

Introduction

West Virginia has always had a low incidence of human West Nile disease, with only six cases reported from 2002 to 2006. This is thought to be in part to the topography of the state which is not conducive to having large areas of standing water. We must however continue to monitor and test mosquito populations for arboviral diseases to prevent outbreaks of human disease. In spring 2007 we proposed a new way to collect large numbers of mosquitoes and to have those mosquitoes tested for arboviral disease.

Methods

Beginning 26 June 2007, three student interns and the public health entomologist initiated mosquito surveillance by setting an array of traps in areas known to breed mosquitoes. These locations would include sewage lagoons, sewage treatment plants for municipal areas and package treatment plants for mobile home parks or housing developments. The array of traps were set on Monday and collections were made from the trap daily from Tuesday to Friday (4 collections). The traps were taken down on Friday and reset in the same location the following Monday. All mosquitoes from a given location were pooled together for virus testing daily.

Trap locations were chosen in areas with considerable West Nile virus (WNV) activity. Parkersburg between 2002 and 2004 had twenty dead birds that were positive for WNV. However, most communities around the Parkersburg area have since been placed on the municipal water system, thus eliminating good trapping locations. Only one trapping site was located in the Parkersburg area: a package treatment plant serving a nursing home on Dupont Avenue (**Photo 1**).

The second choice for trap locations was located south of Parkersburg. The Cottageville area of Jackson County is plagued with many failing sewage systems and several WNV positive dead birds have been found in the area since 2002. Four trapping areas were located in or around the Cottageville area. Two sets of traps were located adjacent to sewage lagoons. One lagoon served a large mobile home park, (**Photo2**) and the other lagoon provided sewage treatment for the county fair grounds (**Photo 3**). A third set of traps was located at a sewage treatment package plant that serves a small mobile home park (**Photo 4**). This location is near the two sites which have sewage lagoons. The fourth set of traps was located at the Cottageville Public Service District waste water treatment facility (**Photo 5**).

In addition to the trap locations in Wood and Jackson counties, two additional trapping sites were identified in Raleigh County. Both sites were sewage lagoons associated with large mobile home parks. One trap site was located in Fairdale, WV and the other was in Shady Spring, WV on opposite sides of the county.

Trapping Method

Mosquitoes were trapped using CDC gravid traps (**Photo 6**) powered by a 6-volt rechargeable battery. The trap consists of a small plastic bin, a fan motor suspended above the bin and a catch bag. Approximately one-half gallon of mosquito “brew” is poured into the plastic bin. The brew is made monthly at the lab and consists of water, brewers yeast, lactalbumin powder and dried chicken manure. Female mosquitoes seeking foul water oviposition sites are attracted by

the brew and are pulled into the collection bag via the fan motor. Catch bags were replaced daily at each site. A handheld GPS receiver (Garmin etrex) recorded the location of each trapping site.

Specimen Handling

All mosquitoes collected daily were returned to the lab in the nets in which they were collected. All collecting nets were then placed in a minus 80 Celsius freezer to the kill the mosquitoes. After all mosquitoes were dead, all samples from a single trapping area were placed in a large six inch Petri dish. Mosquitoes were sorted into two groups: *Culex* and non-*Culex* mosquitoes. All non-*Culex* mosquitoes were discarded or saved; to be used as teaching specimens. All *Culex* mosquitoes (regardless of species) were pooled into groups containing 25 to 100 mosquitoes (dependant on the number of mosquitoes collected). The pooled groups of mosquitoes were placed in 2 ml Sarstedt micro tubes with two copper BBs. A buffer solution was added to the micro tubes and then the tubes were placed on a mixer mill for four minutes. With the addition of the buffer solution and the action of the copper BBs, the mosquitoes are ground into slurry on the mixer mill. The resulting material was centrifuged and extracted. Using RTPCR the sample was analyzed for WNV and Saint Louis encephalitis.

