*Journal of the American Mosquito Control Association,* 23(2):137–148, 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

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ABSTRACT. The effectiveness of $CO_2$-baited Centers for Disease Control and Prevention miniature light traps elevated in the tree canopy (~7.6 m) was compared with light traps placed at ground level (~1.5 m) and grass-sod infused gravid traps for collecting *Culex pipiens, Culex restuans,* and *Culex salinarius* and detecting West Nile virus (WNV) activity in an operational surveillance program that encompassed 12 ecologically diverse sites in Connecticut in 2004 and 2005. More than twice as many *Cx. pipiens* were collected on average in light traps suspended in the tree canopy than in either light or gravid traps placed at ground level. This difference was generally restricted to those collection sites where markedly greater numbers of *Cx. pipiens* were collected with all trapping methods but was not associated with site-specific urbanization indices. *Culex restuans* was not preferentially attracted to light traps suspended in the tree canopy. No differences in the overall abundance of this species were recorded with either of the 2 trapping procedures, but both light traps were more effective than the gravid traps. *Culex salinarius* was significantly more attracted to ground-based light traps than traps suspended in the tree canopy, while gravid traps were ineffective at all sites regardless of the level of urbanization or any other specific land-use characteristic. $CO_2$-baited light traps placed in the tree canopy were generally superior to ground-based light traps for detecting WNV in *Cx. pipiens.* West Nile virus–infected females were collected more regularly, and the frequency of infected pools was significantly greater. Twofold higher minimum field infection rates (maximum likelihood estimation [MLE] = 6.7 vs. 3.0 per 1,000 mosquitoes) were also recorded from canopy collections of this species, and virus was detected in canopy-collected females several weeks before it was detected in collections from light traps at ground level. We conclude that the use of $CO_2$-baited light traps placed in the tree canopy for targeted trapping of *Cx. pipiens* and subsequent detection of WNV are likely to yield better overall results than light traps placed at ground level in this region of the northeastern United States. The virus isolation data obtained from *Cx. pipiens* collected in gravid traps compared favorably both temporally and spatially with results from canopy trap collections. There were no significant differences in the overall frequency of WNV-infected pools or MLEs for *Cx. pipiens,* but fewer total WNV isolations were made from *Cx. pipiens* collected in the gravid traps and virus was detected more infrequently. Results reaffirmed the utility of gravid traps as effective surveillance tools for detection of WNV in *Cx. pipiens* in the northeastern United States. However, findings also demonstrated that $CO_2$-baited light traps placed in the tree canopy provided more consistent results where weekly detection of virus amplification is a critical objective. The comparative effectiveness of ground- and canopy-based light traps for detection of WNV-infected *Cx. restuans* and *Cx. salinarius* was inconclusive owing to the limited number of virus isolations that were made from these species during the 2 years of study. However, WNV virus isolations were made several weeks earlier and more frequently from *Cx. restuans* collected in traps placed in the canopy rather than at ground level in 2004. Results support the view that ground-based light traps are more effective for detection of WNV in *Cx. salinarius*.

**KEY WORDS** West Nile virus, *Culex pipiens*, *Culex restuans*, *Culex salinarius*, surveillance, light trap, gravid trap, elevation

## INTRODUCTION

West Nile virus (WNV) is now endemic throughout most of North America (Hayes et al. 2005). The virus is primarily transmitted by *Culex* mosquitoes, and in the northeastern United States, 3 species, *Culex pipiens* L., *Culex restuans* Theobald, and *Culex salinarius* Coquillett, have been implicated as the principal vectors (Andreadis et al. 2001, 2004; Kulasekera et al. 2001; Ebel et al. 2005; Kilpatrick et al. 2005). Mosquito-based surveillance continues to be a primary tool for monitoring virus activity and quantifying the intensity of virus transmission. This is especially critical in the northeastern region of the United States, where WNV-induced avian mortality, most notably among corvids, has sharply declined and has largely ceased to be a sensitive spatial and temporal indicator for estimating risk of human infection.

Mosquito surveillance for WNV is most commonly conducted using $CO_2$-baited Centers for Disease Control and Prevention (CDC) miniature light traps suspended ~1.5 m above the ground and/or gravid traps baited with various infusions (hay or sod grass) that are placed directly on the ground (Andreadis et al. 2001, 2004; Bernard et al. 2001; Kulasekera et al. 2001; Nasci et al. 2001). Recent studies conducted in the northeastern United States and eastern

Canada, however, have shown that in certain habitats greater proportions of *Cx. pipiens* and *Cx. restuans* (Drummond et al. 2006) and significantly greater numbers of uninfected (Russell and Hunter 2005) and WNV-infected (Anderson et al. 2004, 2006) *Cx. pipiens* can be captured in $CO_2$-baited traps placed in the tree canopy compared with traps placed near the ground. It has been suggested (Anderson et al. 2006) that placement of traps in tree canopies, therefore, might be a useful strategy to augment current surveillance practices for mosquitoes infected with WNV. The effectiveness of targeted trapping using elevated $CO_2$-baited traps has not been evaluated in a large-scale operational surveillance program over a broad geographic region. We, accordingly, integrated the use of elevated canopy traps at 12 ecologically diverse trap sites located in 3 counties in Connecticut as part of our statewide surveillance program (Andreadis et al. 2004) and compared the effectiveness of elevated ($\sim$7.6 m) and ground-level ($\sim$1.5 m) $CO_2$-baited light traps with ground-level gravid traps for collecting *Culex* mosquitoes (*Cx. pipiens, Cx. restuans,* and *Cx. salinarius*) and detecting WNV activity over a 2-year period, 2004–05.

## MATERIALS AND METHODS

### Description of study areas

Mosquito trapping was conducted from June through October of 2004 and 2005 at 12 different surveillance sites located in Fairfield, New Haven, and Hartford counties where there had been a previous history of WNV activity (Andreadis et al. 2004). Land-use characterization was determined for each of the 12 sites using the 1995 land-use land cover classification map from the University of Connecticut Magic Geospatial Data Resources (http://mapserver.lib.uconn.edu/magic/index_lulc.htm). The 28 initial categories were reduced into 5 major classes (developed/urban, agriculture/soil/grass, forest, wetlands, and deep water) following the Anderson Level I classification system (Anderson 1976), and the composition of each land cover category within a 500-m radius of each site was computed using ArcGIS version 9.1 (Fig. 1). The 3 urban environments—commercial/industrial, residential/commercial, and rural/residential were combined into "developed/urban." Turf tree complex, turf grass, pasture hay grass, pasture hay/cropland, pasture hay/exposed soil, exposed soil/cropland, exposed soil, shade-grown tobacco, nursery stock, and exposed ground sand were combined into "agriculture/soil/grass." Scrub shrub, deciduous forest, deciduous forest mountain laurel, coniferous forest, dead dying hemlock, forest/clear cut, and mixed forest were combined into "forest." Shallow water mud flats, nonforested wetland, deciduous shrub wetland, deciduous forested wetland, coniferous forested wetland, low coastal marsh, and high coastal marsh were combined into "wetlands." Deep water was retained as a separate category.

Potential associations between specific land-use classes and the abundance of *Cx. pipiens, Cx. restuans,* and *Cx. salinarius* were determined using Pearson product-moment correlation coefficients (Jandel Corporation 1995), wherein the overall mean number of mosquitoes per trap night for each species using all trapping methods was compared with the proportion of each of the 5 land-use categories (developed/urban, agriculture/soil/grass, forest, wetland, and deep water) found at each of the 12 collection sites.

