Landscape Ecology of Arboviruses in Southern California: Temporal and Spatial Patterns of Vector and Virus Activity in Coachella Valley, 1990–1992

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ABSTRACT Consistent temporal and spatial patterns in the activity of *Culex tarsalis* Coquillett and western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE) viruses were delineated that were useful in developing a stratified surveillance program. Vernal increases in *Cx. tarsalis* abundance typically were associated with flooding of saline marshes along the north shore of the Salton Sea and were followed 6–8 wk later by the onset of WEE and SLE virus activity. Viruses then spread to managed marsh (duck club) and agricultural habitats in the Whitewater Channel flood plain and, depending upon the intensity of amplification, to agricultural and residential areas in the more elevated northwestern portion of the valley. Mean annual *Cx. tarsalis* abundance was correlated inversely with elevation and distance from the Salton Sea. Abundance was greatest at managed marsh habitats. Although spatially correlated with vector abundance among sites, virus transmission rates to sentinel chickens were asynchronous temporally with vector abundance. Seroconversion rates were related to flock location but not flock size (10 versus 20 chickens). Human cases were not detected during the study period, despite elevated transmission rates of both WEE and SLE viruses to sentinel chickens positioned in peridomestic habitats.

KEY WORDS *Culex tarsalis*, arbovirus transmission, landscape ecology

AN IN-DEPTH UNDERSTANDING of the landscape ecology of a zoonosis is critical in developing effective surveillance and control strategies as well as in ascertaining the relative risk of human infection. The California Encephalitis Virus Surveillance (EVS) program determines the level of western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE) virus activity each season using a "best estimate" sampling strategy (Emmons et al. 1991). Using this approach, collaborating mosquito control agencies monitor the abundance and virus infection rates in *Culex* populations and seroconversion rates in flocks of sentinel chickens at sites that are perceived to be most sensitive in detecting virus activity. Although this approach provides the most efficient spatial allocation of sampling resources, sensitivity is contingent upon correct site selection. However, few studies have investigated the small-scale, spatial dynamics of either WEE or SLE virus activity to aid in the selection of sampling sites and assist in the interpretation of surveillance information. Although the California EVS program has accumulated considerable information on wide spread virus activity, these data have not been analyzed in regard to habitat and are confounded by distance among sites.

In the current research, the landscape ecology of WEE and SLE viruses was investigated to identify terrain features associated with the early-season detection of enzootic virus activity. Our previous attempts to address this problem in the San Joaquin Valley were hindered by successive years of little or no virus activity (Reisen et al. 1990) interspersed with years of rapid and widespread epizootic transmission (Reisen 1984, Reisen et al. 1992b). Research described in the current article focused on temporal and spatial patterns in mosquito and arbovirus activity in the southern Coachella Valley, Riverside County, California. A companion article (Reisen et al. 1995) examines, in depth, the patterns of initiation and spread of WEE and SLE viruses to identify possible centers of enzootic virus maintenance.

WEE and SLE viruses have been active intermittently in the Coachella Valley along the northern shore of the Salton Sea since surveillance was initiated in 1977 (Emmons et al. 1978, Durso & Burguin 1988), and arbovirus surveillance activities continue to be concentrated in that area (M.J.W., unpublished data). However, two cases of SLE vi-

Unrefereed interim summaries of this research have appeared previously (Lothrop et al. 1992, 1993; Reisen et al. 1992a). Protocols for the care and use of vertebrate animals in this research were described in Animal Use Protocol R009-0592B and -0693B "Arbovirology Ecology and Vector Competence Studies" approved by the Animal Care and Use Committee of the University of California, Berkeley.

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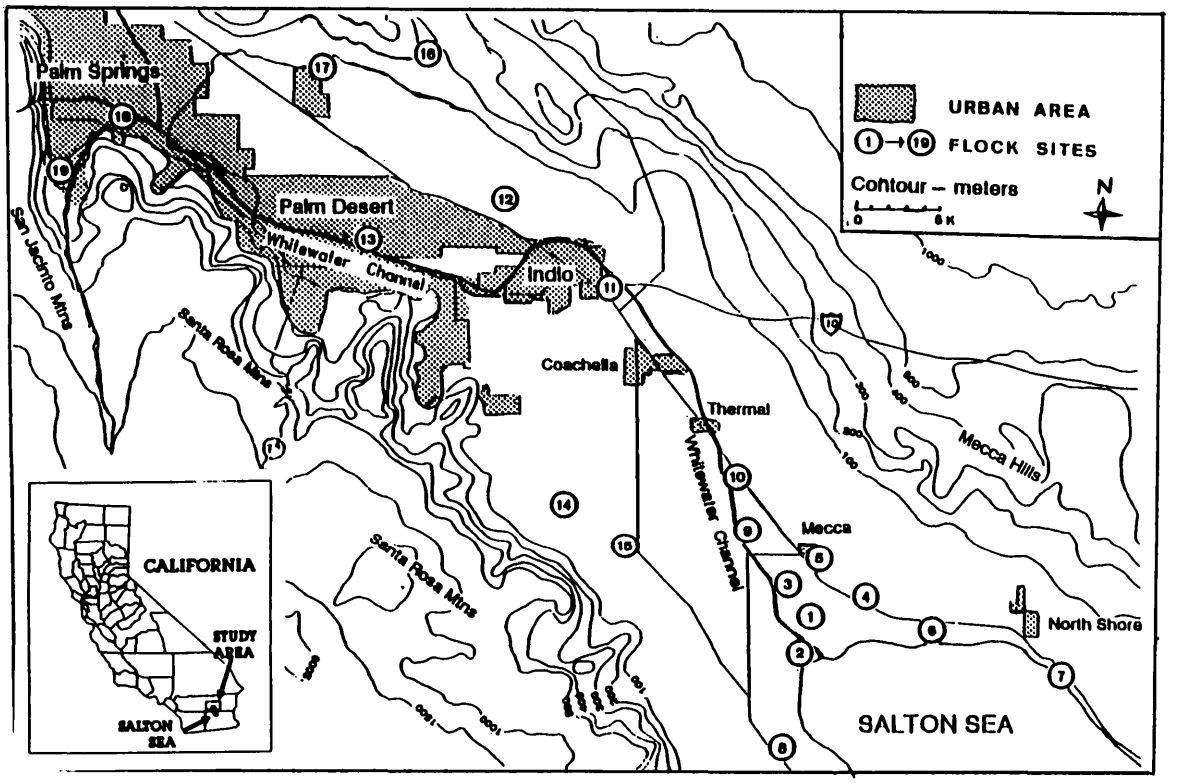

