

## BIONOMICS OF *Aedes atropalpus* BREEDING IN SCRAP TIRES IN NORTHERN INDIANA<sup>1</sup>

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**ABSTRACT.** Eighty new tires were placed in field habitats, half in a junkyard and half in a woodlot. A 300 ml sample of water was taken from alternate tires once weekly for a 12 week period. There were 17,173 larvae collected from 8 different habitats. 90% of the larvae were *Culex restuans*, the most rapid (7 days) and most successful colonizer of new habitats. *Aedes atropalpus* was more common in tires in the open and *Ae. triseriatus* was more common in the shade. *Ae. atropalpus* proved to be a more rapid colonizer than *Ae. triseriatus* (17 days vs. 31 days), probably because of the tendency to oviposit directly on the water surface. The presence of leaf litter did not affect oviposition choice by either *Aedes* species. *Aedes triseriatus* was shown to be bivoltine in northern Indiana.

A total of 2,261 adult mosquitoes in 13 species was collected from tires by suction aspirator during the 14 week summer season. Among these were 190 females and 125 males of *Ae. atropalpus*; about 67% of the *Ae. atropalpus* came from exposed, unshaded tires.

### INTRODUCTION

Discarded tires provide an excellent larval habitat for many species of mosquitoes. The problem of disposal of discarded tires grows annually; an Environmental Protection Agency estimate indicates that 200,000,000 tires are discarded each year (Deese et al. 1981). Rendering of most tires, especially steel belted tires, is very difficult. As a result, discarded tires are frequently stored in large outdoor aggregations. These tire piles are often in close proximity to places where humans live, work or play. Several of the mosquito species inhabiting tires are known to transmit arboviruses to man. Consequently, accumulations of tires can be a significant public health hazard.

In northern Indiana, 8 species of mosquitoes are known to utilize tires as a larval habitat. The most common is *Culex restuans* Theobald; late in the summer it is replaced by *Culex pipiens* L., the primary midwestern vector of St. Louis encephalitis (Luby 1979). *Culex territans* Walker, a frog feeder, also occurs in tires. The second species in abundance is *Aedes triseriatus* (Say), the primary vector of La Crosse virus (Bunyavirus, California serogroup) (Watts et al. 1972). The third most common species is *Aedes atropalpus* (Coquillett); Freier and Beier (1984) have shown that this species may be a potential vector of La Crosse (LAC) virus since it transmits the virus both orally and transovarially at a rate comparable to *Ae. triseriatus*. Other northern Indiana mosquitoes that occasionally occur in tires include *Anopheles barberi* Coquillett, *Or-*

*thopodomyia signifera* (Coquillett) and *Or. alba* Baker.

Special attention should be given to the tire habitat as a source of breeding for *Aedes atropalpus*. This species normally occurs in rockpools near fast-flowing streams and along the rocky shores of lakes. Until recently, its distribution was limited to eastern Canada, rivers and streams associated with the Appalachians south to Alabama, and a westward extension to northern parts of Minnesota, Wisconsin and upper Michigan (Darsie and Ward 1981). In recent years, it has adapted to tire-breeding; Table 1 contains records of tire-breeding which has allowed range extension in Kentucky, Ohio and Indiana, as well as container-breeding at both the eastern and western ends of New York State (Fig. 1). We think that this spread is both recent and fairly rapid. For example, in South Bend, IN, an intensive county-wide survey was made of larval breeding places in the summers of 1976-78 by the St. Joseph County Health Department and the University of Notre Dame. Although over 3,000 collections of larvae were made from tires each year, *Aedes atropalpus* was never found. In 1979, Gregory Lanzaro found 23 larvae (3 collection dates) in one tire pile in a single auto salvage yard (Restifo and Lanzaro 1980). By 1982, the species was common in 4 tire dumps several kilometers apart. It is suspected that a new tire-breeding variety has developed and that shipments of tires will spread this variety over a much wider area.

