West Nile Virus from Female and Male Mosquitoes (Diptera: Culicidae) in Subterranean, Ground, and Canopy Habitats in Connecticut

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ABSTRACT In total, 93,532 female mosquitoes (Diptera: Culicidae) were captured in traps placed in subterranean (catch basin), ground (≈ 1.5 m above ground), and canopy (≈7.0 m above ground) habitats in Stamford and Stratford, CT, during 2003–2005. *Culex pipiens* L. was the most abundant (64.8%) of the 31 species identified. Significantly greater numbers of *Cx. pipiens* were captured in canopy-placed mosquito magnet experimental traps, and significantly greater numbers were collected in catch basin-placed (Centers for Disease Control) CDC traps than in CDC traps placed elsewhere. *Culex restuans* Theobald was captured in significantly greater numbers in traps placed in catch basins. *Aedes vexans* (Meigen), *Aedes cinereus* Meigen, and *Aedes cantator* (Coquillett) were significantly more abundant in ground traps. In total, 429 isolations of West Nile virus (WNV) were made from seven species of mosquitoes from late June through the end of October during 2003 through 2005. Three hundred ninety-eight (92.8%) isolates were from *Cx. pipiens*. Others were from *Cx. restuans* (n = 16), *Culex salinarius* Coquillett (n = 5), *Ae. vexans* (n = 4), *Ae. cantator* (n = 3), *Aedes triseriatus* (Say) (n = 2), and *Ae. cinereus* (n = 1). Multiple isolates from *Cx. pipiens* were made each week, primarily during the later part of July through the end of September. Weekly minimum infection rates (MIRs) were lower in 2004 (highest weekly MIR = 7.1) when no human cases were reported in Connecticut in comparison with 2003 and 2005 (highest weekly MIR = 83.9) when human cases were documented. Frequencies of infected pools were significantly higher in *Cx. pipiens* captured in traps in the canopy and significantly higher in catch basin placed traps than in traps at ground level. The physiological age structure of *Cx. pipiens* captured in the canopy was significantly different from that of *Cx. pipiens* collected in catch basins. Invariably, *Cx. pipiens* captured in the canopy were nulliparous or parous with ovaries in Christophers' stage 2, whereas 58.7% of the females captured in catch basins possessed ovaries filled with mature oocytes in Christophers' stage 5. Our results suggest that females in the canopy are seeking hosts, and after digestion of the bloodmeal and development of mature oocytes, they descend to catch basins for shelter and deposition of eggs. WNV was isolated from three, one, and two pools of male *Cx. pipiens* captured in catch basin-, ground-, and canopy-placed traps, respectively, and from six nulliparous *Cx. pipiens* females collected in the canopy. Weekly MIR ranged from 1.2 to 31.1 per 1,000 male specimens. These data show that mosquitoes become infected by means other than by blood feeding, possibly by transovarial transmission. The placement of traps in tree canopies and in catch basins can be used to augment current practices of placement of traps near the ground for surveillance of mosquitoes infected with WNV and for studies of the ecology of WNV.

KEY WORDS West Nile virus, *Culex restuans*, *Culex salinarius*, *Aedes vexans*, *Culex pipiens*

Culex pipiens L. is the primary enzootic vector for West Nile virus (family *Flaviviridae*, genus *Flavivirus*, WNV) in the northeastern United States (Andreadis et al. 2001, 2004; Nasci et al. 2001b; Anderson et al. 2004). An early study on the natural history of *Cx. pipiens* in eastern United States reported artificial containers above and below ground to be primary oviposition and larval sites (Smith 1905). More recently, the importance of catch basins and flooded basements in sustaining populations of *Cx. pipiens* has been emphasized (Munstermann and Craig 1977, Siegel and Novak 1997, Savage et al. 1999). Adult *Cx. pipiens* are attracted to dark, damp subterranean habitats where eggs are laid on the water in the bottoms of catch basins and basements.

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Surveillance programs for WNV in the United States often use Centers for Disease Control (CDC) miniature light traps baited with CO_2 placed ≈ 1.5 m above ground in urban and rural areas to capture mosquitoes (Andreadis et al. 2001, 2004; Nasci et al. 2001b). Recently, we documented that the placement of traps in tree canopies increased the numbers of WNV-infected and uninfected *Cx. pipiens* compared with traps placed near the ground (Anderson et al. 2004). In our current study to more fully understand the ecology of WNV, we assess minimum infection rates (MIRs) throughout the season; compare the abundance of different species of mosquitoes and the number of isolations of WNV; and report the isolation of WNV from male *Cx. pipiens* captured in traps placed in catch basins, at ground level (≈1.5 m above ground), and within tree canopy level (≈7 m above ground), and from nulliparous female *Cx. pipiens* in the canopy. Additionally, we document the physiological age composition of populations of female *Cx. pipiens* collected in canopies and catch basins in Connecticut.

Materials and Methods

Trapping was conducted in Stamford and Stratford, CT, both in Fairfield County. Stamford is located on the coast of Long Island Sound with an area of 96.6 km^2 and a population of 117,000. It is located ≈40 km northeast of New York City, NY, where the first humans in the New World were infected with WNV in 1999 (Asnis et al. 2001). We selected this city because WNV has been isolated from birds and or mosquitoes since 1999 (Anderson et al. 1999, Andreadis et al. 2004), and five humans became infected with WNV in 2002 and 2003 (Wilcox et al. 2004).

