ANNUAL REPORT

TO THE

NEW JERSEY STATE MOSQUITO CONTROL COMMISSION

"THE SURVEILLANCE OF VECTOR-BORNE ARBOVIRUSES IN NEW JERSEY"

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Submitted by:
Lisa Reed, Scott Crans, Dina Fonseca, Marc Slaff and Randy Gaugler
Center for Vector Biology
180 Jones Avenue
New Brunswick, NJ 08901
Tel 732/932-9565
E-mail *lreed@rci.rutgers.edu*

RUTGERS
New Jersey Agricultural Experiment Station

Introduction

The NJ State Mosquito Control Commission (SMCC) has monitored potential vectors of mosquito-borne encephalitis in New Jersey since 1975 with a vector surveillance program designed to keep health related agencies aware of the potential for human involvement. Eastern equine encephalitis (EEE) was the original target for investigation because of its impact on coastal resorts in the southern portion of the state. West Nile virus (WNV) was added to the program in 2000 following an outbreak in New York City the previous year. County mosquito control personnel were recruited to collect and process specimens. This program functions as a cooperative effort that includes the NJ Department of Environmental Protection, the NJ Department of Health, the NJ Agricultural Experiment Station at Rutgers and the 21 county mosquito control agencies in the state. The goal is a disease surveillance effort that provides mosquito control with information to target vector populations for the prevention of human disease. This report documents the results of virus surveillance efforts during the 2007 encephalitis season.

Methodology of EEE Surveillance

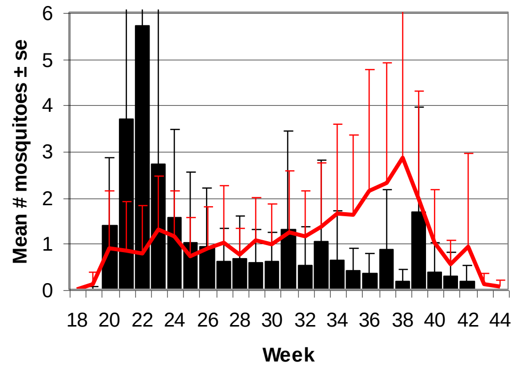
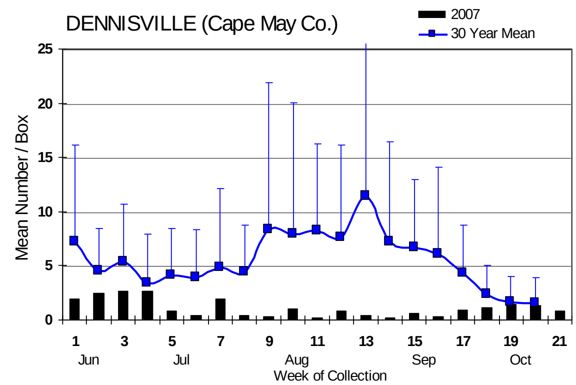
The mosquito, *Culiseta melanura*, is monitored from late May to mid-October as the primary indicator of EEE virus in southern New Jersey. This bird feeding mosquito usually does not bite mammals but can be used to monitor virus levels in local bird populations as the season progresses. Weekly collections of *Cs. melanura* are made from resting boxes at permanent study sites by a team of field staff from Rutgers. The mosquitoes are frozen on dry ice at the collection site and transported to Headlee Research Labs at Rutgers for further processing. The frozen specimens are sorted on a chill table to maintain the cold chain and are identified to species, pooled by stage of blood meal digestion and submitted weekly to the PHEL facility in Trenton for virus tests. Positive pools are detected by Taqman RT-PCR. Information from the investigation is summarized and distributed weekly to mosquito control and public health agencies in New Jersey and the Northeast. The resting box collection sites for 2007 included: Turkey Swamp in Monmouth Co., Green Bank in Burlington Co., Corbin City in Atlantic Co., Dennisville in Cape May Co., Waterford in Camden Co., and, Centerton in Salem Co.