Fig. 1. Map of the southeastern Coachella Valley, Riverside County, California, showing the location of the 19 study sites, 1990–1992.

rus occurred in residents of Indio and Palm Springs (Murray et al. 1985), perhaps indicating the spread of virus activity to the northwestern portion of the valley. Arbovirus surveillance and research in southern California was concentrated in Imperial County during the 1970s to detect the possible northward spread of Venezuelan equine encephalitis virus (Bown & Work 1973, Madon et al. 1974, Workman et al. 1976). Associated research focused on seasonal and small-scale spatial changes in *Culex* abundance and arbovirus infection rates along the Highline Canal (LeDuc 1973), at the Finney-Ramer Refuge (Work et al. 1977a), and along the New River (Work et al. 1977b, Walters & Smith 1980). Collectively, these investigations documented that *Culex tarsalis* Coquillett was the primary vector of both WEE and SLE viruses, that virus infections in mosquitoes were limited to the May–October period, and that both viruses could be detected in mosquitoes at a variety of habitats. However, because these studies focused on testing mosquito pools from specific localities, they failed to provide information on the importance of terrain features on virus transmission. Our studies in southeastern California during 1986–1990 confirmed the consistent widespread summer activity of WEE and SLE viruses and demonstrated low-level winter carry-over of fall virus activity along the lower Colorado River and in Imperial and Coachella valleys (Reisen et al. 1992c).

The primary objective of the present study was to determine if best estimate sampling adequately describes WEE and SLE virus activity levels in different, but adjacent, habitat types. The southern Coachella Valley was selected for study because WEE and SLE viruses have been active consistently in this area and because the landscape presents a variety of vegetative types, land uses, and physiographic features. By sampling a variety of habitats supporting different levels of *Cx. tarsalis* abundance, we also evaluated the impact of vector abundance and seasonality on the level of virus activity.

Materials and Methods

Study Areas and Rationale. Research was concentrated in the southern half of the Coachella Valley from Palm Springs to the Salton Sea (Fig. 1). Fish culture and duck club ponds are concentrated below sea level near the Salton Sea in poorly drained, alkaline soils. Although fish ponds rarely produce mosquitoes, large numbers of *Cx. tarsalis* emerge soon after the duck club ponds are inundated in late summer (Fanara & Mulla 1974). Increased elevation and improved soil type and drainage allow the production of row crops and

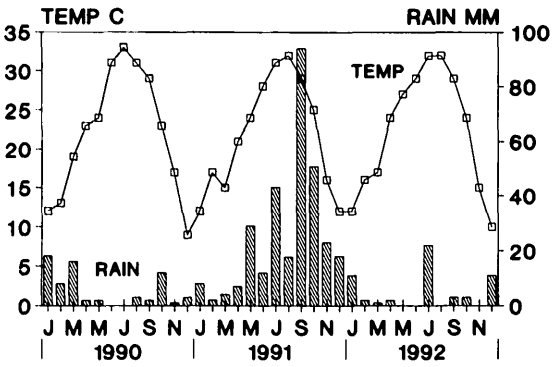

Fig. 2. Mean monthly ambient temperature and total rainfall recorded by the California Irrigation Management Information System at Thermal, Coachella Valley, 1990–1992.

dates to the north and dates, citrus, and grapes to the east and west. Terrain to the north of Palm Springs features well-drained sandy desert that rarely produces mosquitoes. Human population density and per-capita income in Coachella Valley increase progressively from the southeast to the northwest.

Weather is characterized by mild winters followed by hot, dry summers (Fig. 2). Coachella Valley lies in the rain shadow of the San Jacinto Mountains and typically receives infrequent and sparse winter rains, although run-off from rain showers in the arid mountains can cause focal flooding on the valley floor. Occasional summer rain is introduced by southeast monsoons from the Gulf of Mexico. Evening wind patterns consistently are from the northwest to the southeast.

Our pilot observations in 1990 were designed to determine if virus activity at our routine monitoring site at Adohr Farms (Reisen et al. 1992c) in mixed desert scrub, date orchard, and duck club habitat (site 1, Fig. 1) was representative of the surrounding landscape. Four additional study areas within a 7-km radius of site 1 were sampled for virus activity in desert scrub (site 3), date orchards (site 3a, 2 km west of site 1, not shown in Fig. 1), mixed grape and citrus agriculture (site 4), and a residence in the town of Mecca (site 5).

In 1991, sites were positioned to sample prominent physiographic features in the Coachella Valley, including the shore of the Salton Sea and the Whitewater Channel which originates from snow melt in the northern end of the valley, collects municipal and agricultural run-off as it flows southeast, and empties into the Salton Sea (Fig. 1). This channel is well maintained and is dredged periodically to remove emergent vegetation. Prominent habitats in the immediate environs of the 1991 sampling sites are described in Table 2.

Sampling in 1992 was expanded to include additional sites in the northern, more populous portion of the valley (sites 17 and 18) as well as in peripheral canyons to the east (Site 16) and west (site 19, Fig. 1). In addition, site 3 was moved 0.75 km northeast from desert scrub to a farm at which row crops were raised predominantly. Site 14 to the west was deleted because WEE or SLE viruses rarely were active at this site from 1986 through 1991.

Sampling and Processing. Mosquitoes were collected by two to three dry ice-baited CDC traps (Sudia & Chamberlain 1962) that were hung at 1.5 m height on permanent standards at each study area and operated without lights from dusk to dawn biweekly from March through November each year. Selected traps also were operated during the winter. Mosquitoes were transported to the laboratory alive, anesthetized with triethylamine, sorted to species, and counted. Up to 10 pools of 50 *Cx. tarsalis* females per site per biweekly sample were frozen at −70°C for later virus testing.

Virus transmission activity was monitored using sentinel flocks of white leghorn laying hens. In 1990 and 1991, 20 hens were deployed at permanent study sites 1, 15, and 12 (Fig. 1); the remaining flocks in 1990 and 1991 and all flocks in 1992 consisted of 10 hens each. Seronegative hens were deployed in April 1990, February 1991, and March 1992 and then bled by jugular venipuncture every 4 wk in 1990 and 1991 and every 2 wk in 1992. Sentinels that seroconverted during 1991 and 1992 were replaced after a confirmatory second bleed.

