Landscape Ecology of Arboviruses in Southeastern California: Temporal and Spatial Patterns of Enzootic Activity in Imperial Valley, 1991–1994

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ABSTRACT Western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE) viruses were detected in the Imperial Valley during the summers of 1991–1994 by isolation from the primary vector, *Culex tarsalis* Coquillett, and by the seroconversion of sentinel chickens. Enzootic transmission consistently was not detected first each year at sampling sites near specific landscape features such as a heron rookery and other riparian habitats along the New River, sites along the Mexican border, or saline and freshwater marshes along the southern shore of the Salton Sea. Despite mild winter temperatures and the elevated vernal abundance of *Cx. tarsalis*, WEE and SLE activity was not detected until June or July, indicating considerable amplification may be necessary before detection by testing mosquito pools for virus infection or sentinel chicken sera for antibodies. Results did not permit the spatial focusing of early season control efforts or research on mechanisms of virus interseasonal persistence.

KEY WORDS *Culex tarsalis*, arbovirus transmission, landscape ecology, California, western equine encephalomyelitis virus, St. Louis encephalitis virus

IDENTIFYING THE ENZOOTIC maintenance foci of arboviruses is critical to spatially focusing surveillance and control efforts as well as research to elucidate mechanisms that permit persistence between periods of active transmission. In recent years, western equine encephalomyelitis (WEE, Togaviridae, Alphavirus) and St. Louis encephalitis (SLE, Flaviviridae, Flavivirus) viruses have been active consistently in southeastern California, but intermittently throughout the rest of the state. In southeastern California, testing sequential samples from widely distributed study areas failed to detect a south to north pattern in the initiation and spread of enzootic arbovirus transmission along the Colorado River and in the Imperial and Coachella Valleys (Reisen et al. 1992). Subsequent intensive sampling in the Coachella Valley during 1991–1992 revealed consistent patterns in the initiation and dissemination of both WEE and SLE from salt marshes along the Salton Sea to mixed agricultural and managed freshwater marsh habitats within the flood plain of the Whitewater Channel (Reisen et al. 1995a, b).

Previous research in Imperial Valley established that WEE and SLE were widespread spatially (LeDuc 1973; Madon et al. 1974; Work et al. 1977a, b; Workman et al. 1976), active during most summers (Meylan et al. 1989), transmitted principally by *Culex tarsalis* Coquillett (Madon et al. 1974, Workman et al. 1976, Reisen et al. 1992), and infecting farm workers infrequently (Jozan 1977). Because these studies either intensively investigated specific localities (e.g., Work et al. 1977a) or monitored widely dispersed study sites (e.g., Reisen et al. 1992), information was lacking on the landscape ecology of these viruses in the Imperial Valley and the mechanism(s) that may allow their interseasonal persistence.

The current research was aimed at detecting enzootic maintenance foci and describing the patterns of amplification and dissemination of WEE and SLE viruses in the Imperial Valley. Our basic hypothesis was that these arboviruses are amplified initially at sites where virus persists between transmission episodes, that these landscape features or habitats remain consistent over time, and that virus activity can be monitored by testing pools of *Cx. tarsalis* mosquitoes and blood samples from sentinel chickens.

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Protocols for the care and use of vertebrate animals in this research were described in Animal Use Protocol R009-0695B "Arbovirology Ecology and Vector Competence Studies" approved by the Animal Care and Use Committee of the University of California, Berkeley.

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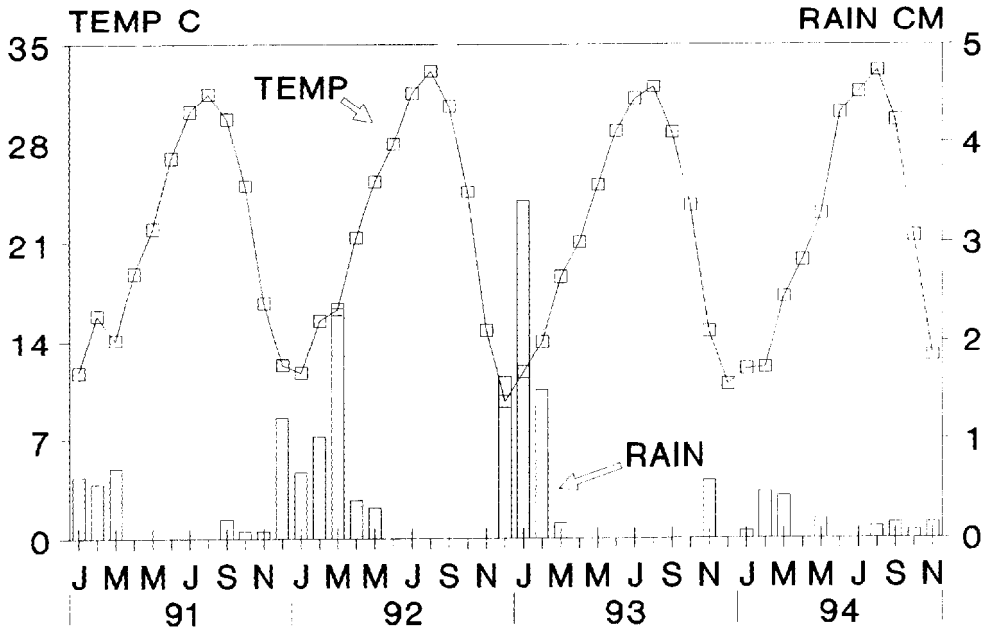

Fig. 1. Mean monthly ambient temperature and total rainfall recorded by the California Irrigation Management Information System at Seeley, Imperial Valley, 1991–1994.

