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SEASONAL OCCURRENCE AND ABUNDANCE OF *AEDES TRISERIATUS* AND OTHER MOSQUITOES IN A LA CROSSE VIRUS-ENDEMIC AREA IN WESTERN NORTH CAROLINA

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ABSTRACT. The species composition and population abundance of mosquitoes were investigated from May to November in 1989 and 1990 on the Cherokee Indian Reservation, an area of western North Carolina endemic for transmission of La Crosse (LAC) virus. Mosquitoes representing 6 genera and 13 species were collected. *Aedes triseriatus* was the most abundant species collected, comprising over 98% of mosquitoes collected by ovitrapping and CO_2-baited suction trapping, and 88% of resting adults collected by vacuuming vegetation and leaf litter. *Culex restuans* and *Anopheles punctipennis* were common but much less frequently collected. Host-seeking and resting *Ae. triseriatus* were most abundant in early to midsummer, whereas gravid *Ae. triseriatus* was most active in the fall. The previous occurrence of cases of LAC encephalitis was concordant with the seasonal activity of gravid but not host-seeking or resting *Ae. triseriatus*.

INTRODUCTION

Aedes triseriatus (Say) is the primary vector and overwintering host for La Crosse (LAC) virus in the midwestern USA (Grimstad 1988). LAC virus, a California serogroup virus, causes an encephalitis in children. In North Carolina, cases of LAC encephalitis generally are reported each year (Szumlas et al. 1996a). North Carolina ranks 8th in the nation with 1.6% of over 1,700 reported cases of encephalitis resulting from California serogroup viruses (Le Duc 1987). All cases have been reported in the Blue Ridge Mountain area of western North Carolina, most prominently from the Cherokee Indian Reservation area of Jackson and Swain counties. Transmission of California serogroup viruses has occurred in the area since at least 1964, when the first encephalitis cases were verified by Kelsey and Smith (1978). Documentation of LAC encephalitis cases on the Cherokee Indian Reservation was provided by Kappus et al. (1983). In this same investigation, LAC virus antibody was found in blood serum samples taken from 2nd grade children on the reservation in 1968 (2%) and 1978 (4.5%), and from high school students in 1979 (11.3%). Kappus and coworkers also made 2 LAC virus isolations from *Ae. triseriatus* mosquitoes that were reared from larvae collected on the reservation near the homes of children previously hospitalized for LAC encephalitis.

On the reservation, exposure of Native Americans to mosquitoes appears to be high. Recently, Szumlas et al. (1996a) reported finding LAC virus antibody prevalence rates of 20.6% in serum samples from the reservation, whereas only 4.7% of samples collected off-reservation were positive for antibody. In backyard container surveys, conducted in 5 of 6 of the principal communities, 11 species of mosquitoes were collected, but 80.9% of all mosquitoes reared from containers were *Ae. triseriatus* (Szumlas et al. 1996b). All communities averaged >6 containers per residence, suggesting that the potential for mosquito production on home grounds was high, and that exposure to virus-infected *Ae. triseriatus* likely occurred on home grounds.

On the Cherokee Indian Reservation, we investigated the population ecology of *Ae. triseriatus* in relation to the occurrence of LAC encephalitis cases. Herein, we report the seasonal occurrence and abundance of host-seeking, resting, and gravid *Ae. triseriatus*.

MATERIALS AND METHODS

Study area: The Cherokee Indian Reservation is located in western North Carolina in the Blue Ridge Mountains. The reservation, which occupies *ca.* 22,600 ha, is located in Swain and Jackson counties at the junction of U.S. highways 19 and 441 (Fig. 1). More than 6,000 Native Americans live in approximately 2,300 residences (Welch 1990). Elevations range from 572 to over 1,667 m, but most residences are situated at elevations ranging from approximately 600 to 900 m. The flora and fauna have been described previously (Szumlas et al. 1996a).

Six sites were established on the Cherokee Indian Reservation in 1989 and 1990 (Fig. 1) for routine sampling. Four sites were established at residences of children who had had LAC en-