Virus and Serological Assays. At the Viral and Rickettsial Disease Laboratory (VRDL) (summer of 1990 only) or the Arbovirus Research Laboratory (ARL), University of California, Berkeley, mosquito pools were thawed, triturated in mosquito diluent, and then screened for virus. At the VRDL, pools were tested for virus infection by using an in situ enzyme immunoassay (EIA) similar to that described by Kramer et al. (1993). At the ARL, pools first were screened for infectious virus using a Vero cell plaque assay (Hardy et al. 1993). Positive Vero cell passage one or two cultures then were identified using the EIA of Kramer et al. (1993) modified by using 3, 3' diaminobenzidine as the substrate for final color development.

Chicken sera were screened during the summer by the VRDL and during the winter by the ARL using an indirect EIA. Sera positive by EIA were confirmed by an indirect fluorescent antibody (IFA) test or by testing repeat bleeds on the same hens.

Statistics. The number of *Cx. tarsalis* females collected per trap night per biweekly sample were transformed by $\ln(y + 1)$ to control the variance and normalize the distribution (Sokal & Rohlf 1981). Abundance within years was compared by a two-way analysis of variance (ANOVA) with study sites and weeks as main effects. Abundance at study sites was evaluated for similar seasonality and compared among landscape features. Sites with similar seasonal patterns were grouped within years using a principal components analysis and by

Table 1. *Cx. tarsalis* abundance and SLE virus activity in the Coachella Valley during 1990

Site	Habitat	Fem/TN[a]	Virus infection		Sero-conversions, %
			Fem (Pools)	Pos	
1	Duck ponds, dates, scrub	106.1a	14,300 (295)	1	40[c]
3	Duck ponds, scrub	132.5a	3,087 (67)	0	80
3a	Dates	38.4ab	1,552 (38)	0	10
4	Grapes, citrus, dates	9.8bc	1,115 (26)	0	60
5	Residential	5.3c	439 (11)	0	0
Totals		32.8	20,493 (437)	1	38

[a] Geometric mean number of *Cx. tarsalis* females/trap night from April through October 1990. Means followed by the same letter are not significantly different ($P > 0.05$) when tested by a least significant range test (Sokal & Rohlf 1981).

[b] Fem, total number of *Cx. tarsalis* females tested for virus infection; pools, number of pools; pos, number of pools positive for SLE virus.

[c] Flock of 20 hens; the rest had 10 birds each.

inspection of time series correlations. Effects of landscape features such as site distance from the Whitewater Channel and the north shore of the Salton Sea, and elevation on *Cx. tarsalis* abundance were examined by regression analyses. Sites also were grouped into nine habitat types and compared by a two-way ANOVA with habitats and years as main effects.

Mosquito virus infections were expressed as minimal infection rates per 1,000 *Cx. tarsalis* females tested (Chiang & Reeves 1962). Comparison of seroconversions among years and sites was complicated by sentinel mortality, sampling frequency, and the replacement of seropositive birds after a confirmatory bleed. In 1990, birds were not replaced, and seroconversions were expressed as the percentage of seroconversions per flock. In 1991 and 1992, seroconversion rates (percentage of new antibody positives/seronegative birds at the onset of the sampling interval) were used in time series comparisons, whereas the percentage of positive sera/sera tested per site per season was used in spatial comparisons. The number of sera tested in 1992 was approximately twice that in 1991, because birds were bled every 2 wk rather than every 4 wk, respectively.

Results

***Culex tarsalis* and Virus Activity, 1990.** During 1990, habitat characteristics and juxtaposition to breeding habitat resulted in significant heterogeneity in *Cx. tarsalis* abundance among sites within a 7-km radius of permanent monitoring site 1 (Table 1). Abundance was highest at sites 1 and 3 within or adjacent to duck ponds and lowest at sites 4 and 5 in mixed agricultural and residential habitats to the east. With the exception of site 5, where a vernal increase in abundance was not observed, the temporal patterns of *Cx. tarsalis* abundance were similar at the remaining four sites ($r > 0.588$, df = 12, $P < 0.01$).

Only a single SLE virus infection was detected, despite the testing of 20,493 *Cx. tarsalis* females (Table 1). However, 23 sentinel chickens (38.3%, $n = 60$) seroconverted to SLE virus at four of the five sites. Because positive sentinel chickens were not replaced, the number of seroconversions per flock was most likely an underestimate of virus transmission activity, especially at site 3, where eight of 10 sentinels seroconverted. Seroconversion rates seemed to be independent of flock size, because the number of seroconversions at site 1 with 20 hens was the same as at nearby site 3 with only 10 hens. WEE virus was not detected in California during 1990 (Emmons et al. 1991).

***Culex tarsalis* Abundance Patterns, 1991–1992.** The pattern and number of *Cx. tarsalis* collected by CO_2 traps were consistent during 1991 and 1992 (Table 2). A two-way ANOVA for each year blocked by sampling date indicated that *Cx. tarsalis* abundance varied significantly among trap sites (Table 2). An analysis of covariance (ANCOVA) with years as the group effect compared females per trap night per site per year as a function of site distance from the shore of the Salton Sea. There was no significant difference ($P > 0.05$) between years or slope values, and the pooled model II regression provided a good fit for the data ($b = -0.11$, df = 31, $t = 10.4$, $P < 0.001$ [Fig. 3A]). The decrease in abundance with distance from the shore of the Salton Sea was curvilinear, because the coefficient of determination calculated using backtransformed means ($R^2 = 0.22$) was considerably lower than that using the $ln(y + 1)$ transformed values ($R^2 = 0.78$). Similarly, *Cx. tarsalis* abundance decreased as a negative curvilinear function of elevation in meters (Fig. 3B); i.e., there was no significant differences ($P > 0.05$) between years or slopes, and the pooled regression equation fit the data well ($b = -0.02$, $R^2 = 0.62$, df = 31, $t = 7.16$, $P < 0.001$). In contrast, there was no significant relationship between *Cx. tarsalis* abundance and site distance from the Whitewater Channel ($R^2 = 0.06$, $P > 0.05$).

Sites were grouped into nine habitat categories based on dominant terrain features or vegetation (Table 2) and were analyzed using a two-way ANOVA with years and habitats as main effects. There was no significant difference between years ($F < 0.12$; df = 1, 15; $P > 0.05$), but abundance varied

Table 2. Habitat characteristics, vector abundance, and virus activity at 19 study sites in Coachella Valley, 1991–1992.