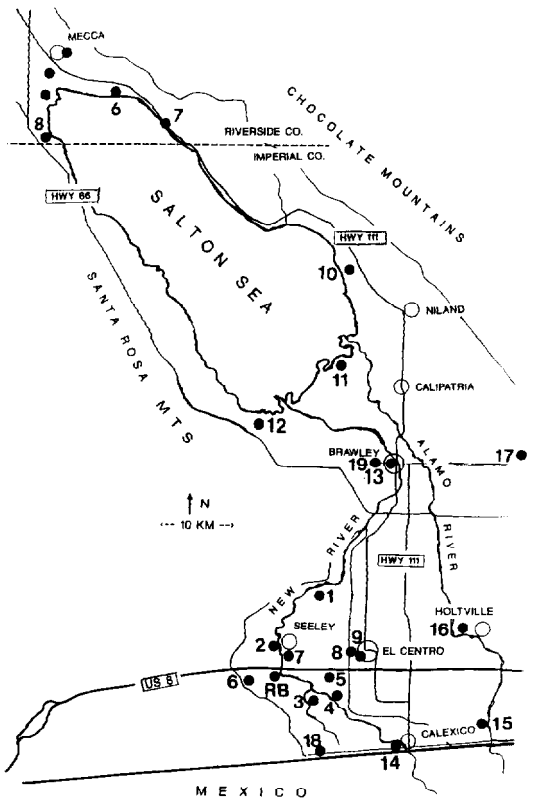

Fig. 2. Imperial Valley, Imperial County, California, location of 19 study sites sampled during 1991–1994. RB, Rio Bend permanent monitoring site on the New River.

Methods and Materials

Study Areas and Rationale. The Imperial Valley lies in the rain shadow of the coastal mountains and typically receives infrequent and sparse rainfall during winter (Fig. 1). Summer rains are introduced infrequently by the southeast monsoon from the Gulf of Mexico. Intensive agricultural irrigation (mostly fodder and row crops), habitat within the New and Alamo river bottoms, and marshes along the Salton Sea provide most mosquito larval habitat. Both rivers originate in Mexico, are heavily polluted, and empty into the Salton Sea below sea level (Fig. 2). Temperatures are mild during winter and hot during summer (Fig. 1).

Study areas in 1991 and 1992 were positioned in the southwest portion of the Imperial Valley to determine the role of a heron rookery at Rio Bend on the New River in the initiation and amplification of enzootic virus activity. In 1991, study sites were positioned to the north (sites 1 and 2) and south (sites 3 and 4) of Rio Bend to determine if virus activity was initiated at Rio Bend or was widespread within riparian habitat (Fig. 2). Sites 5 and 6 were positioned ≈7 km to the east and ≈5 km west, respectively, to determine if virus activity was confined initially to the New River channel. During 1992, sites 2 and 3 near Rio Bend were deleted, and sites within (sites 7 and 9) or adjacent (site 8) to the towns of Seeley and El Centro were added to determine if virus activity initiated within riparian habitat spread to adjacent and distant residential habitat (Fig. 2). Failure of virus to disperse to human population centers would explain, in

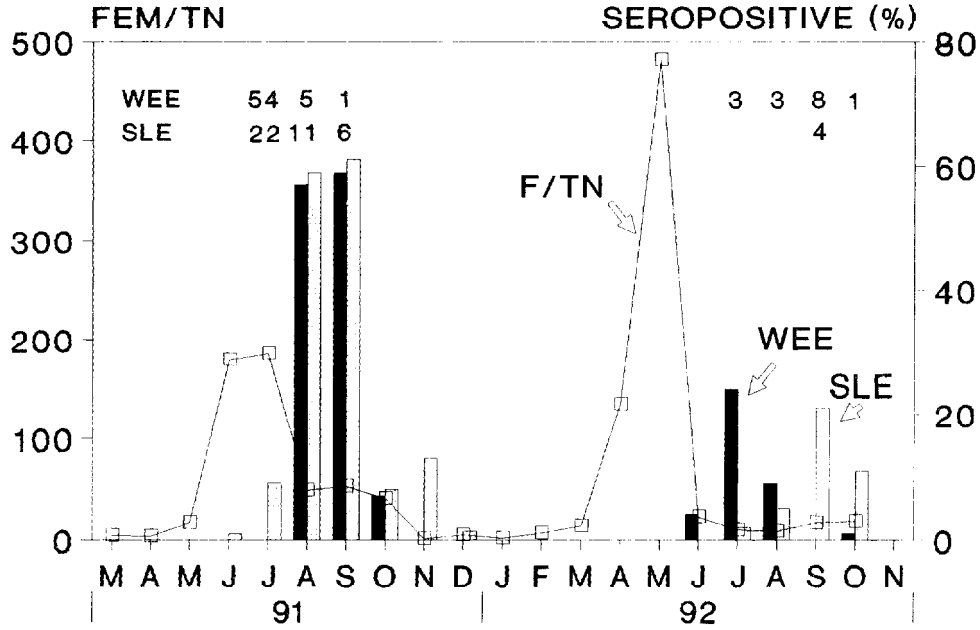

Fig. 3. Seasonal changes in the relative abundance of *Cx. tarsalis* (females per CO_2 trap night per 4 wk [FEM/TN]) and the percentage of sentinels seroconverting to WEE and SLE viruses in the Imperial Valley, 1991–1992. Numbers indicate the number of WEE and SLE virus isolations made per month.

part, the absence of cases in humans reported by the California Department of Health Services.