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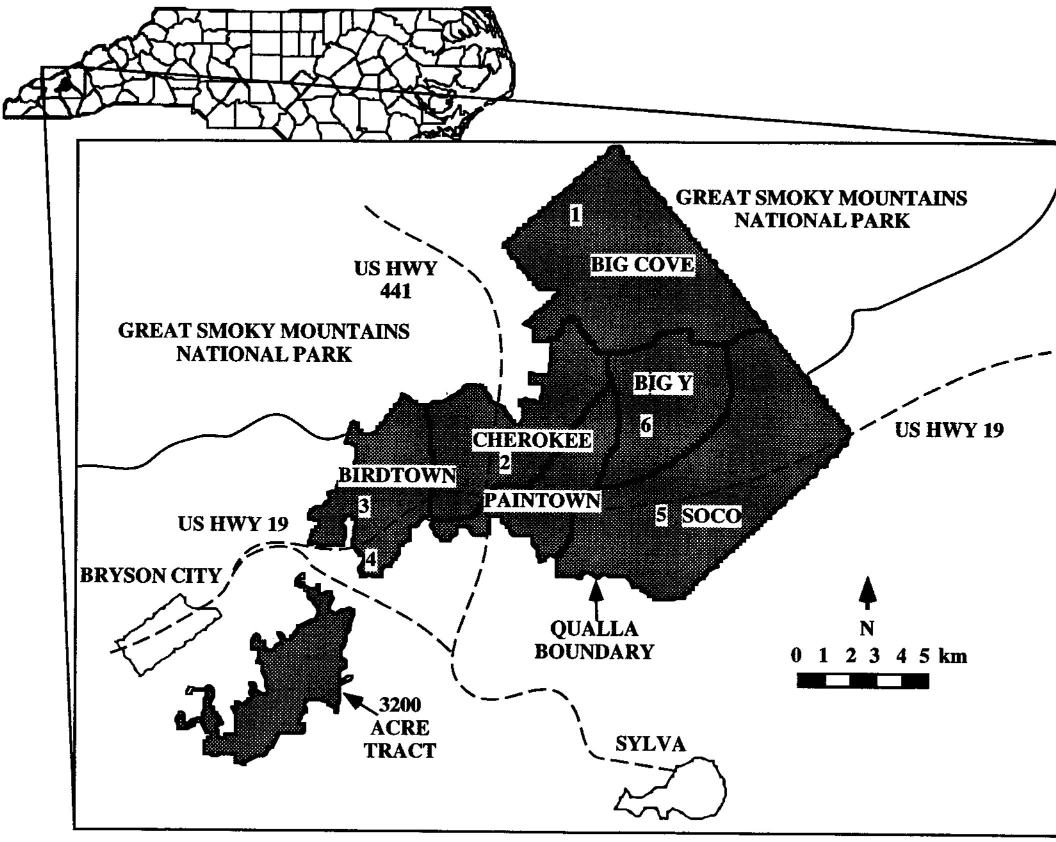

Fig. 1. Cherokee Indian Reservation in western North Carolina. Numbers correspond to the location of study sites.

cephalitis. An additional 2 sites were located at trash dumps where numerous artificial containers provided potential habitats for container-breeding mosquitoes.

Site 1 was located at a residence in the community of Big Cove at 890 m. Numerous food containers (>100) and some tire casings (5–10) were present at this site, all of which were located 10–20 m from the house. The residence was inhabited in 1989, but it was vacant during 1990. Site 2 was a trash dump site located on Yellow Hill in the community of Cherokee at about 730 m. This site was approximately 100 m from the nearest residence. Numerous discarded containers and a few automobile tire casings were found. Site 3 was located at a residence in Birdtown at 585 m. Site 4 was located at a trash dump site, also in Birdtown, at 610 m. Hundreds of discarded trash containers and >1,000 tire casings were present at this site in 1989. In 1990, over half of all the tires and much of the surrounding vegetation were destroyed by fire. The nearest residence to this site was about 25 m away. Site 5 was located at a residence on Blue Wing Creek Road in the community of Soco at about 710 m. Man-made containers were not abundant at this site. The nearest neighbor was at a distance of *ca.* 25 m. Site 6 was at a residence in the Big Y area on Wright's Creek at 790 m. Discarded containers were not abundant.

Sampling larvae: A survey was conducted during the week of July 18, 1990, to characterize conditions at each site and to collect mosquitoes. With the exception of site 4, artificial containers were sampled at all sites. Containers at site 4 were too numerous to sample. All larvae and pupae in flooded containers were removed and placed in Whirl-Pak® bags (Fisher Scientific), placed in an ice chest, and transported to an insectary at North Carolina State University.

Sampling adults: The seasonal activity of females was monitored using oviposition and suction traps (baited with CO_2), and resting males and females were collected by vacuuming vegetation and leaf litter.

Ovitrapping: Oviposition activity of container-breeding mosquitoes was monitored with traps at all 6 sites daily for 23 wk in 1989 from May 31 to November 7, and for 26 wk in 1990, from May 2 to November 8. During both seasons, ovitraps were placed in the same locations. Ovitraps consisted of no. 10 tin cans (*ca.* 4 liters volume) painted glossy black inside and out with Rustoleum®. Holes were drilled 2.5 cm from the top for drainage. Dry oak leaf litter was added to a depth of 3–5 cm, a piece of 4-gauge galvanized metal screen was placed on top of the leaf litter, and tap water was added to fill the can half full. One 2.5 × 15-cm red velour paper strip was clipped to the inside of each trap as an oviposition substrate (ovistrip). Four ovitraps per site were placed at least 50 m apart in the shade on the ground. Clean ovitraps were set out at each site once a week when ovistrips were collected. Each ovitrap was covered with 2-cm-gauge wire screen to prevent animals from drinking. When serviced, the ovistrips were removed, and the contents of each ovitrap were poured into a white enamel pan and carefully examined for early instar mosquito larvae and *Culex* egg rafts. Mosquitoes were collected and placed in labeled Whirl-Paks and transported to the insectary.