Site no.	Group[a]	Habitat[b]	Elevation, m	*Cx. tarsalis* abundance (fem/TN)[c]		*Cx. tarsalis* MIR[d]						Seroconversions, %[e]			
						1991			1992			1991		1992	
				1991	1992	No. (Pools)	W	S	No. (Pools)	W	S	W	S	W	S
1	B	Duck ponds, dates, desert	−67	104.1ab	79.0b	5,890 (124)	0	0	5,744 (117)	0	0	8.9	6.0[f]	0.7	2.7
2	B	Duck ponds, saline marsh	−69	158.3a	247.2a	5,106 (109)	0	0	6,750 (138)	0.3	0.3	7.6	5.2	0.0	2.3
3	B	Scrub (1991), row crops (1992)	−63	106.4ab	38.8bc	3,722 (80)	0.3	0.3	3,129 (69)	0	0	6.5	6.4	0.7	0.7
4	B	Grapes-citrus, dates	−52	16.2de	31.3bc	2,096 (44)	0.5	1.4	3,268 (72)	0	0	1.2	13.2	0.7	1.3
5	B	Residential	−56	6.2e	18.4bc	295 (11)	0	0	1,289 (32)	0	0	2.4	7.5	0.9	0.9
6	B	Dates, grapes, citrus	−60	8.2e	18.2bc	421 (12)	0	0	911 (25)	0	0	3.1	11.0	0.7	6.8
7	A	Saline marsh, desert	−67	26.8cd	33.2bc	1,950 (47)	1.5	0	2,665 (60)	0	0	4.1	9.0	6.0	4.1
8	A	Saline marsh, grapes-citrus	−54	44.7abc	54.6bc	2,792 (62)	2.5	0	3,775 (85)	0.5	0.3	11.7	10.4	2.1	10.3
9	B	Row crops, desert	−53	46.6bc	68.9bc	2,573 (56)	1.5	0	4,902 (102)	0.2	0	9.4	8.7	0.0	1.3
10	B	Dates	−47	7.4e	14.0c	257 (12)	0	0	1,132 (33)	0.9	0	7.5	3.1	0.0	0.0
11	C	Irrigated pasture	−8	1.4fg	3.1d	23 (3)	0	0	263 (17)	0	0	2.1	0.0	0.0	0.0
12	C	Citrus, row crops	12	1.5f	3.6d	97 (4)	0	0	242 (17)	0	0	5.1	0.5[f]	0.0	6.5
13	C	Sewage plant, sod farm	110	0.3g	1.1de	24 (2)	0	0	74 (7)	0	0	0.0	0.0	0.0	0.0
14	C	Dates, irrigated pasture	−24	1.2fg	ND	170 (10)	0	0				2.1	0.0[f]		
15	B	Dates	−27	3.5f	4.3d	50 (5)	0	0	324 (18)	0	3.1	7.5	0.0	0.0	0.0
16	C	Palm oasis (dates)	165	ND	2.0d				132 (11)	0	0			0.0	0.0
17	C	Desert, horse stables	85	ND	0.2e				7 (2)	0	0			0.0	0.0
18	C	Sewage plant, residential	112	ND	0.1e				79 (2)	0	0			0.0	0.0
19	C	Desert, residential	171	ND	0.2e				0 (0)					0.0	0.0
Totals				31.2	29.1	25,466 (581)	0.6	0.2	34,805 (819)	0.2	0.1	5.3	4.6	0.6	1.9

[a] Groups A–C designated by principal components analysis (see Figs. 4 and 5).
[b] Nine main vegetation–habitat categories used in the ANOVA on habitat underlined. Adjacent secondary types are also listed.
[c] Geometric mean number of *Cx. tarsalis* females per trap night from March through October. Means within columns followed by the same letter are not significantly different ($n = 17$, $P > 0.05$; Duncan's multiple range test [Sokal & Rohlf 1981]); ND, not done.
[d] MIR, minimum infection rate per 1,000 *Cx. tarsalis* females; No., number of females tested (pools tested in parentheses); W, WEE virus; S, SLE virus.
[e] Percentage of sentinel chicken sera positive each year for WEE (W) and SLE (S) viruses.
[f] Flock of 20 sentinel chickens.

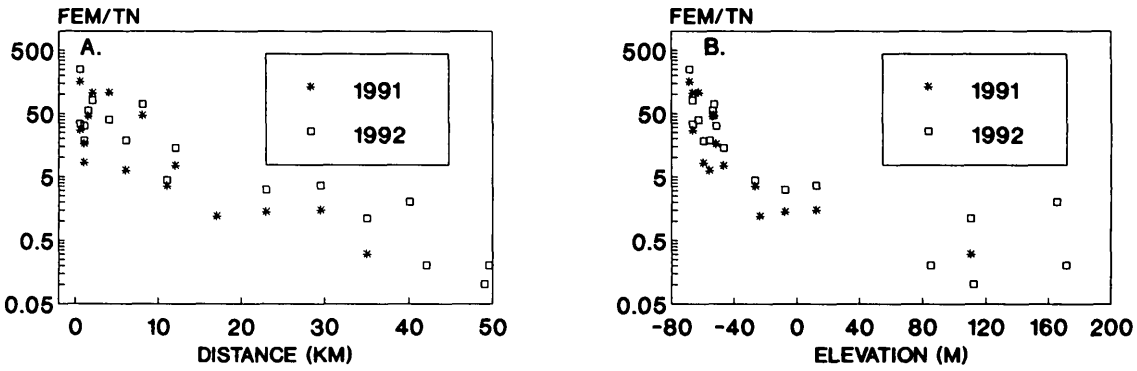

Fig. 3. *C. tarsalis* relative abundance in geometric mean numbers of females per CO_2 trap night per site per season (FEM/TN) plotted as a function of study site (A) distance from the margin of the Salton Sea in kilometers and (B) elevation in meters.

significantly among habitat categories ($F = 12.03$; df = 8, 15; $P < 0.001$). When grouped by an a posteriori multiple range test, abundance was significantly ($P < 0.05$) highest at duck club (back-transformed or geometric mean, 133.8 *Cx. tarsalis* per trap night), row crop (48.9), and saline marsh (38.0) habitats and lowest at sewage treatment plants (0.3).

Sites were divided into groups A–C based on annual *Cx. tarsalis* abundance (Table 2), temporal patterns (principal components analyses [Figs. 4 and 5]), inspection of time series correlations, and geographical proximity (Fig. 1). In general, abundance was highest in groups A and B near the Salton Sea or adjacent agricultural habitat and lowest in group C in the northwestern portion of the valley. The first two principal components explained 61 and 64% of the cumulative variance among the temporal patterns of females at the 15 and 18 sites sampled during 1991 and 1992, respectively.