Because WEE and to a lesser extent SLE activity was initiated concurrently throughout the Seeley area and spatial patterns were not resolved during 1991–1992, the study area was expanded during 1993–1994 and only sites 1 (1993), 8 and Rio Bend were retained. New study sites were added to evaluate the role of saline and managed freshwater marshes along southern shore of the Salton Sea (sites 10–12), the northern intrusion of virus from Mexico along riparian corridors (sites 14, 15, 16, 18), agricultural habitat on the east side of the valley (site 17), and residential habitat near Brawley (sites 13, 19; Fig. 2).

Sampling and Processing. Sampling methods were similar to those described in Reisen et al. (1995a). Briefly, mosquitoes were collected by 2–3 dry ice-baited traps (Sudia and Chamberlain 1962) that were operated biweekly (2-wk intervals) at each site from March or April through November. Collection effort ranged from 16 (1991) to 23

Table 1. Habitat characteristics, *Cx. tarsalis* abundance and virus infection rates, and sentinel chicken seroconversion rates, Imperial Valley, 1991–1992

Site no.	Habitat	Abundance (fem/TN)		Virus infection[a]		Seroconversions[b]	
		1991	1992	1991	1992	1991	1992
				Fem (pools) W–S	Fem (pools) W–S	W–S	W–S
RB	Riparian, residential	205.9a	203.8a	6,145 (126) 18–17	6,405 (134) 11–2	18–20[c]	7–0
1	Fodder crops, riparian	45.1b	40.3b	2,178 (50) 13–4	1,870 (49) 2–1	10–13	5–9
2	Fodder crops	22.3bc	ND	1,108 (29) 5–1		9–14	ND
3	Row crops, riparian	8.6c	ND	334 (13) 1–1		10–8	ND
4	Fodder crops	55.2b	42.0b	2,183 (49) 7–5	2,503 (59) 1–1	10–14	3–8
5	Fodder crops	23.3bc	15.4bc	1,336 (35) 6–5	1,682 (38) 0–0	5–9	5–1
6	Fodder crops	24.2bc	30.6b	1,910 (42) 10–7	1,855 (47) 1–0	8–9	0–3
7	Residential	ND	34.3b		2,046 (46) 0–0	ND	0–0
8	Residential, row crops	ND	13.6bc		1,317 (34) 0–0	ND	3–3
9	Suburban residential	ND	6.1c		383 (15) 0–0	ND	0–0
	Totals	35.2	21.9	15,194 (344) 60–40	18,061 (422) 15–4	70–73	23–24

Geometric mean number of *Cx. tarsalis* females per trap night within columns followed by the same letter were not significantly different using a multiple range test ($P > 0.05$); ND, not done; RB, Rio Bend; TN, trap night.

[a] Number of *Cx. tarsalis* females (Fem) and pools tested for virus; W, WEE, and S, SLE, virus positive pools.

[b] Total number of seroconversions during each year; W, WEE, and S, SLE, antibody positive chickens.

[c] Flock of 20 sentinel chickens.

Table 2. Method, location, and sampling date when WEE and SLE viruses were detected initially in Imperial Valley, 1991–1994

Virus	Year	Method[a]	Site[b]	Date
WEE	1991	Pool	1, 2, 4–6, RB	9 July
		Chicken	all	19 Aug.
	1992	Pool	1, RB	14 July
		Chicken	1, RB, 8	29 June
	1993	Pool	8	19 May
		Chicken	10, RB	28 June
	1994	Pool	10, RB	6 July
		Chicken	16, RB	15 Aug.
SLE	1991	Pool	5, RB	9 July
		Chicken	4	24 June
	1992	Pool	1	9 Sept.
		Chicken	1	27 July
	1993	Pool	RB	28 July
		Chicken	8	28 June
	1994	Pool	RB	6 July
		Chicken	RB	18 July

[a] Pool, ≥1 positive mosquito pool; Chicken, ≥1 sentinel seroconversion.
[b] See Fig. 2 for site locations. RB, Rio Bend.

(1994) trap nights per biweekly sample. Mosquitoes were anesthetized with triethylamine, sorted to species and counted, and ≤10 pools of ≤50 females per site per biweekly sample were stored at −70°C for later testing for virus.

Transmission activity was monitored at each site using flocks of white leghorn laying hens. In 1991, 20 hens were deployed at site Rio Bend; the remaining flocks in 1991 and all flocks in 1992–1994 consisted of 10 hens. Hens were deployed in February 1991 and March 1992–1994, and bled every 4 wk in 1991–1992 and every 2 wk in 1993–1994. Hens that seroconverted were replaced after a confirmatory 2nd bleed.

Virus and Sera Assays. Mosquito pools were screened for virus using a Vero cell plaque assay (Hardy et al. 1993). Virus in Vero cell passage 1 or 2 cultures exhibiting cytopathic effect was identified using the enzyme immunoassay (EIA) described by Kramer et al. (1993) modified by using 3,3'diaminobenzidine as the substrate for final color development.

Chicken sera were screened for antibody using an indirect EIA (Reisen et al. 1994). Sera positive by EIA were confirmed by an indirect fluorescent antibody (IFA) test or by testing repeat bleeds on the same birds.

Statistics. *Cx. tarsalis* abundance in females per trap night per site per biweekly sample were transformed by $\ln(y + 1)$ to control the variance and normalize the distribution (Sokal and Rohlf 1981). Abundance within years was compared by a 2-way analysis of variance (ANOVA) with study sites and weeks as main effects. During 1993–1994 sites with similar seasonal abundance patterns were grouped using principal components analyses, inspection of time series correlation matrices, and study site spatial relationships.

