

Adult Mosquito Surveillance

Introduction

The West Nile Virus survives by circulating between bird and mosquito populations. A female mosquito can acquire the infection by obtaining a blood meal from a WNV-infected bird. After a suitable incubation period, it can then pass the infection by injecting its virus laden saliva into another host (bird, horse, human or other animal) when it takes another blood meal.¹⁰ Once in the new host, the virus multiplies and is able to infect another female mosquito. The virus can also survive over winter in adult female mosquitoes or eggs. When these mosquitoes take a blood meal or the eggs hatch in early spring, a new cycle of WNV transmission can begin.¹⁰

The purposes of mosquito surveillance programs are: to monitor mosquito populations associated with WNV; to determine the level of WNV activity among these species; and to use this information to make decisions regarding the risk of transmission to humans and the need to implement mosquito reduction plans.

Methods

In 2004, Peel Public Health used two types of traps to capture live adult mosquitoes. The first type of trap was the Center for Disease Control (CDC) miniature light trap (Figure 7) which uses carbon dioxide and light to attract host-seeking adult female mosquitoes looking for a blood meal. The second type of trap used was the gravid trap (Figure 8) in which nutrient-rich stagnant water (infusion media) is used to attract gravid female mosquitoes (egg-carrying) searching for a suitable site in which to lay their eggs.



Figure 7: CDC Mosquito Light Trap

Source: Photo taken by Peel Public Health WNV Team

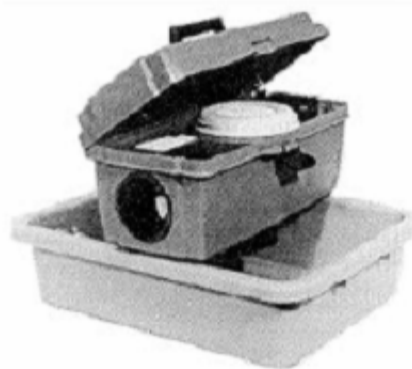


Figure 8: Mosquito Gravid Trap

Source: Photo Courtesy of Central Massachusetts Mosquito Control Project
<http://cmmcp.org/>

Thirty-two CDC traps were deployed in 30 fixed and two temporary locations across Peel from June 14 to September 29, 2004. Adult mosquito traps were located as shown in Figure 9. There were 16 mosquito trapping sites in Mississauga, nine in Brampton and five in Caledon. Two “Hot Spot” CDC light traps were located in Brampton where positive birds were found.

Adult mosquitoes were usually collected once per week at a trap site, refrigerated, and transported alive by Peel Public Health staff to the Department of Biological Sciences at Brock University in St. Catharine’s, Ontario.

In order to ensure the delivery of mosquito specimens to Brock in a timely manner and to assist in other surveillance activities, Peel Public Health leased a truck for a period of six months. Twice a week (Tuesday and Thursday afternoons), a student transported adult mosquitoes in coolers packed with freezer packs. The leasing of this truck is a result of one of the recommendations in the 2003 WNV Prevention and Control Plan Evaluation Report.⁶

At Brock, the trap contents were frozen until such time as they could be examined. At that point, the contents were evenly spread out on a dry ice platform in order to keep them frozen. Large insects (moths, caddis flies, etc.) were removed and then a section of the sample was chosen at random. With the use of a magnifying glass, female mosquitoes were individually removed by the sorter until a count of 100 was reached.² The entire sample of mosquitoes was then counted and separated into males (which feed on nectar and do not take blood meals) and remaining females, which were classified as “extras”. The 100 females were separated into species, recounted and then “pooled” or batched by species, date of collection and location for testing. Batches typically contained 50 or less adult female mosquitoes.²

When species could not be distinguished, they were either classified as a group (e.g. *Culex pipiens/restuans*) or to the level of genus (e.g. *Culex spp.*). Some mosquitoes ended up being classified as “unidentifiable females” because the specimens were either unusable due to the natural aging process, or damaged during collection, shipping or storage.²

Counts of sampled female mosquitoes by species, unidentifiable females, males, extras and total mosquitoes, along with their corresponding date collected and site identification number were entered into a database by staff at Brock University; this information was then posted on the Brock website. Testing for WNV was done by Brock University and positive batch test results were sent electronically to health units often within one week. The results were posted on the Peel WNV website.