Results of EEE Surveillance in 2007

The 2007 mosquito season began with higher than normal levels of *Culiseta melanura* showing up in the Statewide Surveillance light traps of the Pinelands, suggesting the potential of disseminated virus activity later on in the year. However, resting box populations in the Vector Surveillance program only modestly reflected that large first generation (Figure 1) due to the fact that the Vector Surveillance program began (in Week 24) after the initial spring emergence detected by the Statewide Program (week 19). Populations from both types of traps dropped during the second half of the season in comparison to historical data, indicating that there were fewer individuals that comprised the second generation, This generation is responsible for the large increase or amplification of virus in avian hosts during the second half of the season when horse and human cases usually develop. In 2006, virus was detected at only one site and appeared to be poorly disseminated in southern New Jersey. EEE was detected later in New Jersey than in neighboring states. This year, virus detection also occurred earlier in some states to the north (by about 6 weeks in Massachusetts and two weeks in New

Hampshire). No detection occurred in Pennsylvania and Connecticut first detected EEE the week following New Jersey.

Figure 1. Populations of *Culiseta melanura* in two types of traps in southern New Jersey during 2007.

Light trap (Statewide) surveillance in the Pinelands, 2007	Resting box (Vector) surveillance in Dennisville, 2007
	

Eastern equine encephalitis virus was first detected at the Green Bank site, on August 8th, for the second consecutive year. The Mullica River drainage, site for this monitoring area, has traditionally been a primary focus for early season EEE activity. The second site for confirmed activity was at Centerton, suggesting that dissemination had occurred. Further infection detected at four other monitoring sites suggested that virus activity was widespread throughout southern New Jersey.

Table 1. Total number of *Culiseta melanura* tested for EEE by site in 2007, together with positives and earliest isolation dates.

Site Name	Coastal or Inland	Total Pools	Total Mosquitoes	Positive pools	MFIR	Earliest Date
Corbin City	Coastal	50	298		0.000	
Dennisville	Coastal	89	1414	3	2.122	2-Oct
Green Bank	Coastal	73	743	3	4.038	**8-Aug**
Centerton	Inland	77	836	1	1.196	17-Sep
Turkey Swamp	Inland	94	1616	2	1.238	2-Oct
Waterford	Inland	65	1011	1	0.989	3-Oct
Statewide		**448**	**5918**	**10**	**1.690**	**8-Aug**

Four additional pools used for West Nile virus sampling detected EEE virus in *Culiseta melanura*. These occurred in Tabernacle, Burlington County (3 pools, earliest Sep. 25th) and in Millville, Cumberland County (1 pool, Aug 27th).

Cape May County Department of Mosquito Control reported that four chickens from two sentinel flocks turned positive for EEE. One flock was located in the southern portion of the county while the other was in the northern portion. The last positive chicken seroconverted on the 25th of October.

As with last year, no bridge vectors were found positive with EEE. Table 2 lists all species tested for EEE.

Table 2. Total non-*Cs. melanura* species tested for EEE. No positives occurred among potential bridge vectors.

Species	Total pools	Total mosquitoes
Aedes albopictus	7	24
Aedes canadensis canadensis	1	3
Aedes cantator	1	12
Aedes japonicus	8	12
Aedes sollicitans	4	10
Aedes taeniorhynchus	1	4
Aedes triseriatus	2	4
Aedes vexans	24	233
Anopheles bradleyi	4	15
Anopheles crucians	8	29
Anopheles punctipennis	6	27
Anopheles quadrimaculatus	12	42
Anopheles walkeri	1	1
Coquillettidia perturbans	7	21
Culex erraticus	8	44
Culex pipiens	11	176
Culex restuans	2	7
Culex salinarius	4	4
Culex sp.	14	387
Culex territans	2	55
Culiseta inornata	1	1
Psorophora ciliata	1	4
Psorophora columbiae	2	11
Psorophora ferox	1	1
Uranotaenia sapphirina	6	34
Statewide	**138**	**1161**

Horse and Human Involvement with EEE

Veterinary activity was limited to a single confirmed horse case reported in Atlantic County. This was a 6-year old gelding whose onset of symptoms began early October and euthanized 5 October. This horse had been vaccinated in April. The American Association of Equine Practice suggests that horses living in an endemic area for EEE should receive two vaccinations: one spring and one early summer. This horse appears to have only received the spring vaccination.

No human cases occurred.

Methodology of WNV Surveillance

New Jersey's WNV surveillance program in 2007 relies on significant county initiative to conduct meaningful surveillance within their county borders. Counties have various approaches to monitoring West Nile virus activity, ranging from focussing on the enzootic vector, *Culex pipiens* (primarily through the submission of Mixed *Culex* pools) to the submission of a wide range of potential bridge vectors.

The Rutgers program used gravid traps and CO_2 baited traps to collect mosquitoes from areas where human or equine cases required special surveillance investigations. The Rutgers program also conducted WNV surveillance activities for counties that requested assistance.

