AEDES TRISERIATUS: A COMPARISON OF DENSITY IN TREE HOLES VS. DISCARDED TIRES[1]

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ABSTRACT. Pupae of *Aedes triseriatus* were collected from discarded automobile tires and tree holes in St. Joseph Co., Indiana. Peak pupation occurred about 2–3 weeks earlier in discarded tires than in tree holes, probably due to the higher water temperatures in tires. Unshaded and shaded tires produced 42% and 133% as many males and 20% and 91% as many females as tree holes, respectively. There was a decline in body size of adults from shaded tires towards the end of the summer, probably owing to depletion of nutrients and thus increased intraspecific larval competition. Transovarially infected females emerging from discarded tires may initiate La Crosse encephalitis virus amplification as much as a month earlier than females from tree holes.

INTRODUCTION

Discarded tires have long been recognized as a larval habitat for several species of mosquitoes (Horsfall 1955), particularly periodomestic species. Transporation of discarded tires was responsible for *Aedes aegypti* (Linn.) reinvading sections of Texas (Haverfield and Hoffman 1966) and, more recently, Colombia (Groot 1980).

Aedes triseriatus (Say), the eastern tree hole mosquito, has been incriminated as the major vector of La Crosse encephalitis virus in the midwestern USA (Thompson et al. 1972, Berry et al. 1975). Public health workers both in Ohio and Wisconsin (Watts et al. 1974, Berry 1982, Parry 1983) have found an association between discarded tires containing *Ae. triseriatus* and human cases of La Crosse encephalitis. Consequently, there is renewed interest in defining the chemical, physical, biological, and ecological parameters that compose the mosquito-tire system (Beier et al. 1983a, 1983b). The objective of this study was to compare the seasonal pattern of pupation and the production of pupae of *Ae. triseriatus* in both tree holes and tires.

MATERIALS AND METHODS

Pupae of *Ae. triseriatus* were collected from tires located at the Western Iron and Metal Co. in South Bend (St. Joseph Co.), Indiana where thousands of discarded tires are stockpiled (Beier et al. 1983a, 1983b). Most of the tires were continuously exposed to direct sunlight, but some were shaded by tree canopies or large shrubs.

Pupae were collected from automobile tires weekly from May 10 through August 25, 1982. Samples were taken from 20 to 30 randomly selected tires on a single day from at least 3 locations in the salvage yard. An approximately equal number of samples was collected from exposed and shaded tires each week. The contents of the tire were stirred with a soup ladle and a 500 ml sample was removed with a meat baster. As there was a limited number of shaded tires, some tires were resampled beginning the third week of July. Only tires (or tree holes) that contained water were sampled and no container was sampled more than twice.

Pupae of *Ae. triseriatus* were collected from basal tree holes in beech (*Fagus grandifolia*) and maple (*Acer* spp.) located in Bendix Woods (St. Joseph) County Park, Indiana. Between 9 and 14 tree holes of at least 0.3 liter capacity were sampled on a single day each week from May 11 through August 23. The liquid contents of each tree hole were removed with a squeeze-bulb powered pump of the type used for cleaning aquaria. Then the sides of the tree hole were rinsed with 150 ml of water to wash any remaining pupae into the bottom. Finally, the remaining liquid was removed with a meat baster and the pupae were collected. After all the tree holes had been sampled once, the resampling of the tree holes began on the third week of July. The water temperature was recorded just before each of the containers was sampled.

Adults reared from field-collected pupae were anesthetized with ether and the length of one wing from the base of the costa to the tip of the wing margin was measured with an ocular micrometer. Wing length was used as a measure of body size because this character is significantly correlated with dry weight (Clements 1963). Adult female mosquitoes which appeared to be *Aedes hendersoni* Cockerell (a morphologically similar sibling species of *Ae. triseriatus* occasionally found in basal tree holes) were frozen at −70° C. The mosquitoes were identified by electrophoresis with diagnostic enzymes devised by Saul et al. (1977) with

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staining modified for cellulose acetate electrophoresis (R. C. Nasci, unpublished data).