The above procedures were somewhat different than those used in 2002. For example in 2002, a number of traps were set out twice per week at the same site, resulting in a higher number of trapping events. In addition, Brock University

identified all female mosquitoes by species in 2002, rather than taking a portion of the mosquitoes and sampling them as was done in 2003 and 2004. All viral testing of female mosquitoes was conducted at Brock University in 2003 and 2004, whereas in 2002, some of the testing was done at Health Canada's National Microbiology Laboratory in Winnipeg, Manitoba.

Estimates of the total number of mosquitoes for a particular species were derived using the actual number of adult female mosquitoes that had been separated into species as a proportion of the total number of mosquitoes (excluding males). In the tables and charts of this section of the report, certain analyses are based on actual numbers while others are based on estimated numbers of mosquitoes, and are notated accordingly.

Five gravid mosquito traps were used on a trial basis in 2004. The gravid traps were set up once per week at trap sites in Brampton and Caledon from mid-July until early October. The mosquitoes captured in the traps were refrigerated and transported to our in-house mosquito lab in Brampton. The specimens were then frozen prior to being identified by Peel Public Health staff. The mosquitoes that were collected were counted and separated into species. Viral testing of the mosquitoes captured in the gravid traps was not undertaken.

Results

In Peel, approximately 55,800 mosquitoes were trapped over the course of the 2004 season, of which 53,556 were identified as female mosquitoes of various species (Table 5). The total number of mosquitoes trapped in 2004 exceeds the totals for 2002 (41,000) and 2003 (43,500). The increase in mosquito numbers is directly related to the increase in the number of permanent and temporary traps used during the 2004 season. Twenty permanent and three temporary traps were deployed in 2003 while 30 permanent and two temporary traps were used in 2004. Adult females from 38 mosquito species were collected in Peel traps in 2004 (Table 5). As in previous years, *Coquillettidia perturbans* and *Aedes vexans* were the most common mosquito species.

There were 70 WNV positive mosquito batches identified in Ontario in 2004¹¹ (Table 6). Toronto had the highest number of WNV positive mosquito batches with 31. In 2002, there were 598 WNV positive mosquito batches in the province and 135 in 2003.¹¹

In 2004, there was a substantial reduction of WNV positive mosquito batches in Canada (Table 7). In 2004, there were 176 WNV positive mosquito batches in the country compared to 579 in 2003 and 663 in 2002.¹² The WNV positive mosquito batches were located in five provinces: Quebec, Ontario, Manitoba, Saskatchewan and Alberta.¹²

2004 WEST NILE VIRUS IN THE REGION OF PEEL

**Table 5: Estimated Number of Female Adult Mosquitoes Collected
by Species and Municipality, Region of Peel, 2004**

Species Name	Brampton	Caledon	Mississauga	Peel
<i>Coquillettidia perturbans</i> *	1,456	286	18,350	20,092
<i>Aedes vexans vexans</i>	3,674	309	5,092	9,075
<i>Aedes /Ochlerotatus spp.</i> **	2,204	2,431	1,537	6,172
<i>Ochlerotatus stimulans</i>	1,876	1,509	1,026	4,411
<i>Ochlerotatus canadensis</i>	1,893	1,716	139	3,748
<i>Ochlerotatus triseriatus</i>	149	102	2,288	2,539
<i>Culex pipiens/restuans</i>	997	116	1,333	2,446
<i>Ochlerotatus trivittatus</i>	810	50	226	1,086
<i>Culex pipiens</i>	435	36	294	765
<i>Culex restuans</i>	250	92	349	691
<i>Ochlerotatus excrucians</i>	219	200	81	500
<i>Anopheles punctipennis</i>	177	56	223	456
<i>Culex spp.</i>	208	9	186	403
<i>Aedes vexans/cantator</i>	116	9	179	304
<i>Anopheles quadrimaculatus</i>	153	27	17	197
<i>Aedes cinereus</i>	79	65	18	162
<i>Ochlerotatus triseriatus/hendersoni</i>	3	3	94	100
<i>Ochlerotatus dorsalis</i>	57	8	8	73
<i>Ochlerotatus fitchii</i>	7	54	3	64
<i>Aedes vexans nipponi</i>	18	2	41	61
<i>Ochlerotatus japonicus</i>	0	0	45	45
<i>Culiseta morsitans</i>	16	19	3	38
<i>Culex territans</i>	9	1	23	33
<i>Anopheles earlei</i>	14	4	1	19
<i>Anopheles walkeri</i>	15	2	1	18
<i>Anopheles spp.</i>	6	6	5	17
<i>Ochlerotatus euedes</i>	0	7	0	7
<i>Psorophora ferox</i>	0	0	6	6
<i>Ochlerotatus riparius</i>	0	4	1	5
<i>Culex salinarius</i>	3	0	1	4
<i>Culiseta inornata</i>	4	0	0	4
<i>Ochlerotatus abserratus</i>	0	3	0	3
<i>Ochlerotatus cantator</i>	1	0	2	3
<i>Uranotaenia sapphirina</i>	1	0	2	3
<i>Anopheles perplexans</i>	0	1	1	2
<i>Ochlerotatus provocans</i>	0	2	0	2
<i>Culiseta spp.</i>	0	1	0	1
<i>Orthopodomyia alba</i>	1	0	0	1
Total Mosquitoes ***	14,851	7,130	31,575	53,556

