

## Seasonal abundance of *Culex nigripalpus* Theobald and *Culex salinarius* Coquillett in north Florida, USA

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Received 5 October 2001; Accepted 15 January 2002

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**ABSTRACT:** North Florida is a transition zone between widespread *Culex nigripalpus* populations to the south and focal *Culex salinarius* populations to the north. *Culex nigripalpus* is a vector of St. Louis encephalitis (SLE) and eastern equine encephalitis (EEE) viruses in south Florida, while *Cx. salinarius* is a suspected New World vector of West Nile (WN) virus. Abundant vector populations are often a prerequisite for epidemic and epizootic transmission of arboviruses. Extensive SLE transmission has never been reported from north Florida, but sporadic WN transmission was reported there during the summer of 2001. The disparate flavivirus transmission patterns observed in north and south Florida may be due, in part, to the local geographical and seasonal distribution of *Culex* vectors. Here we report that from May 1991 to April 1994, *Cx. salinarius* was most commonly observed during the winter and spring in northeast Florida (Duval County), whereas *Cx. nigripalpus* was most abundant during the summer and autumn. An unusually mild spring in 1991 allowed *Cx. nigripalpus* to reproduce early in the year, resulting in a summer population that emerged more than 8 wks earlier than in 1992 and 1993. The 1991 *Cx. nigripalpus* population persisted through October, when SLE transmission was detected by sentinel chickens. Transmission of SLE was not detected in Duval County during 1992 or 1993. These data indicate that mild winter and spring conditions in north Florida may favor increased abundance and survival of *Cx. nigripalpus* in a region where this species is normally not abundant. A seasonal shift in population structure may increase the transmission risk of arboviruses for which *Cx. nigripalpus* is a competent vector, including SLE, WN, and EEE. **Journal of Vector Ecology 27(1): 155-162. 2002.**

**Keyword Index:** *Culex nigripalpus*, *Culex salinarius*, St. Louis encephalitis virus, West Nile virus, arboviral transmission.

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### INTRODUCTION

During the autumn of 1990, a widespread St. Louis encephalitis (SLE) virus epidemic was reported in central Florida with a total of 226 human cases and 11 deaths (Day and Stark 2000). This epidemic was remarkably similar to an outbreak of SLE reported in central Florida during the summer and autumn of 1977 (Nelson et al. 1983). During both years, Florida mosquito control

programs intensified their vector control efforts with additional larvicide and adulticide applications from the ground and the air (Day 1991, Harden et al. 1991). Mosquito-borne viral epidemics such as these are disruptive and expensive to local and regional economies. A thorough understanding of the biology, ecology, and behavior of mosquito vectors is necessary to help predict, identify, and manage these epidemics. The northern counties of Florida mark a transition zone

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between *Culex nigripalpus* Theobald and *Cx. salinarius* Coquillett populations (Provost 1969). *Culex nigripalpus* is abundant in the southern half of peninsular Florida where it is the epizootic and epidemic vector of SLE (Chamberlain et al. 1964, Shroyer 1991). Indian River County (IRC), located in east central Florida, marks the transition latitude between a southern subtropical climate and a northern temperate climate. Summer field studies from IRC during which 20 min ground aspirator collections were made at a *Culex* daytime resting site frequently yielded more than 5,000 *Cx. nigripalpus* females (Day and Curtis 1989). Major *Cx. nigripalpus* population concentrations are found from central Florida south throughout the New World subtropics (Mitchell et al. 1980, Day and Curtis 1993).

*Culex salinarius* is a temperate species that is locally abundant throughout eastern North America (King et al. 1960). Its blood feeding habits are similar to those of *Cx. nigripalpus*. Both species feed opportunistically on avian and mammalian hosts, leading to the speculation that *Cx. salinarius* may be a potential SLE vector in the southeastern USA, especially Florida (Edman 1974, Mitchell et al. 1980). In addition, because *Cx. salinarius* is abundant and active during the winter months in mild climates, it may provide a mechanism for viral overwintering. Virus may persist through the winter months in continuous, low-level transmission cycles involving infected *Cx. salinarius*. The seasonal distributions of *Cx. salinarius* and *Cx. nigripalpus* populations in central Florida appear to be inversely related; *Cx. nigripalpus* is most abundant during the summer and fall while *Cx. salinarius* dominates during the winter and spring (O'Meara and Evans 1983).