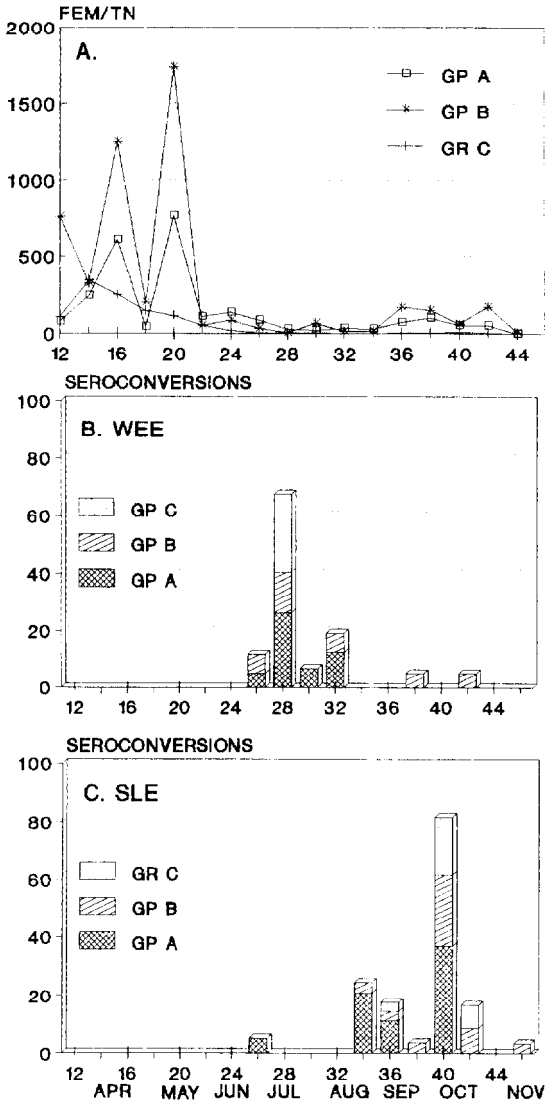

Fig. 4. Mosquito and virus activity during 1993. (A) Seasonality of *Cx. tarsalis* females per trap night (FEM/TN) in groups A (RB, 8), B (10–12), and C (1, 13, 14). (B) Seroconversion rates per biweekly bleeding of sentinel chickens to WEE at group A, B, and C sites. (C) Seroconversion rates per biweekly bleeding of sentinel chickens to SLE at group A, B and C sites.

Mosquito infections were expressed as minimum infection rates per 1,000 *Cx. tarsalis* females tested. Comparison of seroconversions among sites was complicated by our protocol which required the replacement of positive birds after a confirmatory bleed. Seroconversion rates (new antibody positives per seronegative birds at the onset of each sampling interval) were used in time series comparisons. Virus activity for the season per site was expressed simply as the number of birds seroconverting.

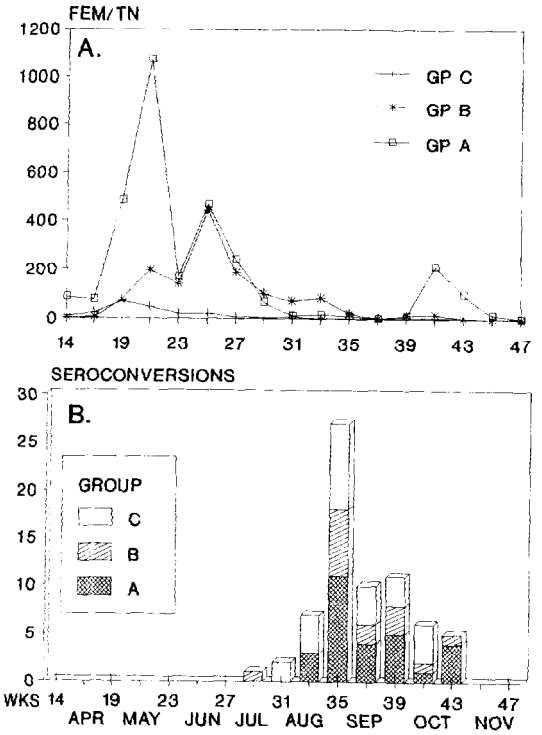

Fig. 5. Mosquito and virus activity during 1994. (A) Seasonality of *Cx. tarsalis* females per trap night (FEM/TN) at group A (RB, 8, 18), B (10–12), and C (14–17, 19) sites. (B) Seroconversion rates per biweekly bleeding of sentinel chickens to SLE at group A, B, and C sites.

Results

1991–1992. *Cx. tarsalis* was more abundant at Rio Bend than at other sites in riparian, agricultural, or residential habitats in southwestern Imperial Valley during 1991 and 1992 (Table 1). Female abundance was lowest in 1991 at site 3, a farm house within row crop habitat near the New River, and in 1992 at site 9, a residence within the city of El Centro. Abundance was unimodal with a single vernal peak (Fig. 3), and this seasonal pattern was similar among sites during both years (time series correlations between paired sites, $r > 0.70$, $P < 0.05$). Although the geometric mean number of *Cx. tarsalis* females collected per trap night per year was not significantly different (1991, 35.2 females per trap night; 1992, 21.9 females per trap night; $F = 0.42$; df = 1, 252; $P > 0.05$), the amplitude of the maximum in July 1991 (185 females per trap night) was only 38% of the maximum of May 1992 (482 females per trap night). However, mean abundance remained >50 females per trap night per month from June to September 1991, but was <20 females per trap night during the same period in 1992.

Western equine encephalomyelitis and SLE were active at all 7 sites in 1991 and at 6 of 8 sites in 1992 (Table 1). Both viruses were most active at site 2 in 1991, but were not detected at site 7 in 1992, even though this site was only 1 km distant from site 2, situated <0.3 km from the New River, and supported comparable abundance levels of *Cx. tarsalis*. Neither WEE nor SLE virus was detected at site 9 on the outskirts of El Centro; however, excessive sentinel mortality during the critical July–August period may have hampered our ability to detect virus activity at this site.

