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EVALUATING SURVEILLANCE METHODS FOR ARBOVIRAL VECTORS OF LA CROSSE VIRUS AND WEST NILE VIRUS OF SOUTHERN APPALACHIA

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ABSTRACT. To monitor mosquito-borne diseases, public health departments conduct mosquito and pathogen surveillance. Our objective was to evaluate mosquito monitoring methods for collecting La Crosse virus (LACV) and West Nile virus (WNV) vectors (*Aedes* and *Culex* mosquitoes, respectively) in southern Appalachia. Centers for Disease Control and Prevention (CDC) light traps baited with carbon dioxide (CO_2), CDC light traps baited with CO_2 and BG lure, BG-Sentinel traps baited with CO_2, gravid traps baited with oak (*Quercus*)–water infusion, and resting traps were compared in eastern Tennessee in 2013. Traps operated at 8 different urban sites throughout Knox County were randomly assigned to and rotated among 6 plots within each site. Results were specific for each vector; the BG-Sentinel trap was the best method for *Aedes triseriatus*, the CDC trap baited with CO_2 and BG lure was the best method for *Ae. albopictus*, and the gravid trap was the best method for *Ae. japonicus*. *Culex erraticus* collections varied by week and trapping method, indicating no single method was best, but the questing traps collected more mosquitoes. There was no significant trapping difference for *Cx. pipiens* complex in this region using the methods tested. The results suggest using a combination of trapping methods when sampling for LACV and/or WNV mosquito vectors in southern Appalachia. Effective trapping methods are necessary to enable accurate surveillance, improve control methods, enhance understanding of dispersal, and use for early detection of vectors and pathogens.

KEY WORDS *Aedes*, Appalachia, *Culex*, La Crosse virus, monitoring methods, West Nile virus

INTRODUCTION

La Crosse virus (LACV) and West Nile virus (WNV) are arthropod-borne zoonotic viruses found in eastern North America, and both are transmitted via the bite of infected mosquitoes (Hollidge et al. 2010). La Crosse virus is transmitted primarily by *Aedes triseriatus* (Say), while *Ae. albopictus* (Skuse) and *Ae. japonicus* (Theobald) act as secondary LACV vectors (Watts et al. 1973, Gerhardt et al. 2001, Bevins 2007, Westby et al. 2015). West Nile virus is transmitted by *Culex* mosquitoes, especially members of the *Cx. pipiens* (L.) complex (Anderson et al. 1999, Nasci et al. 2001, Savage et al. 2008).

Multiple collection methods are available for trapping mosquitoes, and many are designed to capture specific life stages and to quantify mosquito dispersal (Service 1976, Kline 2006, Farajollahi et al. 2009, Cohnstaedt et al. 2012). Questing traps include the Centers for Disease Control and Prevention (CDC) miniature light traps, BG-Sentinel traps, and Fay–Prince traps (Fay and Prince 1970, Kline 2006, Andreadis and Armstrong 2007, Schmaedick et al. 2008). These traps often use an attractant (such as carbon dioxide [CO_2]) to lure and capture host-seeking female mosquitoes seeking a blood meal. Human landing rates monitor actively biting mosquito populations, but the ethical soundness of such methods is debatable in known high-risk regions (Krockel et al. 2006, Trout et al. 2007, Onyango et al. 2013). To collect gravid mosquitoes, gravid traps are baited with bacteria–, grass–, or oak (*Quercus*)–water or other infusions (Williams and Gingrich 2007, Obenauer et al. 2010). Resting boxes and resting traps collect bloodfed mosquitoes that rest on the sides of walls or on vegetation (Panella et al. 2011, Onyango et al. 2013); clay pots are used to monitor resting mosquitoes in Africa (Odiere et al. 2007). Immature mosquitoes are often collected via dipping in stagnant bodies of water (Almiron and Brewer 1994) or setting out ovitraps consisting of artificial containers (plastic cups) baited with water and lined with seed germination paper on which mosquitoes may oviposit (Krockel et al. 2006, Trout et al. 2007). Nonattractant traps, such as malaise traps, are useful for collecting a variety of species, and are often more successful at collecting male mosquitoes than most attractant-based traps (Smith et al. 1965).

The southern Appalachian region includes eastern Tennessee and western North Carolina and accounts for more than a quarter of all La Crosse cases (CDC 2009), with positive virus found in *Ae. albopictus*, *Ae. triseriatus*, and *Ae. japonicus* (Westby et al. 2015). In 2012, Union County, TN, had a fatal case of La Crosse encephalitis that led to an in-depth vector ecology study comparing trapping sites (Trout Fryxell et al. 2015) and LACV strains (Lambert et al. 2015). The field component included a number of different traps targeting both adult and immature mosquitoes to detect LACV in the mosquitoes

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and confirm a field isolate with the fatal isolate. None of the adult traps collected positive mosquitoes; however, LACV was identified from the immature collections (ovitraps). These researchers spent nearly 6 months raising mosquitoes to adulthood in growth chambers, screening them, and amplifying the virus. The time that went into detection of the positive pools was not sufficient to alert nearby communities and was expensive in time, labor, and supplies. The lack of LACV-positive adults (knowing they were in the area since immatures were positive from transovarial transmission) revealed a critical need to evaluate trapping methods for adult vectors in the Appalachian region to determine the optimal method for mosquito monitoring and LACV surveillance. In addition, the constant threat of WNV in southern Appalachia led us to evaluate both LACV and WNV trapping methods, but identifying ideal trapping methods for LACV vectors was the primary objective.

It is essential to identify methods specific for vector surveillance to ensure accurate collection of public health data, including vector frequencies, population densities, disease prevalence, and effectiveness of control methods. Improving these collection methods allows for more accurate monitoring, which can aid in the prevention of vector-borne diseases. The increase of LACV and continued prevalence of WNV in the Appalachian region has created an essential need for the development and understanding of effective adult monitoring techniques. While surveillance methods have been developed and evaluated for both LACV (Williams et al. 2006, Hoel et al. 2007, Meeraus et al. 2008) and WNV (DiMenna et al. 2006, Andreadis and Armstrong 2007, Williams and Gingrich 2007), the changing vector and pathogen distributions justify trapping comparison studies in different regions. We collected mosquitoes using 5 adult trapping methods—a BG-Sentinel trap baited with CO_2 (BG-CO_2), a CDC miniature light trap (with the light removed) baited with CO_2 (CDC-CO_2), a CDC miniature light trap (with the light removed) baited with a combination of CO_2 and BG lure (CDC-CO_2 + BG lure), a CDC gravid trap, and a CDC resting trap—to identify the best monitoring methods for LACV and WNV vectors.