### Trapping techniques

Three trapping procedures were evaluated: 1) a $CO_2$ (dry ice)-baited CDC miniature light trap with an aluminum dome (John W. Hock Co., Gainesville, FL) suspended from a tree branch at a height of approximately 1.5 m, 2) an identical CDC light trap elevated approximately 7.6 m above ground in the tree canopy, and 3) a sod-grass-infused CDC gravid trap (Reiter 1983, Lampman and Novak 1996) placed directly on the ground approximately 10 m from the light traps. One of each trap type was used on each trapping occasion. Trapping frequency was variable but was minimally made once every 10 days at each trap site over the course of both seasons. Traps were placed in the field in the afternoon, operated overnight, and retrieved the following morning. The mean number of trap-nights per site was 24 (range 13 to 31) in 2004 and 20 (range 14 to 52) in 2005.

### Mosquito processing, virus isolation, and data analysis

Adult mosquitoes were transported alive to the laboratory in an ice chest lined with cool packs. Mosquitoes were immobilized with dry ice and transferred to chill tables where they were identified morphologically with the aid of a stereo microscope (90×) using descriptive keys of Darsie and Ward (1981) and Andreadis et al. (2005). Female mosquitoes were pooled in groups of 50 or fewer according to species, trap type and elevation, collection date, and location. Mosquitoes were stored at $-80^\circ C$ until processed for virus.

Viruses were isolated in Vero cell culture growing in 25-$cm^2$ flasks at $37^\circ C$ in 5% $CO_2$. West Nile virus was identified by a real-time reverse–transcriptase polymerase chain reaction assay (Lanciotti et al. 2000) as detailed previously (Andreadis et al. 2004). Infection rates for

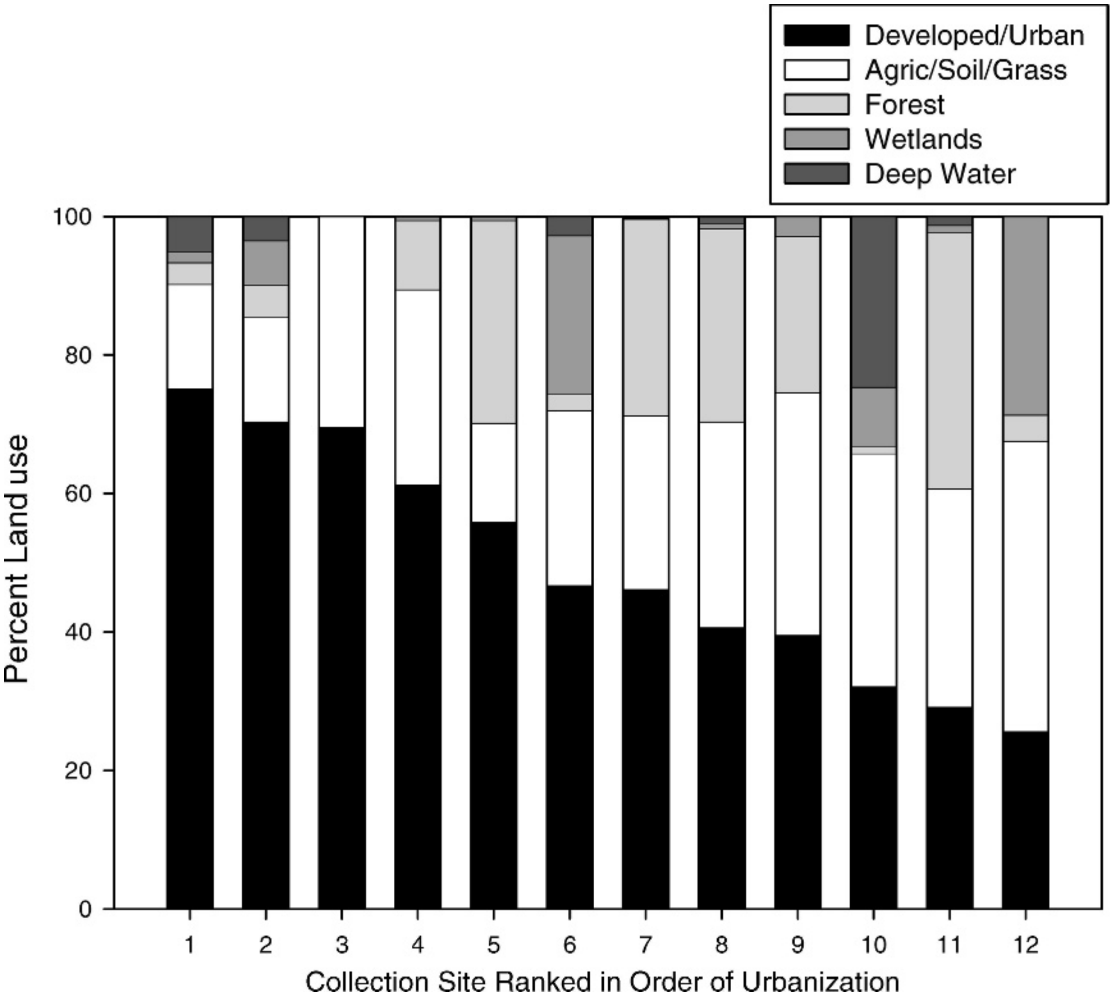


Fig. 1. Proportion of land uses within a 500-m radius of all 12 trapping sites ranked in order of urbanization.

estimating WNV infection per 1,000 mosquitoes were calculated for pooled samples of *Cx. pipiens, Cx. restuans,* and *Cx. salinarius* by trap type, elevation, and locale using the bias-corrected maximum likelihood estimation (MLE) methodology of Biggerstaff (2006). Chi-square analysis using Yates correction for continuity (Jandel Corp. 1995) was used to compare infection rates for each species collected with each of the 3 trapping methods.

The comparative abundance (Dominance Index) of *Cx. pipiens, Cx. restuans,* and *Cx. salinarius* collected with each trap type at each elevation was calculated using the Berger–Parker equation (Magurran 1988). The relative effectiveness of all 3 trapping methods was further evaluated for each species individually at each collection site. This was achieved by analyzing the overall mean numbers of female mosquitoes collected per trap-night for the entire 2-year period by Kruskal-Wallis 1-way analysis of variance (ANOVA) on ranks (Jandel Corp. 1995) for each species collected with each trap type at each of the 12 collection sites.