Results of WNV Surveillance in 2007

During the 2007 mosquito season, a total of 127,356 specimens were tested in 6,708 pools. Results from the surveillance effort produced 345 WNV positive pools, and increase over the previous year. All of New Jersey's 21 county mosquito control agencies participated in the state program during 2007. Table 2 indicates species results from county and Rutgers effort in mosquito collection. The majority of positive pools came from Culex species, either mixed pools or species-identified, with Culex pipiens showing the highest degree of infection at 6.31 mosquitoes/1000 of the three mixed species. Culex restuans was the second most infected species, with an MFIR value of 2.43, and Culex salinarius the third at 1.74. Since the mixed *Culex* pool had an MFIR value greater than *Culex restuans*, it may be that *Culex pipiens* contributed significantly to the mixed pools, raising the MFIR value. This assumes, however, that MFIR values for each species remains static throughout the state and is not a dynamic process.

Table 3. Mosquitoes tested for West Nile in New Jersey during 2007.

Species	Total pools	Total mosquitoes	Positive pools	MFIR
Aedes abserratus	1	2		
Aedes albopictus	926	9642	4	0.41
Aedes atlanticus	4	6		
Aedes atropalpus	8	11		
Aedes canadensis canadensis	53	1212		
Aedes cantator	17	69		
Aedes cinereus	7	25		
Aedes grossbecki	1	1		
Aedes japonicus	499	2338	2	0.86
Aedes sollicitans	41	510		
Aedes sticticus	11	273		
Aedes stimulans	1	3		
Aedes taeniorhynchus	13	118		
Aedes thibaulti	3	4		
Aedes triseriatus	209	553		
Aedes trivittatus	15	94		
Aedes vexans	228	1863		
Anopheles barberi	7	7		

Anopheles bradleyi	92	1712		
Anopheles crucians	11	33		
Anopheles punctipennis	182	819		
Anopheles quadrimaculatus	184	2768		
Anopheles walkeri	8	13		
Coquillettidia perturbans	101	1551	1	0.64
Culex erraticus	113	1468		
Culex pipiens	797	10622	67	6.31
Culex restuans	338	3295	8	2.43
Culex salinarius	208	4606	8	1.74
Culex spp.	2447	82282	255	3.10
Culex territans	76	527		
Culiseta inornata	2	3		
Orthopodomyia signifera	5	8		
Psorophora ciliata	3	7		
Psorophora columbiae	27	179		
Psorophora ferox	31	582		
Psorophora howardii	4	4		
Uranotaenia sapphirina	35	146		
Statewide	**6708**	**127356**	**345**	**2.71**

Table 3 also lists infection rates in potential bridge vectors. In 2004, WNV was limited to bird feeding mosquitoes with no evidence of transfer to any of the bridge vector species that tested positive in prior years. In 2005, single positives were obtained from 4 known mammal biters including: *Aedes vexans*, *Anopheles quadrimaculatus*, *Aedes japonicus* and *Ae. triseriatus*. Last year, infectious bridge vectors were limited to *Ae. albopictus and Ae. japonicus.* This year, *Aedes albopictus* and *Ae. japonicus* were again found positive for WNV. These two species are highly competent vectors as well as aggressive mammalian biters. Although their MFIR values are low, their potential involvement as bridge vectors should not be disregarded. *Coquillettidia perturbans* is a moquito that is an inefficient vector for WNV.

County patterns differed, likely based upon many factors. The degree of urbanization is a significant feature of West Nile virus activity. But, in addition to this, the methods used by the counties to detect WNV activity likely played a significant role. Focusing on submitting mixed *Culex* pools to the PHEL (Public Health Epidemiology Laboratories, where samples were tested) increased the chances of detection. Trapping bridge vectors, those most likely to bite humans and horses, reduced the overall MFIR value for the county. Undoubtedly, the amount of support given to a county agency could potentially limit the amount of trapping a county could do. The total number of mosquitoes caught by a county were correlated with the number of positive pools (Spearman's r = 0.84, n=19, $p<0.05$), indicating that the greater number of mosquitoes submitted by a county, the more likely the county was to find positive mosquitoes. Thus effort was a factor in the results that a county had.

Table 4 indicates the cumulative infection rates in each county by the end of the 2007 season.