Results and Discussion

Between June 26 and Oct 4, a total of 24,434 testable mosquitoes were collected at the seven sites. A total of 420 pools of mosquitoes were submitted for virus testing, of these 36 pools were positive for WNV. Early in the season mosquitoes were pooled into groups of 25 mosquitoes, however as numbers began to increase dramatically that number was increased to 50 mosquitoes and then ultimately 100 mosquitoes per pool. The largest number of mosquitoes was collected in July (**Chart 1**). The majority of mosquitoes were *Culex restuans* and *Culex pipiens*. Both species over winter as gravid mosquitoes which emerge in May and begin egg laying. Larvae of both species have been collected in West Virginia by WVDHHR in late May. Air and water temperatures in July would be conducive to rapid development of mosquitoes.

In Wood County at the Parkersburg location (**Chart 2**) two traps were placed around the package plant at the Love and Care Assisted Living home. The traps yielded a total of 1,632 mosquitoes over the season. A total of 43 pools were submitted for virus testing, with 5 positive pools identified. The first positive pool was collected on 11 July 07.

Three traps were placed around the fairgrounds lagoon in Jackson County (**Chart 3**). These traps collected a total of 4,695 mosquitoes over the collecting season. The mosquitoes were placed in 85 pools and six of those pools were positive for WNV. The local health department asked the County Commission to treat the lagoon with *Bti*, however it never was treated. Four positive pools were identified during the week of 17 July, the week prior to the start of the county fair. An additional pool was collected on 25 July during fair week.

Two traps were placed at the mobile home park lagoon (**Chart 4**) and collected a total of 3,521 mosquitoes. Fifty four pools of mosquitoes were submitted for

testing, with 3 pools being found positive. The local health department suggested to the owners of the property that the lagoon be treated with *Bti*. The first positive pool was collected on 31 July, two weeks after the first positive fair ground positive.

A single trap was placed at the small sewage treatment package plant located in the other mobile home park (**Chart 5**). Only one trap was utilized due to the small size of the facility. A total of 2,043 mosquitoes were collected for the season. Forty two pools were submitted for virus testing, yielding 2 positive pools. The first positive pool from this location on 15 August. This was a small processing plant and never produced large numbers of mosquitoes.

Additionally two traps were located at the Cottageville Public Service District treatment facility (**Chart 6**). The traps collected 7,355 mosquitoes, which were separated into 122 pools. A total of six pools were positive for WNV. The first positive pool was collected on 20 July, with positive pools being collected through September.

In Raleigh County three traps were placed around the lagoon in Fairdale (**Chart 7**) and collected a total of 4,303 mosquitoes. A total of fifty two polls yielded eight positive WNV pools. The first positive pool was found on 26 July and positive pools were found through September.

Three traps were also place at the Gladeview location (**Chart 8**) and collected a total of 796 mosquitoes. This lagoon was aerated by a fountain head spraying water into the air for most of the summer, to control the growth of algae. The constant movement of water likely kept the numbers of mosquitoes low. The mosquitoes were divided into 21 pools. A total of six pools were positive for WNV. The first positive pool was found during first week of August and positive pools were found into September.

Conclusions:

By concentrating our mosquito surveillance around sewage treatment facilities we were able to collect and process large numbers of mosquitoes for arbovirus testing. These facilities are often found near population centers of people and finding positive mosquitoes can give us a heads-up on locations of possible human cases. These sites also could be easily treated with larvacides to control mosquito numbers and possible disease outbreaks. The one limitation to this surveillance is that the summer interns leave in mid-August to return to their classes. This leaves only the public health entomologist to conduct surveillance. At this point surveillance at each site is sporadic as the entomologist must rotate among the various sites. Overall we did find the results were very promising and plan to expand the process to additional counties in 2008.