## RESULTS

### Mosquito collection data

The total mosquito collection data for all species captured in elevated canopy and ground traps for each year are summarized in Table 1. The comparative abundance of the 12 most dominant species collected with each trap type (combined years) is shown in Fig. 2. A total of 89,608 females representing 32 species in 9 genera were collected, identified, and processed for virus isolation. The 3 most dominant species collected in light traps suspended in the tree canopy were *Cx. pipiens* (30.5% of total and 64.6% of all *Culex* species), *Coquillettidia perturbans* (Walker) (27.1% of total), and *Cx. salinarius* (12.3% of

Table 1. Total number of female mosquitoes collected in elevated canopy and ground traps at 12 locations in Connecticut in 2004 and 2005.

| Species | Canopy light | | Ground light | | Ground gravid | | Total |
|---|---|---|---|---|---|---|---|
| | 2004 | 2005 | 2004 | 2005 | 2004 | 2005 | |
| *Aedes cinereus* | 378 | 267 | 3,439 | 1,072 | 156 | 29 | 5,341 |
| *Ae. vexans* | 691 | 1,121 | 8,838 | 3,476 | 42 | 9 | 14,177 |
| *Anopheles barberi* | 9 | 1 | — | — | 7 | 1 | 18 |
| *An. punctipennis* | 5 | 6 | 109 | 196 | 1 | 1 | 318 |
| *An. walkeri* | 3 | — | 8 | 1 | — | — | 12 |
| *An. quadrimaculatus* | 6 | — | 53 | 95 | 4 | 5 | 163 |
| *Coquillettidia perturbans* | 2,481 | 2,794 | 5,404 | 3,047 | 30 | 10 | 13,766 |
| *Culex pipiens* | 3,668 | 2,278 | 1,367 | 1,402 | 805 | 2,044 | 11,564 |
| *Cx. restuans* | 634 | 238 | 506 | 297 | 165 | 463 | 2,303 |
| *Cx. salinarius* | 1,484 | 907 | 5,461 | 1,793 | 76 | 44 | 9,765 |
| *Cx. territans* | 10 | 7 | 14 | 8 | 5 | 4 | 48 |
| *Culiseta melanura* | 49 | 46 | 192 | 184 | 11 | 1 | 483 |
| *Cs. minnesotae* | 30 | 30 | 16 | 8 | — | 5 | 89 |
| *Cs. morsitans* | 9 | 2 | 3 | — | — | — | 14 |
| *Ochlerotatus abserratus* | 1 | 2 | 21 | 5 | — | 1 | 30 |
| *Oc. aurifer* | 26 | 48 | 202 | 308 | 1 | — | 585 |
| *Oc. canadensis* | 99 | 132 | 2,504 | 1,681 | 9 | 7 | 4,432 |
| *Oc. cantator* | 296 | 348 | 2,417 | 2,160 | 6 | 3 | 5,230 |
| *Oc. communis* | — | — | — | 1 | — | — | 1 |
| *Oc. excrucians* | 8 | 6 | 52 | 38 | — | — | 104 |
| *Oc. hendersoni* | 2 | — | — | — | — | — | 2 |
| *Oc. japonicus* | 57 | 29 | 246 | 203 | 61 | 97 | 693 |
| *Oc. sollicitans* | 42 | 84 | 2,257 | 2,301 | 13 | 2 | 4,699 |
| *Oc. sticticus* | — | — | 15 | 32 | — | 1 | 48 |
| *Oc. stimulans* | 21 | 32 | 337 | 225 | 2 | 2 | 619 |
| *Oc. taeniorhynchus* | 321 | 397 | 6,567 | 3,539 | 25 | 48 | 10,897 |
| *Oc. thibaulti* | 2 | 38 | 34 | 7 | — | — | 81 |
| *Oc. triseriatus* | 52 | 83 | 387 | 142 | 53 | 143 | 860 |
| *Oc. trivittatus* | 50 | 22 | 548 | 124 | 2 | 2 | 748 |
| *Orthopodomyia signifera* | 3 | — | — | 1 | — | — | 4 |
| *Psorophora ferox* | 11 | 43 | 867 | 532 | 2 | 2 | 1,457 |
| *Uranotaenia sapphirina* | 37 | 38 | 498 | 473 | 5 | 6 | 1,057 |
| Yearly totals | 10,485 | 8,999 | 42,362 | 23,351 | 1,481 | 2,930 | 89,608 |
| Overall totals | 19,484 | | 65,713 | | 4,411 | | |

total and 26.0% of all *Culex*). *Culex restuans* was the least abundant *Culex* species in the canopy traps, ranking 5th in abundance and representing only 4.5% of the total collection and 9.5% of all *Culex* species.

The most common species collected in the light traps placed at ground level were *Aedes vexans* (Meigen) (18.7%), *Ochlerotatus taeniorhynchus* (Wiedemann) (15.4%), and *Cq. perturbans* (Walker) (12.9%). Among the *Culex* mosquitoes, *Cx. salinarius* was the most dominant (11.0% of total and 67.0% of all *Culex*), followed by *Cx. pipiens* (4.2% of total and 25.6% of all *Culex*), and *Cx. restuans* (1.2% of total and 7.4% of all *Culex*).

*Culex pipiens* was the dominant species collected in the gravid traps, representing 64.6% of the total collection and 79.2% of all *Culex* species. *Culex restuans* ranked 2nd in dominance but was considerably less abundant (14.2% of total and 17.5% of all *Culex*). *Culex salinarius* was infrequently collected in the gravid traps and represented only 2.7% of the total collection and 3.3% of all *Culex* species.

An analysis of the mean number of *Cx. pipiens, Cx. restuans,* and *Cx. salinarius* collected per trap-night in elevated canopy and ground traps at each of the 12 trap sites revealed substantial variation among the sites (Table 2). Overall, when all sites were considered, significantly more *Cx. pipiens* were collected on average in the elevated light traps in the canopy (mean = 15.1 per trap-night, $n$ = 395 trap-nights) than either the light (mean = 6.9 per trap-night, $P = 0.007$) or gravid traps (7.2 per trap-night, $P < 0.001$) placed at ground level. These differences were not universal across all sites, however, and were detected in only 4 of 12 locations (sites 2, 3, 6, 7). These 4 sites were notable in that they produced comparatively greater numbers of *Cx. pipiens* with all trapping methods. There was no significant difference in the overall average number of *Cx. pipiens* collected with the gravid traps when compared with light traps at ground level. The distribution and abundance of *Cx. pipiens* among the 12 collection sites was not significantly correlated ($P > 0.05$) with any