* includes 2 mosquitoes classified as *Cq. perturbans* (pale legs) (not a species type)

** includes 428 mosquitoes classified as *Ochlerotatus* black-legged and 2968 mosquitoes classified as *Ochlerotatus* broad-banded (not a species type)

*** includes adult female mosquitoes, "Unidentifiable Females" and "Extras", but excludes "Males" that were counted

Table 6: Mosquito Surveillance Statistics by Health Unit, Ontario, 2004

Health Unit	Total Positive Batches
Chatham - Kent Public Health Division	3
Durham Region Health Department	2
Halton Region Health Department	5
City of Hamilton - Public Health & Community Services Dept.	8
Hastings & Prince Edward Counties Health Unit	1
County of Lambton Community Health Services Department	2
Middlesex - London Health Unit	2
County of Oxford	1
Regional Municipality of Peel Health Department	4
Perth District Health Unit	2
Timiskaming Health Unit	1
Toronto Public Health	31
Windsor - Essex County Health Unit	7
York Region Health Services Department	1
Ontario Total	70

WNV mosquito surveillance statistics are provided individually to the MOHLTC by Ontario's 37 regional health units. Surveillance statistics are current as of 5:00 p.m. EST Monday through Friday.

Data as of January 4, 2005

Source: Ontario Ministry of Health and Long -Term Care

Available from URL: http://www.health.gov.on.ca/english/providers/program/pubhealth/westnile/wnv_04/wnv_mosquitoes.html

Table 7: Mosquito Surveillance Statistics by Province, Canada, 2004

Province/Territory	No. Confirmed Positive Mosquito Pools
Newfoundland and Labrador	0
Prince Edward Island	0
Nova Scotia	0
New Brunswick	0
Quebec	21
Ontario	70
Manitoba	54
Saskatchewan	30
Alberta	1
British Columbia	0
Yukon Territory	0
Northwest Territory	0
Nunavut	0
Canada Total	176