Photo 1: Package Treatment Facility at Love and Care Assisted Living, Parkersburg WV



Photo 2: Cottageville Public Service District, Cottageville WV



Photo 3: Sewage Lagoon at Jackson County Fair Grounds, Cottageville WV



Photo 4: Mobile Home Park Sewage Lagoon, Cottageville WV



Photo 5: Mobile Home Park Package Treatment Facility, Cottageville WV



Photo 6: CDC Gravid Trap

Culex Mosquito Surveillance for WNV, West Virginia 2007

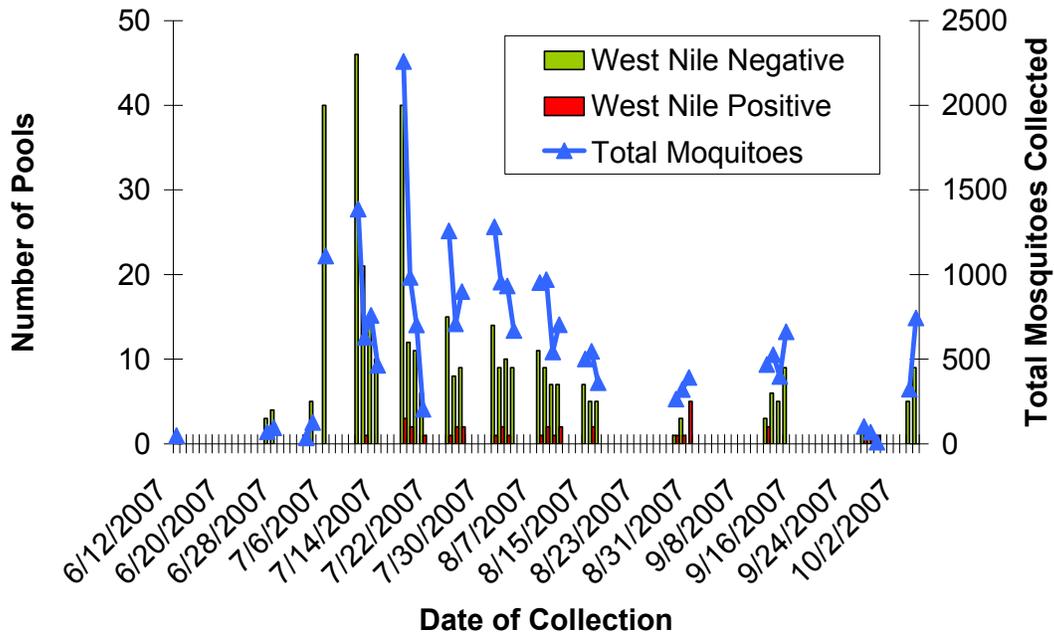


Chart 1: Culex Mosquito Surveillance for WNV Statewide in West Virginia

Culex Mosquito Surveillance for WNV, Love and Care Assisted Living Sewage Package Treatment Plant, Parkersburg, West Virginia 2007