Aspiration sampling: Arbovirus field station (AFS) backpack aspirators (Meyer et al. 1983) were used to collect mosquitoes from vegetation and leaf litter at 5 sites. Site 2 was not sampled because the terrain was too steep to safely operate the aspirators. Four 15-min aspiration samples per site were taken while moving at a slow pace, up to a radius of 100 m from the residence or center of the site. Samples were collected between 1000 h and 1400 h EST. Collected mosquitoes were placed immediately on dry ice, transported to lab facilities, and stored at −70°C until they were processed. Within each site, the same areas were sampled in both years of the study. Aspiration samples were collected weekly from May 17 to October 25, 1989, and from May 2 to November 7, 1990.

Suction trapping: Centers for Disease Control miniature light traps (Hausherr's Machine Works, Toms River, NJ), with light bulbs removed, were used as suction traps to sample host-seeking mosquitoes at sites 1, 4, and 5. Two traps were set up *ca.* 50 m apart at each site. Each trap consisted of a plastic 19.2-liter (5-gal.) bucket (lined with styrofoam) with a 2.5-cm-diam hole cut into the side to allow carbon dioxide to escape. Traps were baited with *ca.* 10 kg of dry ice and operated biweekly for 24 h on each sampling date from June 7 to October 24, 1989, and May 1 to October 30, 1990. After each 24-h sampling, mosquitoes collected were placed on dry ice and transported to lab facilities at North Carolina State University.

Processing mosquitoes: Larval and pupal samples from containers were segregated by site, reared to adults, killed by freezing, sorted on a chill table, identified to species, and counted. Ovistrips were placed on a damp paper towel and held in a sealed plastic container at 27°C under long day length conditions (14 h light : 10 h dark) for 1 wk to assure that all eggs had embryonated. Eggs were hatched and larvae (along with any larvae collected from ovitraps) were reared to the 4th instar, killed in hot water, and preserved in ethanol : glycerin (80:10% by vol.), and subsequently identified to species and counted. Each ovistrip was examined with a microscope at 40× and if <90% of eggs were hatched, the ovistrip was placed back into the container for an additional week and then the strip was reflooded. This procedure was repeated until ≥90% of the eggs had hatched. Mortality of mosquitoes reared from eggs and collected from containers was <1%. Adults collected in aspiration and suction trap samples were sorted on a chill table by site, sample, species, and sex, and enumerated. When aspiration samples were sorted, females were classified as unfed, blood-fed, or gravid.

Weather data: Records of daily precipitation and high and low temperature, collected at the Oconaluftee weather station on the Cherokee Indian Reservation, were acquired from the State Climatological Office in the Department of Marine, Earth and Atmospheric Sciences, North Carolina State University, Raleigh, NC.

Statistical analyses: Weekly rainfall totals were examined by analysis of variance (ANOVA) (Sokal and Rohlf 1994). Differences in the mean weekly number of mosquitoes collected between years, sites, and within sampling methods were tested for significance using ANOVA. Prior to these analyses, the numbers of mosquitoes collected were subjected to square root transformations to normalize variances. Fisher's least-significant-difference (LSD) test was used to segregate significantly different means. Type III mean squares for the year × site × sample interaction were used as error terms in F and LSD tests.

RESULTS

Weather patterns: Air temperature peaked in June, July, and August of both years, and declined rapidly after August (Fig. 2). The averages of maximum and minimum temperatures from May 1 to October 31 were 18.2°C and 18.6°C in 1989 and 1990, respectively. Seasonal patterns of precipitation from February to October for 1989 and 1990 appeared to be markedly different (Fig. 2); however, weekly rainfall