A widespread SLE epidemic was reported in Florida from January through December 1990 (Day 2001). During this epidemic, the two northern-most human SLE cases along east coast Florida were reported in Duval County. The dates of onset for these cases were October 16 and 18, 1990 (Day and Stark 2000), suggesting early October infection dates. Sentinel chicken flocks maintained for arboviral surveillance in Duval County from 1978 through 1996 demonstrated that SLE transmission there was extremely rare (Day and Stark 1996). A high rate (16.3%) of SLE transmission was observed in Duval County sentinel chickens during October 1990. This SLE transmission to sentinel chickens likely occurred coincidentally with the human transmission in Duval County.

Due to the 1990 transmission of SLE virus to humans and sentinel chickens in Duval County, the Navy Disease Vector Ecology and Control Center (NDVECC) located at the Jacksonville Naval Air Station, in cooperation with the Florida Department of Health and Rehabilitative

Services Laboratory (HRS), initiated a 3-yr mosquito surveillance program. This program was designed to provide information about the seasonal distribution and abundance of *Culex* mosquitoes in an attempt to understand the SLE transmission observed in Duval County during 1990.

## MATERIALS AND METHODS

### Study sites and mosquito surveillance

Five mosquito collection sites were selected within the boundaries of the Jacksonville Naval Air Station (NASJAX) (30° 09' N, 81° 42' W) located along the banks of the St. Johns River in the southwest corner of Duval County, FL, USA. All trap sites were located in vegetated sylvan habitats that supported nighttime mosquito flight and served as daytime *Culex* resting sites (Day and Curtis 1993). BioQuip Products, Inc. (Gardena, CA), EVS mosquito traps were used to collect mosquitoes at each site. Traps were placed at all sites once a week at approximately 1500 hr. Traps were retrieved the following morning at about 0730 hr. Each trap held approximately 1 kg of dry ice and was suspended 1.5 m above the ground. Collection bags from each trap were placed separately into a standard household freezer upon return to the NDVECC. The following day, frozen mosquitoes from each collection bag were transferred to labeled holding boxes which were frozen at -70 °C until mosquitoes were separated by sex and identified to species. The study began on May 9, 1991 and ended on April 29, 1994.

Because mosquito populations change over time, catch variances were not independent of their means. Therefore, a logarithmic transformation was done for mosquito catches from each collection and a monthly mean was calculated for each mosquito species over the course of the study ( $n = 36$  mo). The most abundant *Culex* species captured during the study were *Cx. nigripalpus* and *Cx. salinarius*, and they became the focus of this report. The cumulative frequency distributions of the *Cx. nigripalpus* and *Cx. salinarius* abundance curves were compared using the Kolmogorov-Smirnov test for goodness of fit for testing the shape and location of a sample distribution (SYSTAT, Evanston, IL). In addition, the *Cx. nigripalpus* and *Cx. salinarius* collections were divided into winter (January-June) and summer (July-December) cohorts for which intraspecific comparisons were made with the Kolmogorov-Smirnov test for goodness of fit.

### Meteorological conditions

Daily weather data including rainfall, relative humidity and maximum, minimum and mean

temperatures were recorded by the National Weather Service Office at Jacksonville International Airport (30° 30' N, 81° 42' W), 25 km north of the study sites. Weather data were compiled by the Fleet Numerical Meteorology and Oceanography Detachment (37 Battery Ave., Rm. 34, Asheville, NC) and provided to us in electronic format.

Fifty year (1943 to 1992) mean monthly rainfall and mean monthly average temperature data sets were calculated from data reported in *Climatological Data* for the Jacksonville U.S. Weather Bureau recording station (30° 25' N, 81° 30' W). Monthly deviations from normal for rainfall and average temperature were calculated by subtracting observed values from the long term mean monthly values.