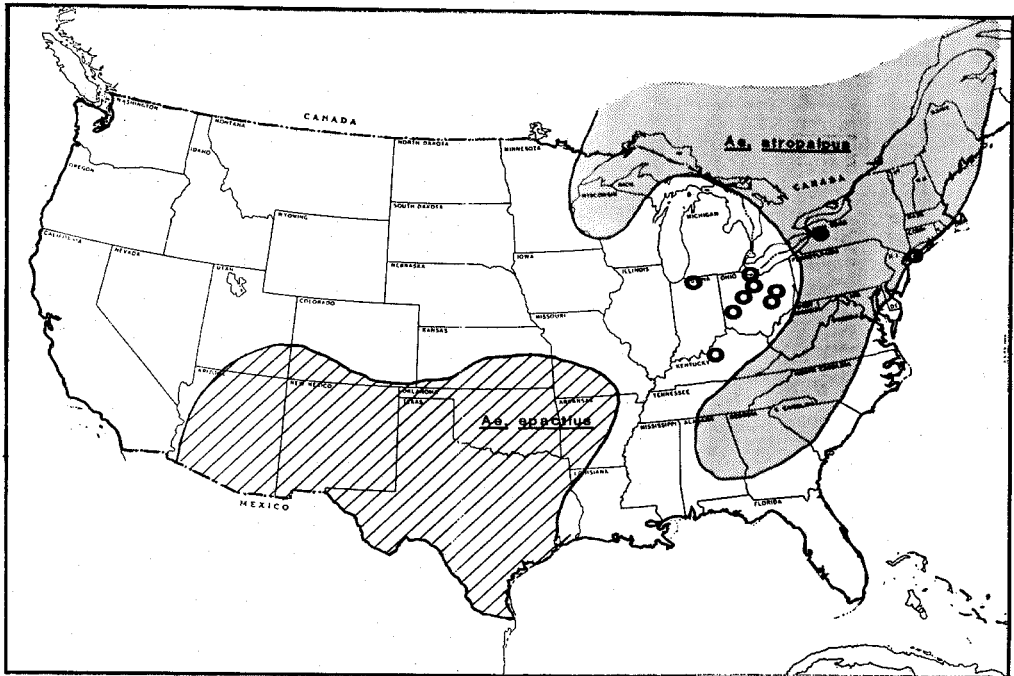
As the problem of discarded tires continues to grow, mosquito breeding in tires will become increasingly important. The purpose of this study was to estimate the amount of time required for mosquitoes to colonize recently discarded tires and to observe the composition of the mosquito community in tires placed at various habitats over the course of the season. Pa-

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Table 1. Reports of *Aedes atropalpus* breeding in tires.

State	County	Remarks	Reference
Kentucky	Jefferson	In abandoned tires behind tire store in downtown Louisville	Covell and Brownell 1979
New York	Suffolk	In discarded tires and containers in a dump on Plum Island, near Long Island	White and White 1980
	Chautauqua	In large tire dump at Sinclairville; 50% of larval samples contained this species	J. A. Berlin, unpublished data 1983
Ohio	Summit	Adults at light trap in Barberton since 1972, Norwalk and Willard since 1977, all near tire dumps; larvae in tire yard at Greenville, 1979	Restifo and Lanzaro 1980
	Huron		
	Darke		
Indiana	Sandusky	Larvae in tire yards in Sandusky, Canton, Finlay	R. A. Restifo, unpublished data 1983
	Stark		
	Canton		
Indiana	St. Joseph	Larvae rare in one auto salvage yard, South Bend, 1979	Restifo and Lanzaro 1980
		Larvae common in four well-separated tire dumps, South Bend, 1982	Beier et al. 1983

Fig. 1. Map showing U.S. distribution of *Aedes atropalpus* (shaded area); range extensions and new records of tire breeding in New York are included.

parameters studied include time of colonization of clean tires, the effect of tire location in sun or shade, the effect of proximity to tires already producing mosquitoes, and the effect of adding organic material to the tire. The capacity of discarded tires to serve as a resting site for adults of tire-breeding mosquitoes was also studied. The parameters studied included the number of mosquitoes collected, the sex ratio of the collections, and age structure of the population.