The wing length distributions of both sexes from each larval habitat were compared statistically with a one-way ANOVA, followed with Duncan's multiple range test (DMRT) to identify differences among the groups (Steel and Torrie 1980). In addition, the wing length distribution of males and females collected in May and June were compared with those collected in July and August to determine if there was a seasonal decline in wing length (ANOVA and DMRT). The seasonal sex ratios for each habitat were compared statistically with a 2 × 2 Chi-squared contingency table (Zar 1974). The water temperatures of the 3 habitats were compared statistically with one-way ANOVA and DMRT.

RESULTS

Of 192 samples from unshaded tires, 16% contained pupae of *Ae. triseriatus* (Table 1); pupae were present from the first week of sampling through the end of August. Peak density for both males (4.2 pupae/500 ml) and females (1.5 pupae/500 ml) occurred in the third week in May (Fig. 1). However, the density of both males and females dropped to very low levels at the end of August.

Of 192 samples from shaded tires, 44% contained *Ae. triseriatus* pupae (Table 1). Pupae were present from the third week in May through the end of August. Peak density for both males (5.2 pupae/500 ml) and females (3.9 pupae/500 ml) occurred in the second week in June (Fig. 1). Density of both sexes declined through the end of August.

Table 1. Occurrence of pupae of *Aedes triseriatus* in discarded tires and tree holes sampled weekly, May–August 1982.

	Pupae of *Aedes triseriatus* present (500 ml sample)					
	Discarded tires					
	Unshaded		Shaded*		Tree holes	
Month	No. sampled	%	No. sampled	%	No. sampled	%
May**	28	43	27	22	39	0
June	69	19	70	63	55	49
July***	42	5	42	36	44	75
August	53	6	53	36	45	60
Totals	192	16	192	44	183	60

* Discarded tires under the canopy of trees or large shrubs.

** Sampling began in the second week.

*** One week's sample from tires was accidentally destroyed.

There were 183 samples collected from basal tree holes; of these, 89% were from beech, 8% from maple, and 3% from stumps. The mean capacity of the tree holes was 1.3 ± 1.0 liters with a range of 0.3–6.3 liters. Approximately 60% of the tree holes sampled contained *Ae. triseriatus* pupae (Table 1), which were present from the second week of June through the end of August. The density of females peaked initially in the third week of July (2.7 pupae/500 ml), but then female density declined, followed by a second peak in late August.

The relative density (total no. pupae/total no. samples) of males in unshaded tires, shaded tires and tree holes for the entire sampling period was 0.5, 1.6 and 1.2 pupae/500 ml, respectively. In other words, unshaded tires and shaded tires produced 42% and 133% as many males, respectively as tree holes. The relative density of females in unshaded tires, shaded tires and tree holes was 0.2, 1.0 and 1.1 pupae/500 ml, respectively. Therefore unshaded tires and shaded tires were 18% and 91% as productive, respectively, as tree holes.

The seasonal male:female ratios in unshaded tires (2.2:1) and shaded tires (1.6:1) were sig-

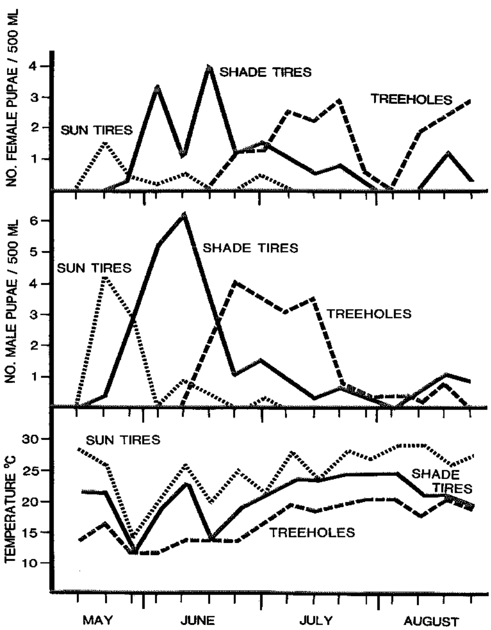

Fig. 1. Mean number of pupae per 500 ml sample and water temperature recorded weekly from discarded tires and tree-holes in St. Joseph Co., IN, May-August 1982.

nificantly higher than that for tree holes (1.2:1) (Chi square, P < 0.01). Although there was a trend toward a higher sex ratio in unshaded tires compared to shaded tires, they did not differ significantly (P > 0.10).