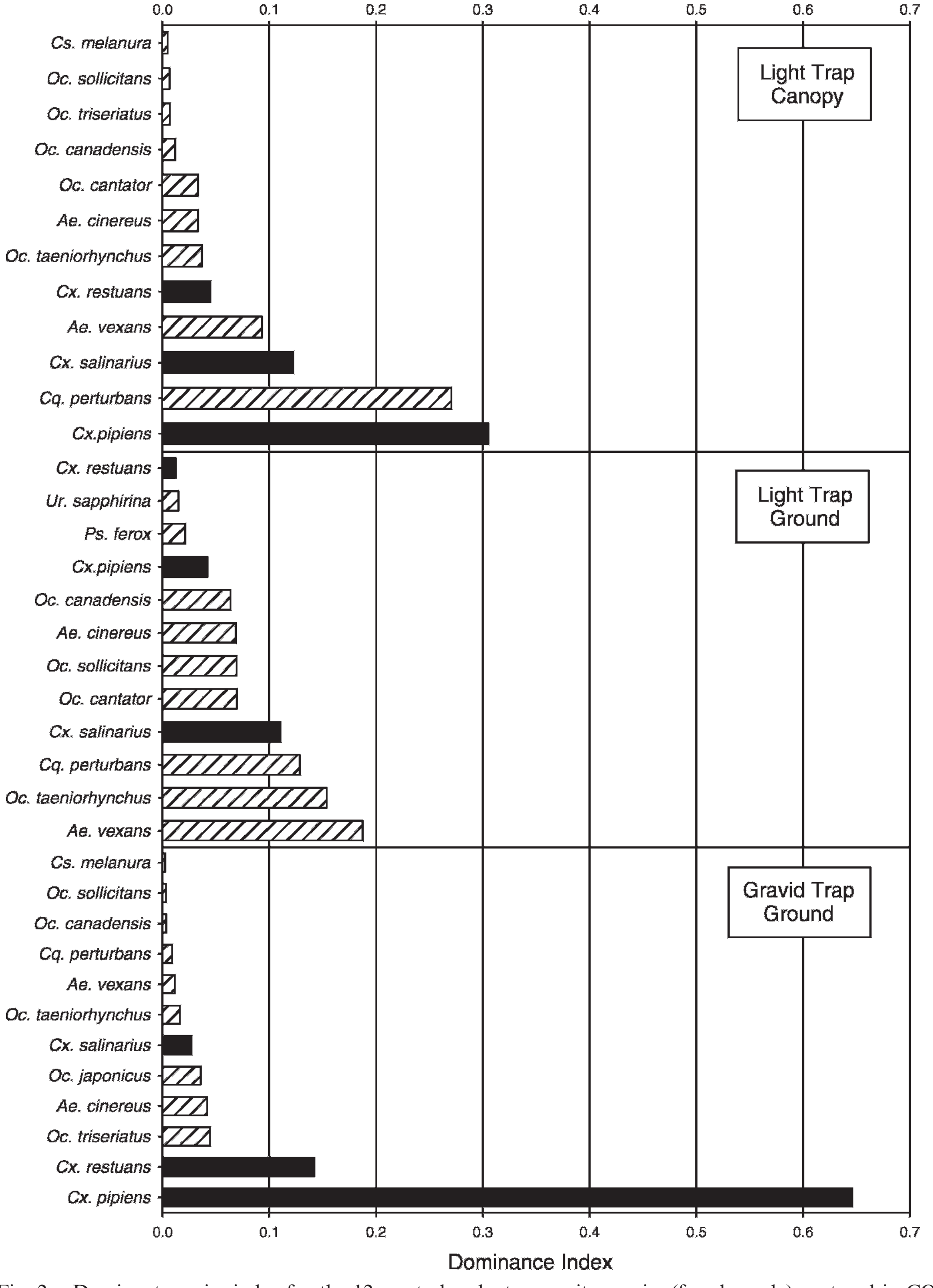


Fig. 2. Dominant species index for the 12 most abundant mosquito species (females only) captured in $CO_2$-baited CDC miniature light traps placed in the canopy and ground and in ground-based gravid traps at 12 locations in Connecticut in 2004 and 2005.

Table 2. Mean number of female *Culex pipiens, Culex restuans,* and *Culex salinarius* collected per trap-night in elevated canopy and ground traps at 12 locations, ranked by percentage urbanized, in Connecticut in 2004 and 2005. Mean numbers within rows followed by a common letter for each species are not significantly different (Kruskal-Wallis ANOVA, $P < 0.05$).

| | | *Culex pipiens* trap type and location | | | *Culex restuans* trap type and location | | | *Culex salinarius* trap type and location | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Trap site | Percentage urbanized | Canopy light | Ground light | Ground gravid | Canopy light | Ground light | Ground gravid | Canopy light | Ground light | Ground gravid |
| 1 | 75.2 | 2.0a | 3.0a | 11.2a | 0.5a | 2.2b | 1.3a | 0.9ab | 1.3b | 0.1a |
| 2 | 70.4 | 35.4a | 18.1b | 7.0c | 8.8a | 3.3b | 1.3c | 6.0a | 27.8b | 0.6c |
| 3 | 69.6 | 27.3a | 3.3b | 6.3b | 5.7a | 0.9a | 6.5a | 3.4a | 3.4a | 0.1b |
| 4 | 61.3 | 2.1a | 5.0b | 5.3ab | 0.5a | 4.1b | 0.9a | 0.2a | 1.6b | 0.1a |
| 5 | 55.9 | 0.6a | 2.4b | 1.1a | 0.3a | 2.4a | 1.1a | 1.8a | 4.5a | 0.1b |
| 6 | 46.8 | 32.3a | 10.5b | 5.0c | 2.9a | 3.4ab | 1.4b | 15.5a | 62.7b | 0.7c |
| 7 | 46.2 | 8.3a | 6.6b | 3.3b | 2.1a | 1.0a | 1.0a | 31.3a | 23.3a | 0.1b |
| 8 | 40.7 | 1.0a | 1.8a | 0.8a | 0.3a | 0.8a | 4.0a | 0.3a | 7.2b | 0.1c |
| 9 | 39.6 | 0.4a | 0.7a | 36.4b | 1.4a | 1.6a | 4.9a | 0.8a | 1.2a | 1.6a |
| 10 | 32.2 | 2.5a | 2.0a | 1.8a | 0.9a | 2.8b | 2.2a | 0.9a | 6.6b | 0.1c |
| 11 | 29.2 | 2.5a | 2.5a | 4.5a | 1.7a | 1.4a | 5.7a | 2.0a | 11.9b | 0.4c |
| 12 | 25.7 | 2.1a | 4.5b | 2.1a | 0.6a | 9.5b | 1.0a | 0.5a | 2.3a | 0 |
| Overall | | 15.1a | 6.9b | 7.2b | 3.2a | 2.9a | 2.3b | 7.9a | 24.0b | 0.4c |

specific land cover category within a 500-m radius of the trap site. (Fig. 1).

Significantly ($P < 0.05$) more *Cx. restuans* were collected per trap-night in light traps placed on the ground than in identical traps placed in the tree canopy at 4 of 12 locations (sites 1, 4, 10, 12). However, when collection data from all 12 sites were combined for analysis, no significant differences ($P = 0.086$, $n = 274$ trap-nights) were found in the overall average number of *Cx. restuans* collected in either the elevated light traps in the canopy (3.2 per trap-night) or at ground level (2.9 per trap-night). Both trapping methods yielded slightly, but significantly ($P < 0.001$) more females on average than the gravid traps (2.3 per trap-night). As with *Cx. pipiens,* the distribution and abundance of *Cx. restuans* among the 12 collection sites was not significantly correlated ($P > 0.05$) with any specific land cover category (Fig. 1).

Significantly ($P < 0.001$, $n = 301$ trap-nights) greater numbers of *Cx. salinarius* were consistently collected (7 of 12 sites) in light traps placed at ground level (overall = 24.0 per trap-night) than in light traps placed in the canopy (7.9 per trap-night). Very few females were collected in the gravid traps (overall = 0.4 per trap-night), and this was observed at almost every trap location. No significant association was found between the abundance of *Cx. salinarius* and any specific land cover category.

## West Nile virus isolation data

The virus isolation data for *Cx. pipiens, Cx. restuans,* and *Cx. salinarius* collected with each trapping method are summarized in Table 3. A total of 71 WNV isolations were obtained from mosquitoes collected at 8 of 12 locations. The majority of the virus isolations were obtained from *Cx. pipiens* (81.7%, $n = 58$ at 8 sites), followed by *Cx. salinarius* (9.9%, $n = 7$ at 3 sites), and *Cx. restuans* (8.4%, $n = 6$ at 4 sites).

