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Research

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Ultra-low volume (ULV) adulticide treatment impacts age structure of *Culex* species (Diptera: Culicidae) in a West Nile virus hotspot

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West Nile virus (WNV) invaded the continental United States over 20 years ago and continues to cause yearly seasonal outbreaks of human and veterinary disease. In the suburbs of Chicago, Illinois, ultra-low volume (ULV) truck-mounted adulticide spraying frequently is performed to reduce populations of *Culex restuans* Theobald and *Cx. pipiens* L. mosquitoes (Diptera: Culicidae) in an effort to lower the risk of WNV transmission. The effectiveness of this control method has not been rigorously evaluated, and evidence for *Culex* population reduction after ULV adulticide spraying has been inconclusive. Therefore, we evaluated the results of 5 sequential weekly truck-mounted adulticide applications of Zenivex® E20 (etofenprox) in 2 paired sites located in Cook County, IL, during the summer of 2018. Mosquito population abundance, age structure, and WNV infection prevalence were monitored and compared between paired treatment and nearby control sites. Adulticide treatment did not result in consistent short-term or long-term reductions in target WNV vector *Culex* abundance. However, there was a significant increase in the proportion of nulliparous females in the treated sites compared to control sites and a decrease in *Cx. pipiens* WNV infection rates at one of the treated sites. This evidence that ULV adulticide spraying altered the age structure and WNV infection prevalence in a vector population has important implications for WNV transmission risk management. Our findings also underscore the importance of measuring these important indicators in addition to abundance metrics when evaluating the efficacy of control methods.

Key words: parity, mosquito, infection prevalence, West Nile virus

Introduction

Since the first detection of West Nile virus (WNV) in New York, USA, in 1999, and its subsequent introduction to Illinois in 2002, controlling *Culex* vector mosquitoes has been the focus of the 4 mosquito abatement districts in the greater Chicago area (Lanciotti et al. 1999, Ruiz et al. 2004, Tedesco et al. 2010). From 2002 to 2018, there were 2,634 human infections of WNV and 176 deaths associated with WNV in the state (IDPH 2019a). Because there have been several large outbreaks of WNV disease, and some communities have a consistent high annual incidence of infection, the city and suburbs of Chicago are considered a "hotspot" for WNV in the Midwest (Bertolotti et al. 2008, Mutebi et al. 2011). By comparison, the annual incidence of WNV human infections in the entire United States from 2009 through 2018 was 1 in 8,156, whereas the incidence in Cook County, IL (which encompasses the city and greater Chicago area) was 1 in 5,433 (McDonald et al. 2021).

In Illinois and surrounding areas, WNV is primarily transmitted by 2 vector species: *Culex pipiens* Linnaeus (Diptera: Culicidae) and *Cx. restuans* Theobold (Diptera: Culicidae) (Hayes et al. 2005). These species are often combined during abundance monitoring and virus testing because of similarities in morphology (Ebel et al. 2005, Harrington et al. 2008, Johnson et al. 2015, Ferreria-de-Freitas et al. 2020). Both species primarily feed on birds and occasionally feed on mammals at similar rates (Molaei et al. 2006, Hamer et al.

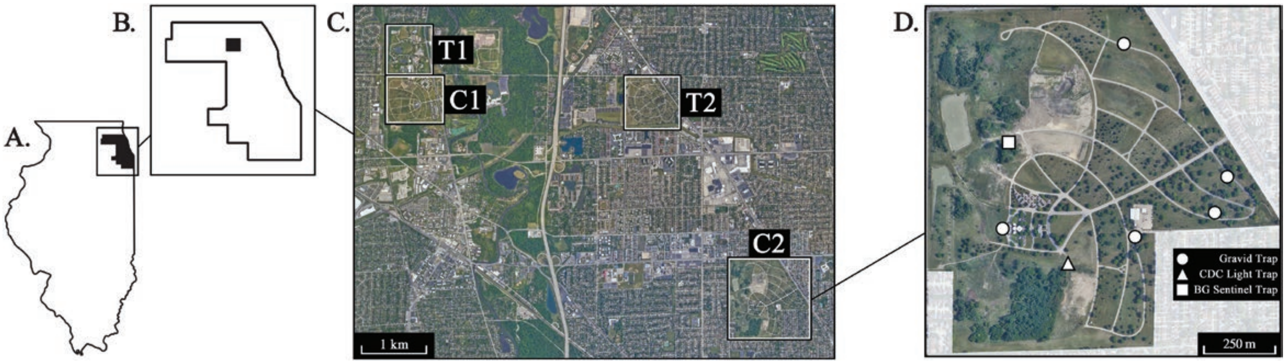

Fig. 1. A) Map of Illinois, USA. B) Map of Cook County. C) Map of study area. T1 is the treatment site of pair 1, C1 is the control site of pair 1, T2 is the treatment site of pair 2, and C2 is the control site of pair 2. D) Example of trap placement in site C2. The square is a BG Sentinel trap, triangle is a CDC miniature light trap, and circles are CDC gravid traps.

