OVIPOSITIONAL BEHAVIOR OF *TOXORHYNCHITES AMBOINENSIS* IN A TIRE YARD[1]

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ABSTRACT. The oviposition behavior of the predatory mosquito, *Toxorhynchites amboinensis,* was examined in a 1.0 ha tire yard in St. Joseph County, Indiana. A total of 129 laboratory-reared *Tx. amboinensis* females was released. Egg production was monitored by checking for eggs daily in 86 marked tires distributed in 4 distinct habitats. Of the 3,134 eggs recovered during the 28-day period after release, 49% were from a transect of 26 tires in a wooded area, 28% were from 20 tires in a completely shaded tire pile, 22% were from 20 tires in a partially shaded pile, and the remaining 1% were recovered from 20 tires in an exposed tire pile. *Toxorhynchites* eggs were found in every available tire sampled in the wooded area and in both types of shaded tire piles, but only 15% of the tires sampled in the exposed tire pile received any eggs. On the 24th day after release, 3rd and 4th instar *Toxorhynchites* larvae were common in shaded tires. The high fecundity and the preferential oviposition in shaded areas indicates that *Tx. amboinensis* should be considered for the control of mosquitoes that develop in shaded tires.

INTRODUCTION

Predatory mosquitoes of the genus *Toxorhynchites* Theobald are promising agents for the control of container-breeding mosquitoes. The National Academy of Sciences, emphasizing the need for new control mechanisms against nuisance and vector populations of container-breeding mosquitoes, recommended *Toxorhynchites* as one means of mosquito control in developing countries (Brown 1973). Studies on the ability of *Toxorhynchites* to effect a reduction in nuisance or vector mosquito populations have seen a variety of successes and failures.

Natural populations of *Tx. brevipalpis* Theobald were able to suppress *Ae. aegypti* (Linn.) populations in a Tanzanian auto dump, but only at the end of the rainy season when the population density of the predator was greatest (Trpis 1973). During the rest of the year, the population levels of the 2 species followed classic predator-prey oscillations. Similarly, introduced populations of *Ae. aegypti* on Seahorse Island, Florida were reduced by the indigenous *Tx. rutilus rutilus* (Coquillett) during the summer and fall but rose again during the winter months when the predator population was diapausing (Focks et al. 1980). Through the hand placement of 6,000 *Tx. brevipalpis* eggs in artificial containers, *Ae. aegypti* populations were substantially reduced on the Caribbean island of St. Maarten (Gerberg and Visser 1978). *Toxorhynchites amboinensis* (Doleschall) was recently introduced into French Polynesia to control vectors of dengue and human filariasis (Riviere et al. 1979). This species had become established, breeding primarily in tires, but apparently has not decreased vector populations.

The frequent failures in releases of *Toxorhynchites* mosquitoes to control vector or nuisance mosquitoes is probably due to several causes. If the initial release does not include enough females, or they are not inseminated, egg deposition will be small and the resultant predator popula-

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tion will be unable to effectively reduce the prey population. Furthermore, the oviposition site selection of the *Toxorhynchites* mosquitoes must sufficiently overlap that of the prey for control to occur.

We are currently evaluating several species of *Toxorhynchites* for controlling populations of *Aedes triseriatus* (Say) in 2 major breeding sites, tree holes and tires. Earlier studies showed that the introduction of 61 laboratory-reared *Tx. r. rutilus* females into a 14 ha woodlot in northern Indiana reduced the *Ae. triseriatus* population by 34% in 100 monitored tree holes (S. L. Durso, unpublished data). The *Toxorhynchites* dispersed throughout the woodlot and oviposited in 75% of the tree holes producing *Ae. triseriatus*.

The present study is a first step in determining the feasibility of using *Tx. amboinensis* to control *Ae. triseriatus* in discarded automobile tires. This species was selected due to its high fecundity relative to most other *Toxorhynchites* species and because the eggs of this species are relatively large[3] and easily recognized in the field. The objectives of this study were to examine the oviposition preferences of *Tx. amboinensis* among several tire habitats, and to determine whether *Tx. amboinensis* larvae can develop in these tires.

MATERIALS AND METHODS

The *Toxorhynchites amboinensis* used were from a colony maintained in the Vector Biology Laboratory at the University of Notre Dame. The colony was founded in May 1979 from first instar larvae obtained from the Pacific Research Unit, Honolulu, Hawaii. The original colony was established from a small collection of larvae and eggs made by Dr. L. Rosen in June 1975 from 2 sites on Oahu, Hawaii.