The frequency of WNV isolations obtained from pools of *Cx. pipiens* collected in light traps in the canopy (35 of 345 pools, MLE = 6.7) was significantly higher ($\chi^2 = 13.01$, df = 1, $P < 0.001$) than the frequency of isolations obtained from pooled females collected in light traps on the ground (8 of 297 pools, MLE = 3.0). However, the frequency of WNV isolations in canopy-collected *Cx. pipiens* pools did not differ significantly ($\chi^2 = 2.40$, df = 1, $P = 0.121$) from those collected in gravid traps (15 of 243 pools, MLE = 5.6), nor was there any significant difference between the frequency of virus isolations from *Cx. pipiens* pools collected with light or gravid traps that were placed at ground level ($\chi^2 = 3.160$, df = 1, $P = 0.075$).

Although more than twice as many WNV isolations were made from *Cx. salinarius* collected in light traps placed at ground level (5 of 335 pools, MLE = 0.7) when compared with isolations obtained from females collected in light traps in the canopy (2 of 198 pools, MLE = 0.8), the MLEs were nearly identical and there was no significant difference in the frequency of isolations with either trapping method ($\chi^2 = 0.006$, df = 1, $P = 0.937$). Similarly, no significant differences were found in the frequency of WNV isolations obtained from *Cx. restuans* collected in light traps placed in the canopy (4 of 147 pools, MLE = 4.7) or on the ground (2 of 181 pools, MLE = 2.5) ($\chi^2 = 0.451$, df = 1, $P = 0.502$).

Table 3. Number of West Nile virus (WNV) isolations and maximum likelihood estimations (in parentheses) obtained from female *Culex pipiens, Culex salinarius,* and *Culex restuans* collected in elevated canopy and ground traps at 12 locations in Connecticut in 2004 and 2005.

| | | *Culex pipiens* trap type and location | | | *Culex salinarius* trap type and location | | | *Culex restuans* trap type and location | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Trap site | No. WNV isolations | Canopy light | Ground light | Ground gravid | Canopy light | Ground light | Ground gravid | Canopy light | Ground light | Ground gravid |
| 1 | 2 | 0 | 0 | 1 (3.4) | 0 | 1 (60.4) | 0 | 0 | 0 | 0 |
| 2 | 25 | 13 (6.9) | 4 (3.5) | 4 (9.3) | 0 | 1 (0.7) | 0 | 3 (6.9) | 0 | 0 |
| 3 | 13 | 11 (16.5) | 0 | 2 (10.1) | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 6 | 1 (12.0) | 1 (4.8) | 2 (9.6) | 0 | 0 | 0 | 0 | 2 (18.3) | 0 |
| 5 | 1 | 0 | 1 (51.7) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6 | 18 | 9 (4.2) | 1 (1.4) | 3 (9.2) | 2 (1.8) | 2 (0.5) | 0 | 1 (7.0) | 0 | 0 |
| 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 9 | 3 | 0 | 0 | 3 (3.0) | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 12 | 3 | 1 (11.8) | 1 (12.8) | 0 | 0 | 1 (4.7) | 0 | 0 | 0 | 0 |
| Overall | 71 | 35 (6.7) | 8 (3.0) | 15 (5.6) | 2 (0.8) | 5 (0.7) | 0 | 4 (4.7) | 2 (2.5) | 0 |

A weekly summary of the temporal distribution of WNV isolations made from *Cx. pipiens, Cx. restuans,* and *Cx. salinarius* collected with each trapping method in 2004 and 2005 is shown in Fig. 3 in relation to the overall abundance of each species. In 2004, WNV isolations were made from *Cx. pipiens* over a 10-wk period, July 21 to September 27. The earliest virus isolations were made from *Cx. pipiens* collected in light traps placed in the canopy and gravid traps placed on the ground nearly 3 wk before the 1st WNV isolation was made from any *Cx. pipiens* collected with the light traps placed at ground level (August 9). Overall, WNV isolations were more regularly obtained from *Cx. pipiens* collected in light traps in the canopy (9 of 10 wk of detectable virus activity), than either the gravid traps (5 of 10 wk) or light traps placed at ground level (3 of 10 wk). Similar findings were obtained with *Cx. pipiens* in 2005. Over a 12-wk period during which WNV activity was detected (July 11 to October 5), WNV isolations were more consistently obtained from *Cx. pipiens* collected in light traps in the canopy ($n$ = 7 wk), than in either gravid ($n$ = 5 wk) or light traps placed at ground level ($n$ = 3 wk). Furthermore, the earliest virus isolations made from *Cx. pipiens* collected in gravid traps and light traps in the canopy were 4 and 2 wk, respectively, prior to the 1st virus isolation made from mosquitoes collected with a light trap at ground level (August 18).

Very similar results were recorded with *Cx. restuans* in 2004. Over the 6-wk period (August 9 to September 15) in which WNV was detected in this species, virus isolations were obtained earlier (August 9) and more frequently ($n$ = 4 wk) from females collected in light traps in the canopy than in light traps placed at ground level (September 13, $n$ = 1 wk). Only a single isolation of WNV was obtained from *Cx. restuans* in 2005, and that was from a female collected in a light trap set at ground level on July 11.

The virus isolation pattern with *Cx. salinarius* was notably different in 2004. Over the 9-wk period (July 29 to September 13) in which WNV was detected in this species, virus isolations were made earlier (July 29) and more frequently ($n$ = 3 wk) from females collected in light traps placed at ground level than in light traps elevated in the tree canopy (August 16, $n$ = 1 wk). Only 2 WNV isolations were made from *Cx. salinarius* in 2005, the 1st from a pool of females collected in light traps in the canopy on August 19, and the 2nd from a pool of females collected in a light trap at ground level 3 days later (August 22).

## DISCUSSION

### *Culex* mosquito abundance

Although site-dependant variation was clearly seen in the numbers of *Cx. pipiens, Cx. restuans,* and *Cx. salinarius* collected with each of the 3 trapping procedures, several notable trends were evident when data from all 12 sites were collectively evaluated over the 2-year sampling period. An analysis of the overall comparative abundance of these 3 species using the dominance index (Fig. 2) showed *Cx. pipiens* to be the dominant species collected in the gravid traps and the most frequently trapped species of *Culex* in $CO_2$-baited CDC light traps suspended in the tree canopy. *Culex restuans* was frequently collected in the gravid traps, ranking a distant 2nd behind *Cx. pipiens,* and was the least dominant *Culex* species collected in light traps