During 1991 when the seasonal minimum infection rates of WEE and SLE were elevated (3.95 and 2.63/1,000 *Cx. tarsalis* females tested, respectively), the number of positive *Cx. tarsalis* pools seemed relatively proportional to the number of sentinel seroconversions. However, during 1992

Table 3. Habitat characteristics, *Cx. tarsalis* abundance and infection rates, and sentinel seroconversion rates, 1993–1994

Site no.	Habitat	Abundance (fem/TN)		Virus infections[a]		Seroconversion[b]	
		1993	1994	1993	1994	1993	1994
				Fem (pools) W–S	Fem (pools) W–S	W–S	W–S
RB	Riparian, residential	297.9a	83.3b	5,855 (119) 8–9	3,216 (68) 0–1	9–8	3– 7
1	Fodder crops, riparian	18.9c	ND	799 (19)		7–5	ND
8	Residential, row crops	11.6cd	8.8f	678 (18) 1–1	511 (14) 0–0	4–7	0– 2
10	Marsh	346.2a	130.1a	4,844 (98) 3–5	3,641 (78) 0–2	0–7	0– 9
11	Marsh	55.8b	58.1c	2,625 (56) 0–0	3,629 (75) 0–0	1–2	1– 7
12	Marsh, fodder crops	17.9c	23.3d	524 (13) 0–0	1,635 (36) 1–0	7–4	0–12
13	Residential, riparian	6.0d	ND	934 (20) 0–0		0–0	ND
14	Riparian, residential	6.3d	4.0h	1,015 (21) 0–0	160 (5) 0–0	0–3	0– 1
15	Riparian, orchard	ND	15.7e		657 (18) 0–0	ND	1–10
16	Riparian, residential	ND	9.9f		368 (11) 0–0	ND	1– 4
17	Fodder crops	ND	6.0g		508 (12) 0–0	ND	0– 4
18	Fodder and row crops	ND	14.6e		991 (24) 0–0	ND	0– 6
19	Residential	ND	1.3i		32 (2) 0–0	ND	0– 7
	Total	31.9	16.1	17,274 (364) 12–15	15,348 (343) 1–4	28–36	6–69

Geometric mean number of *Cx. tarsalis* females per trap night within columns followed by the same letter were not significantly different using a multiple range test ($P > 0.05$); ND, not done. TN, trap night, RB, Rio Bend.

[a] Number of *Cx. tarsalis* females (fem) and pools tested for virus; W, WEE, and S, SLE, virus positive pools.

[b] Total number of seroconversions during each year; W, WEE, and S, SLE, antibody positive chickens.

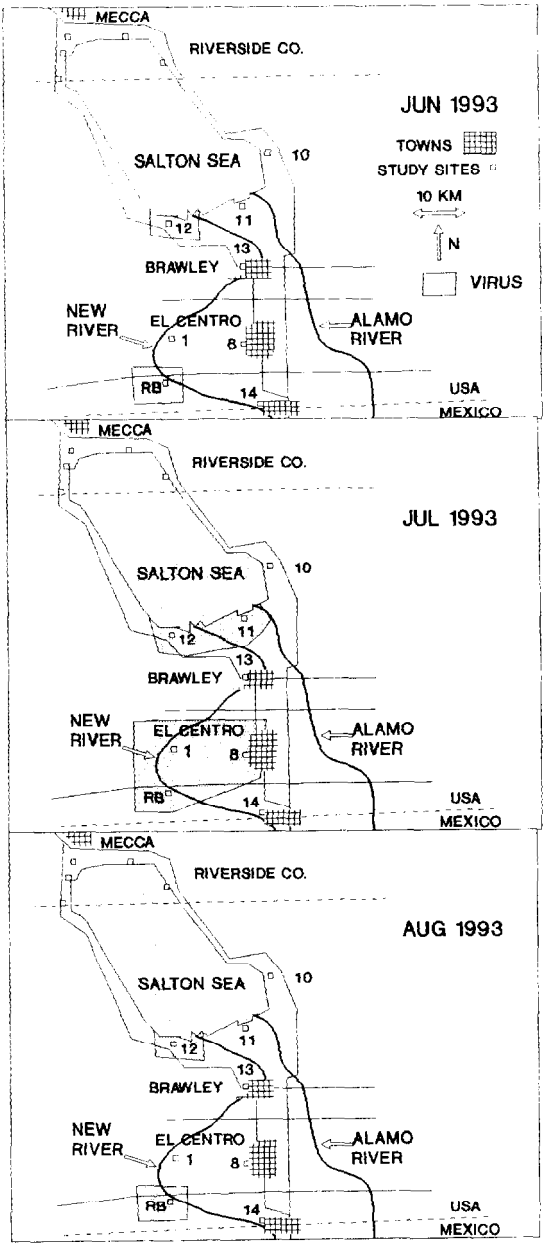

Fig. 6. Monthly changes in the geographical distribution of sampling sites positive for WEE virus during 1993. Positive sites have ≥1 isolation from *Cx. tarsalis* pools or ≥1 sentinel seroconversion. RB, Rio Bend.

when virus activity was less, the seasonal minimum infection rates for WEE and SLE viruses (0.83 and 0.22, respectively) decreased by factors of 4.8 and 12.0, whereas the number of sentinel seroconversions to both viruses decreased by factors of 3.0. These data indicated that sentinel chickens were a more sensitive indicator of virus activity at moderate or low levels than isolation of virus from mosquitoes.

