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ANNUAL EMERGENCE PATTERNS OF *CULEX NIGRIPALPUS* FEMALES BEFORE, DURING AND AFTER A WIDESPREAD ST. LOUIS ENCEPHALITIS EPIDEMIC IN SOUTH FLORIDA[1]

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ABSTRACT. Resting *Culex nigripalpus* females were collected from the same site at least 3 times a week from January 1986 through December 1991. Newly emerged mosquitoes were used to monitor annual emergence patterns that were rainfall driven. Four of the years, 1986–89, preceded the 1990 St. Louis encephalitis (SLE) epidemic in south Florida. The pre-epidemic period averaged 4 emergences per year, with most occurring during July and August. The emergence patterns observed during the epidemic year were unusual in their early start in May and June and in the total number (11) observed. Adult mosquito emergences in 1991 were similar to those in 1990, with an early start and a total of 14 observed, yet no SLE transmission was recorded in Indian River County during 1991. A time series analysis of the 6-year data set showed a significant cross-correlation between the emergence of *Cx. nigripalpus* females and heavy (>50 mm) rains. A significant adult emergence occurred 5–8 days and 12–15 days following a heavy rainfall event.

INTRODUCTION

Culex nigripalpus Theobald is a subtropical mosquito, the local populations of which cycle with seasonal and daily rainfall (Day and Edman 1988). Adult abundance increases annually during the rainy season. Adults become rare during the dry season and during extended droughts. The nightly flight of this species is favored by high relative humidity (Dow and Gerrish 1970). Bloodfeeding (Day and Curtis 1989) and oviposition (Day et al. 1990) are significantly correlated with daily rainfall.

This species was the major vector of St. Louis encephalitis (SLE) virus during the 1962 Tampa Bay, FL, epidemic (Chamberlain et al. 1964, Dow et al. 1964). It was also incriminated as the major vector during the 1990 south Florida SLE epidemic (Shroyer 1991) and is clearly a mosquito of concern relative to its ability to infect humans with SLE virus in Florida.

Increased vector abundance can positively affect the transmission of arthropod-borne viruses (Olson et al. 1979). However, vector abundance, by itself, does not accurately predict epidemic risk. The spatial distribution of mosquitoes during periods of peak viral amplification and their resulting interactions with the virus and amplification hosts is as important as overall abundance. Likewise, the age of females within an existing population has a more direct impact on viral transmission than does the total number of biting mosquitoes. This is evidenced by the 1961 SLE epidemic that occurred in Tampa Bay, FL, from October through December. Mosquito control operations were discontinued throughout the Tampa Bay area in August, well before the beginning of the epidemic, because mosquito abundance, complaints of biting mosquitoes and rainfall were all well below normal (Waters et al. 1963). *Culex nigripalpus* abundance in the Tampa Bay area was low prior to the beginning of the 1961 epidemic. However, the mosquitoes present were old enough to have incubated the virus and to have become part of the human epidemic transmission cycle.

The dynamics of mosquito-borne encephalitis epidemics are complex and poorly understood. Three closely interrelated cycles—a vector cycle, a viral cycle and an amplification host cycle—must be synchronized before epidemic transmission begins (Day and Curtis 1993). Successful viral amplification depends, in part, on the synchronization of vector emergence with susceptible amplification host abundance early in the summer. If mosquito emergences occur too early or too late in the season, the virus will not be amplified.

This paper reports *Cx. nigripalpus* emergence in the years before, during and after an SLE epidemic in Florida and documents how viral transmission during epidemic years is partially linked to the emergence patterns of the main vector of SLE virus in Florida.

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MATERIALS AND METHODS

Mosquito collections: A portable ground aspirator (Day and Carlson 1985) was used to collect resting mosquitoes from a cabbage palm (*Sabal palmetto* (Wolter) Schultes and Schultes f.)–southern live oak (*Quercus virginiana* Miller) hammock 6.4 km SW of Vero Beach, Florida, at least 3 mornings each week. Daily collections were made from August through December during the 1990 SLE epidemic.