Stratford, located ≈39 km east of Stamford on Long Island Sound, has an area of 48.6 km^2 and a population of 49,000. WNV has been present in this city since 1999. We reported previously a relatively intense focus of WNV on City of Stratford Water Pollution Control Authority land, bordering the Housatonic River near where it flows into Long Island Sound (Anderson et al. 2004).

CDC miniature light traps baited with dry ice (model 512, John Hock, Gainesville, FL) were placed inside catch basins located in residential and light industrial/residential areas in Stamford on seven separate dates from 27 August through 4 November 2003. The trap housing, battery, and 1.9-liters (2-qt) container with dry ice were independently attached to the underside of the drain grate. Traps were retrieved ≈24 h after they were set in the field. Traps were placed in each of 22 different catch basins, and the number of nights that traps were placed in each catch basin ranged from one to seven.

In 2004 and 2005, six catch basins in Stamford were selected to receive a CDC miniature light trap baited with dry ice (3.8-liters (4-qt) container) or a mosquito magnet experimental (MMX) trap (American Biophysics Corp., East Greenwich, RI) (Kline 1999). Each catch basin site was assigned a number, and the type of trap used was determined mechanically by using a table of random numbers. CDC traps were used at three sites, and MMX traps were used at three sites. At each catch basin site, traps similar to the trap placed in the catch basin were hung from a branch of a nearby (≈3–9 m) tree growing along the side of the road ≈1.5 m above the ground and ≈7 m above the ground. Trapping was carried out at approximately weekly intervals from 26 May 2004 through 27 October 2004 and 4 May through 19 October 2005.

Three CDC traps and three MMX traps were placed in catch basins in Stratford on Water Pollution Control Authority land and an adjacent industrial private property in 2004 and 2005. As described above, CDC and MMX traps were hung from a branch of a nearby (≈3–9 m) tree growing along the side of the road or parking lot ≈1.5 m above the ground and ≈7 m above the ground. Traps were retrieved ≈24 h after placement in the field at approximately weekly intervals from 19 April 2004 through 30 November 2004 and from 19 April through 7 November 2005.

Captured mosquitoes were anesthetized with dry ice in the field, transferred into vials sealed with a rubber stopper and gas-proof tape, placed on dry ice in the field, and then stored in a −80°C freezer. Female mosquitoes were subsequently identified to species (Darsie and Ward 1981, Andreadis et al. 2005) on a cold platform and were placed into groups by species, sex, date, type of trap, height of trap, and location. Each pool consisted of one to 50 unfed or gravid specimens. Blood was not apparent in the specimens tested. Mosquitoes were kept on wet ice until tested in Vero cells for the presence of virus using methods described previously (Anderson et al. 2004).

Male mosquitoes were tested for virus in 2005. Care was taken to ensure that mosquito pools contained only male mosquitoes and not body parts (leg, abdomen, head, and thorax) of female mosquitoes. Pools of male mosquitoes that were positive for WNV were identified to species by amplification and restriction digestion of the ribosomal DNA internal transcribed spacer (ITS) region by using AgeI restriction endonuclease (Debrunner-Vossbrinck et al. 1996).

Ovaries from 298 and 109 females captured in canopies and in catch basins, respectively, from 1 August through 26 September 2005 were dissected and age graded by procedures described by Kardos and Bellamy (1961). Attempts to isolate WNV were made from individual specimens as described previously. Number 2 insect pins were used to remove the ovaries from each mosquito. New pins were used for each specimen to prevent possible transfer of the virus among specimens.

WNV was identified by a TaqMan reverse transcriptase-polymerase chain reaction (RT-PCR) assay (Lanciotti et al. 2000) or by virus-dilution serum constant-neutralization tests (Hammon and Sather 1969). The MIR per 1,000 specimens was determined for weekly collections (Biggerstaff 2003).

Statistical analyses of collections of mosquitoes were performed as follows. Numbers of specific species of mosquitoes collected at each trap per night were $\log_e$ transformed with zero count data being

Table 1. Total mosquitoes collected by species, elevation, and trap type, Stamford, CT, 2004 and 2005

Species	Trap ht						Total (%)
	Catch basin		Ground		Canopy		
	CDC	MMX	CDC	MMX	CDC	MMX	
Cx. pipiens	1,735	2,066	182	984	209	3,512	8,688 (74.6)
Ae. vexans	8	16	405	464	77	39	1,009 (8.7)
Cx. restuans	321	399	79	31	37	67	934 (8.0)
Cx. salinarius	23	26	68	123	23	66	329 (2.8)
Ae. taeniorhynchus[a]	2	6	111	139	8	5	271 (2.3)
Ae. cantator	2	4	30	75	6	11	128 (1.1)
Ae. japonicus[a]	13	13	27	28	4	5	90 (0.8)
14 other species	9	49	62	44	13	17	194 (1.7)
Total	2,113	2,579	964	1,888	377	3,722	11,643

[a] Author name identified: *Aedes taeniorhynchus* (Wiedemann), *Aedes japonicus* (Theobald).

replaced by a 1. The seasonal average of the logarithms of mosquito counts at each trapping site was used in the analyses. The back-transformed mean of the log-transformed mosquito counts collected at each specific trap throughout the season is, by definition, the geometric mean. Analysis of variance (ANOVA) using Systat 11 (SPSS Inc., Chicago, IL) compared the geometric means of mosquitoes captured in the two different types of traps at different heights. When a trap did not operate on a specific night, data from the other traps at different heights at the same location were discarded and not included in the analysis. The Tukey honestly significant difference (HSD) multiple comparison test was used to determine significant differences. Fisher exact test using Yates correction for continuity (Systat 7, Systat Software, Inc., Point Richmond, CA) was used to compare infection rates among pooled *Cx. pipiens* and *Culex restuans* Theobald collected in catch basins, at ground level, and at canopy level.