2008, 2009). In the Upper Midwest and Northeast United States, *Cx. pipiens* is likely the more important of the 2 vectors for human infection, based on its late-season abundance at times when more amplifying hosts are infected (Andreadis et al. 2001, Ebel et al. 2005, Lampman et al. 2006, Tokarz et al. 2019).

To control *Culex* spp. vectors and prevent human bites, proactive approaches are commonly used (CDC 2020). Proactive control methods used by mosquito abatement districts (MADs) in the Chicago area include public education programs, source reduction, larval surveillance, and larval control in storm water catch basins (Clifton et al. 2019). When risk of human WNV infection is high, reactive control measures such as adulticide sprays are used (Nasci and Mutebi 2019). In the Chicago area, the Vector Index (VI) is used as the decision-making tool for ULV adulticide applications in residential areas (Jones et al. 2011, Nasci and Mutebi 2019). Extensive surveillance of adult mosquitoes is required to acquire vector abundance and WNV infection prevalence metrics to calculate the VI.

Evidence that ground-based adulticide treatments successfully suppress *Culex* spp. vector populations, and that there is an impact on WNV infection and transmission risk, is limited and often contradictory (Beard et al. 2019). Although some studies reported that truck-mounted adulticide spraying in residential settings reduced mosquito numbers (Strickman 1979, Geery et al. 1983, Mutebi et al. 2011, Clifton et al. 2019, Sass et al. 2022), a second body of evidence showed that truck-mounted adulticide treatments were not effective (Reiter et al. 1990, Reddy et al. 2006, Lothrop et al. 2007, 2008). All of these studies emphasized trap counts (mosquito abundance) as the outcome metrics, ignoring WNV infection prevalence, an important aspect of epidemiological risk (Wilson et al. 2015). It is possible that adulticide treatment impacts the age structure of the target mosquito population, either by preferential removal of older, parous females that are more susceptible (Rajatileka et al. 2011, Oliver et al. 2014), or by killing all mosquitoes exposed in combination with a constant emergence of new females. Therefore, measuring parity and infection rates could reveal impacts on the population not detected by measuring abundance alone.

To better understand how truck-mounted sprays are affecting population abundance and virus prevalence in the vector in suburban areas, we evaluated the effect of 5 weekly truck-mounted adulticide sprays, using a pyrethroid-based product, on *Cx. pipiens* and *Cx. restuans* abundance, age-structure, and WNV infection status at 2 study sites in the greater Chicago area during the summer of 2018. We conducted these studies in the Northwest Mosquito Abatement District (NWMAD), Cook County, IL (Fig. 1). The NWMAD covers a 605-km^2 area in the Northwestern corner of Cook County and, at its eastern edge, encompasses areas of high human incidence of WNV (Karki et al. 2020). Our study contributes to knowledge about the impacts of ULV adulticide applications on the risk of WNV transmission that highlight the importance of using multiple methods to assess the vector population.

Materials and Methods

Field Site Description

Four nonresidential study sites were selected in Des Plaines, Cook County, IL, USA within the NWMAD (Fig. 1). Des Plaines is a suburb approximately 32 km northwest from Chicago, IL, USA. Three study sites were cemeteries and the fourth was an educational campus. Each site was approximately 0.4–0.8 km^2 (Fig. 1). Sites were paired geographically and designated as control or treatment sites within each pair. All sites were within a 4 km radius. Prior to this study, these sites had received less than one adulticide treatment per year over the previous decade using either Zenivex® E20 (Central Life Sciences, Schaumburg, IL) or Anvil® 10 + 10 (Clarke, St. Charles, IL) (unpublished data). All storm water catch basins and above ground breeding sites within and around each site were treated with 150-day Altosid XRT briquets (Wellmark International, Schaumburg, IL). Back-checks were completed throughout the summer to ensure larvicide products were effective and water sources were not producing mosquitoes. This study was performed in 2018 for 10 weeks starting in June and ending in August (epidemiological weeks 24–33).