One 20-min collection was made approximately 2 h after sunrise along a track established within the hammock. Markers along the path standardized times by indicating the collector's desired position during each minute of the collection.

At the completion of each sampling period, the sealed aspirator bag was placed into a chilled cooler and returned to the laboratory where the mosquitoes were killed with cold or chloroform, identified by species, categorized according to sex and gonotrophic condition and counted.

The cuticles of newly emerged *Cx. nigripalpus* mosquitoes remain green for up to 30 h after emergence (Day, unpublished data). The neck membrane and ventral thorax are the final tissues to turn from green to brown. Any mosquito with a green neck membrane or lower thorax was recorded as newly emerged.

The day-to-day fluctuations in population trends of newly emerged mosquitoes were smoothed with a 5-day moving average. This analysis provided a clearer graphical representation of the annual and seasonal population trends and greatly enhanced the identification of individual emergence peaks.

Rainfall: Daily rainfall data were recorded at a site 5 km east of the mosquito collection site. We showed previously that rainfall totaling more than 50 mm within a 72-h period stimulated *Cx. nigripalpus* bloodfeeding (Day and Curtis 1989) and oviposition (Day et al. 1990). In the present study, these 50-mm rainfalls are referred to as epic rainfalls. All 3-day cumulative rainfalls equal to or greater than 50 mm are plotted for the years 1986–91 in the bottom half of Figs. 1 through 7. All rainfalls totaling less than 50 mm were deleted from the figures.

Time series analysis: A time series analysis was done to evaluate the relationship between epic rainfall events and the emergence of *Cx. nigripalpus* females. The cross-correlation analysis between the abundance of newly emerged females and rainfall was examined to determine time lag associations. The emergence data were smoothed using a 5-day moving average that removed short-term random noise and allowed the non-random, periodic components of the data to be identified. Rainfall data were modified as described above. These data sets were constructed for the years 1986–91 and cross-correlation comparisons were made using a time series analysis software package produced by Systat, Evanston, Illinois.

Measures of SLE transmission: Sentinel chicken flocks were maintained by personnel from the Indian River Mosquito Control District. Six flocks, with 6 birds each, were placed in the field by mid-June and removed by the end of December, unless viral transmission during the previous season warranted year-round surveillance. A 1.0 ml blood sample was drawn from each bird weekly during peak transmission periods (late August through mid-November) and twice a month for the rest of the year. The blood samples were centrifuged in the laboratory and the sera mailed to the Florida Department of Health and Rehabilitative Services (FDHRS), Tampa Branch Laboratory, for analysis of hemagglutination inhibition antibodies (HAI) to SLE and eastern equine encephalomyelitis viruses. Sera in which there was a 4-fold increase in HAI antibodies for 2 successive blood samples were considered seropositive.

The FDHRS Communicable Epidemiology Branch in Tallahassee is responsible for investigating and reporting suspected human arboviral cases in Florida. They tracked the human SLE cases throughout the 1990 epidemic and reported them in weekly updates. In addition, they investigate and report all confirmed and presumptive cases of arboviral infection during non-epidemic years.

RESULTS

Mosquito collections and rainfall: The smoothed abundance data for newly emerged *Cx. nigripalpus* females collected from 1986–91 are illustrated in the upper half of Figs. 1–6. The vertical axis of each figure is standardized to facilitate year-to-year comparisons. The epic rainfall events for each of the study years are illustrated in the lower half of the figures. Figure 7 illustrates the data set on a continuous time line to allow year-to-year comparisons of mosquito abundance and the timing of annual emergences.

Measures of SLE transmission: Transmission of SLE virus to sentinel chickens and humans in Indian River County (IRC) for 1986–91 is reported in Table 1. No EEE transmission was recorded in the county during the study period.