Results

Diversity and Abundance of Adult Female Mosquitoes. Females were first collected in catch basins in 2003 by using CDC traps placed in Stamford. Ten species were represented among the 458 mosquitoes collected in 104 traps from 27 August to 4 November. *Cx. pipiens* made up 84.5% of the total. Other common species were *Cx. restuans* (5.0%), *Culex salinarius* Coquillett (4.1%), and *Aedes vexans* (Meigen) (3.3%).

In total, 11,643 mosquitoes, representing 21 species, were captured at all heights in Stamford in 2004 and 2005 (Table 1). Numbers of mosquitoes by trap height were 4,692 (40.3%) in catch basins, 2,852 (24.5%) at ground level, and 4,099 (35.2%) in the tree canopy. Diversity of species was greater in traps placed at ground level ($n = 20$) than at catch basin ($n = 15$), or canopy ($n = 15$) levels. *Cx. pipiens, Ae. vexans,* and *Cx. restuans* made up 74.6, 8.7, and 8.0% of the total, respectively. *Cx. pipiens* was the most abundant species captured at all three heights. *Cx. pipiens* and *Cx. restuans* made up 96.4% of the specimens collected in catch basins.

Mosquitoes, including *Cx pipiens,* were much more abundant in Stratford than Stamford. Thirty-one species, totaling 81,431 mosquitoes, were collected in Stratford in 2004 and 2005 (Table 2). Numbers of specimens collected in catch basins, at ground level, and at canopy level were 9,448 (11.6%), 27,630 (33.9%), and 44,353 (54.5%), respectively. Numbers of different species by height were 20, 27, and 26 for catch basins, ground, and canopy levels, respectively. *Cx. pipiens* was the most abundant species, representing 63.3% of all specimens collected and was the most frequently collected species in catch basins and in tree canopies. *Ae. vexans* was the most abundant species collected at ground level.

Captures in CDC and MMX Traps at Three Heights. *Cx. pipiens* collections from Stamford and Stratford, from 2004 and 2005, for both types of traps, and at three different heights were analyzed using a four-way ANOVA (city [2] × year [2] × trap type [2] × height [3]). Multiple comparison tests were then performed comparing trap type and trap height effects based on Tukey's HSD at $P < 0.05$. Significantly greater numbers were collected in MMX traps in the canopy than in MMX traps at ground or catch basin levels, and MMX traps captured more than CDC traps at canopy and ground levels (Table 3). CDC traps placed in catch basins collected significantly larger numbers than CDC traps placed at ground and canopy levels.

The vertical distributions of five other abundant species were determined by combining data from both cities and years and type of trap, because there were no consistent significant differences between the types of trap (Table 4). *Cx. restuans* was significantly more abundant in traps placed in catch basins than in traps placed at ground or canopy levels. *Ae. vexans, Ae. cinereus* Meigen, and *Aedes cantator* (Coquillett) were significantly more abundant at ground level. *Cx. salinarius* was captured in greater numbers at ground positioned traps but not significantly so in comparison with traps in the canopy.

West Nile Virus. WNV was isolated from one pool of *Cx. salinarius* and 10 pools of *Cx. pipiens* collected in CDC traps placed in catch basins in 2003 in Stamford. *Cx. pipiens* had a weekly MIR of WNV of 31.3–44.5 per 1,000 from 27 August through 24 September when geometric means per trap night ranged from 4.2

Table 2. Total mosquitoes collected by species, elevation, and trap type, Stratford, CT, 2004 and 2005

Species	Trap ht						Total (%)
	Catch basin		Ground		Canopy		
	CDC	MMX	CDC	MMX	CDC	MMX	
Cx. pipiens	4,769	1,435	451	4,591	1,197	39,134	51,577 (63.3)
Ae. vexans	457	30	5,896	2,399	308	150	9,240 (11.4)
Ae. cinereus	48	70	1,230	3,730	121	268	5,467 (6.7)
Cx. salinarius	602	69	1,682	1,482	538	903	5,276 (6.5)
Ae. cantator	57	28	1,425	1,484	287	176	3,457 (4.3)
Cx. restuans	1,388	375	183	178	183	581	2,888 (3.6)
Cq. perturbans[a]	6	2	584	251	101	102	1,046 (1.3)
Ae. sollicitans[a]	24	6	582	325	60	42	1,039 (1.3)
Ae. triseriatus	7	1	123	227	9	41	408 (0.5)
Ae. trivittatus[a]	4	0	83	71	9	3	170 (0.2)
Ae. japonicus	12	3	31	48	3	72	169 (0.2)
Ur. sapphirina[a]	10	21	24	104	2	5	166 (0.2)
Ae. taeniorhynchus	3	0	89	39	8	5	144 (0.2)
Ae. stimulans[a]	1	1	51	53	5	5	116 (0.1)
Ae. excrucians[a]	1	0	31	50	0	1	83 (0.1)
16 other species	16	2	60	73	7	27	185 (0.2)
Total	7,405	2,043	12,525	15,105	2,838	41,515	81,431