The activity of WEE virus began abruptly on 9 July 1991, when 16 of 27 pools of *Cx. tarsalis* collected at 6 of 7 sites tested positive for WEE virus (total, 1,252 females tested; minimum infection rates, 12.8 infected females per 1,000); 5 additional pools from 2 sites also were positive for SLE (MIR, 4.0)(Table 2). Two weeks later on 23 July, 38 and 17 of 53 pools from all 7 sites were positive for WEE and SLE (MIRs, 14.7 and 6.6), respectively. Interestingly, chickens bled on 23 July were negative for WEE antibody, but on 19 August, 57% of 75 hens seroconverted at all 7 sites (Fig. 3). Conversely, only 1 sentinel at site 4 bled on 24 June was SLE positive. Seroconversion rates for SLE increased gradually to 61% of 36 hens at 7 of 7 sites by 17 September. These data indicated that SLE activity at moderate levels was detected earlier by testing sentinel chicken sera than pools of *Cx. tarsalis*; however, WEE virus activity at elevated levels was detected 1st by testing mosquito pools. Most likely many of the chickens bled on 23 July were infected with WEE virus, but their antibody titers were not as yet detectable by EIA. These data underscore the importance of frequent sentinel bleeding in surveillance programs.

Sentinel chickens detected virus activity 1st during 1992 when both WEE and SLE viruses were less active (Table 1, 2). WEE virus was detected initially on 29 June when sentinels at 3 of 8 sites seroconverted (Table 2). On 14 July, 2 of 13 pools (425 females tested; minimum infection rate, 4.7/1,000) at 2 sites were WEE positive. Similarly SLE virus was 1st detected on 27 July when 1 sentinel at site 1 seroconverted. Positive *Cx. tarsalis* were not collected at site 1 until 9 September, when 1 of 14 pools was positive (634 tested; minimum infection rate, 1.6).

1993–1994. Because virus activity began concurrently at multiple sites and vector abundance patterns were highly correlated among sites in the southwestern portion of the Imperial Valley during 1991–1992, sampling was expanded to include habitats from the Salton Sea to the Mexican border (8 sites in 1993 and 11 sites in 1994; Table 3, Fig. 2). *Cx. tarsalis* abundance was highest during both years at Rio Bend along the New River and at the Wister Wildlife Refuge (site 10) along the southeastern shore of the Salton Sea. Although principal components analyses explained 82 and 74% of the variability in *Cx. tarsalis* abundance among sites in 1993 and 1994, respectively, sites did not cluster consistently between years or spatially based on habitat type or juxtaposition (data not shown). Therefore, sites were placed into 3 groups by combining principal components results with data on relative abundance and site juxtaposition: group A, sites Rio Bend, 8, and 18 in the SW; group B, sites 10, 11, and 12 along the southern shore of the Salton Sea; and group C, remaining sites in the central, eastern, and southern portions of the valley. *Cx. tarsalis* seasonality at sites in groups A and B were characterized by a rapid vernal increase,

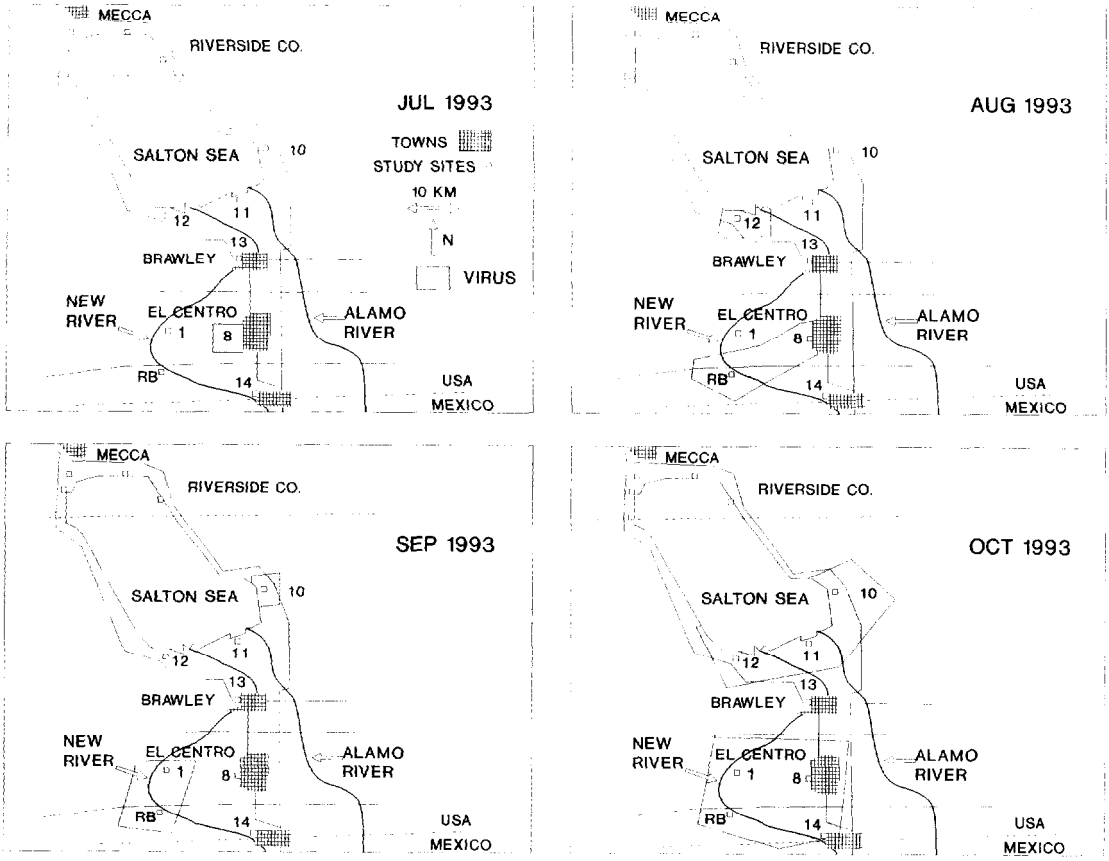

Fig. 7. Monthly changes in the geographical distribution of sampling sites positive for SLE virus during 1993. Positive sites have ≥1 isolation from *Cx. tarsalis* or ≥1 sentinel seroconversion. RB, Rio Bend.