Evaluation of individual study years: Three epic rainfalls during mid-July and early August of 1986 resulted in 5 *Cx. nigripalpus* emergences before the end of August (Fig. 1). Newly emerged

Table 1. St. Louis encephalitis virus transmission to sentinel chickens and humans in Indian River County, Florida, 1986–91.

Year	No. sentinel chicken sero-conversions	Trans. months (to chickens)	Number of human SLE infections	Trans. months (to humans)
1986	3	September—2 November—1	0	—
1987	0	—	0	—
1988	0	—	0	—
1989	14	September—1 October—9 November—4	0	—
1990	99	June—3 July—36 August—25 September—32 October—3	19	June—0 July—2 August—11 September—4 October—2
1991	0	—	0	—

mosquitoes were more abundant in July of 1986 than during any other period of the 6-year data set. Three sentinel chicken seroconversions to SLE virus were recorded; 2 birds were infected in September and one was infected in November.

Culex nigripalpus females were not abundant and SLE transmission did not occur in IRC during 1987 or 1988 (Figs. 2 and 3). The 2-year lull in mosquito production and SLE transmission likely was caused by a severe drought during the

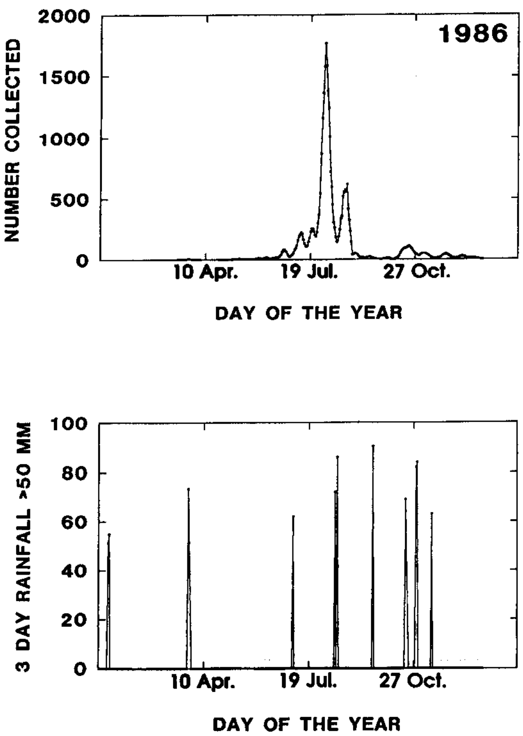

Fig. 1. Abundance of newly emerged *Culex nigripalpus* females and epic rainfall events in Indian River County, FL, during 1986.

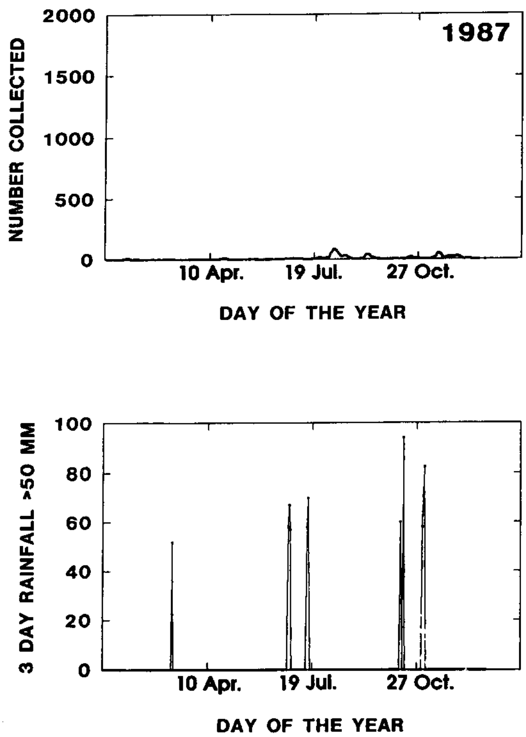

Fig. 2. Abundance of newly emerged *Culex nigripalpus* females and epic rainfall events in Indian River County, FL, during 1987.