There were no significant differences in the wing length distribution of males collected from the 3 habitats (ANOVA, P > 0.10) (Table 2). The females collected as pupae from unshaded tires had the largest mean wing length, followed by those from tree holes, and finally by those from shaded tires (ANOVA, P < 0.001; DMRT, P < 0.05). However, 51% of the females collected from unshaded tires came from only 4 tires. There was a significant decline in size (as measured by mean wing length) of males and female adults reared from pupae collected from shaded tires and male pupae collected from tree holes during the season (Table 3). However, there was no change in the size of females collected from tree holes. Mosquitoes collected from unshaded tires were not included in the preceding analysis because very few were collected in July and August.

The range of observed temperatures was greatest in unshaded tires (9–35° C), followed by shaded tires (9–27° C), and finally by tree holes (12–21° C). The water temperatures recorded at the time of sampling were usually highest in unshaded tires and lowest in tree holes, with the unshaded tires intermediate. In Fig. 1 the points where the temperature lines touch are not significantly different (P > 0.05).

All of the 152 females from basal tree holes suspected of being *Ae. hendersoni* were identified by electrophoresis as *Ae. triseriatus*. Consequently, all of the male pupae were assumed to be *Ae. triseriatus*.

Weather records for St. Joseph Co. obtained from the National Oceanic and Atmospheric Administration (NOAA) indicated that temperature was above normal in May, below normal in June and August, and normal in July. Precipitation was above normal in May and June, below normal in July, and normal in August.

DISCUSSION

Discarded tires provide an excellent larval habitat for *Ae. triseriatus*. Larval densities found in tires may approach those found in tree holes. However, *Ae. triseriatus* in the two habitats have different patterns of peak pupation, seasonal density, and sex ratio.

The relatively high water temperatures found in rain water filled discarded tires appear to be responsible for the early emergence of *Ae. triseriatus* in tires. As with most insects the rate of larval development of *Ae. triseriatus* increases with temperature (Keirans and Fay 1968, Jalil 1974). The unshaded tires usually had the highest temperature at the time of sampling, followed by shaded tires, and then by tree holes. The preceding order was duplicated chronologically for the peak density of both sexes of pupae, which suggests that the higher temperature in tires accelerates larval development.

Table 2. Size of adult *Aedes triseriatus* from pupae collected from discarded tires and tree holes.

	Wing length of adults (in mm)*							
	♀♀				♂♂			
Habitat	No.	Mean	S.D.	Range	No.	Mean	S.D.	Range
Tires								
Unshaded	43	4.00[a]	0.30	3.27–4.48	96	2.90[d]	0.26	2.13–3.42
Shaded	194	3.69[b]	0.52	2.51–4.56	305	2.85[d]	0.31	1.89–3.50
Tree holes	504	3.89[c]	0.29	2.72–4.64	581	2.90[d]	0.22	2.20–3.50

* Means within sexes with different letters are significantly different (P<0.05 or greater).

Table 3. Seasonal decline in size of adult *Aedes triseriatus* from pupae collected from tree holes vs. discarded tires.

		Wing length of adults (in mm)						
		May–June			July–August			Seasonal decrease
Sex of adult	Habitat	No.	Mean	S.D.	No.	Mean	S.D.	in mean
♀♀	Tree holes	64	3.90	0.23	440	3.89	0.30	0.01
	Shaded tires	154	3.73*	0.53	40	3.55*	0.50	0.18
♂♂	Tree holes	262	2.93*	0.24	319	2.87*	0.20	0.06
	Shaded trees	268	2.89*	0.30	37	2.60*	0.29	0.29

* P<0.05 or greater.

The unshaded tires were exposed to direct sunlight and thus had the highest water temperature; the difference between shaded tires and tree holes is less apparent. Both habitats were covered by the canopy of trees or large shrubs, but the shaded tires were located along the margin of a narrow (10–50 m) strip of woods or under isolated trees surrounded by dense shrubbery. In contrast, the tree holes were located in a large mature beech-maple forest with a high canopy, which moderates local temperature. In addition shaded tires lack the soil that surrounds tree holes and buffers rapid temperature changes (Sinsko and Craig 1981). Thus the shaded tires were more exposed to the effects of ambient air temperature and intermittent direct sunlight than were tree holes.