an abrupt crash and resurgence, consistent low level activity during midsummer, and a small increase during fall (Figs. 4 and 5). Although the timing of the seasonal increases was consistent, the magnitude of the vernal resurgence varied between years. Abundance at group C sites remained relatively low throughout, with less amplitude between seasonal maxima and minima.

Virus infection rates in host-seeking *Cx. tarsalis* females were low in 1993–1994 (Table 3) compared with 1991 (Table 1), although comparable numbers of *Cx. tarsalis* females and pools were tested. During 1993 WEE was detected initially in a pool of *Cx. tarsalis* collected at site 8 on 19 May and seroconversions of chickens at sites 10 and Rio Bend on 28 June (Table 2). Activity expanded to adjacent sites during July, but then subsided in August (Fig. 6) and declined to single seroconversions during September and October at group B sites (Fig. 4D). SLE was detected initially on 28 June 1993 in a positive pool of *Cx. tarsalis* collected at Rio Bend and on 28 July by the seroconversion of a sentinel chicken at site 8 (Table 2; Fig. 7). SLE activity then spread to flocks near the Salton Sea during August and September, and became widespread during October (week 40). Most SLE activity was delayed until fall, peaking during week 40 after a small increase in *Cx. tarsalis* abundance during weeks 36 and 38 at group A and B sites (Fig. 4B).

Although relatively widespread, WEE activity was detected at low levels during 1994; 1 isolation from *Cx. tarsalis* from site 12 on 6 July and 6 sentinel seroconversions at sites Rio Bend, 11, 15, and 16. In contrast, although only 2 pools of *Cx. tarsalis* tested positive for SLE virus, 69 sentinels at all 11 sites seroconverted (Table 3). Initial SLE activity was detected by seroconversions at site Rio Bend on 18 July (Table 2). Amplification preceded slowly, but seroconversions were detected at all flocks during both August and September, and in 9 of 11 sites during October and November (Fig. 8). Sentinels were bled for the last time on 11 November, when the project was terminated.

Other Mosquito Species Tested for Virus. Females of several other mosquito species were tested sporadically for virus infection. Overall, 4,439 (106 pools) *Cx. quinquefasciatus* Say, 734 (68 pools) *Aedes dorsalis* (Meigen), 181 (4 pools) *Ae. vexans* (Meigen), 450 (9 pools) *Cx. erythrothorax* Dyar, and 165 (8 pools) of *Culiseta inornata* (Wil-