Data as of January 4, 2005

Source: Health Canada

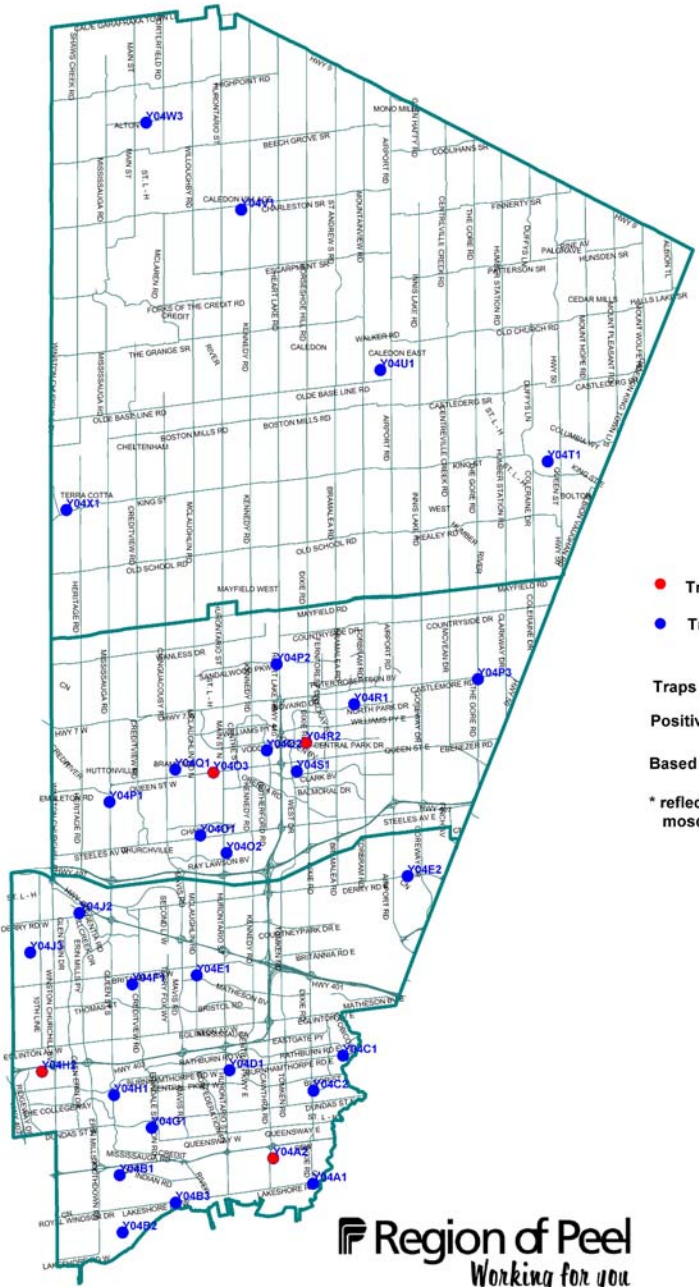
Available from URL:

http://www.phac-aspc.gc.ca/wnv-vwn/pdf_sr-rs/2004/situation_report_102604_mp.pdf

Peel also experienced a reduction of WNV positive mosquito batches in 2004. This year, only four positive batches were found in Peel, two each in Mississauga and Brampton. The first WNV positive mosquito batch in Ontario was found in Peel on July 15 (Week 28) near the Queen Elizabeth Way and Cawthra Road. The last recorded WNV positive batch in Peel was found on August 19 (Week 33) in the area of Queen Street E and Dixie Road in Brampton. As in previous years, no positive mosquitoes were found in Caledon. In 2003, there were 24 positive batches of mosquitoes identified in Peel and 128 in 2002.

A temporal analysis of the WNV positive mosquito batches is shown in Figure 10. Since the numbers of positive batches were low, there was no large peak in the number of batches collected in any one week as was the case in 2002. The highest number of positive batches collected in a given week was in Week 33 (August 15 - August 21) when two positive batches were identified.