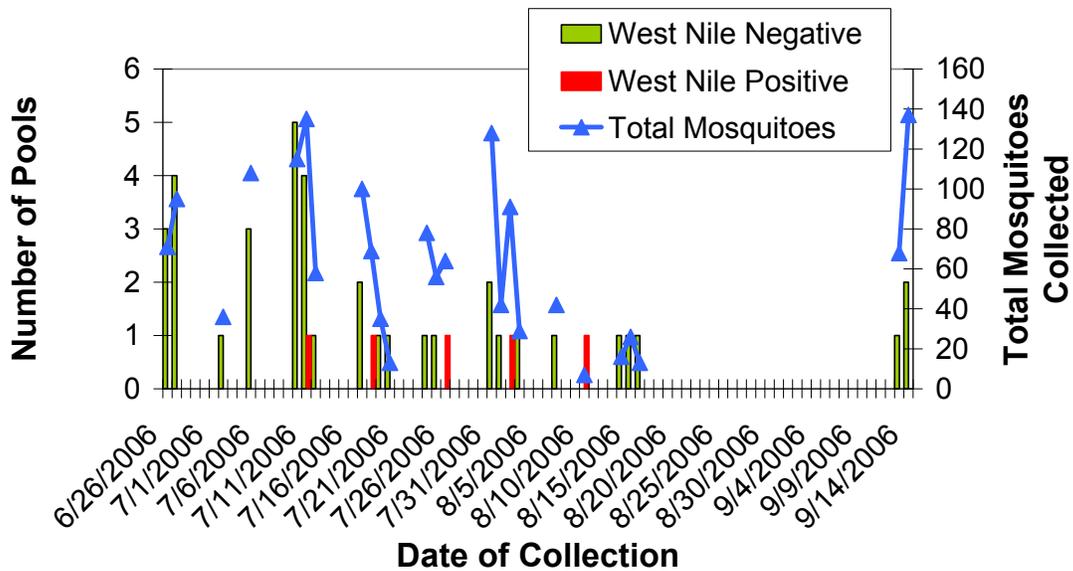


Chart 2: Culex Mosquito Surveillance for WNV, Love and Care Assisted Living Sewage Package Treatment Plant, Parkersburg, West Virginia 2007

**Culex Mosquito Surveillance for WNV Surveillance, Jackson County
Fairgrounds Lagoon, Cottageville, WV 2007**

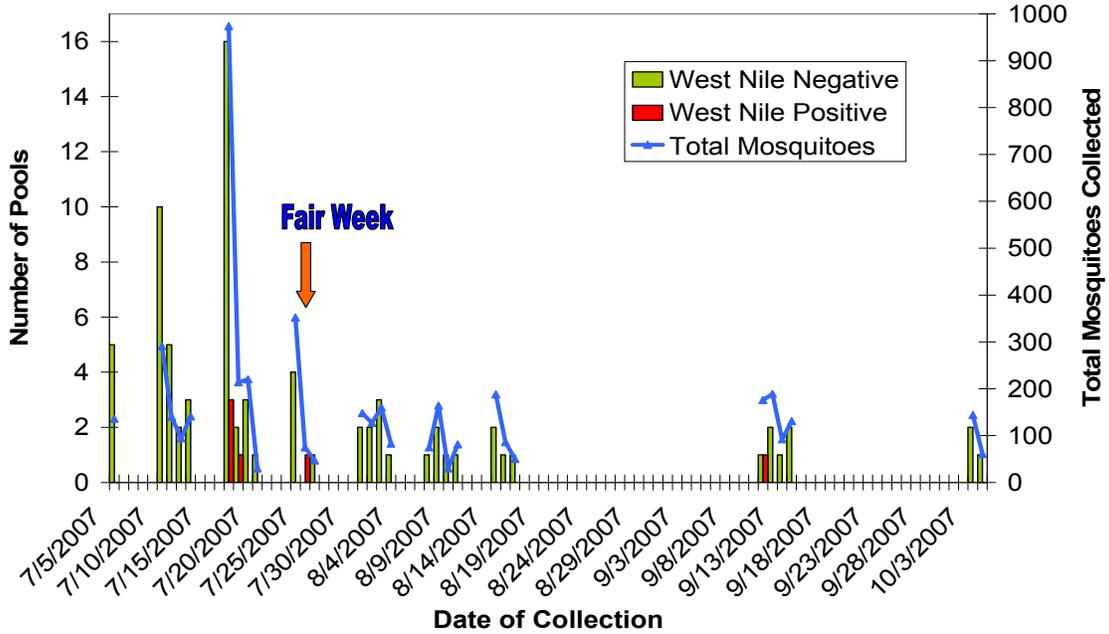


Chart 3: Culex Mosquito Surveillance for WNV Surveillance, Jackson County Fairgrounds Lagoon, Cottageville, WV