[a] Author name identified: *Coquillettidia perturbans* (Walker), *Aedes sollicitans* (Walker), *Aedes trivittatus* (Coquillett), *Uranotaenia sapphirina* (Osten Sacken), *Aedes stimulans* (Walker), *Aedes excrucians* (Walker).

to 11.8. The MIR on 11 September for *Cx. salinarius* was 114.6.

Four hundred-eighteen isolates of WNV from pooled female mosquitoes were made from seven species collected in 2004 and 2005 in Stamford and Stratford (Table 5). Eighty-nine, 44, and 285 isolates were made from mosquitoes captured in traps placed in catch basins, at ground level, and in tree canopies, respectively. Three hundred eighty-eight (92.8%) and 16 (3.8%) of the isolates were from *Cx. pipiens* and *Cx. restuans*, respectively. Isolates also were obtained from *Cx. salinarius* ($n = 4$), *Ae. vexans* ($n = 4$), *Ae. cantator* ($n = 3$), *Aedes triseriatus* (Say) ($n = 2$), and *Ae. cinereus* ($n = 1$).

WNV was initially isolated during the week of 27 June 2004, 1 wk after geometric mean numbers of *Cx. pipiens* reached 13.2 per trap night (Fig. 1). The last of a total of 55 isolates was made during the week of 3 October. Multiple isolations were made from collections during the weeks of 25 July through 19 September. Weekly MIR during this period ranged from 1.1 to 7.1. *Cx. pipiens* was relatively abundant (geometric mean numbers per all traps per night ranged from 6.3 to 26.2) from the week of 6 June through the week of 19 September.

Three isolations from *Cx. restuans* were made from 10 August through 14 October 2004 with MIRs ranging from 7.7 to 49.0. Two isolations of WNV from *Cx. salinarius* were made during the week of 29 August. The MIR was 4.6. Single isolates of WNV were made from *Ae. cantator*, *Ae. cinereus*, and *Ae. triseriatus* during the weeks of 4 July, 11 July, and 8 August, respectively.

The rate of WNV infection in *Cx. pipiens* was greater in 2005 than in 2004. The first isolations were made during the week of 24 July, with multiple isolations made through the week of 2 October (Fig. 2). The weekly MIRs ranged from 2.2 to 83.9. The last isolation from *Cx. pipiens* was made during the week of 30 October. *Cx. pipiens* became abundant >1 mo before WNV was initially isolated. Geometric means for *Cx. pipiens* per trap night ranged from 5.8 to 19.2 during the weeks of 19 June through 2 October.

WNV also was isolated from *Cx. restuans*, *Cx. salinarius*, *Ae. vexans*, *Ae. cantator*, and *Ae. triseriatus* in 2005. These isolations were made during the weeks of

Table 3. Geometric means of *Culex pipiens* collected by type of trap and ht per night

Ht	Trap type	
	CDC	MMX
Catch basin	7.5Aa	6.9Aa
Ground	2.0Ba	6.8Ab
Canopy	2.4Ba	31.3Bb

Means within each column having the same uppercase letter are not significantly different. Means within each row having the same lowercase letter are not significantly different.

Table 4. Geometric mean numbers of females per trap-night of five species of mosquitoes collected at three heights

Species	Ht	Geometric mean
Cx. restuans	Catch basin	2.40A
	Ground	1.36B
	Canopy	1.55B
Cx. salinarius	Catch basin	1.37A
	Ground	2.65B
	Canopy	1.91AB
Ae. vexans	Catch basin	1.14A
	Ground	3.32B
	Canopy	1.36A
Ae. cinereus	Catch basin	1.12A
	Ground	2.28B
	Canopy	1.24A
Ae. cantator	Catch basin	1.09A
	Ground	2.21B
	Canopy	1.33A

Means within each column for a specific species having the same uppercase letter are not significantly different.

Table 5. Total number of WNV isolates from seven *Aedes* and *Culex* species by trap height and trap type, Stamford and Stratford, CT, 2004 and 2005

Species	Trap ht and trap type						Total
	Catch basin		Ground		Canopy		
	CDC	MMX	CDC	MMX	CDC	MMX	
Cx. pipiens	56	20	6	27	8	271	388
Cx. restuans	8	2	1	1	0	4	16
Cx. salinarius	2	0	2	0	0	0	4
Ae. vexans	0	0	0	3	0	1	4
Ae. cantator	1	0	2	0	0	0	3
Ae. triseriatus	0	0	1	1	0	0	2
Ae. cinereus	0	0	0	0	0	1	1
Total	67	22	12	32	8	277	418

24 July–18 September. Weekly MIR for *Cx. restuans* during the weeks of 24 July through 18 September ranged from 8.9 to 101.1.