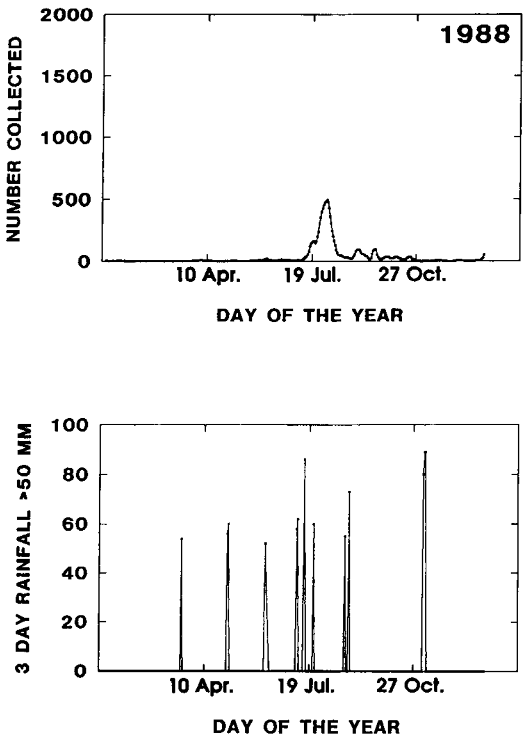

Fig. 3. Abundance of newly emerged *Culex nigripalpus* females and epic rainfall events in Indian River County, FL, during 1988.

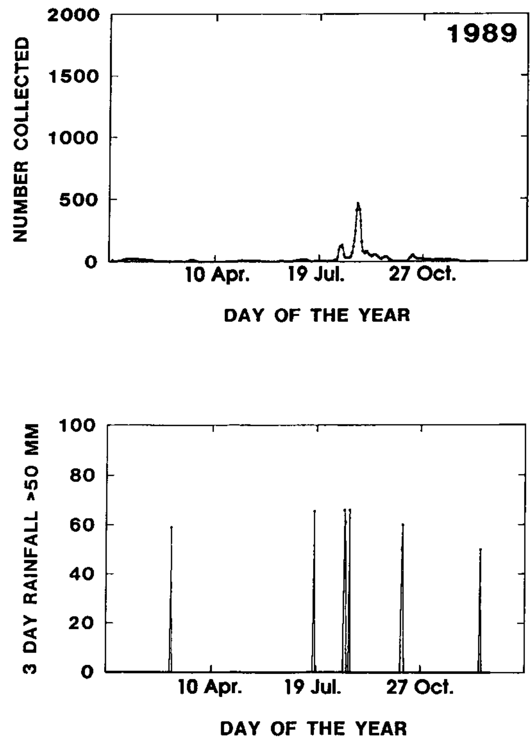

Fig. 4. Abundance of newly emerged *Culex nigripalpus* females and epic rainfall events during 1989, the year before a widespread St. Louis encephalitis epidemic in southern Florida.

spring and summer of 1987 when only 2 epic rainfalls were recorded between April and mid-October (Fig. 2).

In 1988, a cluster of 3 epic rainfalls in mid-July followed by 2 in mid-August resulted in 3 mosquito emergences from late July through September (Fig. 3). However, drought conditions from August through the end of the year once again curtailed viral transmission.

Four epic rainfalls in July, August and October of 1989 produced 3 detectable mosquito emergences: 2 in August and one in October (Fig. 4). A total of 14 sentinel chicken seroconversions to SLE virus was recorded in IRC during 1989: one in September, 9 in October and 4 in November (Table 1).

Culex nigripalpus emergence patterns for the epidemic year were strikingly different from those observed during the 4 previous years (Fig. 7). Five closely spaced epic rainfalls in mid-July and early August provided abundant groundwater and oviposition sites resulting in multiple ovipositions and a series of 6 adult emergences. No additional epic rainfalls were recorded until early October when there were 2, resulting in an additional 5 adult emergences (Fig. 5). Virus transmission to 99 IRC sentinel chickens was recorded from June through October with peak transmission in July, August and September. The 19 human infections occurred from July through October with peak transmission in August (Table 1).