MATERIALS AND METHODS

Site selection

Eight properties within Knox County, TN, were selected for weekly mosquito monitoring. Five properties were the residence of a La Crosse encephalitis–diagnosed child in 2011–12. The remaining 3 properties were within 5 km of at least one of the previous properties, but had no known incidence of LACV or WNV exposure. The sites varied in thickness of vegetation, acreage, and elevation, as well as proximity to mountains, bodies of water, and other homes. Homeowners from all sites were contacted and permission was obtained to monitor mosquito populations during the 2013 mosquito season. Within each site 6 plots were chosen and georeferenced using a handheld global positioning system device (Garmin eTrex Legend, 010-00256-00; Garmin Ltd., Kansas City, MO). Most of the sites chosen had ample vegetation (~800-m^2 to 15,000-m^2 lots) and coverage for setting up traps. Vegetation included trees (*Acer* spp., *Quercus* spp., *Liriodendron tulipifera* L., and *Cornus florida* L.), bushes (*Lornicera* spp., *Juniperus* spp., and *Ligustrum* spp.), and flowering plants (*Lilium* spp., *Solidago* spp., and *Echinacea* spp.). One of the previously positive sites was an exception, as it had <800 m^2 of land and very little vegetation. In this case, traps were deployed both at the house and in vacant lots around the neighborhood. One adult trap plot was placed at this specific homestead and the adjacent tree lines surrounding the neighborhood were used for the rest of the plots (<500 m away). Domestic animals were present at each of the sites as 7 of the properties had dogs, and some of the properties had cows, goats, and chickens. Additionally, each of the sites had active populations of birds (*Aves*), squirrels (*Sciurus*), and chipmunks (*Tamias*)—known amplifying reservoirs of WNV or LACV. Play structures for children, including swing sets, were present at 5 of the sites. All but 2 of the sites had pools, 3 of the sites had a variety of small containers suitable for catching rainwater (including plastic cups, tanks, and buckets), and 1 of the sites had obvious tree holes in which live mosquito larvae were observed by the investigators. The investigators did not alter the sites but did provide mosquito management information to the homeowners. To the investigators' knowledge, none of the homeowners used any of these management recommendations except applying personal protection such as a topically applied mosquito repellent. Environmental conditions (temperature, relative humidity, wind speed) were recorded from the same location at each site during trap setup and takedown.

Adult mosquito monitoring

Five adult trapping methods were compared over 13 wk, starting on July 22 and ending on October 15, 2013. These included three questing traps, a gravid trap, and a resting trap. The 1st questing trap (CDC-CO_2) was a CDC miniature light trap (Model No. 512; John W. Hock Company, Gainesville, FL) with the light removed and baited with ~1 kg of dry ice (for CO_2) in a cooler with holes drilled into the sides and hung from a tree or other structure at ~1.5 m aboveground. The 2nd trap ($CDC-CO_2$ + BG lure) was the same as the CDC trap baited with dry ice and BG lure attached to the trap (BioQuip Products, Rancho Dominguez, CA). The BG lure is comprised of substances found on human skin, including ammonia, lactic acid, and caproic acid. The 3rd questing trap was a BG-Sentinel trap ($BG-CO_2$) (Model No. 2880; BioQuip Products) baited with a tank filled with CO_2 emitted at a rate of 200 ml/min. For this study the BG trap did not include the BG lure; instead, we evaluated CDC traps with and without the lure due to costs, trap accessibility, and results from the literature (Farajollahi et al. 2009). To collect gravid mosquitoes, CDC gravid traps (Model No. 1712; John W. Hock Company) were baited with 2.2 liters of an infusion made from oak leaves and warm water soaked overnight (Obenauer et al. 2010). A CDC resting trap was modified from Panella et al. (2011) using a plastic planter instead of a wood fiber pot and collecting equipment from gravid trap parts, including the plastic portion holding the fan instead of polyvinyl chloride pipe, and the same collection net.

Adult traps operated weekly for a minimum of 18 h starting at 1000 h and ending at 1500 h the following day to collect diurnal, crepuscular, and nocturnal mosquitoes. Sites were visited in the same order each week to ensure similar collection intervals at all sites. At each site, 6 plots were identified and placed a minimum of 6 m apart and traps were randomly assigned each week to a different plot to prevent location bias. Plots varied somewhat in proximity to trees, manufactured structures such as porches and swing sets, and exposure to sunlight, but all plots were located near structures to which CDC miniature light traps could be attached. When traps were retrieved, collecting equipment was removed and specimens were stored in coolers lined with ice packs to keep them alive for identification and to preserve viral RNA. In the laboratory, adult mosquitoes were aspirated from the collecting equipment, transferred to paper cups covered with a square of cheesecloth, and provided a 10% sugar-water source until exposed to triethylamine to knock them down for identification and to keep the mosquito and potential virus alive. The triethylamine-exposed (now paralyzed) mosquitoes were then identified to species and sex (Darsie and Ward 1981).

Statistical analysis

All statistical analyses were conducted in SAS (Statistical Analysis System 2011 version 9.3). Mosquito totals were analyzed using a randomized block ANOVA, with 2 fixed factors: trap and week. Collection site was modeled as a randomized block and the plots within the sites formed replicates. Since traps changed weekly within a plot, week was not considered a repeated measure. All effects in the full factorial design were tested. The ANOVA assumption of normality was tested on model residuals using the Shapiro–Wilk test and the equal variance assumption was tested by Levene's F test. When one or both assumptions were violated, data were log transformed and ANOVA assumptions were retested. All species, except *Ae. albopictus*, were tested using a nonparametric, mixed-model ANOVA based on rank-transformed data (Wittek et al. 2004, Wiggins et al. 2010). The *Ae. albopictus* collections were log transformed to meet assumptions. When a significant effect was present, post hoc comparisons were made using the Tukey method of mean separation. A value of $P < 0.05$ was considered significant for all tests. Means in the results and within the figures were back-transformed for interpretation (raw counts).