Adulticide Application

Five sequential weekly adulticide applications were performed at treatment sites during epidemiological weeks 26–30. Control sites were sprayed once during the study period, in accordance with district policy (Supplementary Table 1). Zenivex® E20 was sprayed in ULV in a 1:1 mineral oil dilution (10% etofenprox solution) at 177.4 ml/min (6oz/min) from a truck-mounted London Fog 18–20 (London Foggers, Minneapolis, MN) at 16 km/h (10 mi/h) at a 45° nozzle angle. Each spray was done no earlier than 30 min before sunset when atmospheric conditions are most stable (Bonds 2012) (Supplementary Table 1). Applications were made from all roads within each site. Temperature, wind speed, and wind direction were recorded from a hand-held anemometer (Ambient Weather WM-4 [Ambient Weather, Chandler, AZ]) at the start of each treatment event and paired with data from a nearby weather station (Supplementary Table 1). Data provided in Supplementary Table 1 show that ULV applications at adjacent sites C1 and T1 did not result in drift into the control site C1, because the wind did not blow in the direction of the control site (Supplementary Table 1).

Mosquito Collection

To evaluate the effectiveness of ULV application on mosquito populations, 28 mosquito traps were deployed. At each site, 5 CDC gravid traps (Model 1712, John W. Hock Company, Gainesville, FL), 1 CDC miniature light trap (Model 512, John W. Hock Company, Gainesville, FL), and 1 BG-Sentinel trap (Biogents, Regensburg, Germany) were operated continuously, and trap contents were collected daily Monday through Friday. CDC light and BG-Sentinel traps were baited with carbon dioxide (CO_2) from 9.07 kg tanks releasing 1.13 kg/day from 19:00 to 07:00 h the following morning. CO_2 release was regulated by a BG counter (Biogents, Regensburg, Germany) for BG-Sentinel traps and a BG-CO_2 timer (Biogents, Regensburg, Germany) for CDC miniature light traps. Gravid traps were baited with an alfalfa pellet infusion. Traps were placed less than 50 m away from roads, and traps of the same type were placed at least 150 m away from one another. Mosquito trapping occurred during week 24 and through week 33. All adult mosquitoes were identified based on morphological characteristics (Siverly 1972) and counted. *Cx. pipiens* and *Cx. restuans* mosquitoes collected from BG-Sentinel and CDC light traps were not differentiated and will hereafter be referred to as target WNV *Culex* vector spp.

Mosquito Dissection—Parity Analysis

To assess the impact of ULV adulticide on population age structure, up to 30 WNV *Culex* vector spp. females from CO_2-baited traps at each site were dissected on the day of collection. Mosquitoes were dissected in saline using minuten pins under a dissecting microscope. Ovaries were removed into a clean drop of saline, gently compressed under a cover slip, and observed with a compound microscope at 100× magnification. Parity was estimated by observing tracheolar coiling in the ovaries and each specimen was recorded as nulliparous or parous (Detinova 1962). Ovaries of that were not clearly identifiable as nulliparous or parous, either due to damage from dissection or autogeny, were rare and not recorded. Mosquitoes from CO_2-baited traps were not frozen before dissection, because freezing obscured tracheolar coiling in preliminary laboratory studies.

WNV Detection

Culex spp. mosquitoes from gravid traps were stored at −20 °C, then identified to species based on morphological characters as part of a concurrent study (Ferreira-de-freitas et al. 2020); some of these specimens were reported in that paper (C1 is labeled as “All Saints Cemetery”). Pools of approximately 50 *Cx. pipiens* females from the same site and day were tested for WNV using the RAMP West Nile virus testing system (Response Biomedical Corp., Vancouver, Canada). Only *Cx. pipiens* from weeks 29–33 were tested because the abundance of *Cx. restuans* was low and very few positive pools of WNV-infected mosquitoes were found within the district before that time (IDPH 2019b).

Data Analysis

Combined mosquito species abundance was calculated as the total number of *Culex* spp. mosquitoes (host-seeking or gravid) captured in each trap at each site per night. On a few occasions, data were not collected due to trap disturbance, severe weather, and battery failure. Each week there was 1 trapping event that ran from Friday to Monday and included the weekend. Mosquito counts were averaged over weekend trapping periods. This longer session occurred 3 days postapplication for the first application and 5 days post-application for applications 3, 4, and 5. Trap count data from the second application were omitted from the analyses, as trap contents the days immediately before and after the spray were not collected.