The frequency and timing of mosquito emergences and epic rainfalls in 1991 were similar to those observed in 1990 (Fig. 7). In fact, 3 midsummer epic rainfalls and the resulting mosquito emergences seemed to produce ideal conditions for viral amplification and transmission (Fig. 6). However, no viral transmission was recorded in IRC during 1991.

Time series analysis: There was a significant cross-correlation between epic rainfall events and the emergence of *Cx. nigripalpus* females on days 5–8 and again on days 12–15 following an epic rainfall event.

DISCUSSION

The 1990 SLE epidemic in southern Florida provided a unique opportunity to document the epidemiology of a widespread SLE outbreak. This was particularly true because of the baseline serological and vector abundance data collected

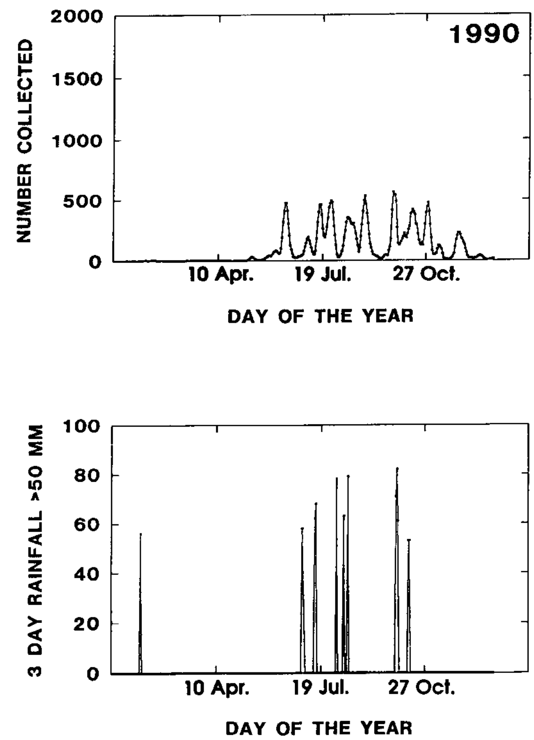

Fig. 5. Abundance of newly emerged *Culex nigripalpus* females and epic rainfall events during the widespread St. Louis encephalitis epidemic of 1990.

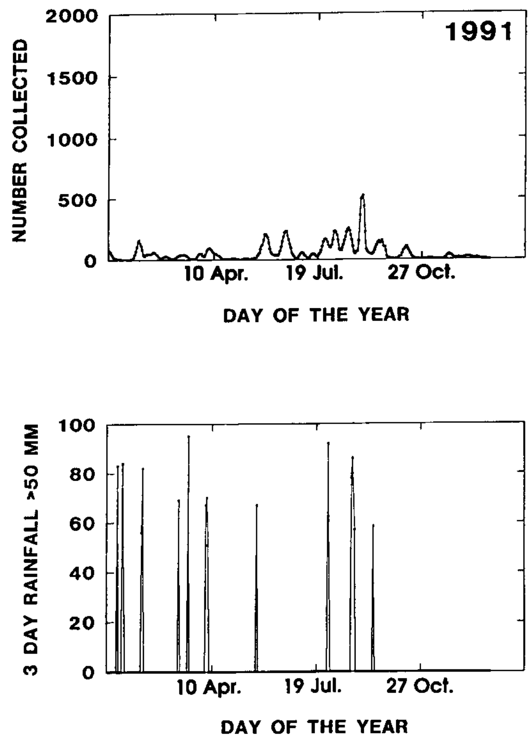

Fig. 6. Abundance of newly emerged *Culex nigripalpus* females and epic rainfall events during 1991, the year following a widespread St. Louis encephalitis epidemic in southern Florida.

during the pre-epidemic period. These data allowed the annual comparison and analysis of *Cx. nigripalpus* emergence patterns and SLE transmission.