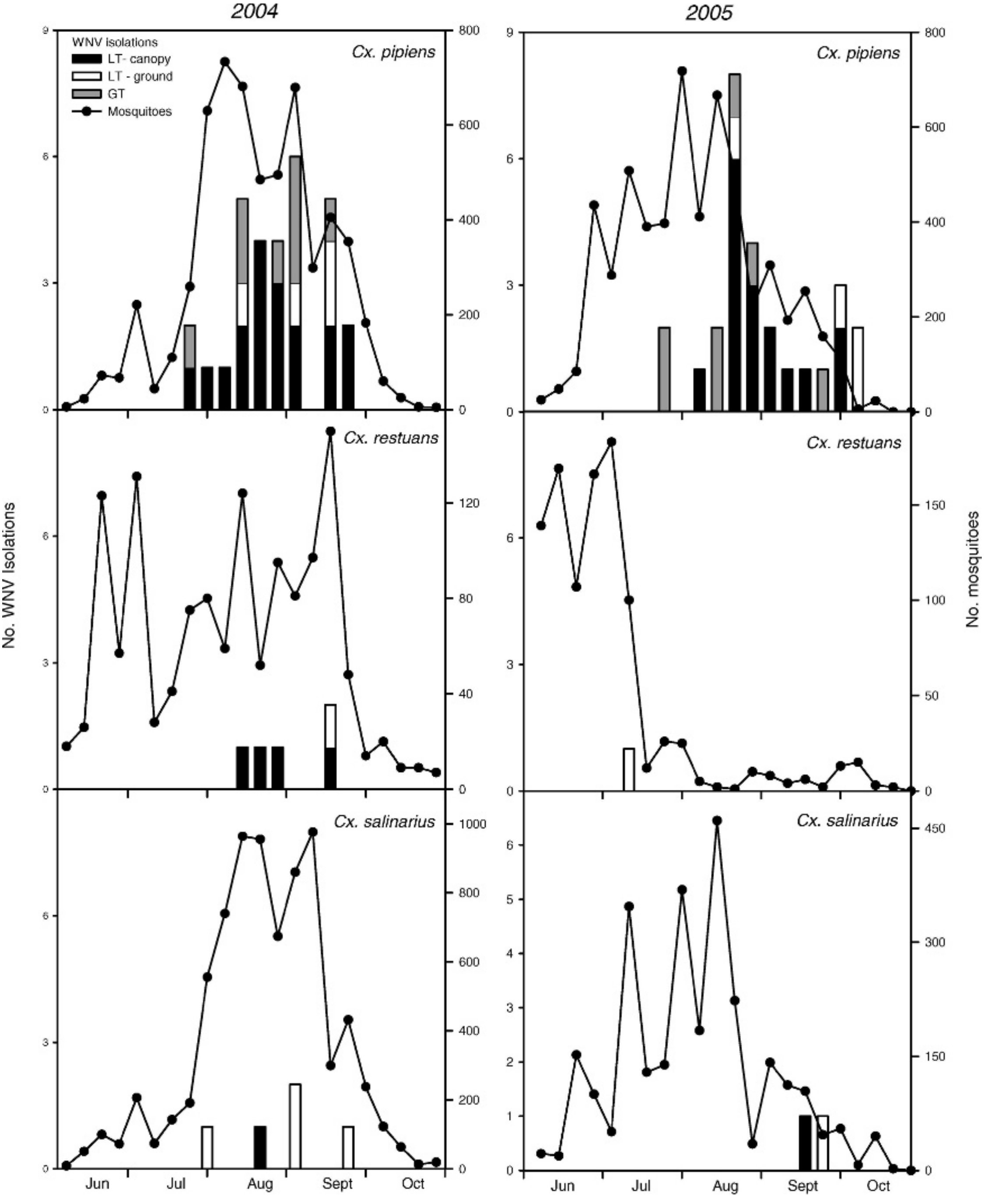


Fig. 3. Total weekly abundance of female *Cx. pipiens, Cx. restuans,* and *Cx. salinarius* collected with all 3 trapping procedures in 2004 and 2005 and number of corresponding West Nile virus (WNV) isolations obtained from females collected with each trapping procedure.

in the canopy. *Culex salinarius* on the other hand, was rarely collected in the gravid traps but dominated all other species of *Culex* in light traps placed at ground level.

An examination of the relative abundance of each species collected with each of the 3 trapping procedures revealed the following. In general, more than twice as many *Cx. pipiens* were collected on average in $CO_2$-baited CDC light traps suspended in the tree canopy than in either light or gravid traps placed at ground level. However, it is important to emphasize that this difference was generally restricted to those collection sites where markedly greater numbers of *Cx. pipiens* were collected with all trapping methods (13.6 vs. 3.3 mean no. mosquitoes/trap-night/site), and presumably higher adult populations were present. It is unclear why these differences were not detected in locations where the overall collections of *Cx. pipiens* were comparatively sparse. This discrepancy did not appear to be directly associated with urbanization or any site-specific land-use characteristic, since no significant associations were found with any of the 5 land-use categories measured within a 500-m radius of each trap site. A similar observation was recently reported by Drummond et al. (2006), who compared elevated and ground-level $CO_2$-baited CDC light traps for 1 season at 6 study sites located in upstate New York. They found that *Cx. pipiens* abundance varied over habitats and was not strongly correlated with site-specific urbanization indices within a 1-mile radius of each trap site. Furthermore, at only 1 trap location, where the overall number of *Cx. pipiens* was nearly 6-fold higher, there were markedly more *Cx. pipiens* collected in elevated traps in the tree canopy than in those on the ground (19.3 vs. 6.8 per trap-night). This particular site was located near a wastewater treatment plant facility similar to the one where Anderson et al. (2004, 2006) also reported capturing significantly greater numbers of *Cx. pipiens* in both $CO_2$- and animal-baited canopy-placed traps. *Culex pipiens* abundance has been previously associated with high human population density in Connecticut (Andreadis et al. 2004) and predictive models using remotely sensed data have identified nonforested suburban areas as likely regions of abundance (Diuk-Wasser et al. 2006). However, within these suburban regions, it is clear that other environmental variables associated with specific breeding habitats are likely to be more critically important.

*Culex restuans,* unlike *Cx. pipiens,* did not seem to be preferentially attracted to $CO_2$-baited light traps suspended in the tree canopy. No differences in the overall abundance of this species were recorded with either of the 2 trapping procedures. However, both light traps did outperform the gravid traps consistent with previous observations (Andreadis et al. 2004). The former finding concurs with other regional studies conducted in the northeastern United States (Anderson et al. 2006, Drummond et al. 2006), which similarly reported capturing equal numbers of *Cx. restuans* in ground and canopy level $CO_2$-baited light traps. Anderson et al. (2006) further collected significantly larger numbers of *Cx. restuans* in $CO_2$-baited CDC light and mosquito magnet experimental traps placed underground in catch basins than in identical traps placed at ground or canopy levels.

We find it noteworthy that *Cx. pipiens* appears to be more preferentially attracted to $CO_2$-baited light traps in the canopy while *Cx. restuans* does not, since studies on the blood-feeding behavior of local populations show that both species predominately feed on avian hosts and focus their feeding activity on similar species of birds (Apperson et al. 2002, 2004; Molaei et al. 2006). The conventional interpretation to explain this presumed preferential host seeking by *Cx. pipiens* at higher elevations has been attributed to host accessibility, since nesting and roosting birds are more likely to be abundant in the tree canopy (Mitchell 1982, Anderson et al. 2004, Russell and Hunter 2005, Drummond et al. 2006). However, other intrinsic and/or host-specific factors unrelated to the attractiveness of light and the emission of a plume of $CO_2$ may be involved in the host-seeking and flight behavior of *Cx. restuans.* Consistent with this hypothesis is the recent finding of Darbro and Harrington (2006), who reported that in rural localities in upstate New York where *Cx. restuans* was the dominant *Culex,* this species exhibited a preference for chicken- and sparrow-baited traps that were elevated in the tree canopy (~9 m) rather than those placed near ground level (~1.5 m). Main et al. (1966) similarly captured 10-fold more *Cx. restuans* in lard-can traps baited with chicks that were hung 7.6 m in the canopy rather than at ground level in a white cedar–red maple swamp habitat in nearby Massachusetts; and Russell and Hunter (2005) collected more *Cx. pipiens*/*Cx. restuans* at higher elevations (5 m) in the forest canopy in a woodlot in Ontario, Canada, using bird uropygial gland odors to attract host-seeking females in combination with $CO_2$ in CDC traps without light. However, this study did not differentiate between the 2 species, so it is difficult to fully interpret the results as they specifically relate to *Cx. restuans.* Nevertheless, these studies suggest that *Cx. restuans* may preferentially seek hosts in the tree canopy as well, but may simply not be as attracted to $CO_2$ and light as is *Cx. pipiens*.