Group A sites 7 and 8 were located relatively near the Salton Sea, but >7 km distant from duck club ponds and >15 km distant from each other. Increased depth of the Salton Sea during winter inundated marginal saline marshes and produced large vernal populations of *Cx. tarsalis* (Figs. 4A and 5A). Increased evaporation and decreased rainfall lowered the depth of the Salton Sea, dried adjacent marsh breeding habitats, and decreased the abundance of the *Cx. tarsalis* population during summer. Populations here remained low during fall and winter.

Culex tarsalis abundance at group B sites increased slightly during spring, similar to abundance at group A sites, but abundance patterns were dominated by a large late summer–early fall peak associated with the flooding of duck ponds in preparation for the fall hunting season (Figs. 4B and 5B). Although ponds remained flooded throughout the winter, peak *Cx. tarsalis* production occurred during the first month after flooding.

Culex tarsalis production at the northern group C sites was intermittent and the result of focal water mismanagement, mostly by agriculture (Fig. 4C and 5C). Abundance at these sites remained low throughout the year.

Virus Activity, 1991–1992. Both WEE and SLE viruses were active in Coachella Valley during 1991 and 1992 (Table 2). Minimum infection rates were low during both years for WEE and SLE viruses: 1991, 0.59 and 0.16; 1992, 0.17 and 0.12 infected females per 1,000 *Cx. tarsalis* tested, respectively. Similar to 1990, virus infection rates in *Cx. tarsalis* seriously under represented the distribution and intensity of WEE and SLE virus activity when compared to sentinel seroconversion rates. *Cx. tarsalis* pools positive for WEE virus were detected at five and four sites, whereas sentinel seroconversions to WEE virus were detected at 14 and 7 of the 15 and 18 localities sampled during 1991 and 1992, respectively. In 1992 when WEE virus activity was comparatively low, pools positive for WEE virus were collected at sites 2, 9, and 10 in the absence of sentinel seroconversions. Similarly, *Cx. tarsalis* pools were positive for SLE virus at only two and three sites, whereas sentinels seroconverted at 11 and 10 sites during 1991 and 1992, respectively.

Seroconversion rates were higher during 1991 than during 1992 for both viruses (Table 2). During 1991 when birds were bled every 4 wk, 87 (5.3%) of 1,653 sera and 77 (4.6%) of 1,669 sera were positive for WEE and SLE viruses, respectively. During 1992 when birds were bled every 2 wk, 16 (0.6%) of 2,671 and 49 (1.9%) of 2,610 sera were positive for WEE and SLE viruses, respectively. The numbers of sera tested reflected the total numbers of seronegative birds bled per site per sample and ranged from 68 to 100 in 1991 and from 117 to 160 in 1992 for flocks of 10 birds each and from 178 to 199 in 1991 for flocks of 20 birds.

During 1991, sentinel flocks at sites 1, 12, and 14 each consisted of 20 birds, whereas the remaining flocks each consisted of 10 birds (Table 2). When tested by one-way ANOVA, the mean number of seroconversions per season at 20 bird flocks

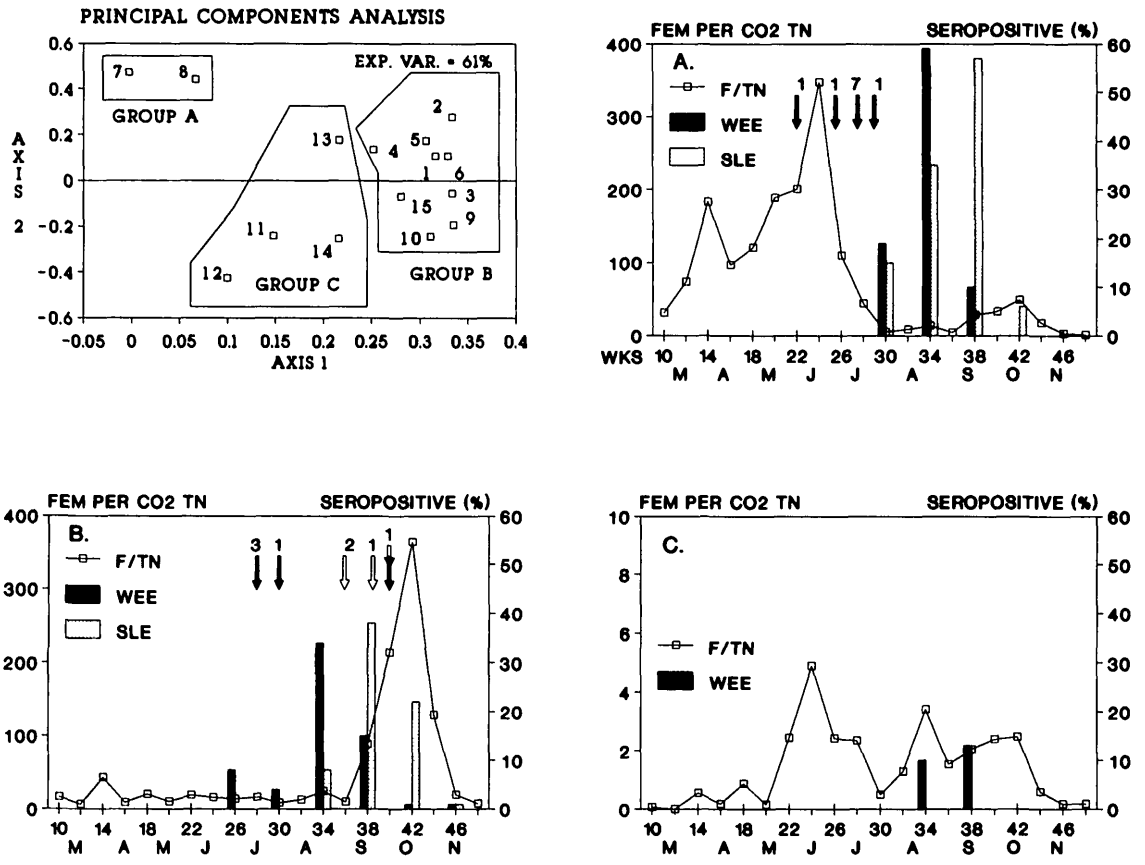

Fig. 4. Principal components analysis segregating 15 study sites in Coachella Valley into groups A–C based on similarities among the seasonal patterns of *Cx. tarsalis* female abundance during 1991 (upper left panel). Also shown are biweekly mean numbers of *Cx. tarsali* females per CO_2 trap night (F/TN) and the percentage of sentinel chickens seroconverting to WEE or SLE viruses on each bleeding date for groups A, B, and C. Arrows indicate the total number of pools of *Cx. tarsalis* females positive for WEE (black arrows) or SLE (white arrows) viruses on each sampling date.