In general, mosquito reproduction and abundance were low for the 3 years (1987–89) before the epidemic. In addition, with the exception of 1985, the transmission of SLE virus to sentinel chickens in IRC was low between 1978 and 1988. In fact, transmission was not recorded in 1987 or 1988 (Day 1989). The SLE transmission observed during 1989 was of interest because, in spite of relatively low vector reproduction and abundance (Fig. 7), virus was transmitted to sentinel chickens at record levels (O'Bryan and Jefferson 1991), yet human cases were not reported.

Culex nigripalpus reproduction from June through December of the epidemic year was persistent and steady with at least 11 emergences (Fig. 5). This reproductive pattern was significantly correlated with the frequency and timing of epic rainfall events. While the mosquito reproduction and rainfall patterns recorded in 1991 were similar to those of 1990 (Fig. 7), no SLE transmission was recorded during 1991. The widespread infection and subsequent immunity of large numbers of avian amplification hosts during the summer of 1990 interrupted the natural cycling of the virus during the following year. In fact, no SLE transmission was recorded in IRC from late October 1990 (when epidemic transmission ended) through the end of 1992.

A depression of SLE transmission following epidemic years has been reported previously, but is poorly understood (Chamberlain 1980, Monath 1980). However, low transmission during years following epidemics is not always the rule. For example, human epidemics occurred during consecutive years in Tampa Bay, FL (1961–62); Corpus Christi, TX; Memphis, TN; and Birmingham, AL (1974–75) (Monath 1980).

The reduced mosquito abundance and SLE transmission recorded in IRC during 1987 and 1988 may have been an important precursor to the 1990 epidemic. Rainfall was low and widespread droughts were recorded throughout southern Florida during those years. This greatly reduced mosquito reproduction. The droughts also affected SLE transmission to wild birds, allowing the buildup of large numbers of seronegative individuals that survived into the spring and early summer of 1990.