RESULTS

Environmental data

The year 2013 had more precipitation than usual (116.71 cm per month), with a mean of 166.42 cm per month from January 1 to December 20, 2013 (Tennessee Climatological Service 2013). Throughout the study temperatures ranged from 16.8°C to 39°C (28.8 ± 0.29°C), RH ranged from 34% to 96%, and wind speeds ranged from 0 to 2.3 mph (0.27 ± 0.03 mph) (= 3.68 km/h [0.43 ± 0.05 km/h]). Weekly environmental data are presented in Fig. 1. No extreme conditions were reported and there was no significant plot or site effect on mosquito collections ($P > 0.05$).

Adult mosquito populations

A total of 4,843 of adult mosquitoes belonging to 20 species were collected and organized into 1,196 pools (Table 1). None of the mosquitoes collected were engorged. The most common species collected were *Ae. albopictus* at 59% (2,846 specimens), *Ae. trivittatus* (Coq.) at 8% (368 specimens), and *Ae. vexans* (Meigen) at 6% (294 specimens). Frequently encountered specimens included 260 *Anopheles punctipennis* (Say), 255 *Ae. triseriatus*, 241 *Cx. erraticus* (Dyar and Knab), 231 *Cx. pipiens* complex, 108 unidentified *Culex* species, 71 *Ae. japonicus*, 60 unidentified *Aedes* species, 17 *Cx. restuans* Theobald, 31 *Psorophora ferox* (Von Humboldt), and 28 *An. quadrimaculatus* Say. The remaining species included fewer than 10 specimens of *An. walkeri* Theobald, *Cx. nigripalpus* Theobald, *Mansonia dyari* Walker, *Orthopodomyia signifera* (Dyar and Knab), *Ps. ciliate* F., *Toxorynchites rutilus*

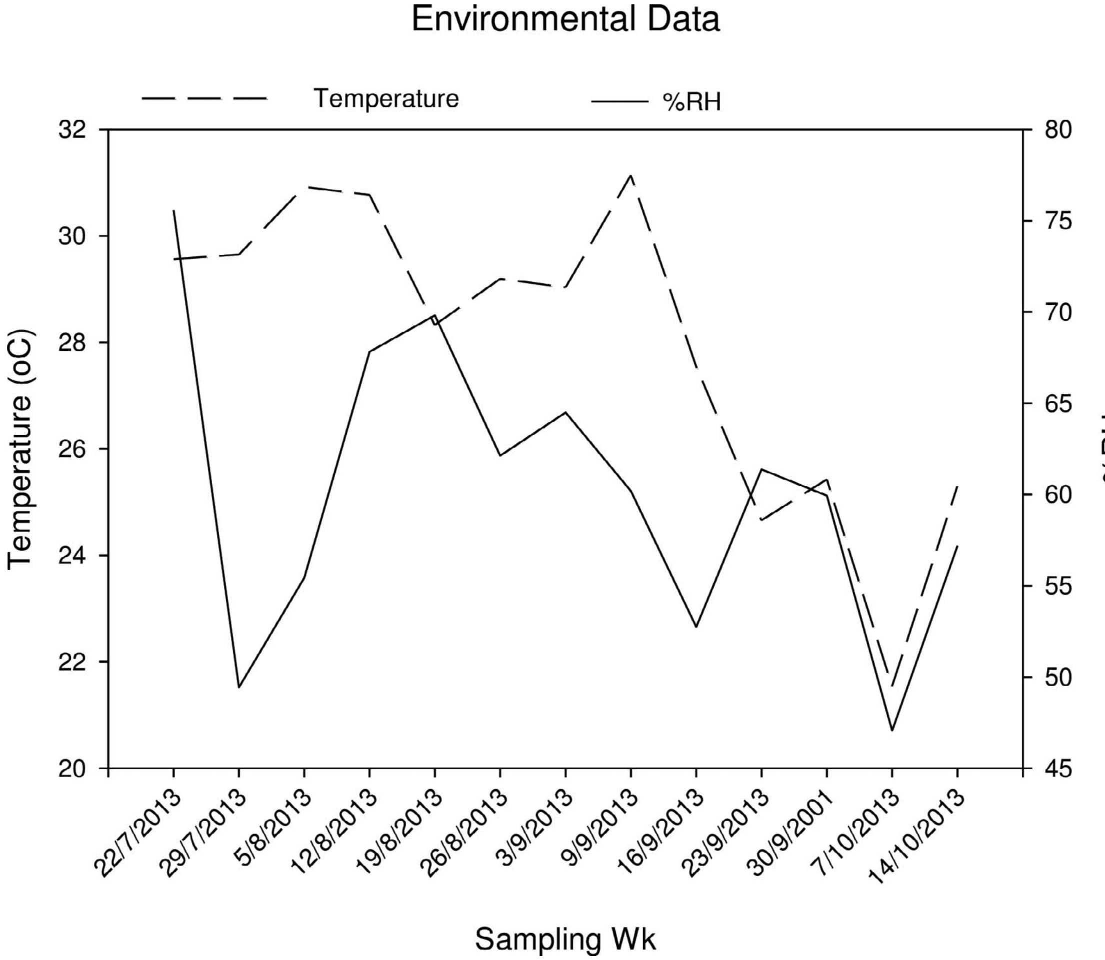

Fig. 1. Weekly environmental data at each site: mean temperature (°C) and RH (%).

septentrionalis (Coq.), and *Uranotaenia sapphirina* (Osten Sacken).

Mosquito populations peaked at the end of July (764 specimens) and were fewest the 1st week of October (72 specimens). The 1st week of August had the most *Ae. albopictus* (7.55 ± 1.19 specimens) per trap, while the 4th week in July had the most *Ae. japonicus* (3 ± 1.00 specimens) and *Ae. triseriatus* (6.67 ± 4.26 specimens) (Fig. 2). The week of August 26 yielded the most *Cx. erraticus* specimens per trap (3.67 ± 0.99) and the week of September 23 yielded the most *Cx. pipiens* specimens per trap (7.375 ± 3.64) (Fig. 3). There were no significant differences among the 5 sites with previous La Crosse encephalitis patients and those from the 3 remaining sites for total mosquito collections (unpaired *t*-test: $t = 0.6481$; df = 5,916; $P = 0.5169$) or LACV vector collections (unpaired *t*-test: $t = 0.4644$; df = 6; $P = 0.6587$). However, there was a significant difference between the site types such that WNV vectors were significantly more likely to be collected from previous LACV case houses (2.76 ± 0.22 specimens) than from the remaining 3 houses (1.5 ± 0.18 specimens) ($t = 2.7326$; df = 303; $P = 0.006653$).