### Virus surveillance

Five to 8 sentinel chicken flocks, each containing 6 adult birds, were maintained in Duval County to monitor arboviral transmission between 1990 and 1993 according to the protocols outlined by Day and Stark (1996). At least 3.0 ml of blood were collected from a wing vein of each chicken biweekly by personnel from the Jacksonville Mosquito Control Division. Blood samples were maintained on wet ice in the field and returned to the laboratory where they were refrigerated overnight at 4° C. Serum was separated from blood cells by centrifugation the next morning. Sera were shipped to the Florida Department of Health and Rehabilitative Services, Tampa Branch Laboratory, where they were tested for hemagglutination-inhibition antibody to SLE and eastern equine encephalitis (EEE) viruses according to the methods described by Day and Stark (1996).

All arboviral-positive chickens were replaced with an adult bird that had been maintained in a mosquito-proof holding facility and had an arboviral-negative baseline blood sample drawn and tested prior to placement of the bird in the field. The number of chickens exposed each year was calculated by multiplying the maximum number of flocks used that year by 6 chickens per flock and adding the number of arboviral-positive chickens that were replaced in all of the flocks during that year. Arboviral-positive sera were confirmed with plaque reduction neutralization tests as described by Day et al. (1996).

## RESULTS

### Mosquito surveillance

*Culex nigripalpus* and *Cx. salinarius* accounted for 81% of the 1991, 84% of the 1992, and 66% of the 1993 mosquito collections made in Duval County. *Culex nigripalpus* were most abundant from late summer

through early winter and population ebbs were recorded from mid-winter through early summer. *Culex salinarius* abundance remained more constant during the study period, with lowest trap catches recorded in August of each year (Figure 1). The cumulative frequency distribution comparison of the *Cx. nigripalpus* and *Cx. salinarius* abundance curves was not significantly different ( $P = 0.102$ , Kolmogorov-Smirnov two-sample test) for the May 1991 through April 1994 sampling period. However, when within species winter (January through June) and summer (July through December) distribution comparisons were made, the abundance curves of both species were significantly different ( $P < 0.000$ , Kolmogorov-Smirnov two sample test), indicating distinct seasonal distribution patterns for these two *Culex* species in north Florida.

### Virus surveillance

Sentinel chickens maintained in Duval County for arboviral surveillance between 1978 and 2000 indicated SLE transmission during only 3 of these years: 1990, 1991, and 2000. In 1990, 10 of 40 (25%) sentinel chickens seroconverted to SLE virus, while in 1991, 4 of 74 (5.4%) seroconverted (Table 1). The SLE transmission observed in Duval County sentinel chickens during the autumn and early winter of 1991 was likely the result of a *Cx. nigripalpus* population that persisted from May through October of 1991, as well as the continued presence of a north Florida SLE transmission focus that was initiated as part of the 1990 epidemic.

### Meteorological conditions

The first half of 1991 was unusually wet in Duval County. Cumulative rainfall for January-July 1991 was 81.03 cm above the expected 50 yr (1943-1992) mean for that 7 mo period (Figure 2, top). By comparison, during the same 7 mo period in 1992 and 1993, the cumulative rainfall amounts were 9.58 cm above normal and 8.36 cm below normal, respectively. The observed monthly average temperatures for 9 mo of 1991 were at least 1.5 °C above the expected 50 yr monthly averages. This was particularly evident for January-September. Overall, 1991 was milder and wetter than the other test years (Figure 2, bottom).

## DISCUSSION

*Culex nigripalpus* and *Cx. salinarius* seasonal population trends observed in Duval County, Florida were consistent with those reported from other regions of the state (O'Meara and Evans 1983, O'Meara et al. 1989). *Culex nigripalpus* is a subtropical species that

Table 1. Duval County sentinel chicken surveillance: 1990 through 1993.