Beginning in February of 1989, we conducted

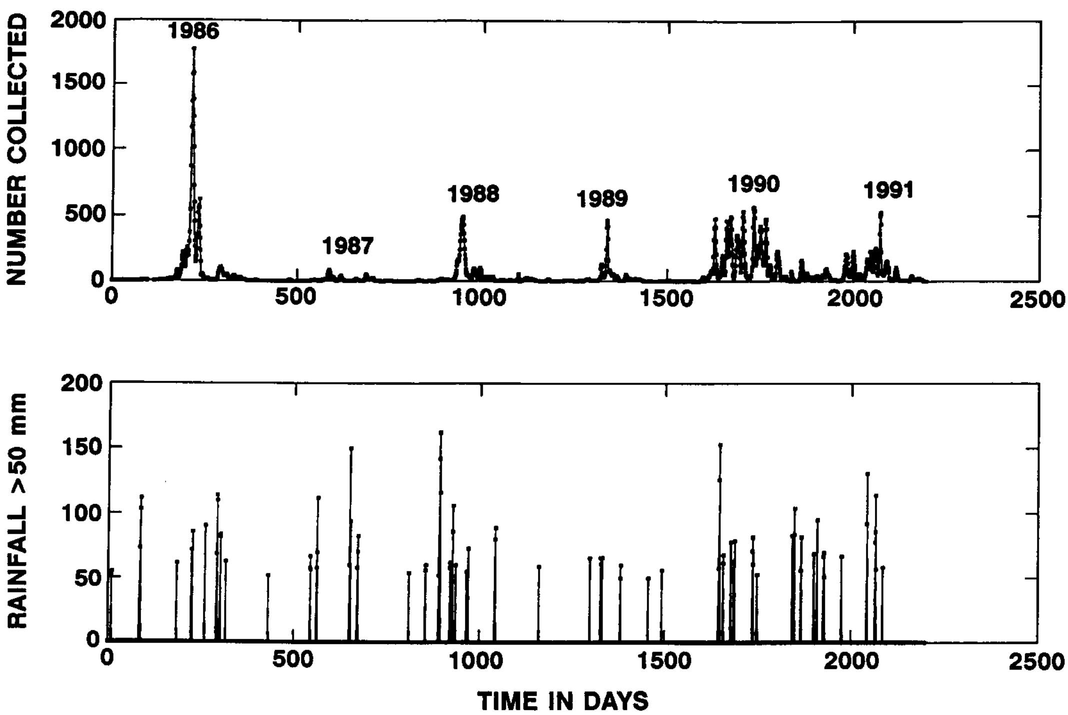

Fig. 7. Abundance of newly emerged *Culex nigripalpus* females and epic rainfall events for 1986–91.

a serosurvey for SLE antibody in wild birds captured throughout Indian River County. Hemagglutination inhibition antibody to SLE virus was found in 2 of 541 birds tested during 1989. Even in the presence of record levels of SLE transmission to sentinel chickens, the majority of the wild birds tested remained seronegative to SLE. This was decidedly not the case during August and September of 1990 when nearly all of the wild birds tested were seropositive for HAI antibody to SLE virus. These seropositive birds survived the epidemic and remained in the IRC area for at least a year, damping 1991 SLE transmission and amplification.

The cause of the sudden reappearance of SLE virus in the absence of large numbers of vector mosquitoes during 1989 is not known. It is possible that SLE amplification that year occurred as the result of 2 closely spaced epic rains in mid-August. Some of the adult female mosquitoes produced by these rains may have bloodfed on SLE-infected wild birds and then survived an extremely dry late August, September and early October without ovipositing (Fig. 4). During the drought, infected females completed the extrinsic incubation of the virus and then transmitted the virus to sentinel chickens following an epic rainfall in mid-October that allowed mass oviposition and synchronization of host seeking and bloodfeeding. It has been proposed by Day et al. (1990) that rainfall patterns in southern Florida serve to synchronize SLE amplification and transmission. Even in the absence of large numbers of vector mosquitoes or abundant virus, the proper meteorological conditions affect the biology of the vector mosquito in such a way that SLE virus can be amplified and transmitted quickly. This was likely the case in the 1961 Tampa Bay SLE epidemic discussed above.

The time series analysis and resulting cross-correlation relationships between epic rainfalls and the emergence of *Cx. nigripalpus* females reported here show significant emergences on days 5–8 and again on days 12–15 following epic rainfall events. Gravid females await epic rainfalls, oviposit as soon as sites become available, then search for hosts and bloodfeed again (Day et al. 1990). The first significant emergence on days 5–8 results from the oviposition of eggs retained by females awaiting the formation of suitable oviposition sites. The second emergence, on days 12–15, results from females that oviposited and bloodfed, or just bloodfed (Day and Curtis 1989), during the rainfall and then developed and oviposited eggs in the still extant oviposition sites 3 or 4 days afterward. It is thus possible for already gravid females to produce at least 2 egg rafts and to take multiple blood meals during the week following an epic rainfall. When infected gravid females complete extrinsic incubation of the virus while awaiting the formation of an oviposition site and are stimulated to oviposit and bloodfeed by heavy rain, viral transmission will be greatly increased during the days immediately following the rainfall.

The work reported here documents *Cx. nigripalpus* reproductive patterns and SLE transmission during a 6-year period that bracketed a widespread SLE epidemic in south Florida. Mosquito reproduction and SLE transmission was reduced until the year before the epidemic when mosquito abundance remained low, but SLE transmission was high. Mosquito reproduction and viral transmission were persistent and steady throughout the epidemic year. Even though similar emergence patterns were observed during the year following the epidemic, SLE transmission was not recorded.

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