*Culex salinarius* was significantly more attracted to ground-based $CO_2$-baited CDC light traps than to traps suspended in the tree canopy. Unlike *Cx. pipiens,* this preference was not associated with overall adult abundance and was detected in locales where *Cx. salinarius* was both abundant and comparatively rare (range = 78.9 to 1.9 mosquitoes per trap-night, all traps). These observations are consistent with Anderson et al. (2004), who similarly collected fewer numbers of *Cx. salinarius* in canopy-placed traps compared with traps placed near the ground at a wastewater treatment facility, and Shone et al. (2006), who found *Cx. salinarius* significantly more attracted to light traps baited with $CO_2$ and octenol at 1.5 m above the ground than to those at 5 m in both freshwater and salt marsh habitats in the Chesapeake Bay area of Maryland. DiMenna et al. (2006) likewise reported that *Cx. salinarius* was significantly more likely to be caught in ground-based light traps than it was in the canopy (10–15 m) in rural areas of New Mexico. These results are in accord with the blood-feeding preferences of *Cx. salinarius,* which unlike *Cx. pipiens* and *Cx. restuans,* feeds much more frequently on ground dwelling mammals in the northeastern United States, especially white-tailed deer (Apperson et al. 2002, 2004; Molaei et al. 2006).

The sod-based gravid traps in our study were very ineffective in collecting *Cx. salinarius,* and this was generally true across all sites regardless of the level of urbanization or any other specific land-use characteristic. Like results were obtained in a prior study that included 91 locations throughout Connecticut (Andreadis et al. 2004). In contrast, DiMenna et al. (2006) found that in New Mexico, standard ground-based CDC light traps and gravid traps baited with a fermentation mixture of horse manure and grass clippings were equally effective in capturing *Cx. salinarius,* especially in urban areas, thus implying regional differences in the oviposition preferences of this species. Nevertheless, we conclude that in the northeastern region, standard ground-based $CO_2$-baited CDC miniature light traps are the trap of choice for surveillance of this species.

## West Nile virus detection

$CO_2$-baited light traps placed in the tree canopy were generally superior to ground-based light traps for detecting WNV in *Cx. pipiens.* West Nile virus–infected females were collected more regularly during the 10- to 12-wk duration of detectable WNV activity, and the frequency of infected pools was significantly greater. Moreover, 2-fold higher MLEs were recorded from canopy collections of this species, and virus was detected in canopy-collected *Cx. pipiens* several weeks before it was seen in mosquitoes collected in light traps at ground level. This phenomenon was observed in both years. Our results are consistent with Anderson et al. (2004, 2006), who likewise recorded significantly greater numbers of WNV isolations from *Cx. pipiens* and higher frequencies of WNV-infected pools from females collected in elevated canopy traps than in those collected at ground level. Therefore, we conclude that the use of $CO_2$-baited light traps placed in the tree canopy for targeted trapping of *Cx. pipiens* and subsequent detection of WNV is likely to yield better overall results than light traps placed at ground level in this region of the northeastern United States.

The number and frequency of virus isolations obtained from *Cx. pipiens* collected in gravid traps was also generally higher than those obtained with light trap collections at ground level and compared favorably both temporally and spatially with the results from canopy trap collections. A comparison with the latter revealed no significant differences in the overall frequency of WNV-infected pools or MLEs for *Cx. pipiens* with either trapping procedure. Furthermore, gravid trapping proved to be just as effective as canopy trapping for early detection of WNV-infected *Cx. pipiens,* since virus isolations were made during the same week (2004) or earlier in the season (2005). On the other hand, fewer WNV isolations were made from *Cx. pipiens* collected in the gravid traps, and virus was detected more infrequently (10 vs. 16 wk, both years). This discrepancy was most likely due to the considerably smaller overall number of *Cx. pipiens* collected in the gravid traps when compared with traps in the canopy, since the MLEs were essentially the same. In a prior study conducted in Connecticut from 2000 to 2003, Andreadis et al. (2004) reported 2-fold greater numbers of *Cx. pipiens* collected in ground-based $CO_2$-baited light traps rather than in gravid traps, but there were no significant differences in non–bias-corrected minimum field infection rates (MIRs) for WNV. Conversely, in New York, Lukacik et al. (2006) reported that mosquito pools submitted from gravid traps (mostly *Cx. pipiens/Cx. restuans*) were 5.7 times more likely to be WNV positive than submissions from ground-based $CO_2$-baited light traps. Our results reaffirm the utility of gravid traps as effective surveillance tools for detection of WNV in *Cx. pipiens* in the northeastern United States. However, our findings also demonstrate that $CO_2$-baited light traps placed in the tree canopy may provide more consistent results where weekly detection of virus amplification is a critical objective.

It is difficult to draw any definitive conclusions concerning the comparative effectiveness of ground- or canopy-based light traps for detection of WNV-infected *Cx. restuans* and *Cx. salinarius* owing to the limited number of virus isolations that were made from these species during the 2 years of study. However, in the case of *Cx. restuans,* WNV virus isolations were clearly made several weeks earlier and considerably more frequently from females collected in traps placed in the canopy rather than at ground level in the 1st year of the study in 2004. This occurred despite the absence of any noticeable differences in the overall numbers of *Cx. restuans* collected with either trapping procedure. Thus, the utility of $CO_2$-baited light traps placed in the tree canopy for detection of WNV in *Cx. restuans* would also appear to be warranted.

Anderson et al. (2004) similarly captured fewer numbers of *Cx. salinarius* in $CO_2$-baited light traps placed in the tree canopy compared with identical traps placed on the ground at 1 location, but noted higher MIRs with WNV in canopy-captured mosquitoes. Our results from a wider variety of collection sites run counter to these findings and support the view that ground-based $CO_2$-baited light traps are effective for detection of WNV in this predominately mammalian biter.


## ACKNOWLEDGMENTS

We gratefully acknowledge the technical assistance of John Shepard, Michael Thomas, and Shannon Finan for identification of mosquitoes and preparation of specimens for virus isolation. We also thank Maria Diuk-Wasser, Department of Epidemiology and Public Health, Yale University School of Medicine, for providing assistance in the analysis of land-use characterization data, and our seasonal staff for their contributions in the laboratory and field: F. Beecher, T. Burke, E. Calandrella, M. Calandrella, J. Capotosto, L. Cash, T. Clohery, S. DeGennaro, R. Duffy, R. Ferrucci, K. Fogg, C. Godman, K. Goheen, D. Gonzalez, K. Hendrickson, D. Krause, L. Meaney-Post, E. McClure, C. McGee, B. O'Neil, C. Pioli, S. Reiff, D. Robertson, C. Secker-Walker, K. Startz, S. Wilby, and P. Zaskey. This work was supported in part by grants from the Centers for Disease Control and Prevention (U50/CCU116806-01-1) and the U.S. Department of Agriculture (58-6615-1-218 and CONH00768).


## REFERENCES CITED


Anderson JF, Andreadis TG, Main AJ, Ferrandino FJ, Vossbrinck CR. 2006. West Nile virus from female and male mosquitoes (Diptera: Culicidae) in subterranean, ground and canopy habitats in Connecticut. *J Med Entomol* 43:1010–1019.