In previous adulticide evaluation studies, changes in mosquito abundance were measured over a wide range of time, between 1 and 14 days (Strickman 1979, Geery et al. 1983, Reiter et al. 1990, Reddy et al. 2006, Lotrhop et al. 2007, 2008, Mutebi et al. 2011, Clifton et al. 2019, Sass et al. 2022). To contextualize our results to other studies, both short-term and long-term impacts of adulticide treatment events were analyzed. To assess short-term impacts, percent reduction was calculated daily for 0–5 days post-treatment using Mulla’s formula (Mulla et al. 1971). This formula provided a quantitative metric of changes in population abundance at treatment sites relative to control sites to account for natural changes in abundance. Positive percent reductions reveal a treatment success, where treatment group abundance is reduced relative to the control group. A negative percent reduction represents a treatment failure, indicating that the treatment site abundance increased after spraying relative to the control site. To analyze long-term effects of treatments, Mulla’s formula was applied to total weekly mosquito abundance from before any treatment occurred (weeks 24 and 25) as compared to abundance each week after (weeks 26–33). In addition, a Kruskal–Wallis rank sum test (Kruskal and Wallis 1952) was used to determine differences in *Culex* spp. abundance between all sites during each epidemiological week. Data from each epidemiological week were separated to control for seasonal population

Table 1. Kruskal–Wallis (Kruskal and Wallis 1952) test for differences in abundance in target WNV *Culex* vector spp. amongst all 4 sites in Cook County, IL, USA, during each epidemiological week of 2018

	Host-seeking				Gravid			
Week	Chi-squared	*n*	*P*-value	Significance	Chi-squared	*n*	*P*-value	Significance
24	16.730	4	8.03e-4	***	10.730	4	0.013	*
25	2.722	4	0.436		18.756	4	3.07e-4	***
26	15.122	4	1.176e-3	**	5.073	4	0.166	
27	12.102	4	0.007	**	11.261	4	0.010	*
28	15.419	4	0.002	**	22.810	4	4.424e-5	***
29	5.7475	4	0.125		8.675	4	0.034	*
30	16.565	4	8.684e-4	***	4.490	4	0.213	
31	22.965	4	4.107e-5	***	9.948	4	0.019	*
32	23.976	4	2.527e-5	***	22.945	4	4.145e-5	***
33	20.923	4	1.092e-4	***	16.143	4	0.001	***

ULV adulticide was applied during weeks 26–30 (shaded gray).

Table 2. Post-hoc Wilcoxon pair-wise comparisons (Mann and Whitney 1947) for significant differences in target WNV *Culex* vector species abundance measured from host-seeking and gravid traps run in Cook County, IL, USA in 2018, as detected with a Kruskal–Wallis test (see Table 1)

	Host-Seeking						Gravid					
	Pair 1			Pair 2			Pair 1			Pair 2		
Week	*P*-value	C1 Mean (± SE)	T1 Mean (± SE)	*P*-value	C2 Mean (± SE)	T2 Mean (± SE)	*P*-value	C1 Mean (± SE)	T1 Mean (± SE)	*P*-value	C2 Mean (± SE)	T2 Mean (± SE)
24	**0.013***	**86.25 (±32.073)**	**17.25 (±8.693)**	**0.027***	**143.75 (±50.301)**	**40.25 (±2.688)**	0.206	201.000 (±77.333)	145.25 (±22.25)	0.046*	**205.249 (±13.916)**	**477.749 (±31.416)**
25	0.840	36 (±13.107)	26.8 (±13.290)	0.720	43.2 (±25.743)	16.8 (±11.372)	0.107	209.142 (±37.073)	117.285 (±15.512)	0.003**	**230.714 (±37.055)**	**587 (±64.458)**
26	**0.041***	**69 (±28.644)**	**123.8 (±39.230)**	0.519	39 (±24.941)	32.2 (±6.755)	0.120	457.285 (±202.26)	124.428 (±29.675)	0.900	213.428 (±44.163)	301.714 (±122.63)
27	**0.009****	**20.25 (±6.651)**	**58.75 (±15.776)**	0.361	57.25 (±19.027)	42.25 (±19.241)	**0.020***	**463.142 (±97.177)**	**150.142 (±11.019)**	0.233	221.142 (±55.541)	430 (±67.552)
28	**0.017***	**12.6 (±4.214)**	**36 (±11.480)**	0.210	36 (±12.029)	19 (±7.028)	**0.004****	**427.571 (±68.492)**	**178.142 (±15.101)**	**0.003****	**1019.42 (±128.38)**	**450.571 (±43.893)**
29	0.900	17.4 (±6.209)	18.6 (±3.529)	0.280	16.2 (±4.831)	45.2 (±30.822)	0.142	704.999 (±169.03)	206.428 (±25.738)	0.124	381.285 (±145.45)	590.714 (±91.046)
30	0.399	23.4 (±4.611)	30.8 (±13.76)	**0.004****	**20.6 (±12.730)**	**63 (±23.588)**	0.310	504 (±74.742)	349.142 (±118.76)	0.440	377.285 (±59.526)	566.285 (±116.33)
31	**0.003****	**9.6 (±2.909)**	**28.4 (±6.005)**	**0.004****	**13.4 (±5.679)**	**63.2 (±27.932)**	0.057	468.678 (±33.253)	145.285 (±16.202)	0.300	738.678 (±99.314)	485.321 (±72.934)
32	**0.003****	**65.8 (±24.344)**	**17.8 (±4.923)**	**0.003****	**44.6 (±12.956)**	**262.2 (±72.269)**	**3.10e-4*****	**276.749 (±34.520)**	**98.7142 (±7.6232)**	0.619	238.892 (±61.738)	416.821 (±36.134)
33	0.007**	**40 (±13.277)**	**14 (±3.361)**	0.012*	**46.6 (±19.561)**	**155.4 (±32.952)**	0.014*	**178.857 (±21.505)**	**76.5714 (±13.712)**	0.014 *	**84.8571 (±22.746)**	**289.999 (±67.828)**