(WEE = 9.7; SLE = 6.4; n = 3) was not significantly greater ($P > 0.07$) than the mean number of seroconversions at 10 bird flocks (WEE = 4.8, SLE = 5.4; n = 12), indicating that the number of seroconversions per flock depended on site location rather than on the number of birds. There also was no significant differences ($P > 0.16$) in the number of seroconversions when the three 20-bird flocks were compared with the nearest flock of 10 birds (sites 3, 15, and 11 [Fig. 1]).

Annual seroconversion rates to WEE virus per site increased significantly as a curvilinear function of *Cx. tarsalis* abundance during 1991 (b = 1.22, R^2 = 0.45, df = 13, t = 3.24, P = 0.006), but not during 1992 (R^2 = 0.11, P = 0.188) when WEE virus activity was comparatively low. In an ANCOVA, both years (F =30.8; df = 1, 30; $P < 0.001$) and regression coefficients (F = 7.7; df = 1, 29; P = 0.009) were significantly different (Fig. 6A). A significant relationship between sentinel seroconversion rates to SLE virus and *Cx. tarsalis* abundance per site was observed during 1991 (b = 1.56, R^2 = 0.41, df = 13, t = 3.00, P = 0.01) and 1992 (b = 0.56, R^2 = 0.18, df = 16, t = 1.86, P = 0.08); years and slopes were not significantly different in an ANCOVA ($P > 0.05$) (Fig. 6B). Based on these data, it was not possible to establish thresholds of *Cx. tarsalis* abundance required for virus transmission to sentinel chickens. During 1991 when seroconversion rates were high, viruses were transmitted actively at all sites having a geometric mean annual abundance >1 *Cx. tarsalis* female per trap night; however, during 1992 when virus transmission levels were lower, viruses only were active at sites having >10 females per trap night (Fig. 6 A and B). During both years, seroconversion rates were somewhat lower for both viruses at sites with vector abundance levels >100 females per trap night.

Because of colinearity with *Cx. tarsalis* abundance, seroconversion rates to SLE virus in 1991 decreased as an inverse function of flock distance from the shore of the Salton Sea (b = −11.98, R^2 = 0.64, df = 13, t = 4.77, $P < 0.01$); however, increased WEE and SLE virus activity at site 12 in 1991 and 1992, and low seroconversion rates to

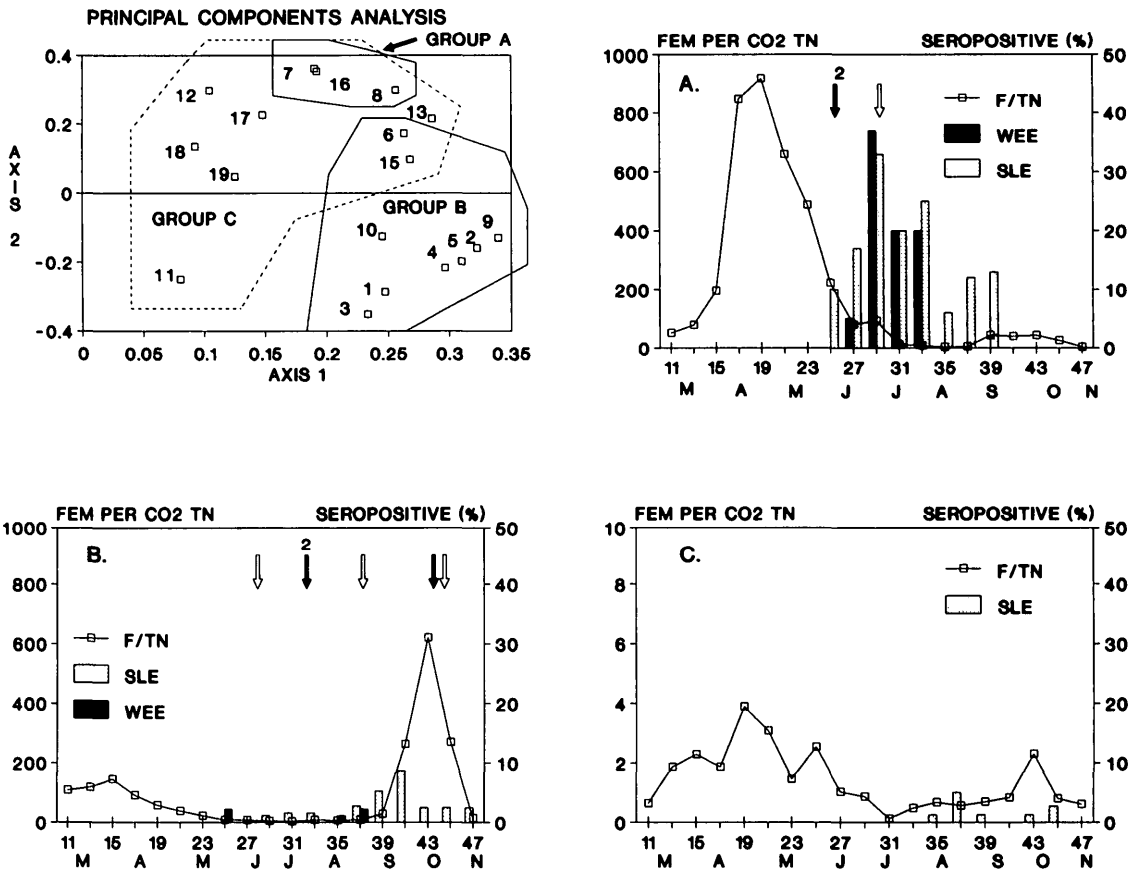

Fig. 5. Principal components analysis segregating 18 study sites in Coachella Valley into groups A–C based on similarities among the seasonal patterns of *Cx. tarsalis* female abundance during 1992 (upper left panel). Also shown are biweekly mean numbers of *Cx. tarsalis* females per CO_2 trap night (F/TN) and the percentage of sentinel chickens seroconverting to WEE or SLE viruses on each bleeding date for groups A, B, and C. Arrows indicate the total number of pools of *Cx. tarsalis* females positive for WEE (black arrows) or SLE (white arrows) viruses on each sampling date.

WEE virus in 1992 led to the nonsignificant correlations of seroconversion rates with distance ($R^2 < 0.18$, $P > 0.05$ [Fig. 6C and D]). The relationship between seroconversion rates to WEE virus and elevation differed between years ($F = 22.3$; df = 1, 30; $P < 0.001$), and explained only a small percentage of the variability in seroconversions among sites ($R^2 < 0.24$). Although the relationship between SLE seroconversions and elevation was similar between years and the overall regression function was significant ($b = -0.03$, $t = 3.16$, df = 31, $P = 0.003$), relatively little variability in the seroconversion rates to SLE virus was explained by elevation ($R^2 = 0.24$).