LACV vector collections

The BG-CO_2 trap collected the majority of LACV vector mosquitoes ($n = 1{,}136$ specimens; 7.1 ± 0.60 specimens per trap) while the resting trap collected the fewest ($n = 197$ specimens; 3.23 ± 0.62 specimens per individual trap). Specifically, a mean of 8.0 (± 0.74) *Ae. albopictus*, 2.14 (± 0.51 per trap) *Ae. japonicus*, and 4.68 (± 0.88 per trap) *Ae. triseriatus* were collected from each BG-CO_2 trap (Fig. 4). There was no significant trap × week interaction effect for *Ae. albopictus* ($F = 1.07$; df = 48, 426; $P = 0.3481$), *Ae. japonicus* ($F = 1.30$; df = 48, 426; $P = 0.0924$), or *Ae. triseriatus* ($F = 0.61$; df = 48, 426; $P = 0.9830$). As mentioned earlier, only *Ae. albopictus* collections were log transformed to meet the statistical

Table 1. Data on adult mosquitoes collected in 5 different trapping methods (BG-Sentinel traps baited with CO_2 [BG-CO_2], Centers for Disease Control and Prevention light traps baited with CO_2 [CDC-CO_2], CDC light traps baited with CO_2 and the BG lure [CDC-CO_2 + BG lure], gravid traps, and resting traps) during July 22 to October 15, 2013 in Knox County, TN.

	Trapping method					
Mosquito species	No. BG-CO_2	No. CDC-CO_2	No. CDC-CO_2 + BG lure	No. gravid	No. resting	Total mosquitoes
Aedes albopictus	976	488	939	251	189	2,843
Ae. japonicus	15	16	6	30	4	71
Ae. triseriatus	145	41	46	19	4	255
Ae. trivittatus	170	94	100	1	3	368
Ae. vexans	122	67	104	0	1	294
Aedes spp.	33	10	15	1	1	60
Anopheles punctipennis	147	57	46	0	10	260
An. quadrimaculatus	4	14	8	1	2	29
Culex erraticus	84	53	66	28	10	241
Cx. nigripalpus	1	1	0	0	0	2
Cx. pipiens complex	42	61	45	70	8	226
Cx. restuans	4	7	3	3	0	17
Culex spp.	40	25	10	29	4	108
Mansonia dyari	1	0	1	0	0	2
Orthopodomyia signifera	1	1	4	3	0	9
Psorophora ciliate	4	0	0	0	0	4
Ps. ferox	26	2	3	0	0	31
Toxorhynchites rutilus septentrionalis	0	0	0	3	0	3
Uranotaenia sapphirina	0	3	2	0	0	5
Totals	1,815	940	1,398	439	236	4,828

assumptions; once transformed there was a significant difference in *Ae. albopictus* collections by trap (F = 19.49; df = 4, 426; $P < 0.0001$). The most effective trap was the CDC-CO_2 + BG lure (2.52 ± 0.22 mean), followed by the BG-CO_2 traps (2.06 ± 0.26), CDC-CO_2 trap (1.48 ± 0.26), gravid trap (1.23 ± 0.2), and resting trap (0.89 ± 0.09). Raw untransformed means of the *Ae. albopictus* data set are presented in Fig. 4. The BG-CO_2 traps had the most *Ae. triseriatus* (4.68 ± 0.88 specimens); the CDC-CO_2 + BG lure trap (2.56 ± 0.33 specimens), and CDC-CO_2 trap (1.71 ± 0.21 specimens) collected significantly more *Ae. triseriatus* than the other traps (F = 7.80; df = 4, 426; $P < 0.0001$) (Fig. 4). The gravid trap collected significantly more *Ae. japonicus* (1.76 ± 0.25 specimens/trap) than any other trap (F = 3.94; df = 4, 426; $P < 0.0038$) (Fig. 4).

WNV vector collections

The CDC-CO_2 trap collected the majority of *Culex* mosquitoes ($n = 284$ specimens; 2.31 ± 0.19 specimens/wk), while the fewest *Culex* mosquitoes were collected in the resting trap ($n = 31$ specimens, 1.19 ± 0.09 specimens/wk). Since 2 of the 137 (1.46%) *Cx. erraticus* pools were positive for WNV (Urquhart, Paulsen, Moncayo, Trout Fryxell; unpublished data), trapping methods were also compared for this species. When sampling for *Cx. erraticus* there was a significant trap × week interaction effect (F = 1.88; df = 48, 426; $P = 0.0006$), such that the most effective trap was dependent on the trapping week (Fig. 5). Overall, the most effective traps for *Cx. erraticus* were BG-CO_2 traps (3.194 ± 0.655 specimens), CDC-CO_2 traps (2.282 ± 0.305 specimens), and CDC-CO_2 + BG lure traps (2.233 ± 0.306 specimens). Gravid traps (1.8 ± 0.224 specimens) and resting traps (1.1667 ± 0.112 specimens) collected few *Cx. erraticus*. The effectiveness of the CDC-CO_2 + BG lure traps varied significantly depending on the week. The least successful trapping weeks were those of August 5, September 23, and October 14. The most successful week for trapping *Cx. erraticus* using CDC-CO_2 + BG lure traps was the week of August 26. There was no significant difference by week for any of the other 4 traps. In the case of the *Cx. pipiens* complex, the resting trap was the least effective (F = 2.66; df = 4, 426; $P < 0.0326$), but the remaining traps collected similar numbers of this species complex (Fig. 4).