Frequencies of pools of *Cx. pipiens* infected with WNV were significantly different by height ($\chi^2 = 42.73$, df = 2, $P < 0.001$). Among heights, frequencies of infected pools of *Cx. pipiens* collected in ground positioned traps were significantly less than those collected in catch basins ($\chi^2 = 10.56$, df = 1, $P = 0.001$) and in the canopy ($\chi^2 = 37.96$, df = 1, $P < 0.001$); *Cx. pipiens* collected in the canopy were significantly more likely to be infected than those collected in catch basins ($\chi^2 = 8.98$, df = 1, $P = 0.003$). Total numbers of WNV isolates from *Cx. pipiens* captured in the canopy were significantly higher than those from mosquitoes collected in traps at ground ($\chi^2 = 321.75$, df = 1, $P < 0.001$) and catch basin ($\chi^2 = 211.86$, df = 1, $P < 0.001$) levels, and total numbers of infected pools from *Cx. pipiens* captured in catch basins were significantly greater than those isolated from *Cx. pipiens* captured in ground-placed traps ($\chi^2 = 18.80$, df = 1, $P < 0.001$). The frequency of WNV isolates collected from *Cx. restuans,* the only other species that WNV was isolated from >10 pools, did not differ significantly by collection height ($\chi^2 = 2.17$, df = 2, $P = 0.34$).

WNV was isolated from male mosquitoes collected in 2005. In total, 2,711 male *Culex* were tested, of which six pools were infected with WNV. Infected males were captured during the week of 7 August through the week of 4 September. Three, one, and two positive

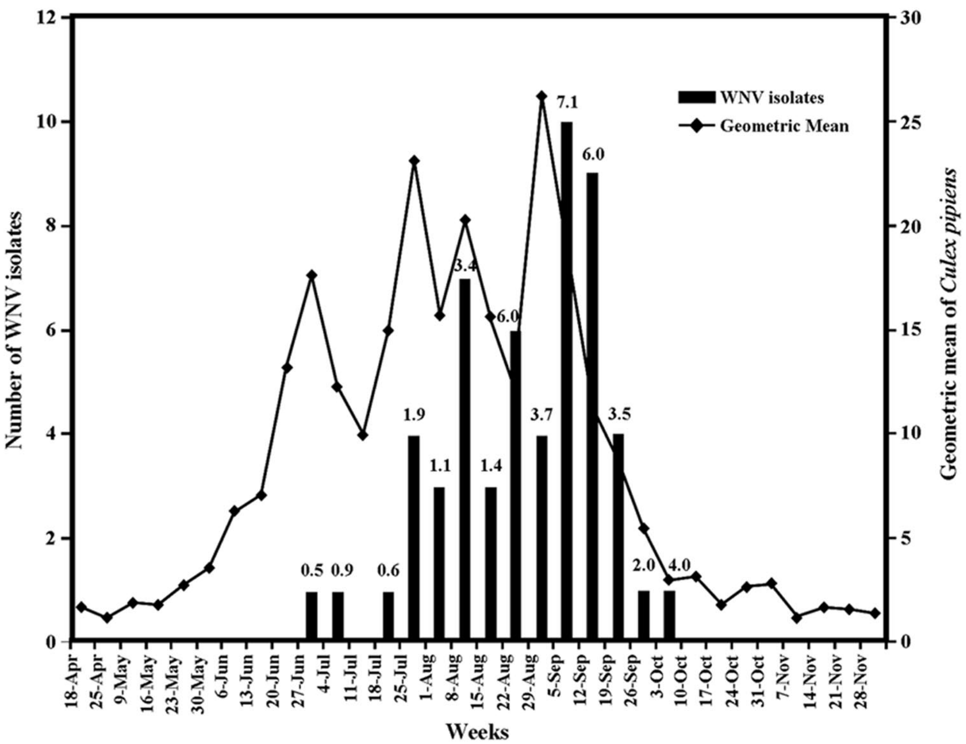

Fig. 1. Geometric mean numbers of *Cx. pipiens* collected per trap night, numbers of WNV isolations, and minimum infection rates (number above each bar) per week, Stamford and Stratford, CT, 2004.