County	Total pools	Total mosquitoes	Positive pools	MFIR
Atlantic	286	3129	8	2.56

Bergen	387	19086	105	5.50
Burlington	449	3830		
Camden	695	6572	16	2.43
Cape May	368	5080	1	0.20
Cumberland	200	4256	3	0.70
Essex	133	1433	4	2.79
Gloucester	265	3038	2	0.66
Hudson	439	13980	70	5.01
Hunterdon	276	11751	10	0.85
Mercer	793	9562	51	5.33
Middlesex	348	8498	23	2.71
Monmouth	757	5913	9	1.52
Morris	173	3416	5	1.46
Ocean	348	6398	10	1.56
Passaic	103	2656	11	4.14
Salem	305	3803		
Somerset	263	4732	4	0.85
Sussex	264	6591	1	0.15
Union	169	2705	12	4.44
Warren	258	8345	1	0.12
Grand Total	**7279**	**134774**	**346**	**2.57**

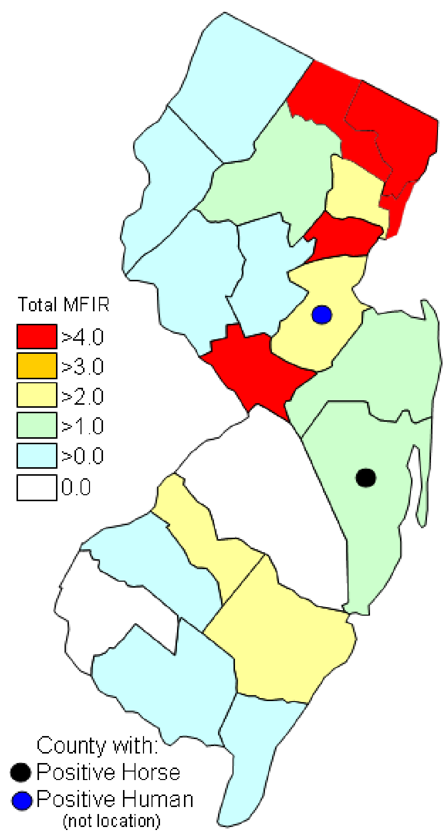
Horse and Human Involvement

During 2007, there was one equine case that occurred in Ocean County with onset of symptoms on September 26th. This 21 year old mare was not vaccinated against West Nile and was euthanized the following day. One human case occurred in Middlesex County. This was an 88 year old male had developed onset of symptoms on 29 August. Acquisition of the virus is unclear as the man also apparently owned farm property in Monmouth County.

Conclusions

EEE virus was detected in *Culiseta melanura* and disseminated throughout southern New Jersey despite low numbers of the primary enzootic vector. No detection was found in other bird feeding mosquitoes nor potential bridge vectors. All of New Jersey's 21 county mosquito control agencies collected and processed specimens for the WNV surveillance initiative. WNV was largely limited to bird feeding mosquitoes in 2007. Positive mosquitoes involved beyond the amplification cycle included 4 pools of *Ae. albopictus*, 2 pools of *Ae. japonicus*, 8 pools of *Culex salinarius* (some of which may have fed on birds) and 1 pool of *Coquillettidia perturbans*.

Figure 2. Cumulative WNV activity by the end of the mosquito season.



West Nile Risk Assessment

In order to produce predictability about how likely disease may be transmitted to humans, we are developing a risk assessment model. This model is used to predict human case occurrence with a variety of factors, including climatic and biotic. Human cases were defined by the NJDHSS.

Creating a Model

Model creation involves two major step: the creation of a predictable model and the fit of this model to a geospatial program. This report focuses on the first step. Human cases from 2000-2005 were put into a multiple regression model, predicted by a number of variables. These variable were lagged by two weeks in order to produce conditions that would increase the probability of an infected mosquito biting a human and subsequent transmission of West Nile. Variables included spring rainfall, temperature, precipitation, degree days, mosquito abundance, and MFIR values of various mosquito groups. Variables were calculated as week averages. Initially, a discriminant analysis was performed to see if there was enough variability to separate human WNV case from no case weeks (Table 5). About 84% of the cases were correctly classified into the predicted group membership (weeks with either cases or no cases) by the variables used.

Table 5. Classification table to find predictive variability in dataset. 1 = weeks with cases, 2 = weeks with no cases during the mosquito season.