DISCUSSION

This study evaluated the effectiveness of 5 different adult mosquito monitoring methods for collecting the vectors of both LACV and WNV specifically in the Appalachian region. Here, the most effective method for monitoring mosquito-borne viruses is to use a combination of traps. The CDC-CO_2 and/or CDC-CO_2 + BG lure traps were significantly more effective than nonquesting traps in more than one situation, suggesting

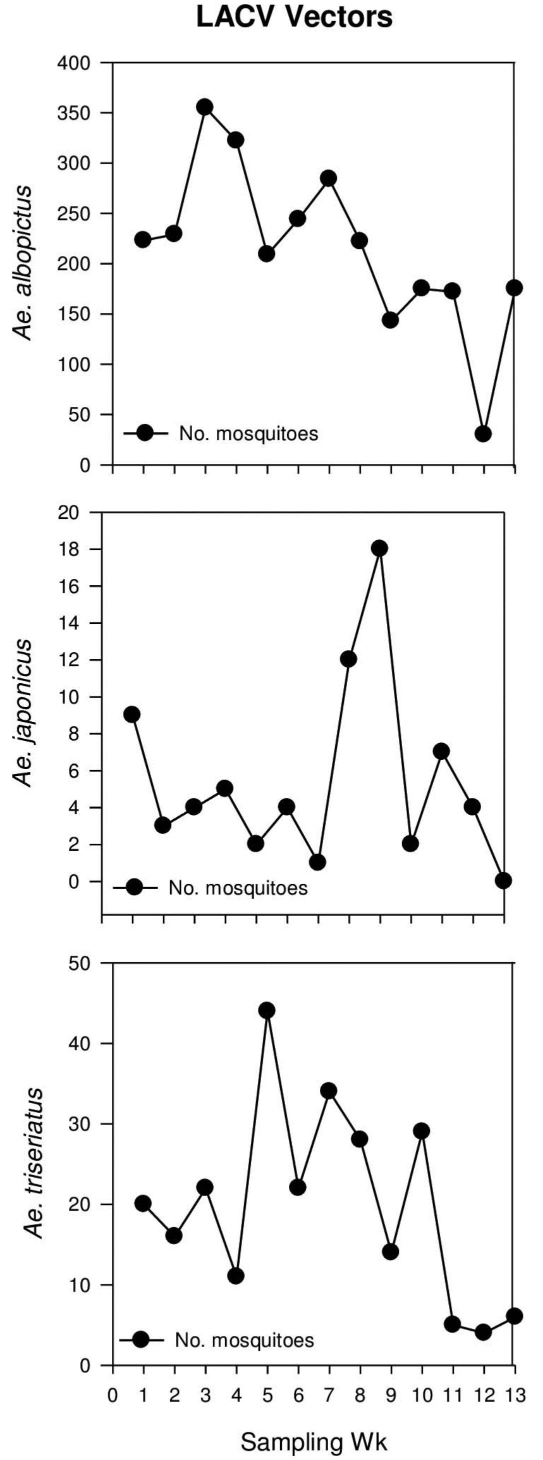

Fig. 2. Total number of La Crosse virus vectors collected using all 5 trapping methods/wk over 13 wk (July 22 through October 18, 2013).

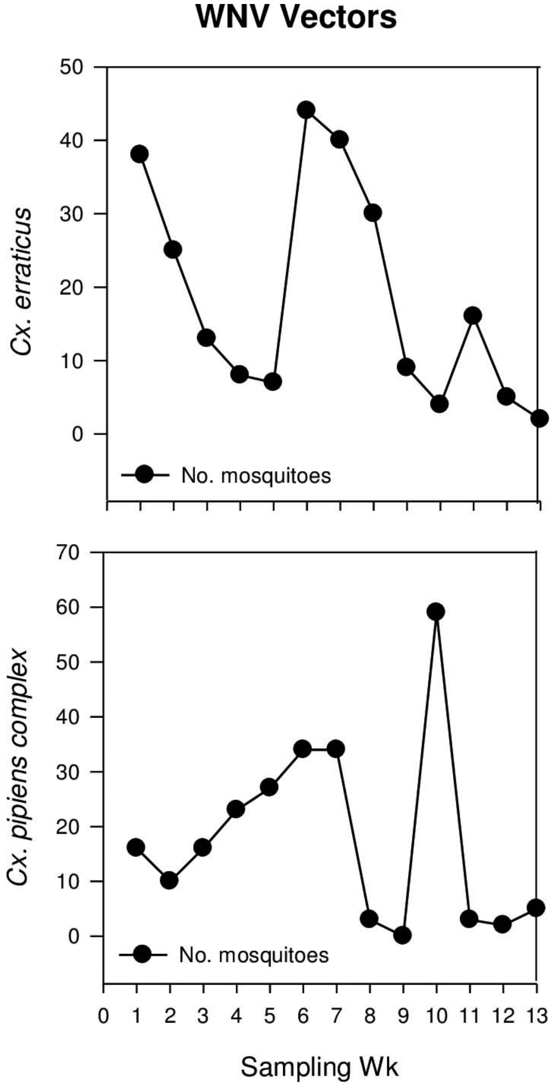

Fig. 3. Total number of potential West Nile virus vectors collected using all 5 trapping methods/wk over 13 wk (July 22 through October 18, 2013).

that either trap would be useful in any mosquito surveillance program. The addition of a gravid trap is also suggested as a supplemental method, especially when *Ae. japonicus* is a target. The resting trap was the least effective trap at collecting mosquitoes and is not necessary; this was likely due to the amount of vegetation and other vertical structures available for resting mosquitoes.

The BG-Sentinel trap is an effective mosquito monitoring method, as demonstrated by this and previous studies (Krockel et al. 2006, Williams et al. 2006); however, it is also expensive, heavy, and comes with a variety of mechanical issues, making it one of the more cumbersome traps available (Crepeau et al. 2013). Krockel et al.