Temporal patterns of WEE and SLE virus activity were consistent during 1991 and 1992. In 1991, WEE virus was first detected near saline marshes at group A sites during week 22 in late May, when one of 17 pools of *Cx. tarsalis* was positive at site 7; however, sentinels did not seroconvert at group A sites until week 30 (Fig. 4A). WEE virus was first detected at group B sites when sentinels seroconverted during week 26, 2 wk before infections were detected in mosquitoes. Seroconversions to WEE virus occurred at all sites in August, except group C sites 11 and 13 to the northwest and site 5 in the town of Mecca. Hens at site 11 seroconverted during September, whereas hens at site 5 seroconverted during October and November after WEE virus activity had disappeared from the rest of Coachella Valley.

Although the overall distribution of SLE virus during 1991 was limited to sites south of site 10, the temporal pattern of SLE virus activity was similar to that of WEE virus (Fig. 4). SLE virus first was detected during week 30, when three of 10 sentinels seroconverted at group A site 7 (Fig. 4A). By week 34 in August, SLE virus had spread to group B sites where both mosquito pools and sentinels were positive. SLE virus remained widespread during September and October and was last detected at site 2 in December 1991 and at sites 6 and 12 in January 1992.

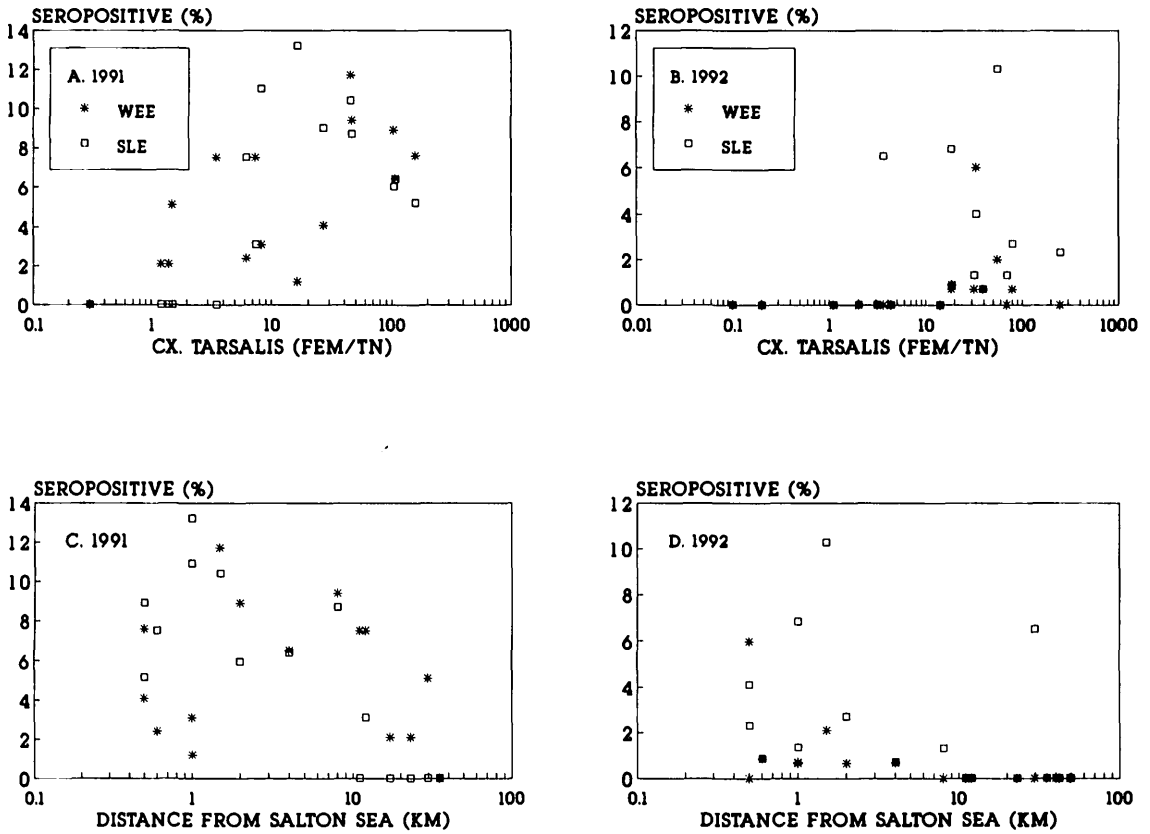

Fig. 6. Sentinel seroconversion rates to WEE or SLE viruses during each year plotted as a function of *Cx. tarsalis* abundance (geometric mean number of females per CO_2 trap night per site per year [FEM/TN]) during (A) 1991 and (B) 1992 or flock distance from the Salton Sea in kilometers during (C) 1991 or (D) 1992.

WEE virus was detected first during 1992 in a *Cx. tarsalis* pool collected at site 9 on 27 February. This winter recovery was considered to be a carryover of virus from 1991, because WEE virus was last active at neighboring site 5 in 1991 and was not detected again until 1 June 1992 when single sentinel seroconversions occurred at sites 5 and 6. WEE virus activity remained at a low level throughout 1992 (Fig. 5) and was restricted to sites near the Salton Sea, with the exception of a single virus-positive *Cx. tarsalis* pool collected at site 10 on 10 August. WEE virus was last detected in a pool of *Cx. tarsalis* collected at site 2 in October.

In 1992, SLE virus was detected first at group A site 8 during week 25, when two sentinels seroconverted (Fig. 5A). SLE virus remained active at group A sites through 7 September. SLE virus appeared first at group B sites during week 27 (Fig. 5B). SLE virus also was focally active at low levels at group C site 12 from 10 August through 19 October (Fig. 5C), and was last detected at site 7 on 15 December.