ULV adulticide was applied during weeks 26–30 (gray shading). Bolded mean abundance indicates significance between treatment and control groups.

fluctuations. When significant differences were detected, a post-hoc pairwise Wilcoxon rank sum test (Mann and Whitney 1947) was used to determine which sites were different from one another.

A generalized linear mixed model (GLMM) with a binomial distribution was used to determine the association between treatment and the proportion of nulliparous females, weighted by the total number of mosquitoes dissected, for each site and day. The response variable was the proportion of nulliparous mosquitoes for each site and day. The explanatory variables tested were treatment (control or treatment site), epidemiological week, and their interaction. To account for the study design, site pair (1 and 2 as random factors) was incorporated in the model. To account for the time series, epidemiological week was included as noncontinuous categorical effect, as there were periods without treatment in the beginning and end of the study. The most parsimonious model, including treatment, was backward selected based on the lowest Akaike's information criterion (AIC) value (Burnham and Anderson 2002). Models within 2 AIC were considered equal (Supplementary Table 4).

WNV infection incidence was calculated as the minimum infection rate per 1,000 females tested (MIR) for each week using the Biggerstaff plug-in for Excel (Table 4; Biggerstaff 2006). The maximum likelihood estimate (MLE) could not be calculated during some weeks when all pools were positive. VI for each week was calculated using the minimum infection rate and mean gravid mosquito abundance (Tables 2 and 4; CDC 2021). *T*-tests were used to detect differences in reported MIR values (Table 4) between each paired treatment and control site. Statistical analyses were performed in R Studio, version 3.5.3 (R Development Core Team 2019) with package "lme4" (Bates et al. 2015).

Results

We did not observe consistent short-term (0–5 days post-treatment) percent reductions in the abundance of target WNV vector *Culex* spp. after truck-mounted ULV treatments in 2 study areas within Cook County, IL, USA, during the summer of 2018 (Supplementary Table 2). Compared to control sites, host-seeking mosquito abundance ranged from a 99% reduction to 616% increase in abundance (Supplementary Table 2). For gravid mosquitoes, abundance after adulticide application ranged from 87% reduction to 1,095% increase (Supplementary Table 2).

Long-term (≥1 wk post-treatment) impacts on adult *Culex* spp. abundance were likewise inconsistent. Population abundance at treated sites for host-seeking mosquitoes ranged from 30% reduction to 2,009% increase (Supplementary Table 3). Gravid mosquito abundance ranged from 82% reduction to 38% increase (Supplementary Table 3). Instances of reduction in gravid mosquitoes were more prevalent than in host-seeking abundance. There were many instances of a significant reduction across all sites in host-seeking mosquito abundance during the treatment period across weeks (Table 1), although many instances were not significantly different between site pairs (Table 2). In host-seeking mosquitoes, significant differences between treatment and control sites were almost always due to higher abundance in treatment sites. Gravid mosquito abundance in the treatment site of pair 1 was often lower, and some of these differences were significant during and after adulticide treatment, but this was not consistent (Table 2). In pair 2, no one site was consistently higher in gravid mosquito abundance, and only 1 week was significantly different during adulticide application (Table 2).