## MATERIALS AND METHODS

Two sites were involved in this study, a junkyard and a small woodlot. The junkyard was the automotive scrap yard behind Steve and Gene's Auto Parts, located south of the city of South Bend, IN. This junkyard has been used for a number of studies concerning tire-breeding mosquitoes (Beier et al. 1983; DeMaio et al. 1981); all the major species inhabiting tires in northern Indiana were found at this site. The junkyard was surrounded by an oak forest on the north and west sides, by a road on the east side and by Western Avenue Iron and Metal (a similar scrap yard) on the south side. Throughout the junkyard there were numerous piles of 200 to 500 tires. Some of the piles were exposed to the sun and others were completely shaded by trees. All of the piles were isolated from each other. The woodlot utilized in the study was located on the campus of the University of Notre Dame, IN. This woodlot was bounded on the south and west by open fields, by a road on the north side and by a row of houses on the east side. The woodlot was 2 km from the nearest known large aggregation of tires. A small clearing in the woodlot was also utilized. La Crosse encephalitis has never been reported in St. Joseph County, although foci are known within 100 km to the east and west (Ft. Wayne, Indiana and South Chicago). Thus, no disease hazard was created by these experiments.

In order to study colonization of new tire habitats, 80 tires were obtained from the Baker Tire and Rubber Company, South Bend, IN. The tires were factory rejects shipped to the area for disposal. They were removed directly from the delivery truck.

On May 24, 1982, 40 of the tires were taken to the junkyard. Twenty of the tires were placed around the edges of an existing pile which was exposed to direct sunlight. The remaining 20 tires were placed around the edges of a pile that was shaded for more than 50% of the day.

The other 40 tires were placed in the woodlot. Twenty of the tires were staked upright in rows in a small clearing. This clearing was ex-

posed to direct sunlight throughout the day, with the exception of the early morning and evening. The remaining 20 tires were staked around the base of a large oak tree (*Quercus alba*). These tires were shaded throughout the day.

Organic material was added to 10 of the 20 tires in each of the sites described. This material consisted of 300 ml of wet maple leaf litter from the previous autumnal leaf fall. Rainfall was the only source of water in the tires.

The tires were sampled by thoroughly mixing the contents and withdrawing 300 ml with a small beaker. Each week, 5 of the 10 tires in each of the 8 treatments were sampled. As a result, each tire was sampled only once every 2 weeks. Large pieces of leaf litter were removed from the sample and replaced in the tire. The mosquito larvae in the samples were counted and identified to species. Only second, third and fourth instar larvae and pupae were counted. Pupae were reared for identification as adults.

In addition, 2 small piles of tires in the junkyard were utilized to study resting by adult mosquitoes. One of these piles was located in the shade and the other pile was exposed to direct sunlight. Twenty-five tires containing larvae were selected and marked in each pile. The tires were screened on one side and placed on the surface of the respective piles to facilitate sampling.

The sampling procedure consisted of removing adult mosquitoes from each of the tires with a lightweight mechanical aspirator (Nasci 1981) once every week. Additional samples were taken in the vegetation surrounding the tire piles so that comparisons between the population resting in the tires and the population resting in the vegetation could be made.

The mosquitoes collected were killed by freezing and identified to species and sex. Females were further separated on the basis of blood engorgement. Unengorged females were dissected and the ovaries and midguts examined. When rehydration was necessary, the mosquitoes were soaked in a solution of soap and water (Ungureana 1972). Parity was determined using the method of tracheolar dilation (Polovodova 1949). Blood meals were identified using the modified capillary precipitin test (Nasci 1982).

## RESULTS

Over the 12-week period from June 3 to August 25, 1982, 17,173 mosquito larvae were collected and identified from the two sites (Table 2, Fig. 2). The most abundant species collected at both sites was *Culex restuans*, ac-

Table 2. Mosquitoes collected from 8 types of tire habitats from June 3 to August 25, 1982.

