SURVEILLANCE OF ST. LOUIS ENCEPHALITIS VIRUS VECTORS IN GRAND JUNCTION, COLORADO, IN 1987

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ABSTRACT. Grand Junction, Colorado, was the site of a St. Louis encephalitis (SLE) outbreak in 1985. Epidemiologic and ecologic investigations in 1985 and 1986 suggested that *Culex tarsalis* may not have been the exclusive vector in the outbreak and that *Cx. pipiens* may have contributed to transmission as an accessory vector. A limited field study in 1987 generally confirmed observations from 1986 that *Cx. pipiens* was more abundant than *Cx. tarsalis* in late summer when SLE virus transmission normally occurs. In both years, infection rates in *Cx. tarsalis* were higher than in *Cx. pipiens*, but in 1987 the only SLE virus isolate from *Cx. pipiens* was obtained early in the season. Truck trap collections showed that *Cx. pipiens* was the principal vector species collected, comprising 86% of the total. Light trap collections underestimated the population of *Cx. pipiens*; gravid trap collections gave a closer approximation of the relative proportions of *Cx. pipiens* and *Cx. tarsalis* in the vector mosquito population after midsummer.

INTRODUCTION

In the rural western United States, St. Louis encephalitis (SLE) virus is believed to circulate perennially in an enzootic cycle among birds and *Culex tarsalis* (Coq.) mosquitoes, resulting in a low level of endemic transmission to the resident human population (Monath 1980, Tsai and Mitchell 1988, Mitchell et al. 1980, Reeves and Hammon 1962). Periodically, small outbreaks have occurred, chiefly in rural areas and often concurrently with outbreaks of western equine encephalitis (WEE) (Monath 1980, Tsai et al. 1987b, Tsai and Mitchell 1988a; Monath and Tsai 1987). *Cx. tarsalis* generally has been considered to be the principal SLE virus vector in these outbreaks (Monath 1980, Mitchell et al. 1980, Tsai et al. 1987b).

An epidemiologic investigation of an SLE outbreak occurring in Grand Junction, Colorado, in 1985 and subsequent entomologic studies there produced evidence that *Cx. pipiens* (Linn.) mosquitoes may have been an accessory vector in that outbreak (Tsai et al. 1987b, 1988). In 1986, mosquito and viral surveillance in Grand Junction indicated that *Cx. pipiens* were far more abundant than *Cx. tarsalis* late in the summer. However, infected *Cx. pipiens* and *Cx. tarsalis* were equally numerous in late summer, especially in urban areas where the 1985 outbreak was focused, suggesting that *Cx. pipiens* could have contributed to transmission of SLE virus to the urban population of Grand Junction (Tsai et al. 1988).

Results of surveillance in 1986 demonstrated the importance of operating both gravid traps and light traps. Far more *Cx. pipiens* were caught in gravid traps than in light traps, and the use of light traps alone would have resulted in an underestimation of the *Cx. pipiens* population (Tsai et al. 1988). In this communication, we report the results of mosquito and viral surveillance in Grand Junction through another season.

MATERIALS AND METHODS

Field studies during 1987 in Grand Junction, Colorado, were limited to three collections during the season. These cross-sectional surveys attempted to estimate seasonal changes in the populations of *Cx. pipiens* and *Cx. tarsalis* mosquitoes to assess their role as potential vectors of SLE virus and to monitor SLE viral infection rates in the vectors.

The study area was described previously (Tsai et al. 1987b, 1988). Arthropods were collected during week 30 (July 26–August 1), week 34 (August 23–29) and week 37 (September 13–19) at nine sites that included eight of the sites used in 1986. The nine sites represented three types of habitat: 1) two rural sites outside the city limits, 2) three urban sites inside the city limits near residences of patients who were cases in the 1985 epidemic and 3) four intermediate sites located on the periphery of the city. Collections were made 3–5 nights in each of the three study periods with CDC light traps baited with CO_2 and CDC gravid traps supplemented with 5- to 10-day-old hay infusion (Reiter 1983). In addition, a truck trap was operated during the evening crepusular hours when weather permitted, beginning 15 minutes before sunset until 1 hour after sunset (Holbrook and Wuerthele 1984). The three truck trap routes were in rural, intermediate and urban areas that permitted unin-

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terrupted 10-minute collections over 3.2 km routes.