The most parsimonious model for examining the effect of adulticide treatment on the proportion of nulliparous females included treatment effect, epidemiological week, and their interactions

Table 3. Final logistic model results of proportion nulliparous WNV *Culex* vector spp. as explained by treatment group, epidemiological week (categorical), and their interactions, weighted by the number of mosquitoes dissected

Variable	Estimate	Standard error	*P*-value	Significance
Intercept	−0.611	0.285	3.21e-02	*
Treatment	−0.039	0.456	9.30e-01	
Epi. week				
25	−0.967	0.357	6.77e-03	***
26	−0.254	0.354	4.73e-01	
27	1.072	0.371	3.82e-03	**
28	0.758	0.351	3.09e-02	*
29	0.728	0.339	3.16e-02	*
30	0.061	0.346	8.58e-01	
31	−0.038	0.384	9.21e-01	
32	0.035	0.319	9.13e-01	
33	0.018	0.323	9.54e-01	
Treatment: epi. week interaction				
Treatment:25	0.394	0.584	4.99e-01	
Treatment:26	−0.016	0.530	9.76e-01	
Treatment:27	−0.105	0.546	8.47e-01	
Treatment:28	1.106	0.542	4.12e-02	*
Treatment:29	1.009	0.535	5.92e-02	
Treatment:30	1.982	0.531	1.90e-04	***
Treatment:31	1.468	0.548	7.41e-03	**
Treatment:32	1.016	0.505	4.44e-02	*
Treatment:33	0.684	0.507	1.77e-01	

ULV adulticide was applied during weeks 26–30 (gray shading). **$P < 0.01$; ***$P < 0.001$.

Table 4. The minimum infection rate (MIR, number of infected females per 1,000) and vector index (VI) of *Cx. pipiens* mosquitoes as calculated from RAMP diagnostic of 5 mosquito pools/week

	Pair 1				Pair 2			
	Treatment 1		Control 1		Treatment 2		Control 2	
Epidemiological week	MIR	VI	MIR	VI	MIR	VI	MIR	VI
29	4.00 (0.00–11.82)	0.763	0.00 (0.00–0.00)	2.136	4.00 (0.00–11.82)	2.251	12.00 (0.00–25.50)	1.866
30	8.00 (0.00–19.04)	1.757	4.00 (0.00–11.82)	2.299	8.00 (0.00–19.04)	2.682	20.00 (2.56–37.35)	1.744
31	15.79 (0.00–33.51)	0.707	10.00 (0.00–23.79)	2.098	0.00 (0.00–0.00)	2.429	15.00 (0.00–31.85)	3.433
32	16.00 (0.45–31.55)	0.395	16.00 (0.45–31.55)	1.231	8.00 (0.00–19.04)	1.324	20.00 (2.65–37.35)	1.125
33	17.17 (0.49–33.85)	0.365	20.33 (2.69–37.96)	0.786	8.00 (0.00–19.04)	1.291	16.00 (0.45–31.55)	0.510

Upper and lower confidence intervals (95%) are specified in parenthesis. Shaded rows indicate ULV adulticide treatment weeks. T-tests revealed sites in pair 1 are not different ($t = -0.466$; df = 7.166; $P = 0.655$), but MIRs in treatment 2 are lower than control 2 ($t = 4.959$; df = 7.987; $P = 0.001$).

(Table 3). Site pair did not improve model fit (Supplementary Table 4). The treatment effect itself was not significant in the model (P = 9.31e-01), but several interactions were. The proportion of host-seeking nulliparous mosquitoes was significantly greater in treated sites during weeks 28, 30, 31, and 32 compared to control sites (P = 4.12e-02; P = 1.90e-04; P = 7.41e-03; P = 4.41e02; Fig. 2, Table 3). Week 29 did not reach the statistical threshold (P = 5.92e-02) but occurred during the treatment period and is operationally important. These epidemiological weeks represent the last 3 weeks of adulticide treatment and 2 weeks after.

The minimum infection rate for WNV for all pools tested (n = 96 pools, 4,769 mosquitoes) ranged from 0 to 20.33 per 1,000 gravid mosquitoes (0–37.96, 95% confidence interval). In the weeks tested, infection rates between treatment and control sites in pair 1 were not different ($t = -0.466$; df = 7.166; $P = 0.655$) (Table 4). However, infection rates in the treatment site of pair 2 were significantly lower than the control site ($t = 4.959$; df = 7.987; $P = 0.001$) (Table 4).

Discussion

Our study provided evidence that ground-based applications of an etofenprox based ULV adulticide affected the age structure of the target WNV *Culex* vector spp. populations assessed herein, although that impact was not evident in a reduction of abundance. We concluded that ULV effectiveness cannot be fully assessed when reduction in adult mosquito abundance is the only outcome parameter—an additional measure is needed. The change in age structure at treatment sites showed that repeated adulticide treatment increased the proportion of nulliparous females in the host-seeking population (Fig. 2). Our observation of a shifted age structure is similar to previous work in Aedine and Anopheline species (Lofgren et al. 1970, Pant et al. 1971, Uribe et al. 1984, Brown et al. 1991, Raghavendra et al. 2011, Ponlawat et al. 2017, Gunning et al. 2018) and 2 studies of *Culex* species (Reisen et al. 1984, 1985). The frequency of ground ULV adulticide application needed to consistently produce these results remains unclear and depends on emergence and immigration rates.