Year	Month	Number of sites monitored	No. chickens exposed during test month	No. HI-positive		% HI-positive	
				SLE	EEE	SLE	EEE
1990	June	5	30	0	0	0	0
	July	5	30	0	0	0	0
	August	5	30	0	0	0	0
	September	5	30	0	0	0	0
	October	6	43	7	0	16.3	0
	November	6	38	2	0	5.3	0
	December	6	37	1	0	2.7	0
<b>No. of chickens exposed in 1990:</b>			<b>40</b>				
1991	June	6	44	0	8	0	18.2
	July	6	43	0	7	0	16.3
	August	8	48	0	0	0	0
	September	8	50	1	1	2.0	2.0
	October	8	51	2	1	3.9	2.0
	November	7	42	0	0	0	0
	December	8	50	1	1	2.0	2.0
<b>No. of chickens exposed in 1991:</b>			<b>74</b>				
1992	June	8	48	0	0	0	0
	July	8	49	0	1	0	2.0
	August	8	50	0	2	0	4.0
	September	8	48	0	0	0	0
	October	8	48	0	0	0	0
	November	8	48	0	0	0	0
	December	None	0	--	--	--	--
<b>No. of chickens exposed in 1992:</b>			<b>51</b>				
1993	June	8	48	0	0	0	0
	July	8	48	0	0	0	0
	August	7	44	0	2	0	4.5
	September	7	42	0	0	0	0
	October	7	43	0	1	0	2.3
	November	7	42	0	0	0	0
	December	None	0	--	--	--	--
<b>No. of chickens exposed in 1993:</b>			<b>51</b>				

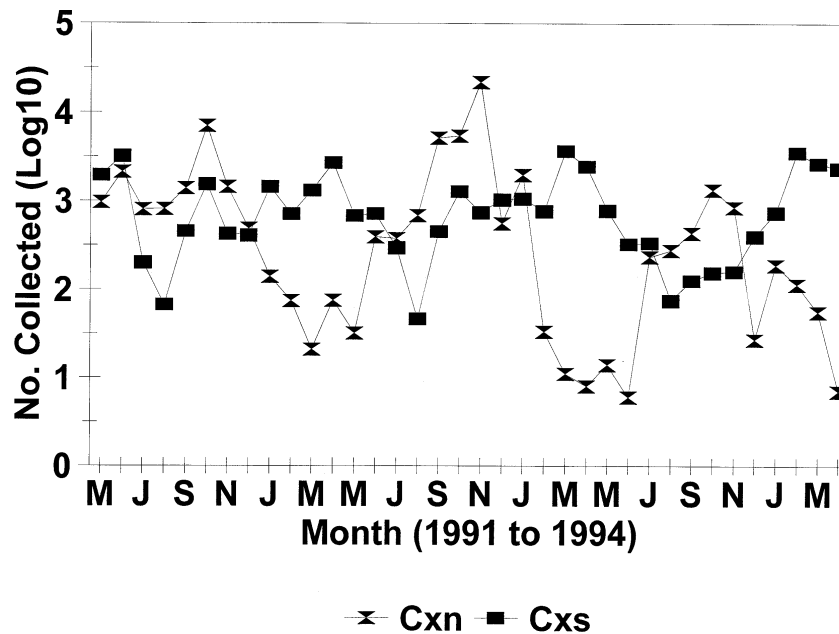


Figure 1. Cumulative monthly means (transformed to  $\text{Log}_{10}$ ) for *Culex salinarius* and *Culex nigripalpus* females captured at 5 study sites from May 1991 through April 1994 at the Jacksonville Florida Naval Air Station.

thrives under the hot, humid conditions commonly reported in central and south Florida during the summer and autumn months. *Culex salinarius*, by contrast, is a species that is most abundant in Florida during cooler months (O'Meara et al. 1989). The abundance of *Cx. nigripalpus* in northeast Florida, as in other parts of the state, is closely linked to rainfall (Day and Curtis 1989, 1994). Above normal monthly temperatures and rainfall were reported in Duval County during 1991. These conditions facilitated an early season buildup of *Cx. nigripalpus* by providing superior flight conditions and abundant oviposition sites. As rainfall-produced surface pools accumulated, an accompanying expansion of oviposition sites occurred resulting in a sustained, elevated *Cx. nigripalpus* population. Extended periods of drought in 1992 and 1993 resulted in a reduction in the number of *Cx. nigripalpus* females.

By contrast, *Cx. salinarius* was the predominant species captured from late winter to early summer. This species was most abundant between March and May of each study year. As temperatures increased, the number of *Cx. salinarius* captured declined, reaching lowest levels in August of each year.