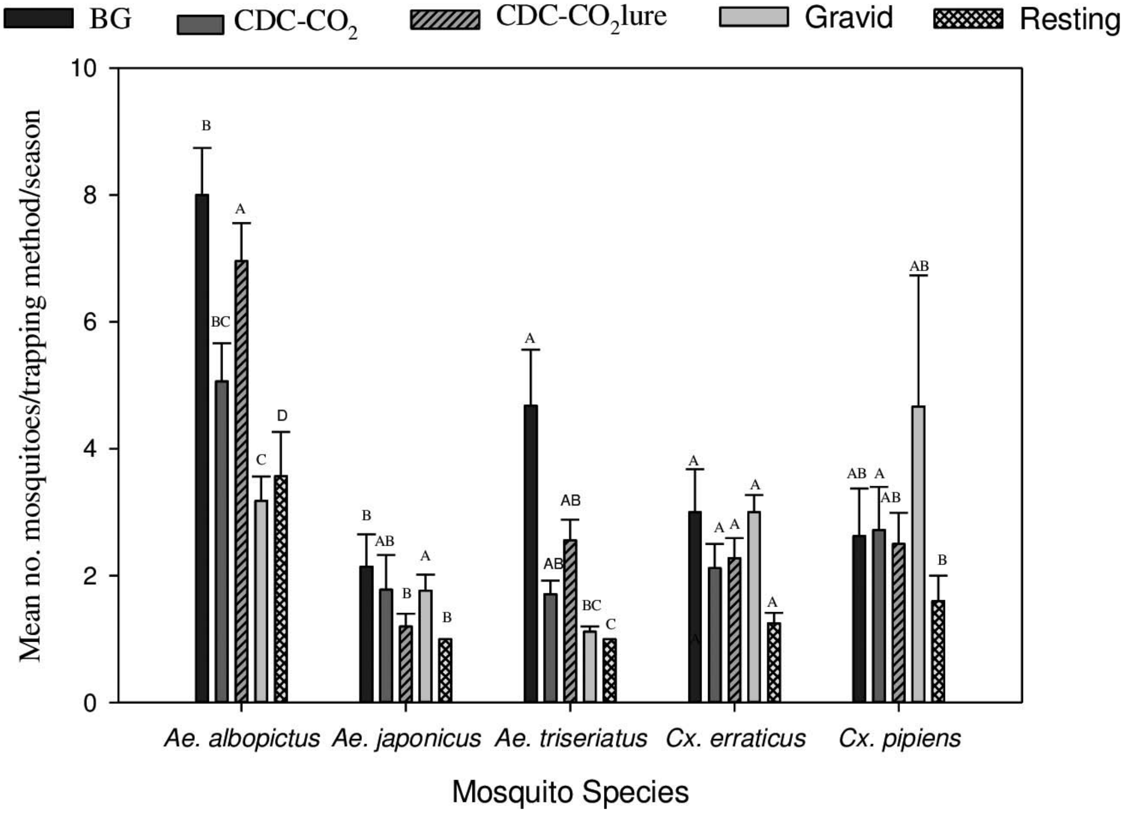

Fig. 4. Untransformed means (± SE) number of La Crosse virus and West Nile virus vectors collected with BG-Sentinel traps, Centers for Disease Control and Prevention (CDC) light traps, CDC light traps baited with the BG lure, gravid traps, and resting traps over 13 wk in eastern Tennessee (July 22 to October 15, 2013). Values with different letters were significantly different within comparisons of each species ($\alpha = 0.05$), as determined by Tukey's test.

(2006) combined the BG lure with the BG-Sentinel trap to demonstrate trap effectiveness at collecting *Ae. aegypti*, but they did not combine any other questing traps with the BG-lure. Farajollahi et al. (2009) combined the BG lure with both CDC questing traps and BG traps, and demonstrated that the BG trap with the BG lure was most effective at collecting *Ae. albopictus* in New Jersey, but that the BG lure combined with either questing trap was more effective than using only CO_2. The present study supports Farajollahi et al. (2009); the CDC-CO_2 + BG lure trap was more effective than using only CO_2 for collecting *Ae. albopictus* and *Ae. triseriatus* and demonstrates the potential for the BG lure to be used as a supplement with other more economical and mechanically simple traps. A future study comparing the effectiveness of different questing traps when combined with the BG lure would be beneficial to establish whether the trap structure makes a significant difference in this region or if the lure, combined with any trap, would be effective.

Evaluating monitoring methods for WNV vectors is important especially since it is prevalent throughout the USA. Evaluations should include assessing methods in areas with different vectors, varying habitats, climates, and hosts. In this study, LACV vectors were targeted over WNV vectors so an oviposition substrate similar to one found in oak trees was used, which is known to specifically target *Aedes* mosquitoes and others that prefer to oviposit in and around oak trees (Trexler et al. 1998). *Culex* mosquitoes are typically collected in greater abundances with gravid traps baited with other materials such as grass–water infusions (Mboera et al. 2000, Lee and Kokas 2004) or bacteria infusions (DiMenna et al. 2006, Beehler et al. 2008, Ponnusamy et al. 2009), but here questing traps were more effective at collecting WNV vectors. Again, this was likely due to the gravid trap's oviposition substrate. Future studies and management techniques should employ gravid traps with each infusion, one with oak-leaf for *Ae. japonicus* and one with grass-infusion for *Cx. pipiens,* to ensure both vectors are collected in areas where both species are important. This is especially true for eastern Tennessee since these vectors are sympatric.