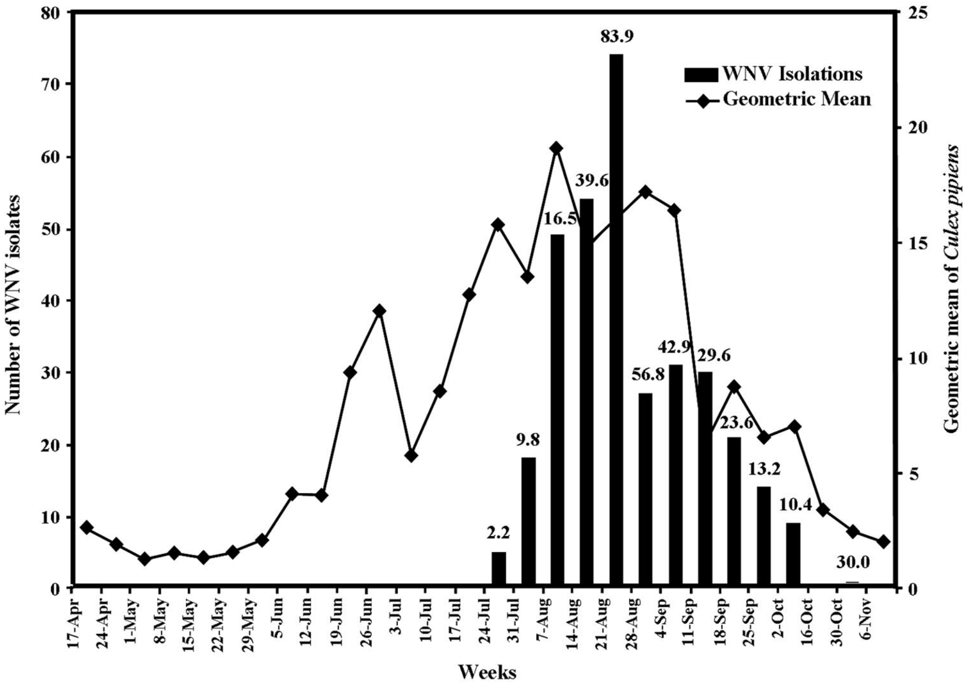

Fig. 2. Geometric mean numbers of *Cx. pipiens* collected per trap night, numbers of WNV isolations, and minimum infection rates (number above each bar) per week, Stamford and Stratford, CT, 2005.

pools were made up of specimens captured in catch basin-, ground-, and canopy-placed traps, respectively. The MIRs for the weeks of 7 August, 14 August, 21 August, and 4 September were 1.2, 31.1, 3.0, and 9.1, respectively. AgeI restriction digests of the single-stranded (ss) rDNA ITS region confirmed that the specimens in the six pools were *Cx. pipiens* (Fig. 3). Digestion patterns for the six pools of field-collected *Culex* infected with WNV (lanes 5–10) expressed a prominent double band near 750 bp that was identical to the known *Cx. pipiens* specimen (lane 2) and distinctly different from the patterns for *Cx. restuans* (lane 3) and *Cx. salinarius* (lane 4) specimens. Although the AgeI digests of the field-collected specimens (lanes 5–10) showed a faint residual double band near 1,500 bp, the bands were larger than the band of *Cx. salinarius* (lane 4).

The physiological age composition of female *Cx. pipiens* in the canopy was significantly different from *Cx. pipiens* captured in the catch basin. Two hundred ninety-seven of the 298 *Cx. pipiens* females (99.7%), which were captured in the canopy and were dissected to determine parity, contained ovaries with oocytes in Christophers' stage 2 (Table 6; Fig. 4A). These specimens were made up of nulliparous (61.1%) and parous (38.9%) mosquitoes (Table 6). In contrast, only 45 of 109 (41.3%) of the *Cx. pipiens* from catch basins possessed ovaries with oocytes in Christophers' stage 2. These specimens consisted of 12.8% nulliparous and 28.4% parous mosquitoes. The remaining 64 (58.8%) females captured in catch basins had ovaries filled with fully formed eggs, Christophers' stage 5 (Fig. 4B). The frequency of gravid females in catch basin traps was significantly higher than in canopy-placed traps ($\chi^2 = 203.8$, df = 1, $P < 0.001$). WNV was isolated from six nulliparous females (Fig. 4C), 14 parous females (Fig. 4D), and two gravid females (Table 6).

Discussion

Isolations of WNV from mosquitoes collected in catch basins suggest that surveillance programs for arboviruses may wish to include subterranean habitats as part of a sampling program. Epidemic vectors of WNV in the northeastern United States have not been specifically identified, but *Cx. pipiens, Cx. restuans* (Kilpatrick et al. 2005), and *Cx. salinarius* (Andreadis et al. 2004, Molaei et al. 2006) have been suggested as principle species responsible for human infection. The isolation of WNV from *Cx. pipiens, Cx. restuans,* and *Cx. salinarius* collected in catch basins indicates that these artificial subterranean sites are important habitats for *Culex* mosquitoes infected with WNV.

Fifteen, zero, and six humans cases of WNV disease were reported in Connecticut in 2003, 2004, and 2005, respectively (Wilcox et al. 2004) (http://www. cdc. gov.ncidod/dvbid/westnile/sur&controlcasecount05_detailed.htm). The relative differences in reported incidence of human WNV disease among the 3 yr also were reflective of infection rates in *Cx. pipiens.* The MIRs were considerably higher in 2003 and 2005 than in 2004. Augmenting standard mosquito surveillance programs with the placement of traps in tree canopies

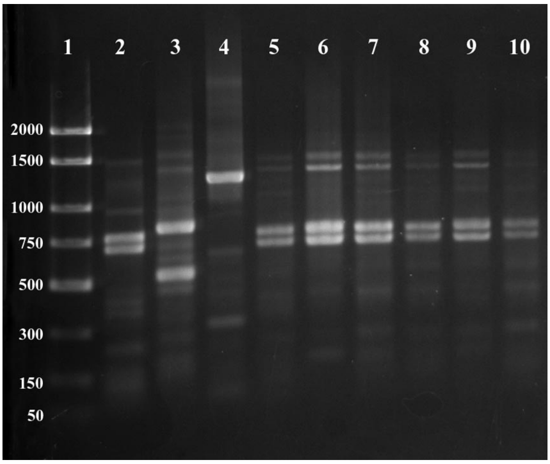

Fig. 3. Two percent agarose gel electrophoresis of restriction digests of ssrDNA ITS region (AgeI) of mosquitoes. Lane 1 is a size standard (exact Gen low range DNA latter, Fisher, Pittsburgh, PA). Lanes 2, 3, and 4 are single specimens of *Cx. pipiens*, *Cx. restuans*, and *Cx. salinarius* cut with AgeI, respectively. Lanes 5–10 are different pools of field-collected male *Cx. pipiens* infected with WNV and cut with AgeI. The known specimens of *Cx. pipiens* (lane 2) and *Cx. salinarius* (lane 3) were from laboratory colonies. The *Cx. restuans* specimen (lane 4) was collected in the field and identified by ssrDNA sequencing.

and in catch basins will increase numbers of isolations and may improve forecasting the risk of spread of WNV to humans and the need to enact mosquito control measures.