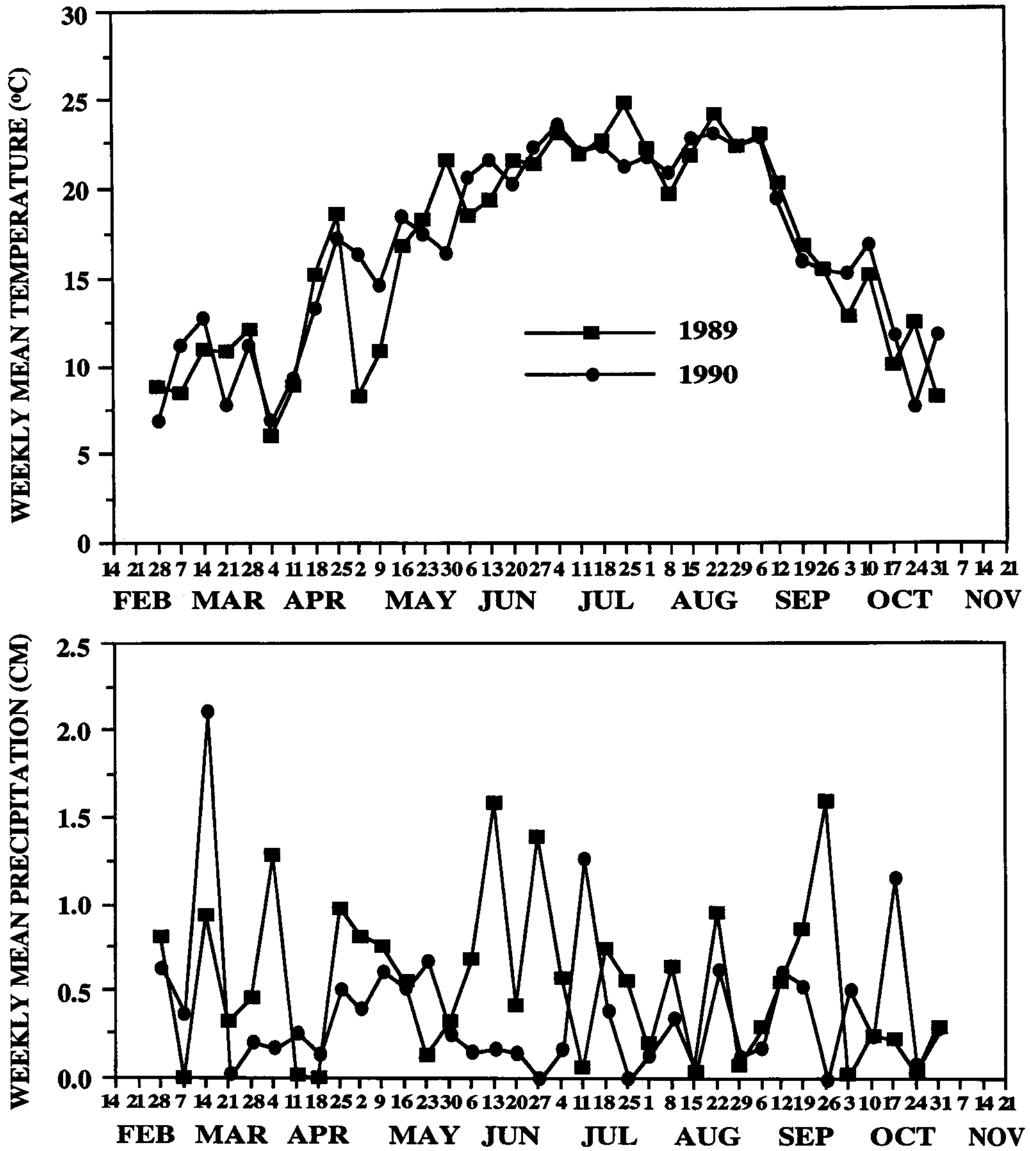

Fig. 2. Air temperature and precipitation measurements recorded at the Oconaluftee weather station of the Cherokee Indian Reservation in 1989 and 1990.

totals from May 1 to October 31 were only marginally different between years (F = 3.69; df = 1; *P* = 0.06). For this period, rainfall totaled 101.6 and 66.9 cm for 1989 and 1990, respectively.

Container surveys: The number of containers found at each study site ranged from 15 at site 5 to >1,000 at site 4 (Table 1). Containers at site 4 consisted mainly of discarded automobile tire casings. Over 90% of the containers were flooded at the time of the survey except for site 6, where only 67% of containers were flooded. Prevalence of flooded containers infested with *Ae. triseriatus* larvae ranged from 15 to 41%. Mean standing crop of larvae in containers was lowest at site 2 (4.3 immatures/container) and highest at site 5 (71.5 immatures/container).

Aspiration sampling: In 1989 and 1990, 11 mosquito species and a total of 6,046 adults were collected in aspiration samples. Aspiration sampling detected more mosquito species than the other 2 survey methods. Almost twice as many adults of all species were collected in 1989 (*n* = 4,024) relative to 1990 (*n* = 2,022),

Table 1. Container and site characteristics for 6 mosquito sampling sites on the Cherokee Indian Reservation, including 4 residences of previous La Crosse encephalitis patients.

Site	Distance (m) to nearest residence	Distance (m) to woods	Containers examined: Total no.	Flooded: No.	Flooded: %	With mosquitoes: No.	With mosquitoes: %	Immature *Aedes triseriatus* collected[1]: Total no.	Immature *Aedes triseriatus* collected[1]: Mean no. per container
1	>100	10	105	96	91.4	39	40.6	273	7.0
2	>100	0	30	27	90.0	7	25.9	30	4.3
3	>100	10	22	20	90.9	3	15.0	73	24.3
4	25	0	>1,000	—[2]	—	—	—	—	—
5	25	10	15	14	93.3	4	28.6	286	71.5
6	>100	20	27	18	66.7	4	22.2	21	5.3

[1] Containers surveyed for mosquito immatures on July 18, 1990.
[2] Containers were not sampled.

with means of 8.4 and 3.6 adults collected per 15-min aspiration sample, respectively. Although 11 species were sampled, males and females of only 5 species were collected regularly. *Aedes triseriatus* was the most abundant species ($\bar{x}$ = 4.8 adults collected per sample), comprising 88.4% of all specimens collected. Other less common species (overall means of <0.4 adults per sample) collected included *Culex restuans* Theobald (6.5%), *Aedes vexans* (Meigen) (2.1%), *Culex territans* Walker (1.6%), and *Culex pipiens* Linn. (1.2%). In 1989 and 1990, 84.1 and 68.4%, respectively, of the total number of mosquitoes were collected from site 4.

CO_2-baited suction trapping: Eight species and 5,438 females were collected (Table 2), but only *Ae. triseriatus* and *Anopheles punctipennis* (Say) were well represented throughout the season. *Aedes triseriatus* was the most abundant species, constituting 98% of all specimens collected, with means of 61.5 and 15.3 females per suction trap sample in 1989 and 1990, respectively. *Anopheles punctipennis* averaged 0.7 and 0.3 females per 24-h trap sample in 1989 and 1990, respectively. For 6 other species, 27 specimens were collected, and each species accounted for <0.3% of the total collected during both years.