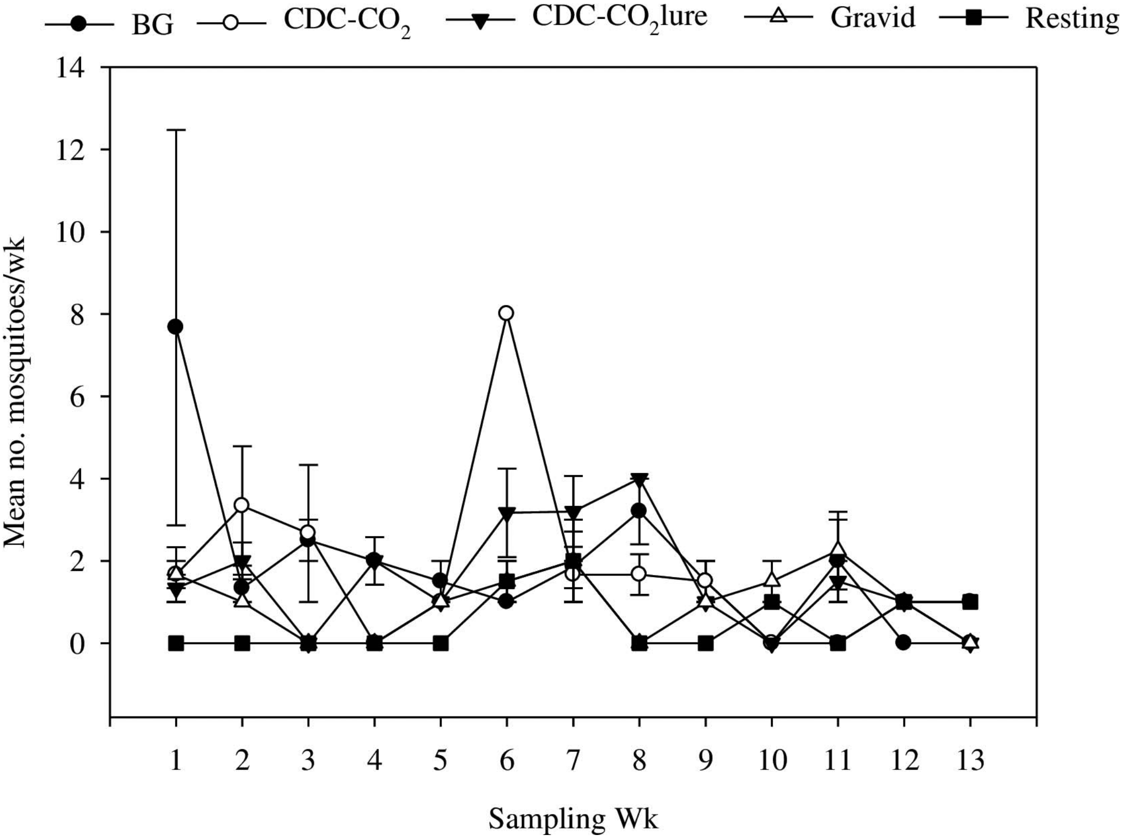

Fig. 5. Weekly differences in mean number (± SE) of *Culex erraticus* for each trapping method during July 22 through October 18, 2013 ($F = 1.88$; $df = 48, 426$; $P = 0.0006$).

It will also be necessary to reevaluate traps at regular intervals (e.g., every 5 years) to ensure that collection methods remain as effective as possible.

The changing habitats and developing vector–host–virus adaptations merit a continuing need for adequate and innovative mosquito surveillance methods. While many of the existing methods are useful and effective, the fluctuations in vector species, populations, densities, and habitats create additional trapping challenges that merit evaluations. For instance, the urban sprawl of humans into forested areas has been associated with an increase in anthropophilic feeding by *Ae. triseriatus* and may contribute to increasing LACV cases in the future (Barker et al. 2003). Likewise, the introduction of the secondary vectors *Ae. albopictus* and *Ae. japonicus* has likely expanded the distribution of LACV from the rural environment to the urban and suburban environment as both species have been recovered in field collections as infected with LACV (Gerhardt et al. 2001, Harris et al. 2015, Westby et al. 2015). Previous surveillance studies have suggested a change in the composition of *Ae. triseriatus* and *Ae. albopictus*, which may be due to the use of different trapping methods or could indicate a change in the ratio of these vector species (Westby et al. 2015). Continued monitoring of these species and evaluation of different trapping methods are necessary for answering those questions. Additionally, using the appropriate trapping methods, public health officials will be better able to monitor and control vectors.

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REFERENCES CITED

Almiron WR, Brewer ME. 1994. Immature stages of mosquitoes (Diptera: Culicidae) collected during the autumn-winter period in Cordoba Province, Argentina. *Mem Inst Oswaldo Cruz* 89:625–628.

Anderson JF, Andreadis TG, Vossbrinck CR, Tirrell S, Wakem EM, French RA, Garmendia AE, Van Kruiningen HJ. 1999. Isolation of West Nile Virus from mosquitoes, crows, and a Cooper's hawk in Connecticut. *Science* 286:2331–2333.

Andreadis TG, Armstrong PM. 2007. A two-year evaluation of elevated canopy trapping for *Culex* mosquitoes and West Nile virus in an operational surveillance program in the northeastern United States. *J Am Mosq Control Assoc* 23:137–148.

Barker CM, Paulsen SL, Cantrell S, Davis BS. 2003. Habitat preferences and phenology of *Ochlerotatus triseriatus* and *Aedes albopictus* (Diptera: Culicidae) in southwestern Virginia. *J Med Entomol* 40:403–410.

Beehler JW, Millar JG, Mulla MS. 2008. Protein hydrolysates and associated bacterial contaminants as oviposition attractants for the mosquito *Culex quinquefasciatus*. *Med Vet Entomol* 8:381–385.

Bevins SN. 2007. Establishment and abundance of a recently introduced mosquito species *Ochlerotatus japonicus* (Diptera: Culicidae) in the southern Appalachians, USA. *J Med Entomol* 44:945–952.

CDC [Centers for Disease Control and Prevention]. 2009. *Information on arboviral encephalitides* [Internet]. Atlanta, GA. CDC, Division of Vector Borne Diseases [accessed July 7, 2014]. Available from: http://www.cdc.gov/lac/.

Cohnstaedt LW, Rochon K, Duehl AJ, Anderson JF, Barrera R, Su N, Gerry AC, Obenauer PJ, Campbell JF, Lysyk TJ, Allan SA. 2012. Arthropod surveillance programs: basic components, strategies, and analysis. *Ann Entomol Soc Am* 29:177–180.

Crepeau TN, Unlu I, Healy SP, Farajollahi A, Fonseca DM. 2013. Experiences with large-scale operation of the Biogents Sentinel Trap. *J Am Mosq Control Assoc* 29:177–180.

Darsie RF Jr. Ward RA. 1981. Identification and geographical distribution of the mosquitoes of North America, north of Mexico. Gainesville, FL: Univ. Press of Florida.

DiMenna MA, Bueno R Jr. Parmenter RR, Norris DE, Sheyka JM, Molina JL, LaBeau EM, Hatton ES, Glass GE. 2006. Comparison of mosquito trapping method efficacy for West Nile virus surveillance in New Mexico. *J Am Mosq Control Assoc* 22:246–253.

Farajollahi A, Kesavaraju B, Price DC, William GM, Healy SP, Gaugler R, Nelder MP. 2009. Field efficacy of BG-Sentinel and industry-standard traps for *Aedes albopictus* (Diptera: Culicidae) and West Nile virus surveillance. *J Med Entomol* 46:919–925.

Fay RW, Prince WH. 1970. A modified visual trap for *Aedes aegypti*. *Mosq News* 30:20–23.

Gerhardt RR, Gottfried KL, Apperson CS, Davis BS, Erwin PC, Smith AB, Panella NA, Powell EE, Nasci RS. 2001. First isolation of *La Crosse Virus* from naturally infected *Aedes albopictus*. *Emerg Infect Dis* 7:807–811.

Harris MC, Dotseth EJ, Jackson BT, Zink SD, Marek PE, Kramer LD, Paulson SL, Hawley DM. 2015. La Crosse virus in *Aedes japonicus japonicus* mosquitoes in the Appalachian region, United States. *Emerg Infect Dis* 21:646–649.

Hoel DF, Kline DL, Allan SA, Grant A. 2007. Evaluation of carbon dioxide, 1-octen-3-ol, and lactic acid as baits in mosquito magnet pro traps for *Aedes albopictus* in North Central Florida. *J Am Mosq Control Assoc* 23:11–17.