**Culex Mosquito Surveillance for WNV, Mobile Home Park
Sewage Lagoon, Cottageville, WV 2007**

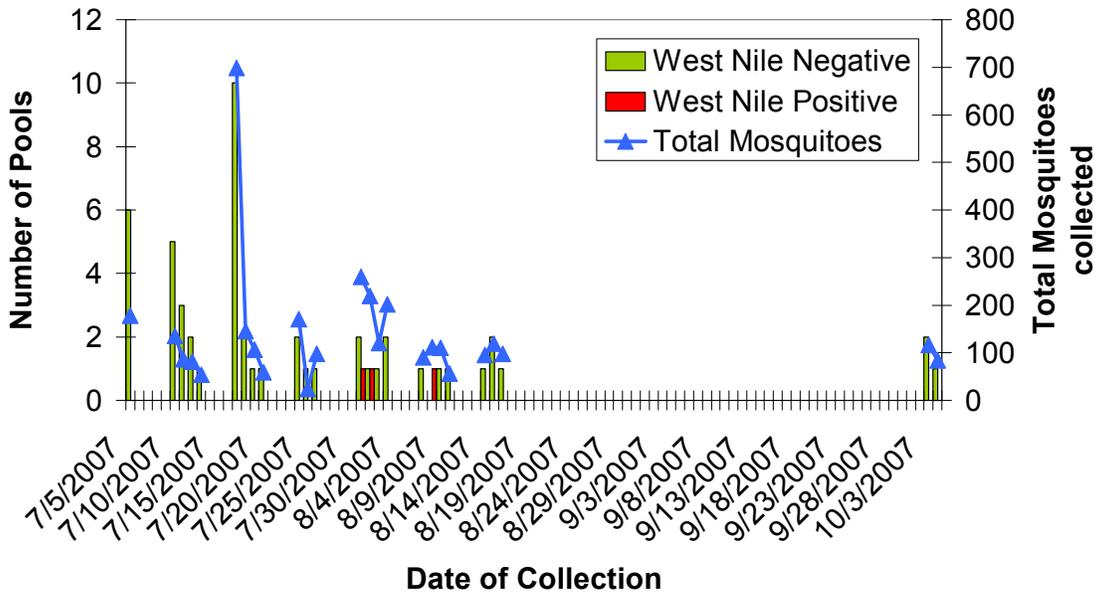


Chart 4: Culex Mosquito Surveillance for WNV, Mobile Home Park Sewage Lagoon, Cottageville, WV

Culex Mosquito Surveillance, Cottageville Mobile Home Package Plant, Cottageville, West Virginia, 2007

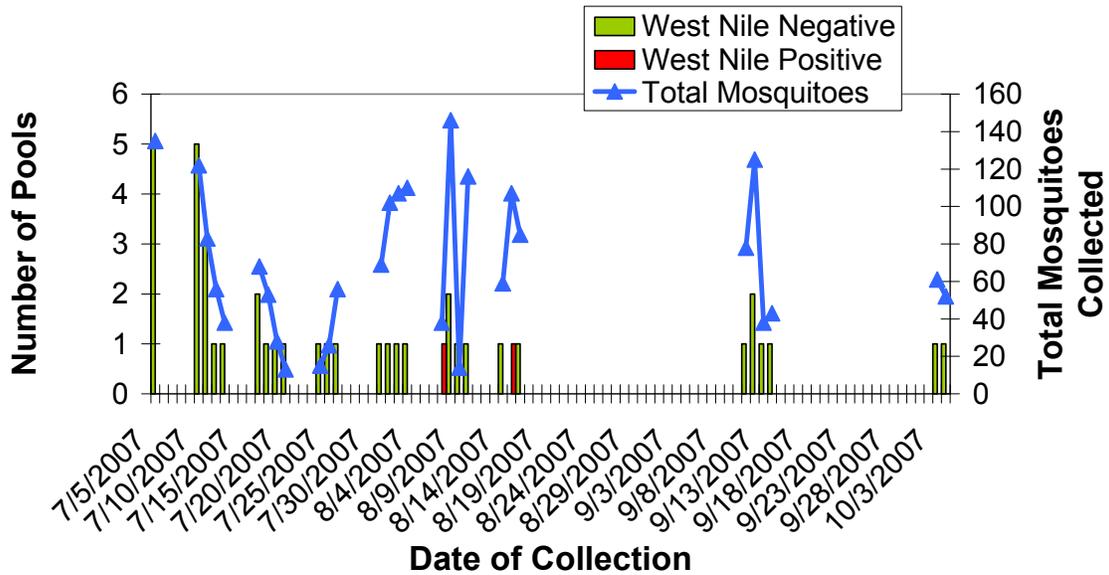


Chart 5: Culex Mosquito Surveillance, Cottageville Mobile Home Package Plant, Cottageville, West Virginia