Tire habitat*	No. tires	Percent of the larvae collected**				
		<i>Culex</i>			<i>Aedes</i>	
		<i>restuans</i>	<i>pipiens</i>	<i>territans</i>	<i>atropalpus</i>	<i>triseriatus</i>
		(5,816)	(14)	(79)	(508)	(462)
Tireyard						
Exposed						
Litter	10	26	100	15	36	6
No litter	10	2	—	1	42	3
Shaded						
Litter	10	55	—	38	6	48
No litter	10	17	—	46	16	42
		(9,716)	(54)	(41)	(0)	(483)
Woodlot						
Exposed						
Litter	10	34	83	78	—	4
No litter	10	14	15	—	—	2
Shaded						
Litter	10	24	—	—	—	53
No litter	10	28	2	22	—	41

\* Each tire sampled once every 2 weeks.

\*\* Figures in parentheses indicate the total number of larvae of each species collected in the tireyard or the woodlot.

counting for 90% of all the larvae collected. *Culex restuans* larvae accounted for 85% of the larvae collected in the tireyard. Of these, 73% were collected from tires in the shade and 81% were from tires containing leaf litter. *Culex restuans* larvae comprised 90% of the larvae collected in the woodlot, 58% from tires containing leaf litter and 52% from tires in the shade. It was also the most rapid colonizer. Larvae were collected in 30 of the 40 tires sampled on June 3, 10 days after the tires were placed in the field.

*Culex pipiens* larvae accounted for only 0.4% of the total collection. This species was collected primarily from tires in the sun and containing leaf litter (86%). The first larvae of this species were collected on July 16. *Culex territans* larvae accounted for 0.7% of the total and were found to increase late in the season.

*Aedes atropalpus* larvae were found in the tireyard but not in the woodlot. This species was first recovered on June 10, 17 days after the experiment was initiated. Over the course of the season, 78% of the *Ae. atropalpus* larvae were found in the tires exposed to sunlight. Tires containing leaf litter contained 42% of the *Ae. atropalpus* larvae collected.

*Aedes triseriatus* larvae were found in all treatments. Ninety-three percent of the larvae of this species were collected from shaded tires and 55% were from tires containing leaf litter. *Aedes triseriatus* larvae were first collected in the shaded tires in the junkyard after 31 days of exposure, on June 24. Larvae of this species

were first collected from shaded tires in the woodlot after 45 days of exposure, on July 7.

Over the course of the season *Cx. restuans* was the dominant species in the new tire habitats. The population of this species peaked in mid-June and began to decline by mid-July in all sites (Fig. 2). *Aedes atropalpus* and *Ae. triseriatus* increased in numbers through the season. Late in the season, *Ae. triseriatus* was the predominant species in the shaded tires in both the woodlot and the tireyard while *Ae. atropalpus* was predominant only in the sun tires in the tireyard.

A total of 2,261 adult mosquitoes were collected from tires by suction aspirator over the 14-week collecting period from May 25 to August 26. Thirteen species were collected from shaded tires and 7 from exposed tires. On the average, 78% of the shaded tires harbored mosquitoes each week. Only 36% of the exposed tires harbored mosquitoes each week. In the shade, tires harbored an average of 74 mosquitoes per tire as compared to 16.4 mosquitoes per tire in the sun.

Only 2 species, *Ae. atropalpus* and *Ae. triseriatus* were collected in sufficient numbers for any trends to be noted (Table 3). Most (98%) of the *Ae. triseriatus* adults were collected in the shaded tires. Approximately 2/3 of the *Ae. atropalpus* adults were collected from exposed tires with the remaining third being collected from shaded tires over the course of 3 weeks from July 21 to August 3.

Seasonal sex ratio variation was detected in

Table 3. Comparison of exposed and shaded tires as a resting place for *Aedes triseriatus* and *Ae. atropalpus*.