Ovitrapping: Five species were detected with ovitraps but more than 98% of the eggs collected were deposited by *Ae. triseriatus.* Mean density of eggs deposited by *Ae. triseriatus* in ovitraps was 83.3 eggs per trap-week. In contrast, mean numbers of *Cx. restuans, Aedes hendersoni* Cockerell, *Aedes albopictus* (Skuse), and *Cx. pipiens* varied from <0.1 to 0.2 eggs per trap-week.

Seasonal abundance and distribution of Aedes triseriatus*:* Resting adults exhibited peaks of abundance in June and late July–early August in 1989; and peak numbers of adults were collected in early June, July, and August, and in mid-September in 1990 (Fig. 3). Phenology of males closely paralleled that of females, but males de-

Table 2. Mosquitoes collected in CO_2-suction traps at 3 sites on the Cherokee Indian Reservation in 1989 and 1990.

Species	Total no. adults collected	Percent of total	Mean no. adults collected per 24-h suction trap: 1989 (n = 66)	1990 (n = 84)	Total (n = 150)
Aedes triseriatus	5,343	98.30	61.50	15.30	35.60
Aedes atlanticus	1	0.02	0.01	0.00	0.01
Aedes trivittatus	1	0.02	0.00	0.01	0.01
Aedes vexans	3	0.06	0.02	0.06	0.02
Anopheles punctipennis	68	1.30	0.70	0.30	0.40
Culex pipiens	14	0.25	0.00	0.17	0.09
Culex restuans	5	0.09	0.03	0.04	0.03
Coquilletidia perturbans	3	0.06	0.03	0.01	0.02

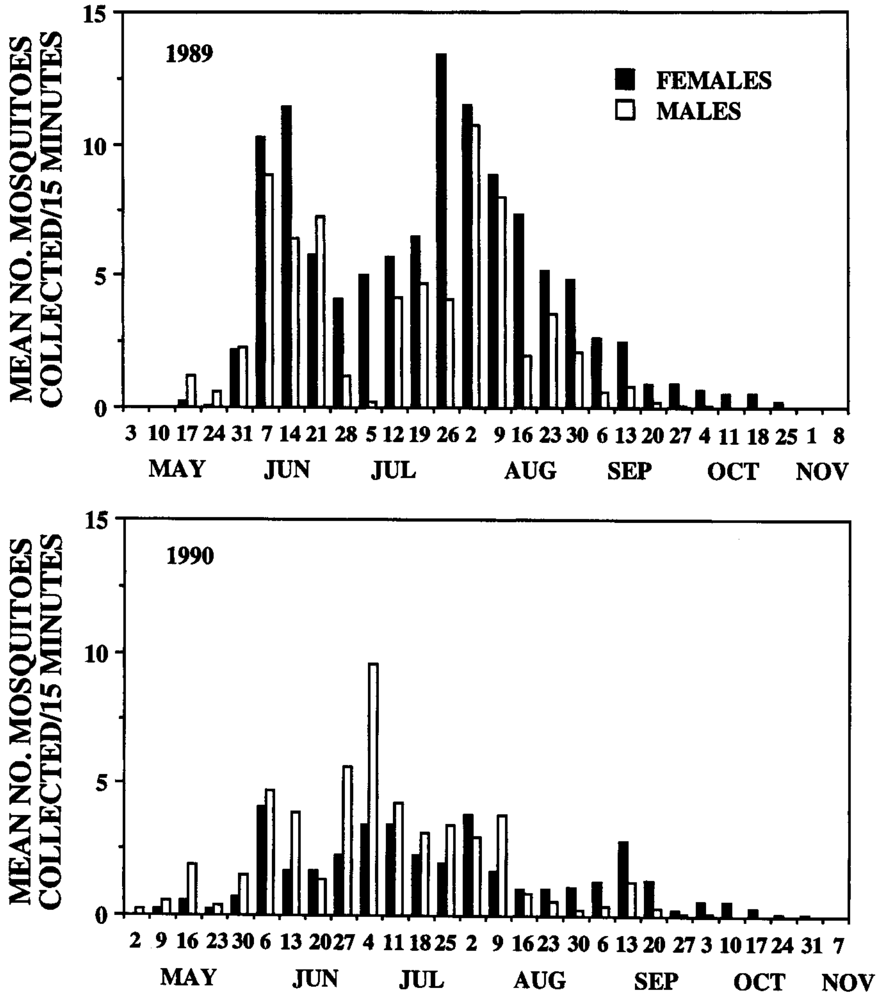

Fig. 3. Mean number of resting male and female *Aedes triseriatus* collected from vegetation per 15-min aspiration sample in 1989 and 1990 at 5 sites on the Cherokee Indian Reservation.

creased precipitously before females at the end of summer. In 1989, females were more abundant, but in 1990, males were generally collected in greater numbers than females up until early August (Fig. 3). Bloodfed and gravid *Ae. triseriatus* were collected throughout the season but only in low numbers (<5) per sample even when populations were at peak numbers. Small peaks in the numbers of bloodfed females collected per sample occurred in late June and July, and early August, and again in mid-September. Gravid females increased in number in our collections from July 4 to September 19, reaching peak numbers on August 29.