Culex Mosquito Surveillance for WNV, Cottageville Municipal Sewage, Cottageville, WV 2007

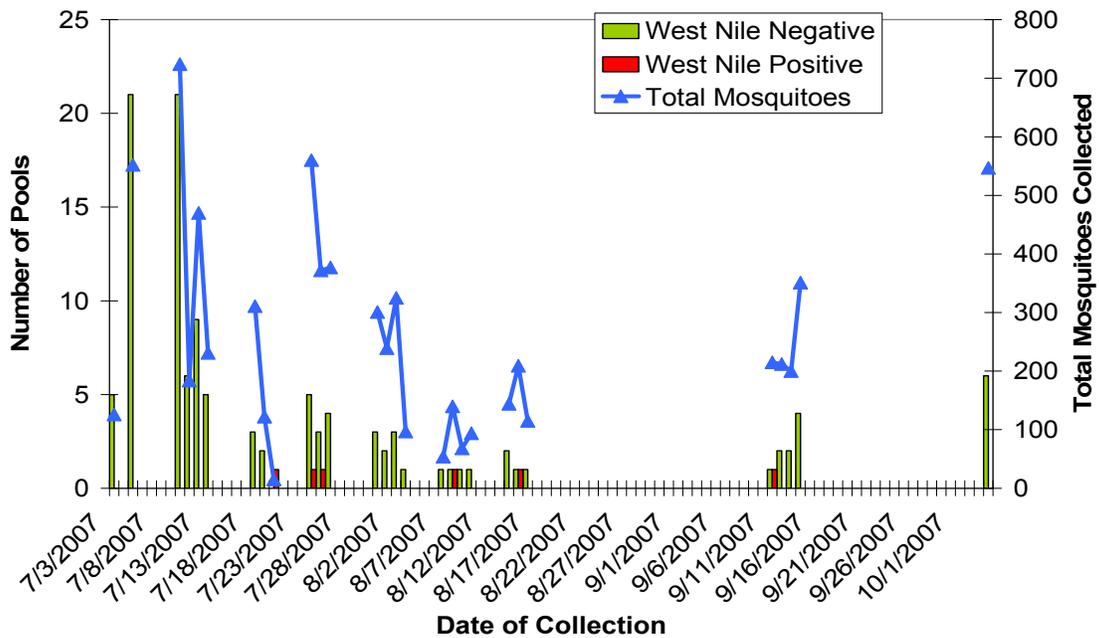


Chart 6: Culex Mosquito Surveillance for WNV, Cottageville Municipal Sewage, Cottageville, WV

Culex Mosquito Surveillance for WNV, GA Mobile Home Park Sewage Lagoon, Fairdale, WV 2007