Tire location	No. tires	Tires sampled*		Adult mosquitoes collected			
				<i>Aedes triseriatus</i>		<i>Aedes atropalpus</i>	
		No.	% positive	No.	% ♀	No.	% ♀
Exposed	25	350	32	40	60	218	50
Shaded	25	350	74	1614	84	97	80
% from shaded				98		31	

\* Each tire sampled once per week for 14 weeks with a suction aspirator.

the collections of adult *Ae. triseriatus* (Fig. 3). Males outnumbered females early in the season but represented a gradually decreasing percentage of the population as the season progressed. No sex ratio variation was detected in *Ae. atropalpus* (Fig. 3).

Unengorged females were dissected to determine parity status. Throughout the season the majority of the *Ae. triseriatus* females collected in the shaded ground cover were nulliparous (Fig. 4). The *Ae. triseriatus* females collected resting in tires consisted primarily of

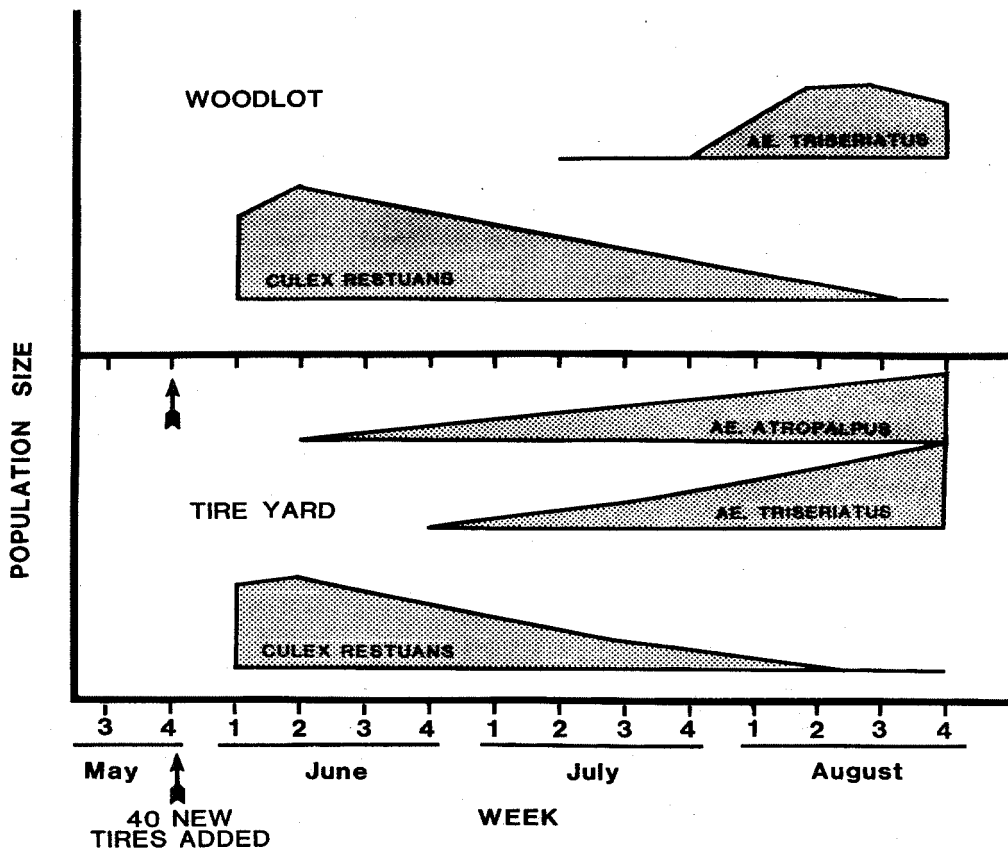


Fig. 2. Diagram of occurrence of larval mosquitoes in recently discarded tires.