Mean number of *Ae. triseriatus* adults collected in aspiration samples in 1989 (6.4 adults per sample) was greater ($F = 18.08$; $df = 1$; $P = 0.001$) than in 1990 (3.1 adults per sample). A larger number of females ($F = 37.89$; $df = 1$; $P = 0.0001$) was collected in 1989 ($\bar{x} = 4.0$ mosquitoes per sample) relative to 1990 ($\bar{x} = 2.4$ mosquitoes per sample). In contrast, the number of males collected in 1989 ($\bar{x} = 1.3$ mosquitoes per sample) and 1990 ($\bar{x} = 1.8$ mosquitoes per sample) was not different ($F = 2.45$; $df = 1$; $P = 0.14$). Adult collections varied significantly among the 5 sites sampled ($F = 108.7$; $df = 4$; $P = 0.0001$), with nearly 87% of all *Ae. triseriatus* collected in both years taken at site 4 (Table 3). The number of adults collected at this site in 1989 ($\bar{x} = 29.5$ adults per sample) was greater ($P < 0.05$) than the number collected in 1990 ($\bar{x} = 12.0$ adults per sample). The abundance of *Ae. triseriatus* was much lower at other

Table 3. Resting *Aedes triseriatus* adults collected from vegetation with backpack aspirators at 5 sites on the Cherokee Indian Reservation in 1989 and 1990.

	No. adults collected per 15-min aspiration sample							
	1989		1990		Total		Totals	
Site	x̄	SE	x̄	SE	x̄	SE	No.	%
4	29.48a[1]	6.57	11.97a	2.77	20.73a	3.80	4,644	86.9
3	0.83b	0.21	1.13b	0.27	0.98b	0.18	220	4.1
6	0.75b	0.21	1.01b	0.25	0.87b	0.17	197	3.7
5	0.54b	0.13	0.84b	0.21	0.69b	0.13	155	2.9
1	0.38b	0.10	0.77b	0.32	0.58b	0.18	129	2.4
Overall	6.40	1.62	3.14	0.67	4.77	0.59	5,345	100.0

[1] Means with the same letter within each column are not significantly different ($P = 0.05$) by an LSD test.

sites, approximately one adult *Ae. triseriatus* per sample, ranging from 2.4% of the total *Ae. triseriatus* collected at site 1 to 4.1% at site 3. Adult abundance did not vary significantly ($P > 0.05$) between years at these other sites, or among these sites during the same year.

After being collected in highest numbers in suction traps in June and July, *Ae. triseriatus* females declined from August to October (Fig. 4). In 1989, high numbers of females were taken in the first collection in June, indicating that *Ae. triseriatus* was not sampled at the beginning of its breeding season. Females seemed to be more abundant in 1989 than in 1990, but high variation among sites made differences between means of each year insignificant ($F = 1.00$; df $= 2$; $P = 0.50$). Each year, more than 94% of all *Ae. triseriatus* collected were from site 4, with means of 178.9 and 49.4 females collected per 24-h suction trap sample in 1989 and 1990, respectively (Table 4).

Aedes triseriatus eggs were collected from early May to early November, with *ca.* 4 peaks in oviposition activity detected each year (Fig. 5). More *Ae. triseriatus* eggs were collected in 1990 from ovitraps (x̄ = 88.9 eggs per trap-week) than in 1989 (x̄ = 36.6 eggs per trap-week) ($F = 102.48$; df $= 1$; $P = 0.0001$) (Table 5). There were differences in mean densities of eggs collected among sites ($F = 5.91$; df $= 5$; $P = 0.003$), but not between years at the same site ($F = 2.14$; df $= 5$; $P = 0.12$) (Table 5). Generally, mean numbers of *Ae. triseriatus* eggs collected per trap-week for sites 4 and 1 were significantly lower ($P = 0.05$) than for other sites.

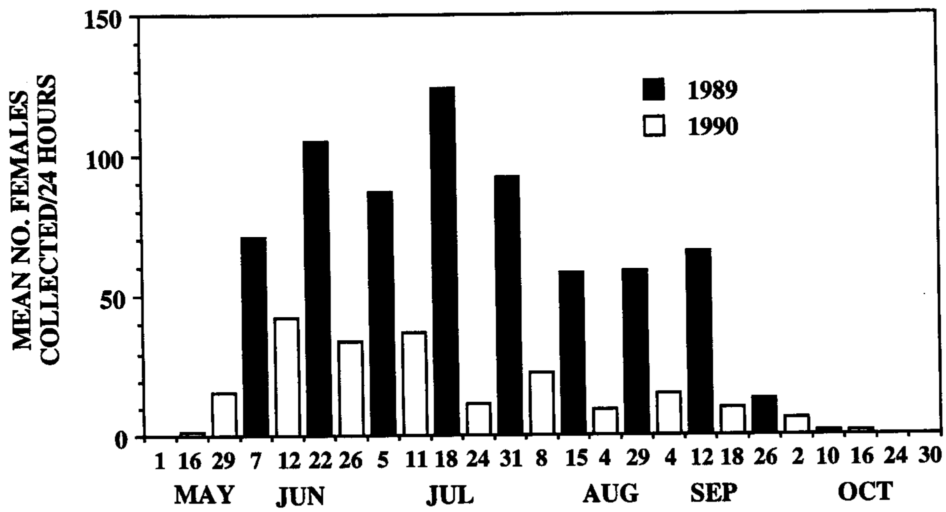

Fig. 4. Mean number of host-seeking *Aedes triseriatus* collected per 24 h in CO_2-baited suction traps at 3 sites on the Cherokee Indian Reservation.