The local abundance and vector competence of mosquito species involved in virus transmission, along with the availability of susceptible vertebrate amplification hosts are important epidemic precursors (Day 2001). St. Louis encephalitis and WN viruses are

transmitted primarily by *Culex* mosquitoes (Mitchell et al. 1980, Hayes 1988). The north Florida *Culex* species captured during our study that were most likely involved in SLE and WN virus transmission were *Cx. nigripalpus*, *Cx. salinarius*, *Cx. quinquefasciatus* Say, and *Cx. restuans* Theobald (Andreadis et al. 2001). Of these, only *Cx. nigripalpus* and *Cx. salinarius* were abundant at our trap sites. We collected 56,000 *Cx. nigripalpus*, 39,000 *Cx. salinarius*, 1,600 *Cx. quinquefasciatus*, and 100 *Cx. restuans* during the 36-mo study period. *Culex quinquefasciatus* and *Cx. restuans* were both rare during the July to September period when SLE and WN viruses are most commonly transmitted in Florida (Day and Curtis 1999).

Large numbers of human SLE or WN cases have not been reported from north Florida, possibly because important *Culex* vectors are not abundant during the right time of year. It is not unusual to collect 10,000 *Cx. nigripalpus* females during a single trap night in central Florida (Day and Curtis 1993). The lack of north Florida flavivirus epidemics may be because *Cx. nigripalpus* rarely attains high population levels there during the spring and summer months when viremic amplification hosts, such as nestling birds, are abundant (Monath and Tsai 1987, Day and Stark 1999). Likewise, *Cx. salinarius* females are abundant during the spring arboviral amplification phase (Day and Curtis 1999), but they do not survive to transmit virus later in the