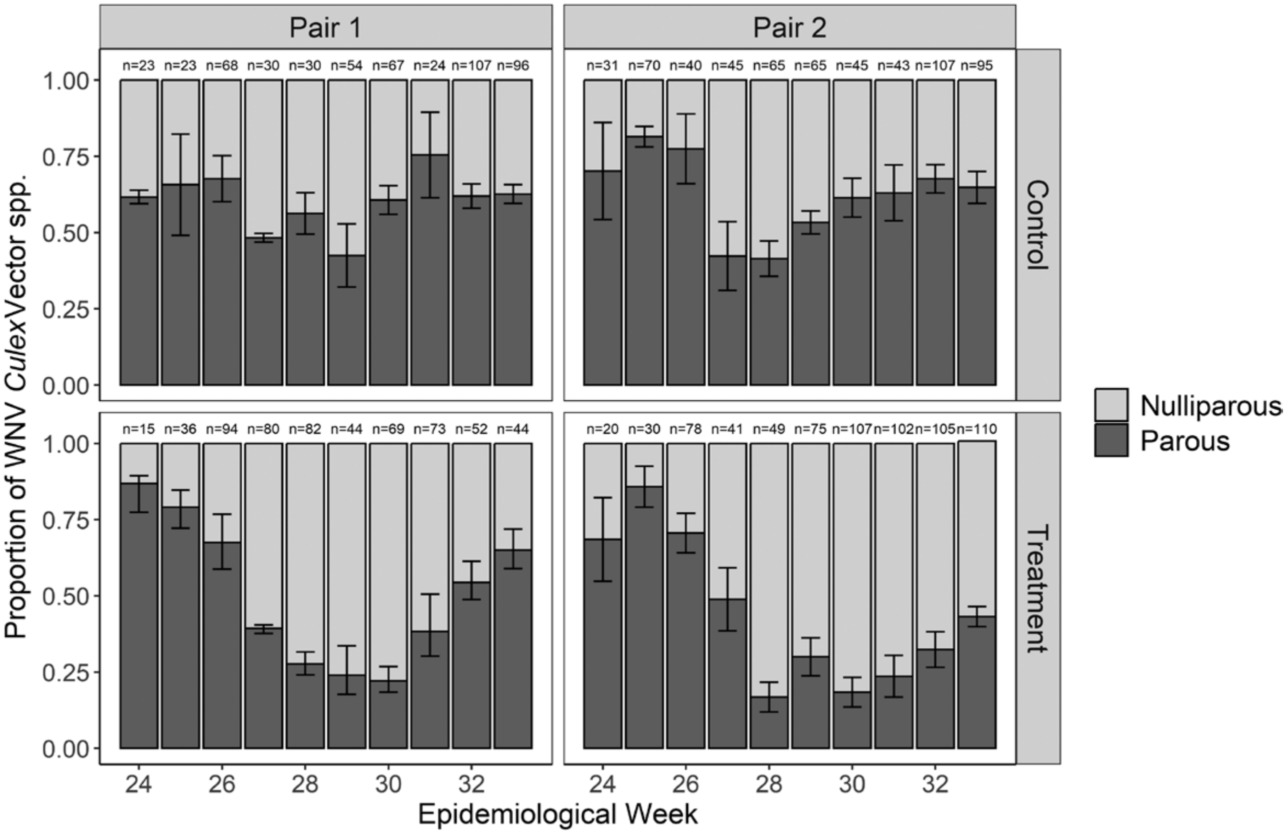

Fig. 2. Mean proportions of parous and nulliparous target WNV *Culex* vector species during each epidemiological week in 2018. Sample size (number of mosquitoes dissected each week at each site) is annotated above each week. Error bars represent standard error. ULV adulticide application occurred during weeks 26–30.

The ultimate goal of adulticide application is to reduce transmission of pathogens. For reduction of WNV transmission it is beneficial for a large proportion of the mosquito population to be nulliparous, even if population abundance remains high. Transmission should be reduced even if biting pressure remains constant, as a large proportion of bites will be uninfected because those females have not consumed a previous blood meal. WNV infection rates were lower in one of our treatment sites compared to the control site, where the proportion of nulliparous females was greater (Fig. 2, Table 4). The sample size was small, but a significant difference was observed.