2004 WEST NILE VIRUS IN THE REGION OF PEEL



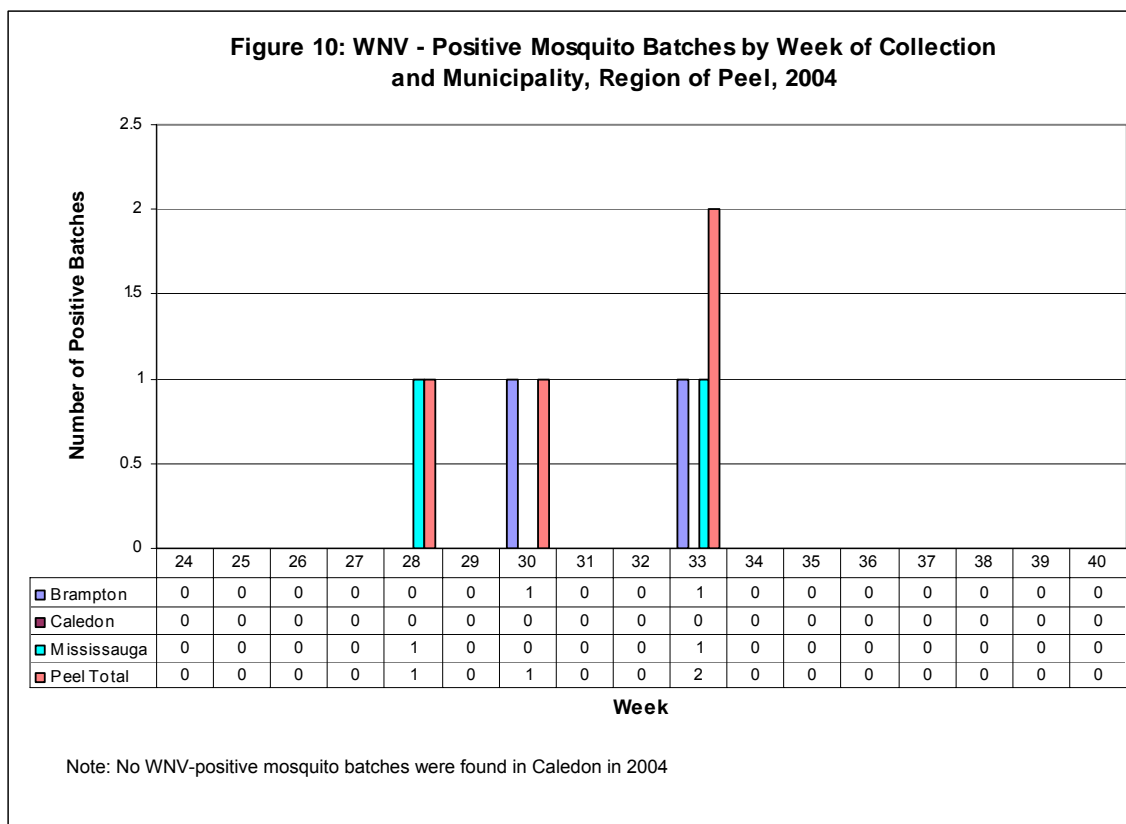
● Trap with WNV-Positive Results (s)*
 ● Trap

Traps = 32
 Positive Traps = 4

Based on data as of September 30, 2004

* reflects locations where WNV-positive mosquitoes collected

Region of Peel
 Working for you



Peel had four positive species identified in 2003 and 11 in 2002. Only three species or groups of mosquitoes were found to be WNV positive in Peel in 2004. The *Culex* group of mosquitoes accounted for all the positives this year. The WNV positive mosquitoes were *Culex restuans*, *Culex pipiens/restuans* and *Culex spp.* mosquitoes which were not able to be identified as specific *Culex* species. *Culex* mosquitoes accounted for 8% of the total female mosquitoes collected, but represented 100% of the positives.

The minimum infection rate (MIR) is used as an indicator of the prevalence of WNV, transmission intensity and thus the risk to human disease.² MIRs of WNV in certain species, expressed as the number infected per 1,000 mosquitoes tested, are shown in Table 8. Higher MIRs are usually indicative of greater WNV activity among a given species, but can be unreliable based on sample size.¹³

In 2004, the only MIR on a sample of more than 1,000 mosquitoes in Peel was observed among *Culex pipiens/restuans* (0.97 per 1,000). The MIR for *Culex spp.* was 2.95 and 1.71 for *Culex restuans*. These results are based on samples of less than 1,000 mosquitoes and are more likely to be unreliable.

The MIRs from 2004 are lower than those from 2003 and much lower than those noted in 2002. This suggests a lower prevalence of WNV in the mosquito population and thus a lower risk of humans contracting the disease. In 2004,

Peel had no reported human cases; this is entirely consistent with low infection rates in Peel's mosquito population.