Collections were processed using standard methods (Sudia and Chamberlain 1967). Collections were stored on dry ice; and, weekly, specimens were transferred to mechanical freezers at the Division of Vector-Borne Viral Diseases Laboratory, Centers for Disease Control, Fort Collins, Colorado. Mosquitoes from each collection were pooled by species, and after trituration they were inoculated into newborn mice for arboviral isolation. Pools of *Culex* mosquitoes also were inoculated into C6/36 cell cultures. After 5 days, C6/36 cell culture fluids were passed blindly into Vero cell cultures for plaque assay. *Culex* pools from weeks 34 and 37 also were assayed for SLE viral antigen using an enzyme immunoassay (EIA) (Tsai et al. 1987a).

RESULTS

Light traps collections resulted in a diversity of mosquito species (Table 1). Collections were balanced between *Aedes* species, which constituted 56% of the collection, and *Culex* mosquitoes, comprising 40% of the total. Gravid traps were more selective in the mosquito species that they attracted, with *Cx. pipiens* composing 85.5% of the collection (Table 1). *Culex pipiens* also was the principal species collected in truck traps, comprising 86.5% of the SLE vectors collected (Tables 1 and 2). All but 12 of the 224 *Cx. pipiens* collected by truck were from the routes through rural-intermediate habitat.

A clear trend was seen in light and gravid trap collections toward a decline in the *Cx. tarsalis* population from midsummer (after week 30) (Table 2). The number of female *Cx. tarsalis* collected per trap night in light and gravid traps declined monotonically in the three collection intervals. Although the numbers collected were small, *Cx. tarsalis* in the truck trap collections declined by >50% from week 30 to week 37. A significant decline between weeks 34 to 37 was not seen (Table 2).

The population of *Cx. pipiens*, estimated by light and gravid traps, was stable during the first two collection intervals but it also fell in week 37, although the decline was to a lesser extent than the decrease in numbers of *Cx. tarsalis*

Table 1. Summary of arthropods collected in Grand Junction, Mesa County, Colorado, 1987.

Collection method	Genus and species	No. tested	Pools tested
CDC light trap	*Aedes dorsalis*	589	31
	Ae. nigromaculis	86	19
	Ae. vexans	2516	69
	Culex pipiens	1243	62
	Cx. restuans	55	2
	Cx. tarsalis	977	54
	Culiseta inornata	134	29
	Other species (5)	88	33
		Total 5688	299
Gravid trap	*Ae. dorsalis*	9	3
	Ae. vexans	51	11
	Cx. pipiens	1331	69
	Cx. tarsalis	110	23
	Culex (*Culex*) species	1	1
	Cs. inornata	30	8
	Other species (2)	25	10
		Total 1557	125
Truck trap	*Ae. dorsalis*	28	14
	Ae. nigromaculis	6	5
	Ae. vexans	39	13
	Cx. pipiens	224	32
	Cx. tarsalis	35	17
	Culex (*Culex*) species	2	2
	Cs. inornata	4	4
	Anopheles freeborni	11	6
		Total 349	93
		Grand total 7594	517

(Table 2). *Culex pipiens* in truck trap collections were similar in the first two collection periods but, contrary to the trend seen in light and gravid traps, increased 70% from week 30 to week 37.

Compared with the results of truck trap collections, light traps consistently underestimated the abundance of *Cx. pipiens* (Table 2). The proportion of *Cx. pipiens* in gravid trap collections, however, was within the 95% confidence interval of the estimate provided by the truck trap in each week. Combining abundance data from gravid and light traps led to an underestimation of *Cx. pipiens* except in week 34.

Only four SLE virus strains were recovered, one from *Cx. pipiens* and three isolates from *Cx. tarsalis* (Table 3). The minimum infection rate (MIR) for *Cx. tarsalis* was 2.8/1000 (3/1087), and for *Cx. pipiens* it was 0.4/1000 (1/2574) ($P < 0.01$ Poisson distribution). St. Louis encephalitis virus was recovered from *Cx. pipiens* only in week 30 (MIR, 2.4/1000; 13.5 infected mosquitoes/1000 trap nights). SLE virus strains from *Cx. tarsalis* were recovered in the later two collection periods, (MIRs of 6.8 and 14.7/1000, respectively). The number of infected *Cx. tarsalis* per trap night was highest in mid-season (16.6 infected mosquitoes/1000 trap nights) and fell in week 37 (9.7 infected mosquitoes/1000 trap nights), despite a greater infection rate in the *Cx. tarsalis* population. The reduced numbers of infected *Cx. tarsalis* collected per 1000 trap nights in week 37 reflected the small size of the population in late summer. All four SLE virus strains recovered in 1987 were detected only in newborn mice. A prior amplification step

Table 2. *Culex pipiens* and *Cx. tarsalis* collections by method, Grand Junction, Colorado, 1987.

