. In other words, assuming Cx. pipiens is the major amplification and potential bridge vector to mammals in Illinois, its earlier appearance could provide a longer period for transmission to build to a threshold where incidental hosts become involved. Meteorologic factors or indices (e.g., drought or water table depth) have been suggested as important for bringing vectors in close association with avian hosts.

The model for crossover of Cx. restuans and Cx. pipiens presented here is probably relevant for much of the eastern United States; however, latitudinal variation in crossover is poorly defined, thus making it difficult to test this hypothesis from the literature. Culex restuans oviposition activity in Illinois, Iowa, and Indiana begins between mid-April and May, and it becomes the dominant Culex species until June or early July. As the number of Cx. restuans decrease, Cx. pipiens oviposition increases, peaking between August and early September. In southern Canada, the temporal pattern of abundance is similar; Cx. restuans is most abundant during the spring and early summer, whereas Cx. pipiens reaches its peak abundance in late summer to early fall.

An experimental application of these models as an operational/forecasting mode was performed using temperature data for 2004. The approach used to test the models (see equations 4 and accompanying discussion) can provide an estimate of the crossover date on any day prior to the crossover. Implicit in such an estimate is that the remainder of the year, up to the crossover day, can be characterized by average temperature conditions, although additional information can be provided using historical climate data. Specifically, we assumed that the past climate history provides an envelope of what may happen during the rest of the current year. Furthermore, we assumed that the probability of what will happen during the rest of the current year is equal to the past frequency of conditions. Each year in the historical climate database is assumed to be one scenario for the outcome of the remainder of the year. To apply this concept, the temperature time series for one scenario was assumed to be the combination of the actual observed data for 2004 up to the current date plus the observed temperature data from some past year for all days after the current date. An estimated crossover date was calculated using equation 4 stepping forward from the current date until \( E_{\text{cross}} \leq j \). This process was repeated 104 times using each year from 1900 to 2003 as a possible scenario for the remainder of the year. These 104 values were sorted from earliest to latest day of year. The result is a probability distribution of crossover dates; thus, this provides an estimate of both the variance and the mean of estimated crossover date. The earliest, latest, median (average of rank 52 and 53 values), the 90% probability of exceedance (rank 10), and the 10% probability of exceedance (rank 94) values were extracted and plotted (Figure 6).

Probability distributions were generated for each week of 2004 beginning with May 1 (Figure 6). For estimates made during May, the range of estimated crossover days of year is large (187–250), reflecting the fact that Cx. pipiens population buildup is in its early stages and the future weather is the determining factor. As the season progresses and the weather for an increasing fraction of the year has been realized, the range of the thermal measure narrows. Or, in a more physical sense, the mosquito population dynamics has progressed closer to crossover and the uncertainty about its timing has lessened. Eventually, all of the curves converge to a single value (236), the final estimated value of crossover for 2004. Another interesting feature is the upward trend in the median and other curves (Figure 6). This is a result of weather conditions during the summer of 2004, which was the fifth coolest since 1889. Because of the cool temperatures, the probability distribution shifted to later crossover dates as the summer progressed. The final estimated value (236) was within the range of the initial (May 1) probability distribution, but on the upper end of the range. The information presented in Figure 6 provides insights into the uncertainties in crossover date as the season progresses.

Integrated mosquito management typically focuses on larval control; however, when this is inadequate, adult control measures may become necessary. Our model provides a method for the timing of adult control near the crossover date, which our research has shown precedes the peak infec-

![Figure 6](image_url)

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