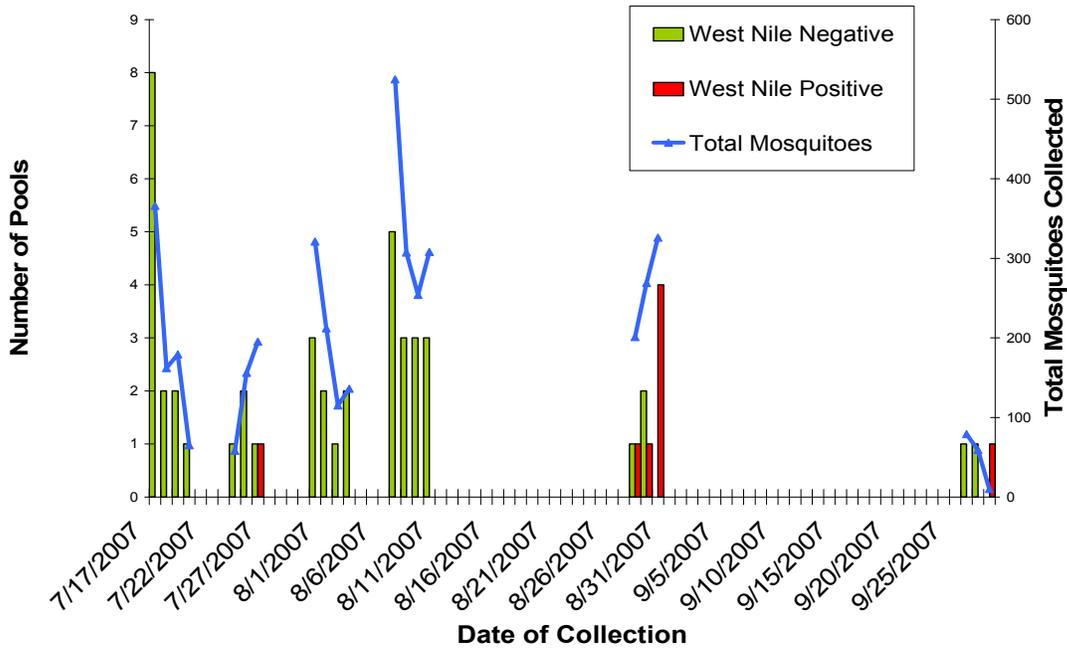


Chart 7: Culex Mosquito Surveillance for WNV, GA Mobile Home Park Sewage Lagoon, Fairdale, WV

Culex Mosquito Surveillance for WNV, Gladeview Sewage Lagoon, Shadyspring, WV 2007

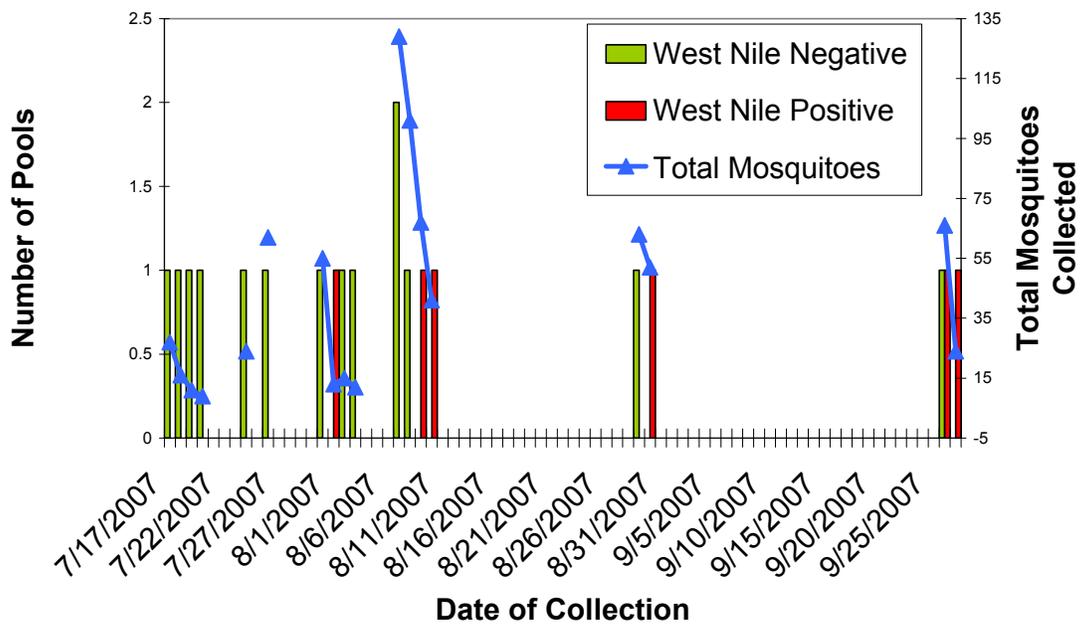


Chart 8: Culex Mosquito Surveillance for WNV, Gladeview Sewage Lagoon, Shadyspring WV