Collection			Female mosquitoes collected		*Cx. pipiens* as a proportion of total (%)
Method	Wk	Trap nights or hr[a]	*Cx. tarsalis*	*Cx. pipiens*	
CDC light trap	30	49	615 (12.6)[b]	424 (8.6)	40.8
	34	78	294 (3.8)	620 (7.9)	67.8
	37	67	68 (1.0)	199 (3.0)	74.5
Total		194	977 (5.0)	1243 (6.4)	56.0
Gravid trap	30	25	82 (3.3)	353 (14.1)	81.1
	34	42	24 (0.6)	674 (16.0)	96.6
	37	36	4 (0.1)	304 (8.4)	98.7
Total		103	110 (1.1)	1331 (12.9)	92.4
Truck trap	30	3.5	20 (5.7)	62 (17.7)	75.6 ± 9.3[c]
	34	3.9	9 (2.3)	73 (18.7)	89.0 ± 6.8
	37	2.8	6 (2.1)	89 (31.8)	93.7 ± 4.9
Total		10.2	35 (3.4)	224 (21.6)	86.5 ± 4.2

[a] Hr are shown for truck trap collections.
[b] Female mosquitoes per trap night for light and gravid traps; female mosquitoes per hr for truck trap collections.
[c] 95% confidence intervals on the proportion.

Table 3. Virus strains recovered from mosquito collections Grand Junction, Colorado, July 29–September 19, 1987.

Virus[b]	Species	Pool size	Collection[a]			Test results		
			Wk	Date	Habitat	nbm[c]	C6/36	EIA[d]
SLE	*Cx. pipiens*	12	30	7/29	Intermediate	+	+	nt[e]
	Cx. tarsalis	11	34	8/25	Urban	+	–	–
	Cx. tarsalis	17	34	8/25	Rural	+	+	+
	Cx. tarsalis	24	37	9/19	Rural	+	+	+
WEE	*Cx. tarsalis*	50	30	7/29	Rural	+	+	
TUR	*Cx. tarsalis*	27	30	7/29	Intermediate	+	–	
	Cx. pipiens	19	34	8/25	Urban	+	–	

[a] All viral isolates were made from light trap collections.
[b] SLE (St. Louis encephalitis); WEE (Western equine encephalitis); TUR (Turlock).
[c] nbm = newborn mice.
[d] Enzyme immunoassay.
[e] nt = not tested.

in C6/36 cell cultures did not increase the frequency of virus recovery.

DISCUSSION

The results of mosquito surveillance in Grand Junction in 1987 generally were similar to the observations from earlier studies in 1986 (Tsai et al. 1988); however, only limited interpretations can be placed on the data because collections were made during only three weeks of the season.

Light traps were more successful than gravid traps in attracting *Cx. tarsalis*. Light traps provided 89% and 90% of the *Cx. tarsalis* collected in 1986 and 1987, respectively. Gravid traps collected slightly more *Cx. pipiens* than light traps (57% and 52% in the two years, respectively). In both years, the difference in numbers of female *Cx. pipiens* collected from gravid and light traps was most pronounced in late summer.

Truck traps were operated in an attempt to collect engorged specimens for blood meal identification and to provide a nonattractant sampling estimate of *Culex* mosquito populations. Since the numbers of specimens collected were small, interpretation of the data is necessarily limited. The total of the truck trap collections disclosed that *Cx. pipiens* predominated over *Cx. tarsalis* throughout the season, accounting for 86% (224/259) of the total collections of these two species (95% confidence interval ±4%) (Table 2). This proportion was approximated most closely by the results of gravid trap collections, which showed that *Cx. pipiens* comprised 92.4% of the vectors collected. Moreover, the relative numbers of *Cx. tarsalis* and *Cx. pipiens* were similar to their proportions in truck trap collections at every point in the study period. These observations support our speculation that in Grand Junction, light traps underestimate the true abundance of *Cx. pipiens* and that gravid traps should be operated in conjunction with light traps to assess more accurately the population of *Cx. pipiens* and its role as an SLE virus vector.