Discussion

Patterns of Virus Activity. Consistent patterns emerged from the spatial and temporal heterogeneity in vector and virus activity. WEE and SLE virus transmission was related closely to the dynamics of *Cx. tarsalis* populations, which, in turn, were related to the creation of natural and manmade larval habitats during spring and late summer. Vernal increases in *Cx. tarsalis* abundance at group A sites and, to a lesser extent, group B sites were associated with the annual rise in the depth of the Salton Sea. Winter rain, vernal snow melt, and reduced evaporation increased the depth of the Salton Sea during the December through May period, inundating adjacent low-lying areas and creating a belt of saline marsh habitat near group A sites. Even though salinity levels exceeded 9,000 ppm (W.K.R., unpublished data), these marshes still produced large populations of *Cx. tarsalis*. Reduced run-off and increased evaporation during summer lowered the depth of the Salton Sea, dried the salt marshes, and caused *Cx. tarsalis* populations to decrease markedly and to remain low for the remainder of the year. Elevated *Cx. tarsalis* vernal abundance was followed by virus amplification, and both WEE and SLE viruses were detected first at group A sites during both years. Although elevated *Cx. tarsalis* abundance seemed critical for virus amplification, both WEE and SLE

viruses appeared during or after the periods when *Cx. tarsalis* populations decreased and were in comparatively low abundance.

The autumnal increase in *Cx. tarsalis* at group B sites was associated with the late summer through fall flooding of duck ponds for migratory waterfowl. Secondary vernal *Cx. tarsalis* population increases in this area were dependent on winter rainfall, flooding by the Whitewater Channel, or agricultural irrigation. Arbovirus activity typically commenced in this area after detection at group A sites, but before the autumnal increase in *Cx. tarsalis* abundance.

Culex tarsalis abundance and virus activity remained lower and appeared later in the summer at group C sites than at group A or B sites during both 1991 and 1992, with the possible exceptions of site 14 during 1991 and site 12 during 1992. Disjunct virus activity at these sites may be related to either mosquito movements or virus dissemination by infected birds. Virus activity appeared to be dependent upon focal mosquito production from agricultural sources.

Surveillance. Results of our research were useful in developing surveillance programs to monitor the enzootic transmission of WEE and SLE viruses. At low or moderate levels of WEE or SLE virus activity, monitoring seroconversion rates in sentinel chickens provided a more sensitive indication of virus activity than testing pools of mosquitoes for virus infection. However, bleeding frequency was critical to the timely detection of activity, especially when virus activity was elevated. Bleeding every 4 wk was unsatisfactory, because detection of transmission may be delayed by as much as 8 wk after the sentinel was bitten by an infectious mosquito (e.g., seroconversions to WEE virus at group A sites in 1991).

Flock location rather than the number of birds per flock was most critical in detecting virus activity. Flocks placed relatively near breeding habitats in vegetation attractive to host-seeking *Cx. tarsalis* had more seroconversions than those placed away from breeding habitats or in habitat(s) less attractive to host-seeking *Cx. tarsalis*. Previous studies indicated that *Cx. tarsalis* feeds most frequently on birds (Reeves et al. 1963) and hunts blood meals most frequently along vegetative ecotones or in tree canopy rather than within dense riparian vegetation or in open pasture (Meyer et al. 1991).

Flocks of 20 hens did not have more seroconversions than adjacent flocks of 10 hens. However, further studies are needed to determine the threshold below which further reductions in flock size will reduce flock attractancy to host-seeking *Cx. tarsalis* females. The Florida arbovirus surveillance program, for example, uses flocks of six chickens that are bled weekly (O'Bryan & Jefferson 1991). The catch of host-seeking *Cx. tarsalis* was shown to decrease when CO_2 release rates were decreased from 2,500 to 25 ml/min (Reeves 1953, Pfuntner et al. 1988); therefore, below a critical threshold, small flocks may attract fewer females than large flocks.

Sampling in Coachella Valley should be stratified spatially to monitor virus activity during vernal amplification and summer dissemination. Placing sentinels along the Salton Sea would monitor early-season amplification, near duck clubs would monitor virus dissemination and summer maintenance, and in residential areas (e.g., the towns of Mecca, Coachella, and Indio) would monitor the risk of human infection as well as the epizootic spread of transmission. Sampling intensity and the allocation of resources to each stratum will depend upon funding levels, the risk of transmission to humans, and the lead time required to muster adult control efforts to interrupt epidemic transmission. Our research failed to establish thresholds of *Cx. tarsalis* abundance or virus infection rates necessary for virus transmission to sentinel chickens. In Coachella Valley, WEE virus transmission in 1991 occurred at sites with a geometric seasonal mean abundance of >1 *Cx. tarsalis* females per CO_2 trap night, but in 1992 this minimum increased to >10 females per trap night. Transmission of SLE virus required >5 females per trap night during both years. Similar to Olson et al. (1979), the relationship between vector abundance and virus transmission rates to sentinel chickens was not linear, and sites with very high *Cx. tarsalis* abundance exhibited lower seroconversion rates than sites with intermediate abundance. The elevated abundance of host-seeking females may reduce the efficiency of enzootic virus transmission among birds by causing them to exhibit mosquito avoidance behaviors (Edman et al. 1972, Nelson et al. 1976), resulting in a shift in *Cx. tarsalis* host selection to include proportionately more mammals (Reeves 1971). In addition, virus activity levels appeared to be independent of both the timing and amplitude of vernal and autumnal increases in *Cx. tarsalis* abundance, similar to previous observations (Reisen et al. 1992c). Therefore, although seasonal sentinel seroconversion rates were correlated spatially among sampling sites with *Cx. tarsalis* seasonal abundance, it was not possible to establish thresholds in vector abundance that were realistic targets for vector suppression or were predictive of virus transmission activity. Other factors that may be important in determining the level of virus activity include the impact of temperature on the duration of the virus extrinsic incubation period and modulation (Reisen et al. 1993) and the duration of the gonotrophic cycle (Reisen et al. 1992d) in the vector, vector and reservoir susceptibility to infection (Hardy & Reeves 1990a, b), herd immunity in bird populations, and the virulence of the virus strain(s) currently circulating in the area (e.g., Bowen et al. 1980, Monath et al. 1980, Mitchell et al. 1983, Hardy & Reeves 1990b).

Despite elevated epizootic transmission rates of WEE and SLE viruses, human or equine cases were not detected in Coachella Valley by the EVS

program during 1990–1992. In fact, only three human SLE and no equine or human cases of WEE were detected in Coachella Valley during the past 10 yr. SLE cases were reported from Banning, Palm Springs, and Indio in Coachella Valley in 1984 (Murray et al. 1985). This lack of clinical disease was unexpected, because comparable seroconversion rates in sentinel chickens historically have been associated with human illness elsewhere in California (Reeves 1990). Possible explanations for the absence of human cases include the following: (1) humans are not being infected, because they infrequently contact infected mosquitoes; (2) virus strains circulating in southeastern California are attenuated and infections rarely produce central nervous system disease; or (3) cases remain undetected or are listed as undiagnosed aseptic meningitis or viral encephalitis. These topics currently are being addressed by our on-going research.

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