Table 8: Minimum Infection Rates by Mosquito Species and Municipality, Region of Peel, 2004

Municipality	Vector Species	Actual Number Tested	Positive Batches	MIR *
Mississauga	<i>Culex pipiens/restuans</i>	1,102	1	0.91
	<i>Culex restuans</i>	307	1	3.26 †
Brampton	<i>Culex spp.</i>	164	1	6.10 †
	<i>Culex pipiens/restuans</i>	861	1	1.16
Caledon	(none) **			
Peel	<i>Culex spp.</i>	339	1	2.95 †
	<i>Culex pipiens/restuans</i>	2,061	2	0.97
	<i>Culex restuans</i>	584	1	1.71 †
All Species Total		18,036	4	

* the Minimum Infection Rate (MIR) is calculated as the number of positive batches of infected mosquitoes of a given species divided by the total number of mosquitoes of a given species that were tested for the presence of the virus, expressed per 1,000

** 14 different species from Caledon were tested (254 batches; 1,685 specimens), but none were WNV-positive

† MIRs based on numbers < 1000 are more likely to be unstable than those based on numbers ≥ 1000 (bolded)

The gravid trap was used on an experimental basis in limited numbers as it incorporated new design features from previous gravid traps. The initial findings indicate that these traps were labour intensive and did not capture sufficient numbers of gravid mosquitoes of the *Culex* species. The WNV team will conduct a short study in 2005 and compare the older style and new design gravid traps using a new recipe for the infusion media in both traps. The infusion media is designed to simulate the smell of stagnant water the gravid female mosquitoes prefer for egg laying.

Comparison of 2002, 2003 and 2004 Mosquito Trap Data

Although approximately 40 species of mosquitoes are found in Peel, only a few are important in the transmission of WNV. The vectors most responsible for the bird-mosquito amplification cycle in Peel are members of the genus *Culex*. The larviciding program targets *Culex* mosquitoes because of their role in the spread of the disease in the environment.

Table 9 shows numbers of female *Culex* mosquitoes collected and positive mosquito batches in Peel in 2002, 2003 and 2004. In 2002, it is estimated that *Culex* mosquitoes accounted for 30% of mosquitoes collected in Peel traps. A

total of 77% (98/128) of WNV positive batches in 2002 were attributed to *Culex* mosquitoes. In 2003, 13% of the mosquitoes collected in Peel traps were *Culex*: they represented 96% (23/24) of WNV positive mosquito batches. The percentage of *Culex* species captured in Peel traps dropped to 8% in 2004 and accounted for 100% (4/4) of the WNV positive mosquito batches.

Table 9: Comparison of Female Culex Mosquitoes by Year, Region of Peel, 2002-2004

Year	Female Mosquitoes Collected			Positive Mosquito Batches		
	Total Number	Number of Culex	% Culex	Total Number	Number of Culex	% Culex
2002	24,269	7,278	30%	128	98	77%
2003	41,212	5,326	13%	24	23	96%
2004	53,556	4,305	8%	4	4	100%

One of the factors reviewed at the weekly risk assessment by the WNV Working Group was vector abundance. *Culex pipiens*, *Culex restuans*, *Culex pipiens/restuans* and *Culex spp.* were incorporated into these measurements. Since the number of traps in the adult mosquito surveillance program increased each year, the method employed to determine vector abundance was to review the average *Culex* captured per trap event on a weekly basis (Figure 11). With the exception of weeks 24, 25, 26 and 32, vector abundance was higher each week in 2002 than during comparable weeks in 2003 or 2004. In 2002, during weeks 27-29 and 33-37, the trap averages were much higher than in the two years that followed. The average number of *Culex* per trap event from week 24-39 was 25.1 in 2002, 12.9 in 2003 and 8.6 in 2004.

A further analysis was undertaken of the relative abundance of *Culex* mosquitoes that were collected in the 13 traps that were located at the same sites during the same time period in each of 2002, 2003 and 2004 (Figure 12 and Table 10). As a proportion of total mosquitoes, *Culex* mosquitoes were lower every week in which mosquitoes were captured in 2004 than in 2002, with the exception of week 30 when the numbers of *Culex* captured were identical. In 2004, weeks 30, 31 and 32 were the only weeks in which the proportion of *Culex* mosquitoes exceeded 2003 trap counts.

Table 10 compares the top 12 mosquito species collected from the 13 common trap sites from 2002 to 2004. This shows the number of *Culex* that were ranked in the top 12 species count decreased from 2,644 in 2002 to 1,816 in 2003 to 1,121 in 2004 at the selected sites (note that *Culex* species were not included in the top 12 species in 2004).