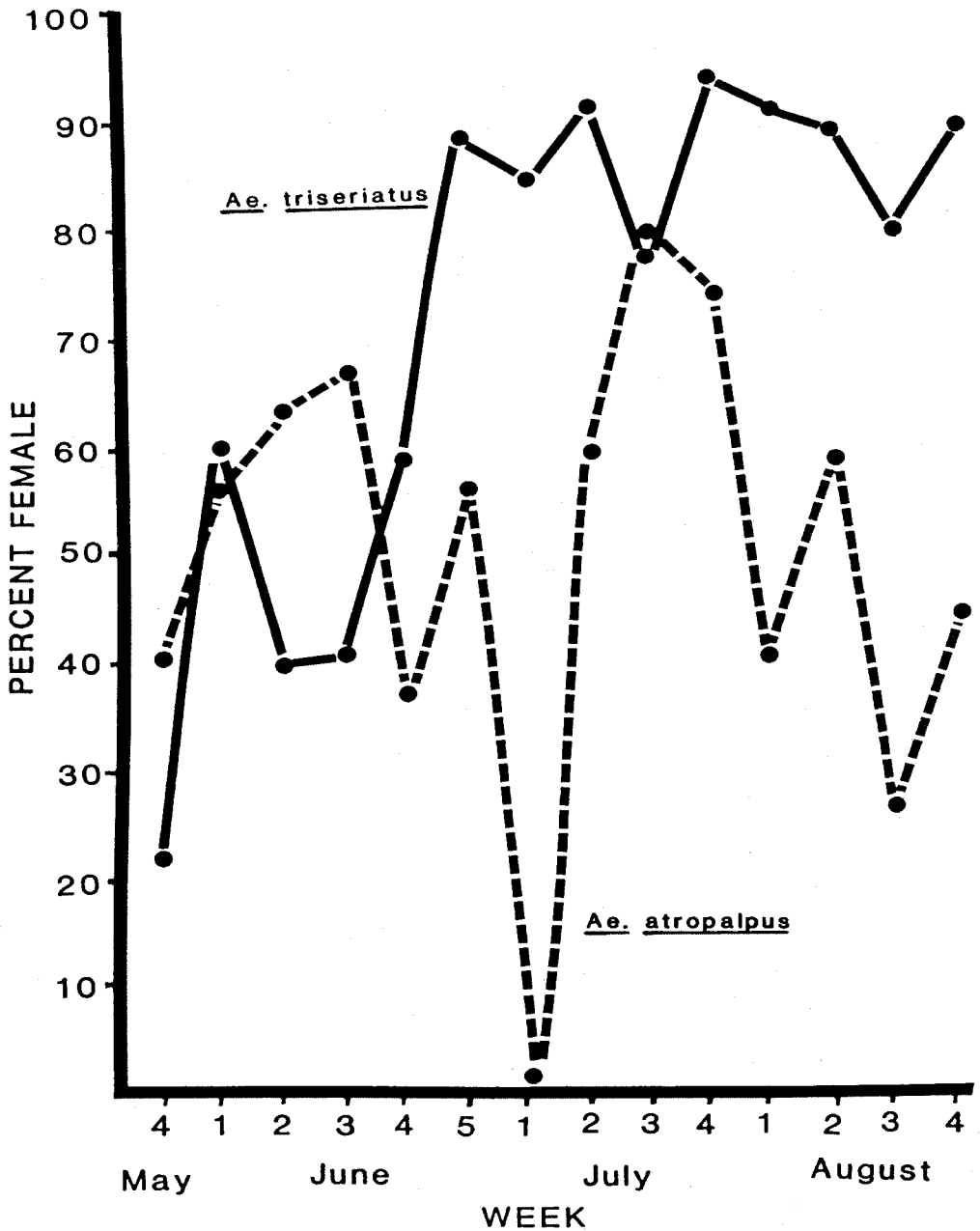


Fig. 3. Weekly variation in the sex ratio of adult mosquitoes collected resting in tires in a junkyard.