Classification Results [b,c]

			Predicted Group Membership		
		disnum	1.00	2.00	Total
Original	Count	1.00	62	9	71
		2.00	6	19	25
	%	1.00	87.3	12.7	100.0
		2.00	24.0	76.0	100.0
Cross-validated [a]	Count	1.00	62	9	71
		2.00	6	19	25
	%	1.00	87.3	12.7	100.0
		2.00	24.0	76.0	100.0

a. Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.

b. 84.4% of original grouped cases correctly classified.

c. 84.4% of cross-validated grouped cases correctly classified.

Because of its ease in the use of GIS applications, a linear regression was then performed to see if the same variables also predicted number of human cases (as it would be expected to given the general linear model that both analyses are based on). First examined were tolerance and VIF values were examined for multicollinearity. Multicollinearity can make a linear regression equation unstable and unpredictable. This can be reduced by eliminating the correlations among variable that are over 0.75. This was done when one of those highly correlated pairs was dropped. Regression analysis was recalculated and all collinearity was resolved. The significance of the equation increased and stabilized. The resulting equation included:

number of human cases = -10.925 (a constant)
+ 0.709 * (*Culex* MFIR two weeks prior)
+ 0.632 * (Spring Rainfall)
- 0.492 * (Cumulative Degree Days)
- 0.318 * (MFIR “other” feeders two weeks prior)
+ 0.328 * (Percent positive dead birds two weeks prior)

($F_{14,56}$=9.545, p<0.000, R^2 = 0.76; significance of all variables p<0.01, except positive dead birds, where p=0.048.)

Discriminant analysis was performed again to identify the degree of error through the production of a classification table. This table would indicate the degree of error through 1) prediction of human cases when none occurred and 2) prediction of no human cases when some occurred.

Table 6. Classification table to find predictive variability in dataset. 1 = weeks with cases, 2 = weeks with no cases during the mosquito season. (Number of weeks decreased as a result of the elimination of several variables from the previous step.)

Classification Results [b,c]

			Predicted Group Membership		
		disnum	1.00	2.00	Total
Original	Count	1.00	44	4	48
		2.00	4	20	24
	%	1.00	91.7	8.3	100.0
		2.00	16.7	83.3	100.0
Cross-validated [a]	Count	1.00	41	7	48
		2.00	7	17	24
	%	1.00	85.4	14.6	100.0
		2.00	29.2	70.8	100.0

a. Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.

b. 88.9% of original grouped cases correctly classified.

c. 80.6% of cross-validated grouped cases correctly classified.

Overall, the predictability of the general linear model improved.

Applying a GIS Layer

The next step in the risk assessment model is to take the current multiple regression equation and apply it to a spatial analysis using GIS. As this will involve taking a smaller landscape view (from statewide to county wide), it is expected that the model may change. Variables that were not critical in the past may become incorporated into the model while others that were predictive at the state level may be dropped.