If the early emergence of *Ae. triseriatus* is temperature dependent, the unusually warm temperatures in early May, followed by cool temperatures in June would have accentuated the difference between peaks of pupation in tires and tree holes. The cool temperatures in June of 1982 may have delayed pupation in tree holes 1–2 weeks when compared to the date for 1975 recorded by Sinsko and Craig (1981). However, even if pupation was delayed in tree holes in 1982 it was still 2–3 weeks behind peak pupation in shaded tires and 1 month behind unshaded tires. Thus adults of *Ae. triseriatus* would have been emerging from tires about 1 month before those in tree holes even if temperatures were normal in May and June.

Larvae of *Ae. triseriatus* in both discarded tires and tree holes are dependent on decaying leaf litter as a substrate for saphrophytic fungi upon which larvae feed (Fish and Carpenter 1982, Carpenter 1982, Beier et al. 1983a). Fallen leaves collect in tires and tree holes in fall and winter, thus the feeding of many larvae early in the season would reduce the trophic resources available to later hatching larvae. The preceding hypothesis is supported by two observations. First, the relative density of both male and female pupae declined sharply from June to July even though rain filled tires and presumably flooded recently oviposited eggs. Secondly, in shaded tires there was a decrease in the wing length of both males and females during July and August. Perhaps the feeding of many larvae in the tires during the early summer depleted much of the limited trophic resources available to larvae later in the season, which in turn reduced the body size of late emerging adults.

The ratio of relative density of pupae in shaded versus unshaded tires was about the same as that observed by Beier et al. (1983a) for larvae of *Ae. triseriatus*, about 3:1. However, the magnitude of pupal density in tires was only about 3–4% of that observed by Beier et al. (1983a) for larvae, whose study was done in part at the same site. This suggests that larval mortality in tires is heavy, probably owing to inter- and intraspecific trophic competition, as is the case in tree holes (Fish and Carpenter 1982, Carpenter 1982).

Intersexual trophic competition may occur. Unshaded tires, which receive little leaf litter, had the highest male:female ratio. When larvae of *Ae. triseriatus* are reared in the laboratory on a limited ration, the sex ratio of adults shifts in favor of males because they require less energy to complete development[3]. Consequently, male larvae would have a higher probability of completing development on the limited trophic resources in unshaded tires. In addition the proportion of males emerging from shaded tires was lower than unshaded tires, but higher than tree holes. Consequently, intersexual trophic competition may have been less severe in shaded tires, which received large quantities of leaf litter (Beier et al. 1983a).

The discarded tire habitat has important implications for the *Ae. triseriatus*-La Crosse virus system. The trend towards smaller females emerging late in the season could be of epidemiological significance because female larvae of *Ae. triseriatus* reared on reduced ratios yield females which transmit La Crosse virus at significantly higher rates than adults which had received adequate rations (Grimstad and Haramis 1984). Thus tires may not only produce females earlier in the season than tree holes; they may also produce smaller females that transmit La Crosse virus at elevated rates. Secondly, transovarially infected females emerging from discarded tires located in woods may initiate La Crosse virus amplification as much as a month earlier than if only tree holes were present, since adults do not normally appear in the northcentral states until late June (Sinsko and Craig 1981, Haramis and Foster 1983). Tires are often discarded in small numbers along the margins of suburban woodlots. These small woodlots may only be a row along a fence and thus have few mature deciduous trees that maintain tree holes that produce *Ae. triseriatus*. However, discarded tires located under young trees are volumetrically about as productive as beech basal tree holes. Consequently, a few tires can produce a source of *Ae. triseriatus* where none existed previously.

Mosquito control agencies should constantly emphasize to the public the importance of

[3] Mc Combs, S. D. 1980. Effect of differential nutrition of larvae on adult fitness of *Aedes triseriatus*. M. S. thesis. University of Notre Dame, IN 46556, 123 p.

eliminating the presence of discarded tires near residences. Stockpiles of discarded tires should be stacked in steep pyramids so that only the exterior tires receive rainwater (Beier et al. 1983a). It is particularly important to prevent vegetation from covering the tires since fallen leaves and plant debris provide nutrients for the development of larvae. Finally, mosquito control agencies must adjust their larviciding schedules to prevent early emergence of adults from discarded tires.

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