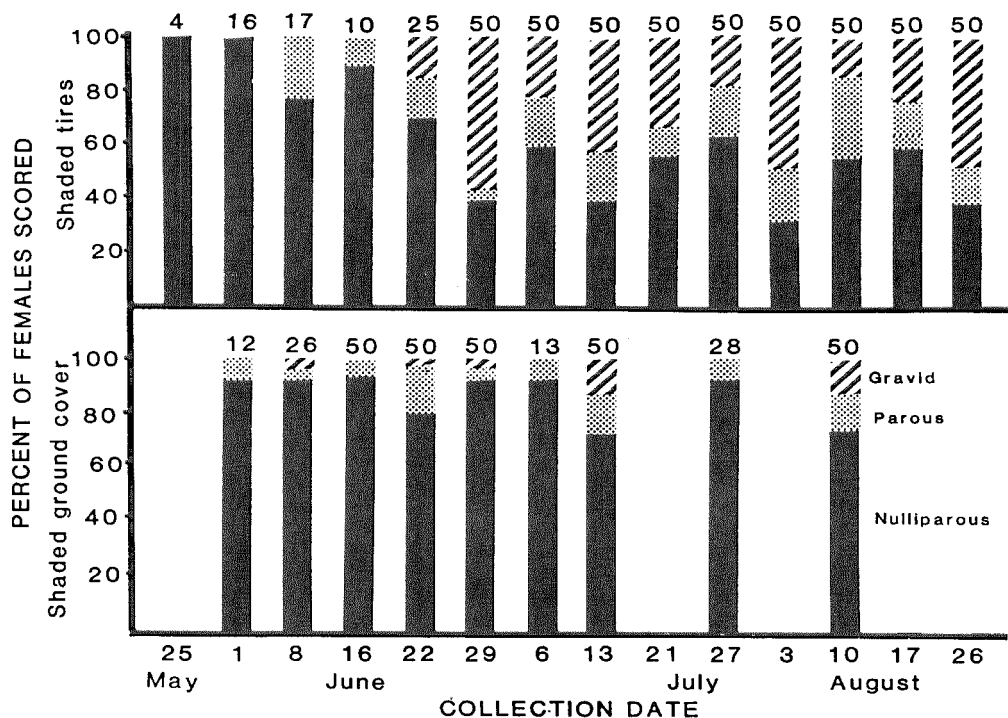


Fig. 4. Population structure of adult female *Aedes triseriatus* resting in shaded tires or in shaded ground cover in the vicinity of tires. (Numbers at the top of the bars indicate the number dissected; females scored per gravid, parous or nulliparous.)

nulliparous and gravid individuals (Fig. 4). *Aedes atropalpus* females were also examined for parity. In this species, midguts must be examined because of autogeny. Due to an undetermined step in the procedure, many of the midguts were unreadable. Many of the female *Ae. atropalpus* (55%) were either gravid or nearly gravid, presumably developing autogenous eggs.

Though the sample size was too small for any conclusions, engorged *Ae. atropalpus* females were collected for blood meal analysis. Hosts utilized were humans, deer (*Odocoileus virginianus*) and cats.

#### DISCUSSION

*Culex restuans* was the most rapid and the most numerous colonizer of the new tire habitats. This species and *Cx. pipiens* as well showed a tendency to utilize tires which contained leaf litter. This trend is consistent with the natural tendency of these species to utilize

water that is high in organic content. The *Cx. restuans* population in the tires peaked early and then collapsed. Identification of the *Culex* mosquitoes collected in 16 New Jersey light traps, distributed throughout St. Joseph County, IN, over the course of the season showed a similar trend for the adult population of *Cx. restuans*. However, the light trap data indicate that the *Cx. pipiens* population rises later in the season, replacing *Cx. restuans*. The tires utilized in this study did not reflect this trend although large numbers of *Cx. pipiens* larvae were collected from older tires placed in a woodlot (L.B. Leiser, unpublished data). Perhaps as a tire ages it becomes more attractive to *Cx. pipiens*.

*Aedes atropalpus* colonized tires in the sun more readily than tires in the shade. Previous observations on larval occurrence (Beier et al. 1982) are consistent with these data. In its natural habitat, this species is found in rockholes in sunny areas. The females must fly across open areas in order to find the oviposition site. The females in the tireyard seem to show a similar

behavior by primarily colonizing tires in the sun.