Cx. pipiens was the most abundant mosquito captured in Stamford in 2003 through 2005 and in Stratford in 2004 and 2005. Significantly greater numbers were captured in MMX traps placed in the canopy and CDC traps placed in catch basins than in CDC traps placed at ground or canopy levels. The capture of larger numbers in tree canopies has been reported by others (Main et al. 1966, Novak et al. 1981, Anderson et al. 2004, Darbro and Harrington 2006). The CDC trap is less efficient than the MMX trap in capturing *Cx. pipiens* at ground and canopy levels in the northeastern United States. These findings suggest that greater numbers of *Cx. pipiens* can be captured in traps placed in the tree canopy, particularly when MMX traps are used, and in catch basins than in CDC traps placed at ground level.

Catch basins also are relatively good habitats for collecting *Cx. restuans.* Significantly larger numbers of *Cx. restuans* were collected in catch basins than at ground or canopy levels.

Certain species of mosquitoes tend to fly and feed relatively close to the ground (Clements 1999). Species collected in significantly greater numbers at ground level included *Ae. cinereus*, *Ae. vexans*, and *Ae. cantator.* Larger numbers of *Cx. salinarius* were collected at ground level but not significantly more than those caught in the canopy. West Nile virus was isolated from these four species and from *Ae. triseriatus. Cx. pipiens* and *Cx. restuans* tend to have an ornithophilic feeding preference (Tempelis 1974, Magnarelli 1977, Apperson et al. 2002), although *Cx. pipiens* is known to feed on mammals, including humans (Apperson et al. 2004, Molaei et al. 2006). *Cx. salinarius* feeds readily on both mammals, including humans, and birds (Edman 1974, Tempelis 1974, Molaei et al. 2006). The *Aedes* tend to feed primarily on mammals and can often be severe pests of humans, but on occasion they are known to feed on birds (Tempelis 1974). The tendency of *Aedes* and *Cx. salinarius* to feed near ground level will bring them into relatively proximity to humans. This characteristic, their documented natural infection with WNV, their demonstrated vector competency to transmit WNV (Turell

Table 6. Physiological age of and WNV isolations from *Cx. pipiens* collected in the canopy and in catch basins, Stratford, CT, 2005

Trap ht	Date collected	No. dissected	No. females with oocytes in: Christophers' stage 2			WNV isolates/total tested[b]
			Nulliparous	Parous[a]	Christophers' stage 5	
Canopy	1 Aug.	6	5	1	0	0/6
	8 Aug.	30	23	7 (2)	0	1/30
	16 Aug.	30	16	14 (2)[c]	0	1/30
	22 Aug.	30	20	10 (1)	0	2/20 (1)
	29 Aug.	30	18	12 (1)	0	5/20 (2)
	6 Sept.	40	23	17 (2)	0	3/33 (1)
	12 Sept.	29	16	13 (1)	0	1/29 (1)
	19 Sept.	44	15	29	0	3/44 (1)
	26 Sept.	59	46	13 (2)	0	0/59
Catch basin	1 Aug.	9	0	3 (2)	6	0/10
	8 Aug.	11	0	6 (2)	5	0/11
	22 Aug.	32	6	8	18	1/22
	29 Aug.	20	6	4	10	4/10
	6 Sept.	18	2	6 (1)	10	1/17
	12 Sept.	9	0	3	6	0/9
	19 Sept.	9	0	1	8	0/9
	26 Sept.	1	0	0	1	0/1

[a] Values in parentheses are number of females with 1–12 retained eggs.
[b] Values in parentheses are number of isolations from nulliparous females.
[c] Oocytes in one female were in Christophers' stage 3.