Hollidge BS, Gonzalez-Scarano F, Soldan SS. 2010. Arboviral encephalitides: transmission, emergence, and pathogenesis. *J Neuroimmune Pharmacol* 5:428–442.

Kline DL. 2006. Traps and trapping techniques for adult mosquito control. *J Am Mosq Control Assoc* 22:490–496.

Krockel U, Rose A, Eiras AE, Geier M. 2006. New tools for surveillance of adult yellow fever mosquitoes: comparison of trap catches with human landing rates in an urban environment. *J Am Mosq Control Assoc* 22:229–238.

Lambert A, Trout Fryxell RT, Lanciotti R, Freyman K, Ulloa A, Paulsen D, Moncayo A. 2015. Comparative sequence analysis of a La Crosse virus strain isolated from patient with fatal encephalitis, Tennessee, USA. *Emerg Infect Dis* 21:833–836.

Lee JH, Kokas JE. 2004. Field evaluation of CDC gravid trap attractants to primary West Nile virus vectors, *Culex* mosquitoes in New York state. *J Am Mosq Control Assoc* 20:248–253.

Mboera LEG, Takken W, Mdira KY, Pickett JA. 2000. Sampling gravid *Culex quinquefasciatus* (Diptera: Culicidae) in Tanzania with traps baited with synthetic oviposition pheromone and grass infusions. *J Med Entomol* 37:172–176.

Meeraus WH, Armistead JS, Arias JR. 2008. Field comparison of novel and gold standard traps for collecting *Aedes albopictus* in northern Virginia. *J Am Mosq Control Assoc* 24:244–248.

Nasci RS, Savage HM, White DJ, Miller JR, Cropp BC, Godsey MS, Kerst AJ, Bennett P, Gottfried K, Lanciotti RS. 2001. West Nile virus overwintering in *Culex* mosquitoes, New York City, 2000. *Emerg Infect Dis* 7:742–744.

Obenauer PJ, Allen SA, Kaufman PE. 2010. *Aedes albopictus* (Diptera: Culicidae) oviposition response to organic infusions from common flora of suburban Florida. *J Vector Ecol* 35:301–306.

Odiere M, Bayoh MN, Giminig J, Vulule J, Irungu L, Walker E. 2007. Sampling outdoor, resting *Anopheles gambiae* and other mosquitoes (Diptera: Culicidae) in Western Kenya with clay pots. *J Med Entomol* 44:14–22.

Onyango SA, Kitron U, Mungai P, Muchiri EM, Kokwaro E, King CH, Mutuku FM. 2013. Monitoring malaria vector control interventions: effectiveness of five different adult mosquito sampling methods. *J Med Entomol* 50:1140–1151.

Panella NA, Kent Crockett RJ, Biggerstaff BJ, Komar N. 2011. The Centers for Disease Control and Prevention Resting Trap: a novel device for collecting resting mosquitoes. *J Am Mosq Control Assoc* 27:323–325.

Ponnusamy L, Wesson DM, Arellano C, Schal C, Apperson CS. 2009. Species composition of bacterial communities influences attraction of mosquitoes to experimental plant infusions. *Microbiol Aquat Syst* 59:158–173.

Savage HM, Anderson M, Gordon E, McMillen L, Colton L, Delorey M, Sutherland G, Aspen S, Charnetzky D, Burkhalter K, Godsey M. 2008. Host-seeking heights, host-seeking activity patterns, and West Nile virus infection rates for members of the *Culex pipiens* complex at different habitat types within the hybrid zone, Shelby County, TN, 2002 (Diptera:Culicidae). *J Med Entomol* 45:276–288.

Schmaedick MA, Ball TS, Burkot TR, Gurr NE. 2008. Evaluation of three traps for sampling *Aedes polynesiensis* and other mosquito species in American Samoa. *J Am Mosq Control Assoc* 24:319–322.

Service MW. 1976. *Mosquito ecology: field sampling methods*. Essex, United Kingdom: Applied Science Publishers.

Smith GE, Breeland SG, Pickard E. 1965. The malaise trap, a survey tool in medical entomology. *Mosq News* 25:398–400.

Trexler JD, Apperson CS, Schal C. 1998. Laboratory and field evaluations of oviposition responses of *Aedes albopictus* and *Aedes triseriatus* (Diptera: Culicidae) to oak leaf infusions. *J Med Entomol* 35:967–976.

Trout RT, Brown GC, Potter MF, Hubbard JL. 2007. Efficacy of two pyrethroid insecticides applied as barrier treatments for managing mosquito (Diptera: Culicidae) populations in suburban residential properties. *J Med Entomol* 44:470–477.

Trout Fryxell RT, Freyman K, Ulloa A, Hendricks B, Paulsen D, Odoi A, Moncayo A. 2015. Cemeteries are effective sites for surveillance of La Crosse virus (LACV) and these environments may play a role in LACV infection. *PLoS ONE* 10:e0122895.

Watts DM, Pantuwatana S, DeFoliart GR, Yuill TM, Thompson WH. 1973. Transovarial transmission of La Crosse (California encephalitis group) in the mosquito, *Aedes triseriatus*. *Science* 182:1140–1141.

Westby KM, Fritzen C, Paulsen D, Poindexter S, Moncayo A. 2015. La Crosse encephalitis virus infection in field collected *Aedes albopictus*, *Aedes japonicus*, and *Aedes triseriatus* in Tennessee. *J Am Mosq Control Assoc* 31:233–241.

Wiggins GJ, Grant JF, Lambdin PL, Ranney JW, Wilkerson JB, Reed A, Follum RA. 2010. Host utilization of field-caged native and introduced thistle species by *Rhinocyllus conicus*. *Environ Entomol* 39:1858–1865.

Williams CR, Long SA, Russell RC, Ritchie SA. 2006. Field efficacy of the BG-Sentinel compared with CDC backpack aspirators and CO_2-baited EVS traps for collection of adult *Aedes aegypti* in Cairns, Queensland, Australia. *J Am Mosq Control Assoc* 22:296–300.

Williams GM, Gingrich JB. 2007. Comparison of light traps, gravid traps, and resting boxes for West Nile virus surveillance. *J Vector Ecol* 32:285–291.

Wittek T, Constable PD, Fürll M. 2004. Comparison of abomasal luminal gas pressure and volume of the perfusion of the abomasum in dairy cows with left displaced abomasum or abomasal volvulus. *Am J Vet Res* 65:597–603.