Table 4. Host-seeking *Aedes triseriatus* collected with CO_2-suction traps at 3 sites on the Cherokee Indian Reservation in 1989 and 1990.

	No. females collected per 24-h suction trap							
	1989		1990		Total		Totals	
Site	$\bar{x}$[1]	SE	$\bar{x}$	SE	$\bar{x}$	SE	No.	%
4	178.9	44.4	49.4	12.9	114.2	24.9	5,034	94.2
5	3.3	1.0	4.6	1.0	3.9	0.7	172	3.2
1	2.4	0.6	3.9	0.8	3.1	0.5	137	2.6
Total	61.5	17.8	19.3	5.0	40.4	9.4	5,343	100.0

[1] Means are not significantly different ($P = 0.05$) by an LSD test.

DISCUSSION

Seasonal occurrence and abundance of mosquito species: Aedes triseriatus was the most abundant mosquito on the Cherokee Indian Reservation in aspiration, CO_2-suction trap, and ovitrap samples. The seasonal profiles of resting males and females collected in aspiration samples (Fig. 3) and host-seeking females sampled with suction traps (Fig. 4) were similar. However, gravid females exhibited a different seasonal activity pattern (Fig. 5). Egg densities were much greater in 1990, and peak numbers were collected from mid-August to mid-September. Resting and host-seeking adults were more abundant in 1989 and generally reached peak numbers in July.

The contrasting phenologies most likely resulted from differences in the seasonal patterns of rainfall for these years. Ovitraps compete for eggs with natural and other man-made oviposition sites (Craig 1983, Hanson et al. 1988). High levels of rainfall would fill containers, diverting gravid females from using ovitraps. Consequently, if there are few natural or artificial containers present in areas where ovitraps are placed, a disproportionately larger number of eggs would be deposited in ovitraps compared to locales where numerous alternative oviposition sites are available. On the reservation, the seasonal occurrence of rainfall had a greater impact on oviposition activity than did the total amount of rainfall in 1989 and 1990. Weekly mean densities of eggs in ovitraps were greater in 1990, but total rainfall was lower than in 1989. In 1989 and 1990, precipitation in April and May would have flooded containers, stimulating production of *Ae. triseriatus* adults. However, in June and early July, 1990, gravid females from these cohorts would have been recruited to ovitraps in relatively high numbers because the decline in rainfall during this period of this year would have

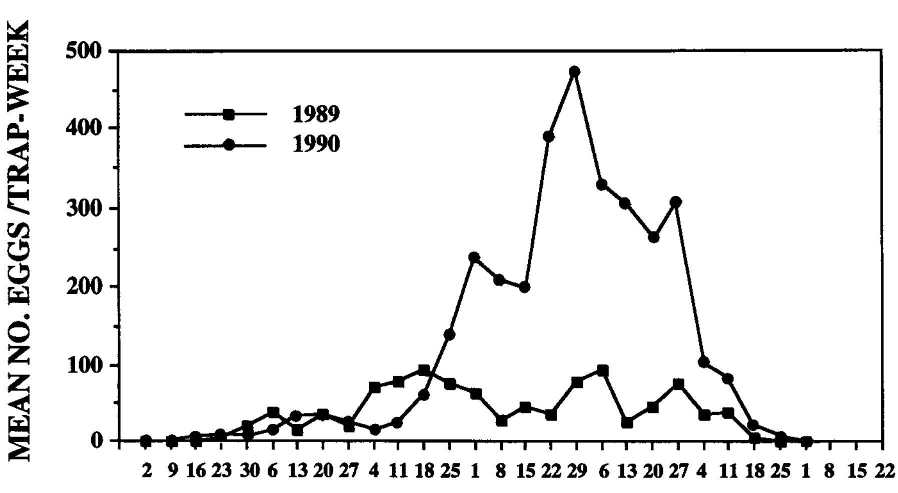

Fig. 5. Mean number of *Aedes triseriatus* eggs collected per trap-week in ovitraps at 6 sites on the Cherokee Indian Reservation.

Table 5. Mean numbers of *Aedes triseriatus* eggs collected from ovitraps on the Cherokee Indian Reservation in 1989 and 1990.