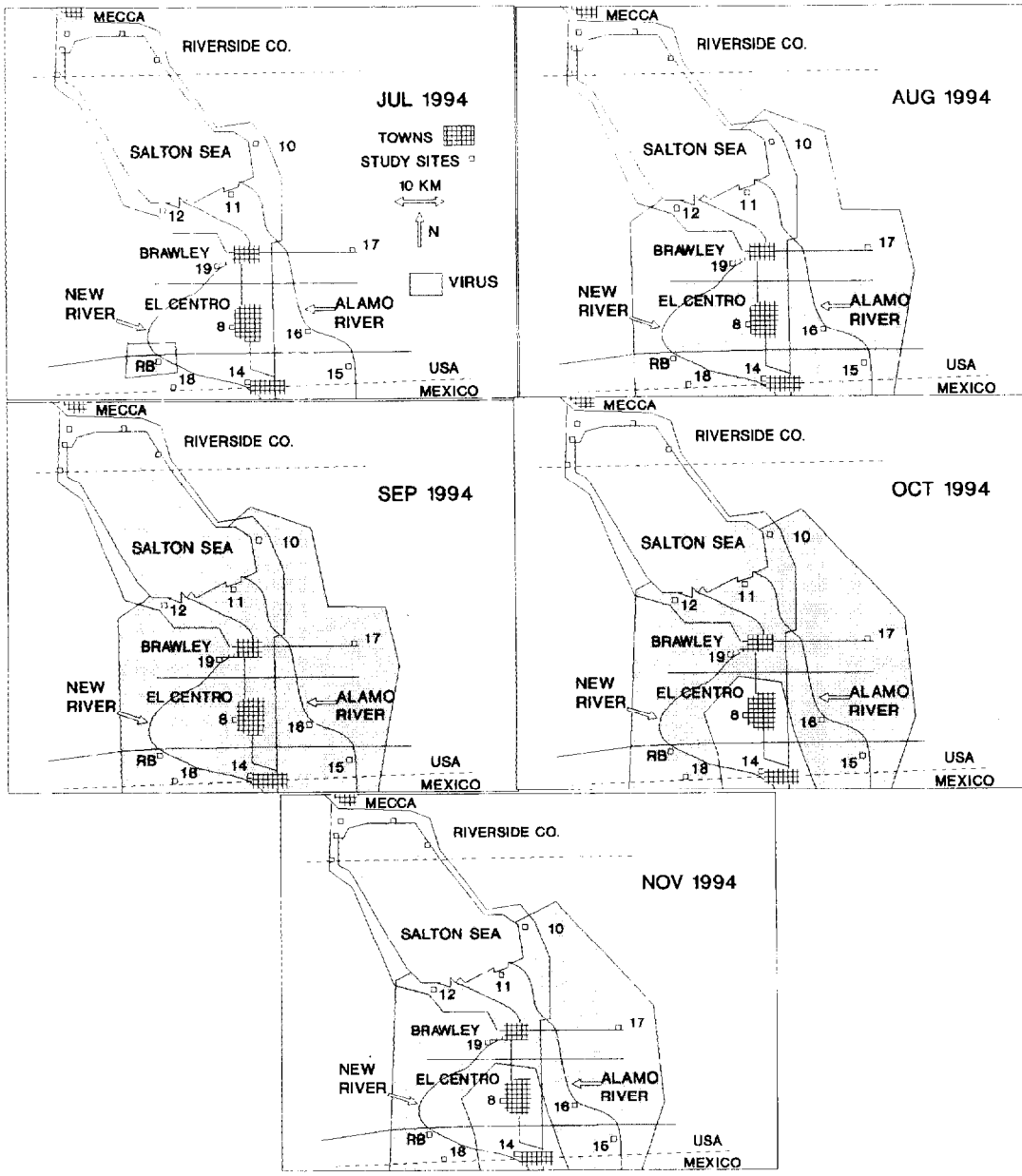

Fig. 8. Monthly changes in the geographical distribution of sampling sites positive for SLE virus during 1994. Positive sites have ≥1 isolation from *Cx. tarsalis* or ≥1 sentinel seroconversion. RB, Rio Bend.

liston) were tested by plaque assay with negative results.

Discussion

Patterns of early season WEE and SLE detection did not support our hypotheses that virus persisted at the large heron rookery at Rio Bend or within the New River riparian corridor. During 1991 and 1992 when sampling was restricted to the southwestern portion of the valley, WEE and SLE initially were detected at multiple sites along the New River north (sites 1 and 2) and south (site 4) of Rio Bend and at sites outside the river channel (sites 5 and 8). When sites were distributed throughout the Imperial Valley, WEE was detected initially at site 8 in 1993 and at sites 10 and Rio Bend in 1994, and SLE was detected initially at sites 8 and Rio Bend in 1993 and Rio Bend in 1994.

Activity of WEE or SLE viruses was not detected 1st at marshes along the southern shore of the Salton Sea without concurrent activity at Rio Bend, even though site 10 at the Wister Wildlife Refuge

frequently supported large spring populations of *Cx. tarsalis* and our study area at the Salton Sea State Park in Riverside County consistently had the earliest virus activity in the Coachella Valley (Reisen et al. 1995a). Neither did virus appear to move north from Mexico or overwinter at sites along the Alamo River or on the east side of the valley. Border sites 18, 14, and 15 never showed early virus activity, even though site 18 was placed with sites Rio Bend and 8 in group A, site 14 was situated adjacent to the New River channel, and site 15 was adjacent to the Alamo River.

Interpretation of these results was based on the hypothesis that mosquito-borne arboviruses are detected initially at sites where they persist between transmission periods or are introduced repeatedly. Unfortunately, information on the sensitivity of our sampling system is not available; that is, we do not understand the geographical size of a virus focus or the intensity of transmission among mosquitoes and wild birds necessary for detection in mosquito pools or by sentinel seroconversion. Quite possibly, enzootic transmission occurred at or near sampling sites, but below the level of sampling sensitivity. In the current study, initial virus detection always occurred 5–6 mo after *Cx. tarsalis* terminated diapause in late December–early January (Reisen et al. 1995c), and several months after permanent and summer resident birds resumed reproductive activity. In agreement with our earlier observations (Reisen et al. 1992), WEE and SLE always were detected initially in late spring or early summer after the onset of hot summer temperatures and after the spring peak of mosquito abundance. Similar temporal patterns were reported in neighboring Coachella Valley (Reisen et al. 1992, 1995a). Even though virus activity was detected after the vernal peak in *Cx. tarsalis* abundance, sites such as Rio Bend with elevated vernal host-seeking abundance frequently had the earliest and most intense levels of virus activity. Hot summer temperature presumably contributed by increasing virus amplification rates by decreasing virus extrinsic incubation and the duration of the gonotrophic cycle in the vector (Reisen et al. 1995d).

Despite extensive enzootic transmission of WEE and SLE at rural farm houses and suburban habitat throughout the Imperial Valley during 1991–1994, only 1 case in humans and none in horses were reported to the state-wide Encephalitis Virus Surveillance Program of the California Department of Health Services (Emmons et al. 1992, 1993, 1994; Reilly et al. 1995). The single human case of SLE was diagnosed in a resident of San Diego County who possibly was infected in August 1993 during a fishing trip to the Imperial Valley. Previously, Jozan (1977) found that 1.5 and 12.2% of mostly hispanic farm workers tested positive for antibodies to WEE and SLE viruses, respectively; comparable to recent results from the Coachella Valley (Reisen et al. 1995e). Because 2 SLE strains isolated from the Imperial Valley in 1978 and 1993 were similar genetically to strains from Los Angeles isolated during the 1984 outbreak (L. D. Kramer, unpublished data) and 1 WEE strain was intermediate in experimental mouse assays of neurovirulence and neuroinvasiveness (J.L.H., unpublished data), we concluded that the infrequent occurrence of cases in humans must be related to decreases in risk rather than changes in virus strains. In Kern County, decreased risk of arbovirus infection was associated with changes in human behavior such as increased viewing of television and staying in air-conditioned housing during peak mosquito host-seeking periods (Gahlinger et al. 1986).

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