2004 WEST NILE VIRUS IN THE REGION OF PEEL

The downward trend in *Culex* mosquito activity could be attributed to the 2003 and 2004 larviciding program which primarily directed at the reduction of *Culex* mosquitoes. Other factors that may have impacted *Culex* numbers were breeding site source reduction and weather conditions.

Figure 11: Proportion of *Culex* Species per Trap Event by Week, Region of Peel, Week 24-39, 2002 to 2004

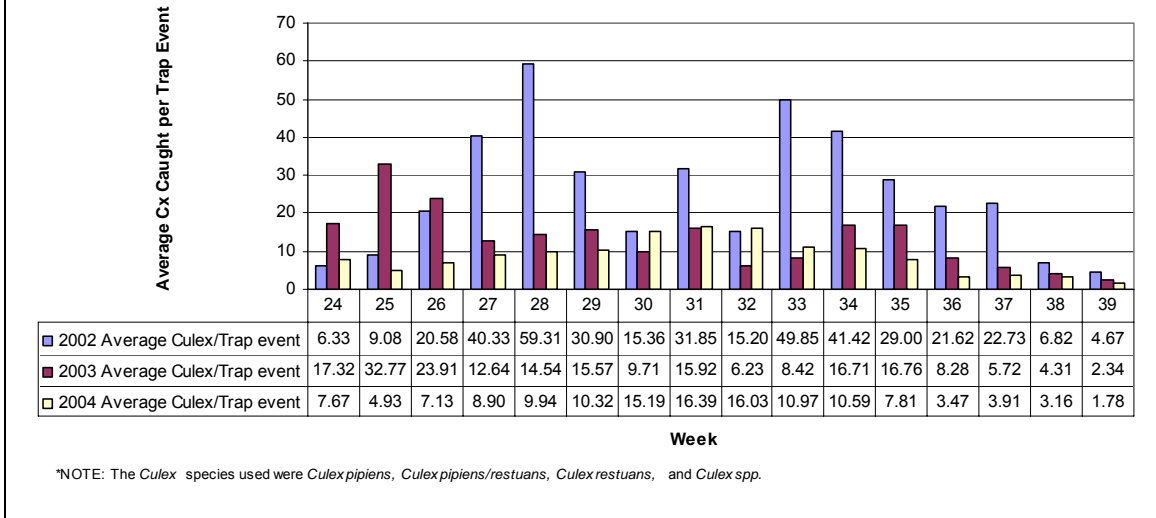
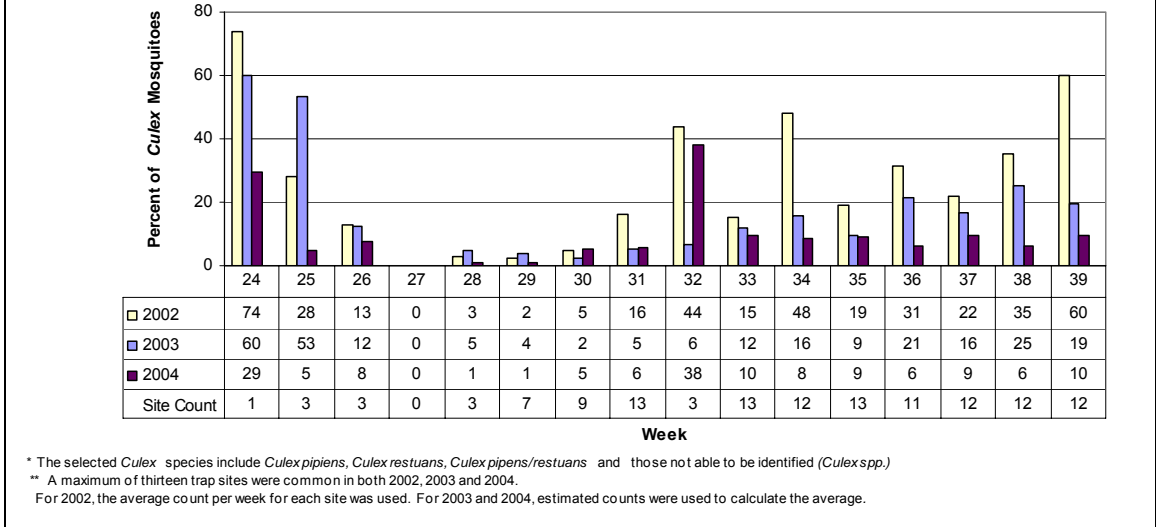