	Mean no. eggs collected per ovitrap-week							
	1989		1990		Total		Totals	
Site	x̄	SE	x̄	SE	x̄	SE	No.	%
5	40.6a[1]	5.3	134.9a	19.4	91.5a	11.1	27,460	23.6
3	54.0a	6.3	96.1b	13.8	76.8a	8.1	23,029	19.8
2	38.9a	5.2	87.4bc	17.6	65.7a	10.1	19,253	16.6
6	45.5a	5.6	81.0bc	15.7	64.6a	8.9	19,387	16.7
4	15.9b	2.8	72.0bc	9.9	46.2b	5.7	13,863	11.9
1	22.8b	3.0	62.1c	10.0	44.0b	5.7	13,205	11.4
Overall	36.6	2.0	88.9	6.1	64.8	3.5	116,197	100.0

[1] Means with the same letter within each column are not significantly different ($P = 0.05$) by an LSD test.

reduced the number of alternative oviposition sites. In contrast, comparatively higher levels of rainfall occurred during June and July, 1989, which would have filled containers diverting some females from ovipositing in ovitraps.

The effects of container abundance on use of ovitraps by *Ae. triseriatus* is evident at site 4. Densities of eggs in ovitraps at this trash dump site generally were lower than at other sites. Containers were predominantly discarded automobile tires, which are highly suitable breeding sites for *Ae. triseriatus* (Haramis 1984). The abundance of host-seeking and resting adults was highest at this site. A decrease in numbers of host-seeking and resting mosquitoes collected at site 4, observed in 1990 relative to 1989, was undoubtedly due to the partial destruction of tires and resting sites by fire. At other sites, similar changes in habitat did not occur, and the numbers of adults collected were not markedly different between years.

At site 1, the other trash dump site, aspiration and suction trap sampling yielded the lowest number of *Ae. triseriatus* adults, which was unexpected. At this site, 105 containers were examined, and more than 40% of flooded containers were positive for larvae, with a mean of 7 larvae per positive container. The low abundance of adults suggests that few adults emerged from these containers. The majority of containers consisted of rusted food cans. In Florida, O'Meara et al. (1992) reported finding few *Ae. albopictus* larvae in bronze cemetery vases that leach copper ions into the water. Metal ions may have similarly limited the survival of *Ae. triseriatus* larvae in rusted containers at site 1.

The abundance of *Ae. triseriatus, Cx. restuans, Ae. albopictus,* and *Ae. vexans* decreased in aspiration samples in 1990 relative to 1989. Amounts of rainfall in May are comparable for both years, but mosquitoes were unexplainably less abundant in June and throughout much of the breeding season in 1990. Only one *Ae. albopictus* larva was recovered during the backyard container surveys in 1990. *Aedes albopictus* was unexplainably not sampled in ovitrap collections made in 1990. Collection of *Ae. albopictus* in 1989 may have resulted from an introduction of this species in tires or other translocated man-made containers the same year. Its decline in 1990 could have been due to its inability to overwinter. Hawley et al. (1989) found that the overwinter-survival of several strains of diapausing and nondiapausing *Ae. albopictus* was lower than that of *Ae. triseriatus* in northern Indiana.

Seasonality of Ae. triseriatus *and the occurrence of LAC encephalitis:* The majority of reported LAC encephalitis cases (48 of 54) in North Carolina from 1964 to 1992 have been reported in August and September (Szumlas et al. 1996a). Seasonal abundance patterns of *Ae. triseriatus* estimated by both aspiration and suction trapping in 1989 and 1990 do not correspond with the seasonal incidence of previously reported LAC encephalitis cases. Population levels of *Ae. triseriatus* declined during August and September, at times when most LAC encephalitis cases in NC have been reported. In contrast to our results, Clark et al. (1985a) reported *Ae. triseriatus* biting rates in a LAC virus-endemic area of northcentral Illinois, to be highest in August and September when LAC encephalitis cases were most frequently reported (Clark et al. 1983, 1985b). On the reservation, however, seasonal oviposition activity did appear to coincide with the reported occurrence of LAC encephalitis cases. In 1989 and 1990, peaks in oviposition activity were observed in August and September. It is possible that the high abundance of host-seeking females in June and July results in virus amplification and higher infection rates of mosquitoes in the latter half of the season. Some mosquitoes, collected from containers and with ovitraps in late summer and early fall of 1990 and 1992, were tested for virus. A total of 16,227 adults and 581 pools of mosquitoes representing 12 species were processed. *Aedes triseriatus* was the predominant mosquito processed, accounting for 93.8 and 78.7% of the total number of mosquitoes and mosquito pools processed, respectively. La Crosse virus was isolated from 2 pools of 56 female and 36 male *Ae. triseriatus* that were reared from eggs collected in ovitraps placed from August 7 to 21, 1990. The minimum field infection rate was estimated to be 0.26 infected adults per 1,000 *Ae. triseriatus* adults (Szumlas et al. 1996b).

The connection between oviposition activity and LAC virus transmission becomes more apparent when considering the availability of breeding sites near residences on the reservation. After *Ae. triseriatus* females become infected by transovarial (Watts et al. 1973) or venereal transmission (Thompson and Beaty 1977), they acquire a blood meal, develop eggs, and seek oviposition sites. Because tree holes are rare on the reservation, and artificial containers are abundant near residences, gravid females would be attracted to peridomestic containers. After ovipositing, these females would seek another blood meal. La Crosse virus would be transmitted to residents through the biting activity of these infected mosquitoes during subsequent gonotrophic cycles.

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