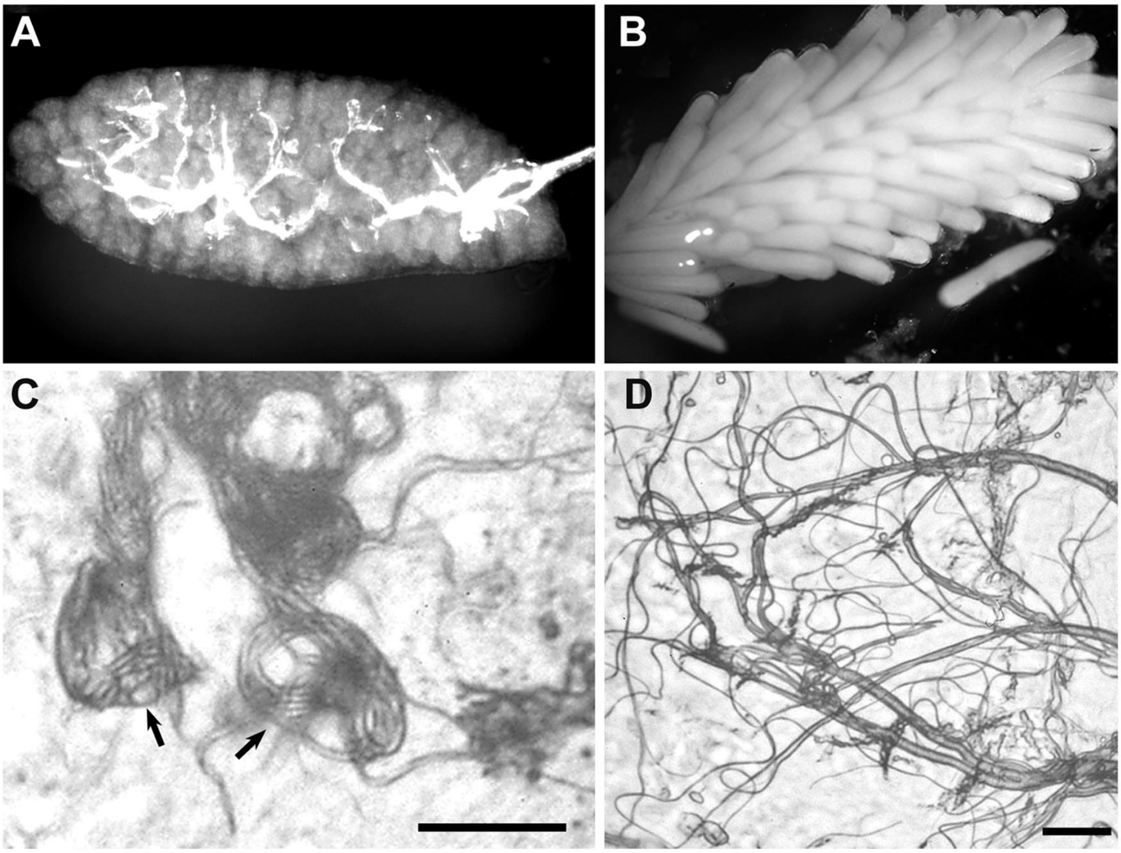

Fig. 4. Photomicrographs of ovaries and portions of ovaries from *Cx. pipiens.* (A) Ovary from specimen captured in the canopy. Oocytes are in Christophers' stage 2. Two hundred ninety-eight of 299 specimens examined had ovaries with oocytes in Christophers' stage 2. Original magnification, 48×. (B) Ovary from specimen captured in a catch basin. Sixty-four of 109 specimens examined from catch basins were gravid and had ovaries packed with mature oocytes in Christophers' stage 5. Forty-five females had ovaries with oocytes in Christophers' stage 2. Original magnification, 27×. (C) Nulliparous ovary with tightly coiled skeins (note arrows). Scale bar = 25 μm. WNV was isolated from the female with this ovary. (D) Parous ovary with open skeins. Scale bar = 25 μm. WNV was isolated from the female with this ovary.

et al. 2005), their aggressiveness in feeding on humans, and their relative abundance suggest to us that all need to be considered as possible bridge vectors of WNV to humans.

The isolation of WNV from male *Cx. pipiens* and from nulliparous females collected in August and September clearly indicates that mosquitoes also are becoming infected by means other than blood feeding. Vertical transmission of WNV in mosquitoes has been reported in laboratory experiments (Baqar et al. 1993, Goddard et al. 2003), suggested by finding the virus in diapausing and presumably unfed females collected in the field during winter (Nasci et al. 2001a), and reported as occurring naturally in *Culex univittatus* (Theobald) complex in Kenya (Miller et al. 2000). Infection of *Cx. pipiens* via means other than feeding on infected vertebrate hosts, possibly by transovarial transmission, probably contributes to the increasing incidence of infected mosquitoes during August and September when humans are most likely to contract West Nile virus disease (Asnis et al. 2001).

The physiological age composition of female *Cx. pipiens* in the canopy is significantly different from that of in females captured in the catch basin. Females in the canopy invariably had ovaries classified as Christophers' stage 2 and most likely were seeking host animals upon which to feed. The 38.9% rate of parity of the *Cx. pipiens* captured in the canopy in our studies was similar to the 32.6% parity rate of *Cx. pipiens* collected in canopy and ground placed traps in New York state (Drummond et al. 2006). In contrast, 58.8% of the females captured in catch basins had ovaries packed with fully formed eggs. These females had already fed on blood, and, after their ovaries had matured, they descended to catch basins for shelter intent upon laying eggs on water in the bottom of the basin. Normally, gravid females are not attracted to CO_2 and light traps, but they are clearly captured in these traps in the close confines of the catch basin. Parous females captured in the catch basin may have recently deposited eggs, whereas the nulliparous females may have recently emerged and were captured before they exited the catch basin or they could have emerged from the catch basin and returned for shelter before obtaining a bloodmeal. The relatively high rates of infection of *Cx. pipiens* in the canopy and catch basin are likely the result of transovarial transmission of virus and the relatively high proportion of parous or gravid females that have previously fed on infected avian hosts.

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