Figure 12: Proportion of *Culex* Species* to Total in Selected Sites Region of Peel, 2002, 2003 and 2004**



2004 WEST NILE VIRUS IN THE REGION OF PEEL

Table 10: Comparison of the Top 12 Mosquito Species Collected from Common Trap Sites and Time Periods, Region of Peel, 2002, 2003, and 2004

2002				2003				2004			
Rank 2002	Species (Actual)	Average Actual Number 2002*	Per Cent	Rank 2003	Species (Estimated)	Average Estimated Number 2003**	Per Cent	Rank 2004	Species (Estimated)	Average Estimated Number 2004**	Per Cent
1	<i>Coquillettidia perturbans</i>	4,147	38.8	1	<i>Cq. perturbans</i>	11,991	58.0	1	<i>Cq. perturbans</i>	14,608	66.4
2	<i>Aedes vexans</i>	2,301	21.5	2	<i>Ae. vexans</i>	3,646	17.6	2	<i>Ae. vexans vexans</i>	3,708	16.8
3	<i>Culex pipiens/restuans</i>	1,173	11.0	3	<i>Oc. canadensis</i>	1,423	6.9	3	<i>Oc. trivittatus</i>	756	3.4
4	<i>Culex spp.</i>	610	5.7	4	<i>Cx. pipiens/restuans</i>	1,284	6.2	4	<i>Cx. pipiens/restuans</i>	709	3.2
5	<i>Culex pipiens</i>	604	5.7	5	<i>Ae./Ochlerotatus spp.</i>	568	2.7	5	<i>Ae./Oc. Spp.</i>	529	2.4
6	<i>Aedes vexans/cantator</i>	445	4.2	6	<i>Oc. trivittatus</i>	319	1.5	6	<i>Oc. canadensis</i>	453	2.1
7	<i>Aedes/Ochlerotatus spp.</i>	319	3.0	7	<i>Oc. stimulans</i>	237	1.1	7	<i>Cx. pipiens</i>	286	1.3
8	<i>Culex restuans</i>	257	2.4	8	<i>Cx. restuans</i>	216	1.0	8	<i>Oc. triseriatus</i>	269	1.2
9	<i>Ochlerotatus excrucians</i>	177	1.7	9	<i>An. punctipennis</i>	195	0.9	9	<i>Oc. Broad-banded †</i>	187	0.8
10	<i>Ochlerotatus trivittatus</i>	175	1.6	10	<i>Cx. spp.</i>	169	0.8	10	<i>An. punctipennis</i>	147	0.7
11	<i>Ochlerotatus canadensis</i>	120	1.1	11	<i>Cx. pipiens</i>	147	0.7	11	<i>Cx. restuans</i>	126	0.6
12	<i>Ochlerotatus triseriatus</i>	93	0.9	12	<i>Ae. vexans/cantator</i>	98	0.5	12	<i>Oc. stimulans</i>	111	0.5

* 2002 data are based on average, actual numbers collected per trap event at 13 common trap sites

** 2003 and 2004 data are based on average, estimated numbers collected per trap event at the same trap sites

† not a true species

Summary

The adult mosquito surveillance program was used to monitor the seasonal abundance of mosquito populations and the detection of the virus.

Mosquitoes were collected weekly from mosquito traps at 30 permanent and two temporary locations throughout Peel. An estimated total of 53,556 female mosquitoes comprising 38 species were identified in Peel in 2004; however, as in previous years, only a small number were likely to be important in the transmission of the virus to humans. Three species from the genus *Culex* accounted for all four positive batches. Two positive batches were found in both Brampton and Mississauga. As in previous years, no positive mosquito batches were found in Caledon.

In 2004, the MIR calculations showed that the infection rates in the *Culex* species were lower when compared to 2003 and 2002 data.² The analysis of trapping results demonstrated lower proportions of *Culex* mosquitoes were collected than in previous years. This may be due to larval reduction measures that were undertaken in Peel which primarily targeted *Culex* mosquitoes.