Although few mosquitoes were captured in truck collections, the truck trap was more efficient (per unit of effort) than gravid or light traps in collecting *Cx. pipiens*. Each hour of a truck trap collection was a night's effort; an average of 21.6 *Cx. pipiens* was collected per night in the truck trap and an average of 6.4 or 12.9 *Cx. pipiens* was collected per trap night in light and gravid traps, respectively (Table 2). The truck trap was less efficient than light traps but more efficient than gravid traps in collecting *Cx. tarsalis*. Yields per unit of effort in truck traps are highly dependent on the flight activity periods for each species and the truck trapping schedule. Our truck collections were made in the evening, from 15 minutes before sunset to 1 hour after sunset, corresponding to the peak period of biting activity for *Cx. tarsalis* (Beadle, 1959). Although less is known of the biting habits of *Cx. pipiens*, it seems unlikely that the proportionately smaller number of *Cx. tarsalis* caught in the truck collections was biased by the period when the truck trap was operated.

The large yields of *Cx. pipiens* in relation to *Cx. tarsalis* in the truck trap collections were unexpected. *Cx. pipiens* comprised 75.9–93.7% of the potential SLE vectors, outnumbering *Cx. tarsalis* 3–15-fold in the three collections. Grand Junction lies in a rural agricultural valley, and the city is surrounded by irrigated fields and pastures that typify the habitat of *Cx. tarsalis* in the West. The truck trap routes sampled one urban and two rural-intermediate areas at the perimeter of the city's residential and business districts. The large proportion of *Cx. pipiens* in these collections suggests that *Cx. pipiens* is breeding extensively in the city and surrounding areas. These observations indicate that *Cx. pipiens* should not be disregarded as a potential vector of SLE in small towns and cities in the rural West.

In both 1986 and 1987 the peak *Cx. tarsalis* population was in midsummer, and the *Cx. pipiens* population peaked somewhat later. These seasonal differences in abundance resulted in a far larger population of *Cx. pipiens* in relation to *Cx. tarsalis* after week 34 (mid to late August). The SLE viral infection rates in the 2 years were similar, 0.96/1000 in 1986 and 1.20/1000 in 1987. Species-specific infection rates in *Cx. tarsalis* and *Cx. pipiens* in the 2 years also were similar. The infection rates in both years, however, were low; and the number of mosquitoes collected was relatively small; therefore, little confidence can be placed in our ability to detect a true difference in the species specific or total infection rates in the 2 years. Unlike 1986, when there was a progressive increase in infection rates in both *Culex* species, in 1987, the only SLE virus isolate from *Cx. pipiens* was recovered relatively early in the season.

A principal object in operating the truck trap was to obtain engorged *Cx. pipiens* mosquitoes for blood meal identification. The host preferences for *Cx. pipiens* in Grand Junction have not been studied previously. We recognize that before this species can be implicated as a SLE virus vector in Grand Junction, data to prove its anthropophilic feeding habits are needed. Unfortunately, in 1987 we collected only three blood-engorged mosquitoes by truck trap. We had considered, but discarded the idea of conducting human landing-biting collections. We could not ethically subject humans to the potential for exposure to vectors carrying SLE and WEE viruses.

Only longitudinal studies of vector populations and viral infection rates will provide the data needed to understand how changes in these conditions favor epidemic transmission. Our studies in two postepidemic years in Grand Junction were similar in their estimates of the seasonal abundance of *Cx. tarsalis* and *Cx. pipiens*, and the overall infection rates in the two species. In the second year of surveillance, however, infections in *Cx. pipiens* were not observed in late summer, when human infections during an epidemic typically occur. The introduction of SLE virus from the rural *Cx. tarsalis* cycle to an urban *Cx. pipiens* cycle previously has been proposed as the mechanism by which urban SLE outbreaks in the West occur (Luby 1979).

Adequate mosquito data were not available for the outbreak year, 1985, and we can only speculate on the relative importance of the two major *Culex* species as vectors of human disease. The absence of human cases in Grand Junction in 1986 and 1987 may have been related to a relative decline in late summer SLE virus activity among urban *Cx. pipiens* in these years compared with 1985, or conversely the *Cx. tarsalis* population in these nonepidemic years may have remained below a critical level necessary to support epidemic transmission.

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