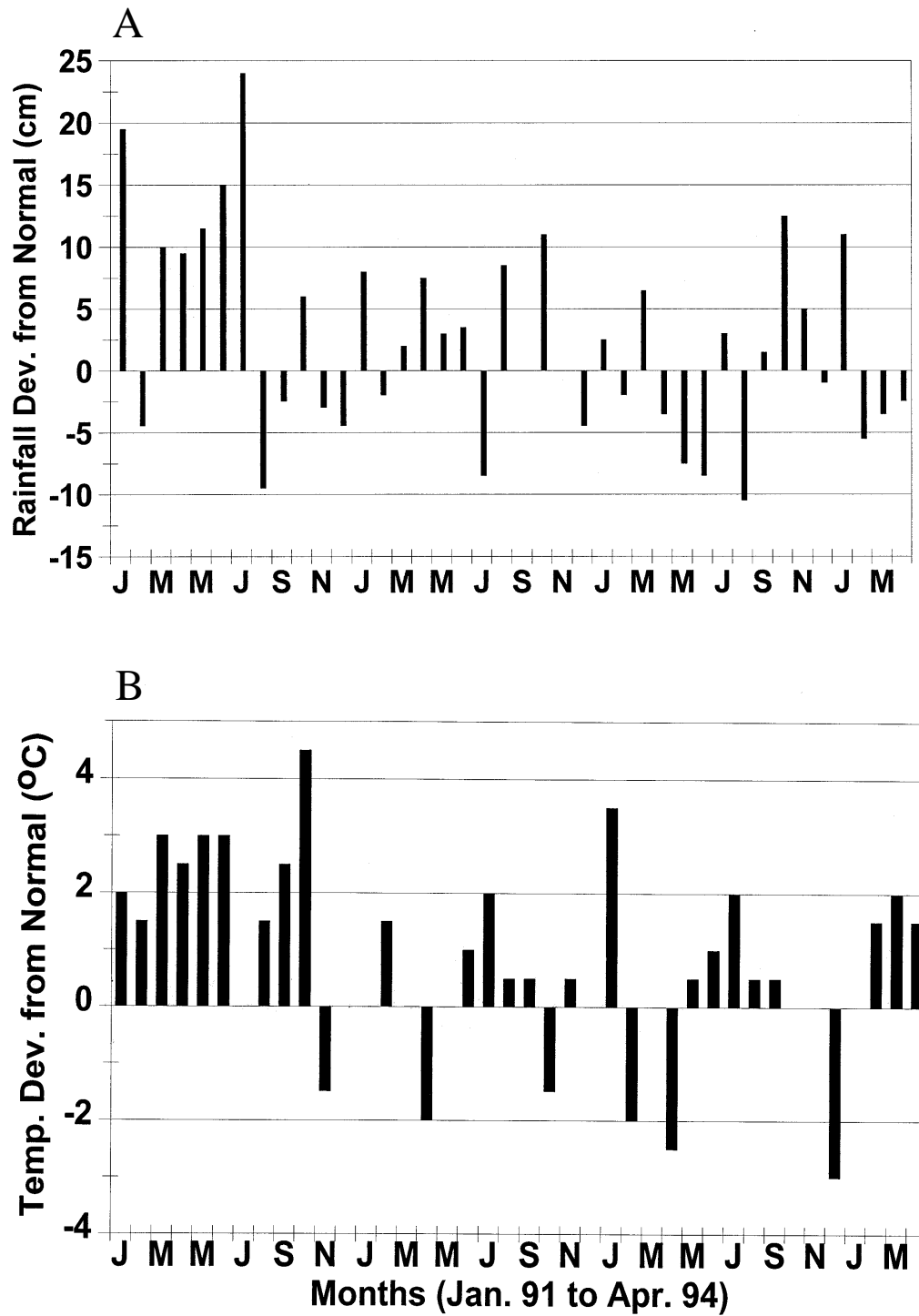


Figure 2. Jacksonville, Florida monthly rainfall (A) and average temperature (B) deviations from normal for January 1991 through April 1994. Observed monthly rainfall and average temperature values were subtracted from a 50 year mean, calculated from data recorded at the Jacksonville U.S. Weather Station between 1943-1992, to determine deviations from normal.

summer. However, when early season mild temperature and excessive rainfall pervade in north Florida, as in 1991, *Cx. nigripalpus* populations increase quickly (Day et al. 1990, Day and Curtis 1999). The SLE transmission observed in Duval County sentinel chickens during 1990 and 1991 was most likely transmitted by *Cx. nigripalpus* in a cycle similar to that observed in other parts of the state (Day 2001).

Transmission of EEE virus is frequently reported in north Florida, but is rarely observed in the central and southern regions of the state. Transmission of EEE virus to horses, humans, and sentinel chickens is generally reported during the spring and early summer months in contrast to SLE and WN viruses that are generally transmitted between July and November (Day and Stark 1996). Transmission of EEE in north Florida is more focal than that of SLE in central Florida. Elevated EEE transmission rates were observed in Duval County sentinel chickens during June and July of 1991 when 18.2 and 16.3%, respectively, of the sentinel chickens seroconverted to EEE virus (Table 1). This was during a period when *Cx. nigripalpus* and *Cx. salinarius* were both abundant (Figure 1). *Culex salinarius* abundance began to decline in early July 1991, suggesting an aging mosquito population during a period when EEE transmission was high. Either of these *Culex* species may be involved in early season EEE transmission in north Florida. The EEE virus has been isolated from both species in the southeastern USA (Scott and Weaver 1989).

The amplification of SLE virus between mosquitoes and wild birds occurs mainly during the avian breeding season in Florida (Day and Stark 1999). The spring to summer amplification of SLE virus essentially removes *Cx. salinarius* as a serious enzootic and epidemic SLE vector because amplification occurs during a period when this species is least abundant. This is not to say that *Cx. salinarius* is unimportant in the epidemiology of SLE and WN viruses in north Florida, and elsewhere in North America. It may serve as an overwintering vector by keeping low-level transmission active during periods when the major epizootic and epidemic vector is not abundant. However, the known distribution of SLE virus transmission cycles in Florida is coincident with that of *Cx. nigripalpus*, indicating that the presence of this mosquito species may be required for epidemic SLE transmission. A similar pattern may be observed in future years for WN virus as it becomes established in central Florida.

#### Acknowledgments

We thank John Mulrennan for his support and

interest which initiated this project. Preventive Medicine Technicians at NDVECC, especially Petty Officers John Roarty, Dave Wolfert, Paul Klimkowski, and John Callhan, were instrumental for the completion of this study. This research was supported by the Florida Agricultural Experiment Station and approved for publication as Journal Series No. R-08421.

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