The absence of consistent short-term and long-term population suppression in host-seeking after spraying is evidence that mosquito abundance at our study sites was not affected by a 5-week, weekly treatment regimen of ULV adulticide application. Short-term and long-term population suppression was observed in gravid mosquitoes using Mulla's formula. Reductions in the proportion of parous mosquitoes are likely directly related to observed reductions in gravid mosquitoes. Similar treatments were implemented in the Chicago area by Clifton et al. (2019) and Mutebi et al. (2011). Clifton et al. (2019) reported short-term reductions in mosquito abundance, followed by a rebound. Mutebi et al. (2011) reported long-term reductions in gravid mosquito abundance, although seasonal abundance was modest. The absence of reduction in mosquito abundance in our study could be due to the adulticide used, high mosquito density, or variation in the number of mosquitoes trapped between sites, traps, and within the same trap. Sites in this study were much smaller than sites utilized in other adulticide evaluation studies, making immigration a particular concern. The number of trapped mosquitoes is likely influenced by immigration of *Cx. pipiens* and *Cx. restuans* mosquitoes from outside of our treatment areas (Reeves et al. 1948, Cui et al. 2013, Hamer et al. 2014). It is established that an abundance of oviposition sites, common in cemeteries, can produce a constant emergence of nulliparous females (Meara, Evans, and Gettman 1992, Pons et al. 2008). After treatment was stopped, we saw a strong increase of mosquito abundance within the treatment site of pair 2, which could be indicative of some degree of population suppression during the treatment period (Table 2). This rebound effect has been documented previously (Clifton et al. 2019). However, we did not observe this rebound in both site pairs.

An absence of clear treatment impact on abundance also could be associated with ULV droplet and mosquito contact issues. ULV applications are highly dependent on weather conditions such as wind speed, a common limiting factor in this area (Irwin et al. 2022). For example, if a spray is lethal to 90% of the population but only reaches 10–20% of the mosquito population because the product doesn't drift, a 9–18% reduction in adult abundance is expected, if there is no emergence and immigration. Adulticide applications kill mosquitoes in flight, including nulliparous and parous individuals, but with notable differential impacts depending on access, age and physiological state. Older (typically parous) host-seeking mosquitoes are more susceptible to insecticides (Rajatileka et al. 2011, Oliver et al. 2014). Effectiveness may be further diminished due to physiological changes such as increased pyrethroid tolerance after blood meal ingestion (Reiter et al. 1990). Additionally, blood-fed and gravid mosquitoes may not be exposed to ULV spray while sheltering during blood meal digestion as observed for *Ae.*

aegypti (Focks et al. 1987, Perich et al. 1990); however, all physiological stages of *Cx. tarsalis* egress at dusk to sugar feed nightly in arid conditions (Reisen et al. 1986). To the best of our knowledge, *Cx. pipiens* and *Cx. restuans* nightly questing activity and behaviors in the Upper Midwest have not been described. The effect of adulticide spray on the gravid population can be expected to be delayed by approximately the duration of the gonotrophic cycle. *Culex* species females can take as little as 4 days to complete the gonotrophic cycle (Eliason et al. 1990), therefore the absence of females that would have taken a blood meal the night of application would be observed after this time period. However, we did not observe a consistent decrease in gravid mosquito populations 3- or 5-days postspray (Supplementary Table 2).

Insecticide resistance is another possible complicating factor for successful ULV adulticide treatment effectiveness. Resistance has been detected across many *Culex* spp. throughout the United States (Richards et al. 2017). Using the CDC bottle bioassay, potential pyrethroid resistance has been detected in Illinois (PRI 2017, Noel 2019, Dubie et al. 2022) and within the NWMAD (Burgess et al. 2022). Although resistance was not evaluated for mosquitoes from these specific sites, reduced susceptibility to etofenprox has been detected in *Cx. pipiens* populations less than 0.5 km away (unpublished data). Further work is needed to fully understand the insecticide resistance status of local *Culex* spp. populations and how insecticide resistance affects control efforts.

Our data show that relying only on mosquito trap counts and focusing on reduction in abundance may yield conflicting, highly variable, and difficult to interpret results. We expected that repeated adulticide sprays would reduce mosquito populations and suppress the overall population when timed appropriately. Although we did not achieve these expected results, we note that by changing population age structure, and thus the probability of WNV infection in biting mosquitoes, infection risk should be lower. These results highlight the need for parity analyses and arbovirus testing in unraveling the intricacies of adult mosquito populations post-adulticide application. Research into these issues in larger-scale studies is necessary.

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Supplementary Material

Supplementary material is available at *Journal of Medical Entomology* online.

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