Anderson JF, Andreadis TG, Main AJ, Kline DL. 2004. Prevalence of West Nile virus in tree canopy-inhabiting *Culex pipiens* and associated mosquitoes. *Am J Trop Med Hyg* 71:112–119.

Anderson JR. 1976. A land use and land cover classification system for use with remote sensor data. Washington, DC. U.S. Government Printing Office. Geological Survey Professional Paper 964.

Andreadis TG, Anderson JF, Vossbrinck CR. 2001. Mosquito surveillance for West Nile virus in Connecticut, 2000: isolation from *Culex pipiens, Cx. restuans, Cx. salinarius*, and *Culiseta melanura*. *Emerg Infect Dis* 7:670–674.

Andreadis TG, Anderson JF, Vossbrinck CR, Main AJ. 2004. Epidemiology of West Nile virus in Connecticut: a five-year analysis of mosquito data 1999–2003. *Vector Borne Zoonotic Dis* 44:360–378.

Andreadis TG, Thomas MC, Shepard JJ. 2005. Identification guide to the mosquitoes of Connecticut. *Bulletin of The Connecticut Agricultural Experiment Station* 966:1–173.

Apperson CS, Harrison BA, Unnasch TR, Hassan HK, Irby WS, Savage HM, Aspen SE, Watson DW, Rueda LM, Engber BR, Nasci RS. 2002. Host feeding habits of *Culex* and other mosquitoes (Diptera: Culicidae) in the Borough of Queens in New York City, with characters and techniques for identification of *Culex* mosquitoes. *J Med Entomol* 39:777–785.

Apperson CS, Hassan HK, Harrison BA, Savage HM, Aspen SE, Farajollahi A, Crans W, Daniels TJ, Falco RC, Benedict M, Anderson M, McMillen L, Unnasch TR. 2004. Host-feeding patterns of established and potential mosquito vectors of West Nile virus in the eastern United States. *Vector Borne Zoonotic Dis* 4:71–82.

Bernard KA, Maffei JG, Jones SA, Kauffman EB, Ebel GD, Dupuis AP II, Ngo KA, Nicholas DC, Young DM, Shi PY, Kulasekera VL, Eidson M, White DJ, Stone WB, NY State WNV Surveillance Team, Kramer LD. 2001. West Nile virus infection in birds and mosquitoes, New York State, 2000. *Emerg Infect Dis* 7:679–685.

Biggerstaff BL. 2006. *PooledInfRate, Version 3.0: a Microsoft® Excel Add-In to compute prevalence estimates from pooled samples.* Centers for Disease Control and Prevention: Fort Collins, CO.

Darbro JM, Harrington LC. 2006. Bird-baited traps for surveillance of West Nile mosquito vectors: effect of bird species, trap height, and mosquito escape rates. *J Med Entomol* 43:83–92.

Darsie RJ, Ward RA. 1981. Identification and geographic distribution of mosquitoes of North America, north of Mexico. *Mosq Syst* 1 Suppl:1–313.

DiMenna MA, Bueno R Jr, Parmenter RR, Norris DE, Sheyka JM, Molina JL, LaBeau EL, 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.

Diuk-Wasser M, Brown HE, Andreadis TG, Fish D. 2006. Modeling the spatial distribution of mosquito vectors for West Nile virus in Connecticut, USA. *Vector Borne Zoonotic Dis* 6:283–295.

Drummond CI, Drobnack J, Backenson PB, Ebel GD, Kramer LD. 2006. Impact of trap elevation on estimates of abundance, parity rates, and body size of *Culex pipiens* and *Culex restuans* (Diptera: Culicidae). *J Med Entomol* 43:177–184.

Ebel GD, Rochlin I, Longacker J, Kramer LD. 2005. *Culex restuans* (Diptera: Culicidae) relative abundance and vector competence for West Nile Virus. *J Med Entomol* 42:838–843.

Hayes EB, Komar N, Nasci R, Montgomery SP, O'Leary DR, Campbell GL. 2005. Epidemiology and transmission dynamics of West Nile virus disease. *Emerg Infect Dis* 11:1167–1179.

Jandel Corporation. 1995. *SigmaStat 2.0 for Windows, version 2.0.* Jandel Corporation: San Rafael, CA.

Kilpatrick AM, Kramer LD, Campbell SR, Alleyne EO, Dobson AP, Daszak P. 2005. West Nile virus risk assessment and the bridge vector paradigm. *Emerg Infect Dis* 11:425–429.

Kulasekera VL, Kramer L, Nasci RS, Mostashari F, Cherry B, Trock SC, Glaser C, Miller JR. 2001. West Nile virus infection in mosquitoes, birds, horses and humans, Staten Island, New York, 2000. *Emerg Inf Dis* 7:722–725.

Lampman RL, Novak RJ. 1996. Oviposition preferences of *Culex pipiens* and *Culex restuans* for infusion-baited traps. *J Am Mosq Control Assoc* 12:23–32.

Lanciotti RS, Kerst AJ, Nasci RS, Godsey MS, Mitchell CJ, Savage HM, Komar N, Panella NA, Allen BC, Volpe KE, Davis BS, Roehrig JT. 2000. Rapid detection of West Nile virus from human clinical specimens, field-collected mosquitoes, and avian samples by a TaqMan reverse transcriptase-PCR assay. *J Clin Microbiol* 38:4066–4071.

Lukacik G, Anand M, Shusas EJ, Howard JJ, Oliver J, Chen H, Backenson PB, Kauffman EB, Bernard KA, Kramer LD, White DJ. 2006. West Nile virus surveillance in mosquitoes in New York State, 2000–2004. *J Am Mosq Control Assoc* 22:264–271.

Magurran AE. 1988. *Ecological Diversity and Its Measurement*. Princeton, NJ. Princeton University Press.

Main AJ, Tonn RJ, Randall EJ, Anderson KS. 1966. Mosquito densities at heights of five and twenty-five feet in southeastern Massachusetts. *Mosq News* 26:243–248.

Mitchell L. 1982. Time-segregated mosquito collections with a CDC miniature light trap. *Mosq News* 42:12–18.

Molaei G, Andreadis TG, Armstrong PM, Anderson JF, Vossbrinck CR. 2006. Host feeding patterns of *Culex* mosquitoes and West Nile virus transmission, northeastern United States. *Emerg Infect Dis* 12:468–474.

Nasci RL, White DJ, Sirling H, Oliver J, Daniels TJ, Falco RC, Campbell S, Crans WJ, Savage HM, Lanciotti RS, Moore CG, Godsey MS, Gottfried KL, Mitchell CJ. 2001. West Nile virus isolates from mosquitoes in New York and New Jersey, 1999. *Emerg Infect Dis* 7:626–630.

Reiter P. 1983. A portable, battery-powered trap for collecting gravid *Culex* mosquitoes. *Mosq News* 43:496–498.

Russell CB, Hunter FF. 2005. Attraction of *Culex pipiens/restuans* (Diptera: Culicidae) mosquitoes to bird uropygial gland odors at two elevations in the Niagara region of Ontario. *J Med Entomol* 42:301–305.

Shone SM, Glass GE, Norris DE. 2006. Targeted trapping of mosquito vectors in the Chesapeake Bay area of Maryland. *J Med Entomol* 43:151–158.