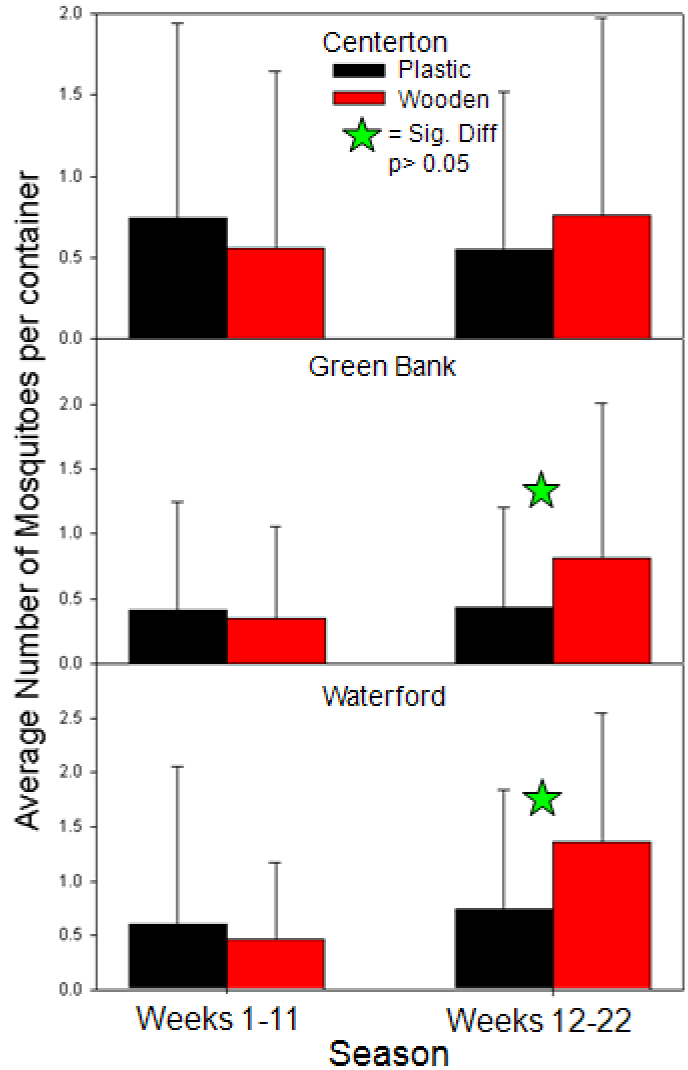
BG Trapping of *Aedes albopictus*

Standard surveillance traps in North America for adult *Aedes albopictus* (Skuse) (Diptera: Culicidae), an invasive biting pest with public health implications, are currently ineffective. We compared the efficacy of the BG-Sentinel trap (BGS) with and without lures (BG-lure, octenol, and carbon dioxide), the Centers for Disease Control and Prevention light trap (CDC) with and without lures, and the gravid trap (GT) for *Ae. albopictus* collection in two urban sites in New Jersey, U.S. The BGS with or without lures collected more *Ae. albopictus* compared to other trap configurations and was more specific for *Ae. albopictus*. In Camden County, the BGS with lures collected three times more *Ae. albopictus* than the CDC (with carbon dioxide only) and five times more than the GT. In Mercer County, BGS with lures collected the most mosquitoes, with three times more Ae. albopictus than the CDC with all lures and fifty times more than the GT. The BGS collected more male *Ae. albopictus* than other traps in both counties, providing further population monitoring. The GT and BGS provided a relative measure of the enzootic activity of West Nile virus in Culex spp. and the potential epidemic activity of WNV in *Ae. albopictus*. The BGS provides effective chemical and visual cues for host-seeking *Ae. albopictus* and should be utilized as a part of existing surveillance programs targeting this mosquito.

Waste Containers in Comparison with Traditional Wooden Resting Boxes for Trapping *Culiseta melanura*

We compared the use of standard office heavy plastic containers in the efficacy of attracting rest-seeking *Culiseta melanura* (Coquillett) (Diptera: Culicidae) mosquitoes with the wood resting boxes. Plastic containers were modified by placing a 2.5 inch access hole on the side of the containers and covered the holes with screening. At three EEE sites each (Centerton in Salem County, Green Bank in Burlington County and Waterford in Camden County), 10 modified plastic containers were placed in a regular pattern through historical wood box set-up. A 3-way ANOVA (unequal variance model used throughout analysis) using box (wood or plastic), site (Centerton, Green Bank and Waterford) and seasonality (1st half and 2nd half) revealed significant interactions among both seasonality and box type ($F_{1,11}$=22.50, $p < 0.001$) as well as seasonality and site ($F_{1,11}$=6.97, $p = 0.001$). Therefore box type was analyzed separately for each season at each site. Wood resting boxes attracted significantly more mosquitoes than did plastic containers at Green Bank and Waterford during the critical second half of the season (Green Bank: t_{227}=-4.19, p=0.000. Waterford: t_{328}=-4.36, p<0.000) (Fig. 3). Both wood and plastic containers performed equally at all three sites during the first half of the season and at Centerton in the second half of the season. Recommendations: In recent years, mosquito populations at critical vector surveillance sites have decreased in New Jersey. Fortunately, positive mosquito pools have continued to be detected. But the threshold where positive pools become undetectable due to low population levels is unknown. Because wood boxes attracted more mosquitoes at two out of the three surveillance sites during the critical transmission portion of the EEE cycle, our recommendation is to continue with the use of the wood box. Possible reasons for the better performance of wood boxes are higher retention of moisture in wood boxes or higher retention of TEA in plastic containers.

Figure 3. ANOVA results for waste container comparisons to traditional wooden resting boxes seasonally at three sites. Wooden resting boxes caught significantly more mosquitoes during the second half of the season at two of the three sites.



Presentations

2007 Northeastern Mosquito Control Association, Plymouth Massachusetts: *Vector and Mosquito Population Surveillance in New Jersey, 2007.*

2007 New Jersey Mosquito Control Association, Atlantic City, New Jersey: *Vector and Mosquito Population Surveillance in New Jersey, 2007.*

Publications

NJMCA proceedings: New Jersey Vector Surveillance Program, 2007 (*in prep*)