The presence or absence of leaf litter did not affect oviposition by *Ae. atropalpus*. This suggests that organic content is not a cue for oviposition by female *Ae. atropalpus*. The presence of developing fourth instar larvae (Kalpage and Brust 1973) and the presence of pupal exuviae tends to increase oviposition by *Ae. atropalpus* under laboratory conditions.

Collection of *Ae. atropalpus* in the junkyard and not in the woodlot indicates that this species may have a limited flight range and that extension of its range may depend upon shipment of tires from one junkyard to another. Thus far, the small piles of tires behind service stations in the South Bend area remain uncolonized by this species.

*Aedes triseriatus* was found most frequently in the shaded tires in both the junkyard and woodlot areas. This species is primarily a woodland inhabiting species and seems to be reluctant to leave wooded areas. However, oviposition (Beier and Trpis 1981) and biting (DeFoliart and Lisitz 1980) by this species have been reported in open areas. Larvae collected in fully exposed tires lend support to the hypothesis that at least a portion of the *Ae. triseriatus* females have a preovipositional dispersal behavior (Beier et al. 1982) and will leave shaded areas in order to find oviposition sites.

Collection of *Ae. triseriatus* larvae in the tires indicates that this species is at least bivoltine in northern Indiana. Scholl and DeFoliart (1978) indicate that factors such as sex ratio variation, coupled with the early onset of diapause, may be sufficient to prevent the occurrence of a second generation in Wisconsin. In northern Indiana there is a sufficient period of time between emergence and diapause for a second generation to occur in both tireyards and woodlots. Due to the possibility of transovarial transmission of LAC virus (Watts et al. 1973), multiple generations could serve to increase the number of infected mosquitoes present in an area in a short period of time. The first adults of the season emerge earlier in tireyards than in woodlots (L. D. Haramis, unpublished data) and the time of colonization reflects this difference. The first larvae of *Ae. triseriatus* were detected in the tireyard 14 days earlier than in the woodlot. Nevertheless, 2 generations were achieved in both instances, increasing the seasonal vector potential of both populations.

*Aedes atropalpus* was able to utilize new tires as a larval habitat more rapidly than *Ae. triseriatus* (17 vs. 31 days). A number of factors, such as autogeny, are probably involved in the difference in the rates of colonization. However,

the most likely explanation for this difference is that *Ae. atropalpus* reared on a long day photoperiod lays some of its eggs directly on the surface of the water (Anderson 1968). *Aedes triseriatus* oviposits on the inside surface of tires above the waterline. Consequently, the eggs of *Ae. atropalpus* hatch as soon as embryonation is completed while the eggs of *Ae. triseriatus* must wait for reflooding during the next rainstorm. Oviposition behavior enables *Ae. atropalpus* to exploit new habitats more rapidly than *Ae. triseriatus*.

Shaded tires are more attractive to resting mosquitoes than exposed tires. A greater number of individuals as well as a greater diversity of species were collected from the shaded tires. The tires probably provide a high humidity environment which is attractive to adult mosquitoes seeking a resting site.

Shroyer and Craig (1981) demonstrated that differential responses to hatching stimuli among eggs of different sexes led to a seasonal variation in the sex ratio of *Ae. triseriatus* in woodlots. The same trend appears to occur in tire-inhabiting *Ae. triseriatus*. The males emerge early and represent a gradually decreasing percentage of the population over the course of the season.

No seasonal sex ratio variation was noted in *Ae. atropalpus*. Because the eggs are often placed directly on the surface of the water, hatching stimuli could not cause a deviation in the sex ratio, assuming a 1:1 sex ratio in the eggs.

The parity analysis indicated that a very low percentage of the females in this area were successful in obtaining a blood meal, possibly because of the high densities of females in the area. The analysis also indicated that gravid females preferentially rest in the tires. This observation suggests that the females, after locating a host, move around until they locate a suitable oviposition site, where they remain until they have deposited their eggs.

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