In rearing the adults for release, *Tx. amboinensis* eggs were collected over a 3 day period. After hatching, 20 first instar larvae were placed in enameled pans containing 1 liter of water. *Aedes triseriatus* (WALTON strain) larvae were exclusively used as prey with care being taken to match prey size to predator size. A prey to predator ratio of approximately 20:1 was maintained to ensure rapid development and to minimize cannibalism among the *Toxorhynchites* larvae.

As the *Tx. amboinensis* pupated, they were placed in paper cups containing 300 ml of tap water. Adults were allowed to emerge into a 1.0 m^3 nylon screened cage. The cage was enclosed in plastic to maintain high humidity. Honey-soaked cellucotton and water were provided. Adults were held in the cage for at least 5 days to allow adequate time for mating. The adults were sexed and counted through the examination of exuviae after emergence.

The site chosen for the release was General Tire Co., a 1.0 ha tire yard located in South Bend, Indiana. The site was bordered on the east, west, and south sides by public roads and on the north by a large corrugated steel building (Fig. 1). This site was chosen because the physical barriers on all 4 sides would presumably limit mosquito migration. Furthermore, it offered a chance to compare ovipositional preferences among several different tire pile habitats. The first habitat (I) was beneath the tree canopy and consisted of approximately 150 tires. It was termed the "shade site" since every tire was in the shade during the entire day. The second habitat (II) consisted of approximately 40 tires and was termed the "partial shade site" since all tires were surrounded by tall vegetation and shaded for part of the day by the nearby canopy. The third site (III) consisted of approximately 400 tires and was termed the "exposed pile" since it was out in the open with no surrounding vegetation. This site received minimal shading during the early morning from the adjacent building but was exposed to direct sunlight during the rest of the day. Twenty tires were randomly chosen from each tire pile, numbered, and marked

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[3] Crown, M. R. 1979. Unpublished M.Sc. Thesis, Louisiana State University Medical Center, New Orleans, LA.

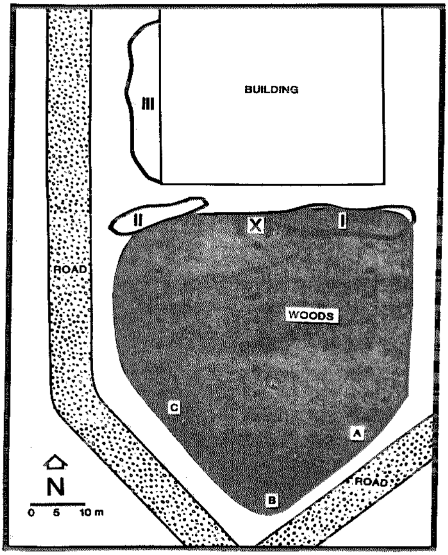

Fig. 1. Diagram of the study site showing the relationship between three tire pile habitats (I,II,III), transects of tires in the woods (A,B,C), and the release point (X).

with orange flagging. Additionally, 3 transects with tires spaced 4.6 m apart were set up in the adjoining woodlot (Fig. 1). All 26 tires in the woodlot were braced upright against trees.

On 14 July 1981, 129 female and 141 male *Tx. amboinensis* were released at the release point (Fig. 1). All marked tires, as well as those in the transects, were checked daily for eggs beginning 24 hours after the release and continuing for 28 days. The tires were inspected for *Toxorhynchites* eggs using a flashlight. All eggs were counted and removed using a wire loop with a nylon screen stretched over it.

To determine the ability of *Tx. amboinensis* to develop in the tires, 10 unmarked tires were randomly chosen from each of sites I and II on the 24th day. All water from these tires was removed using a Foley turkey baster and a soup ladle, and examined for *Toxorhynchites* larvae. The developmental stage of the *Toxorhynchites* larvae and the presence or absence of other larvae were noted for each of the 20 tires.

RESULTS

There was an initial high rate of oviposition during the first 24 hours after release followed by a steadily diminishing rate which fluctuated at a low level after the second week (Fig. 2). The average number of eggs recovered each day dropped from 187 per day during the first 2 weeks of the study to 37 per day during the last 2 weeks. The 2 extremely low points of oviposition (days 12–15 and day 23) were associated with unusually heavy rainfall on the preceding days. The rainfall was so severe during days 11–14 that the entire wooded area and much of sites I and II were under 25 cm of flood water on day 14.

Of the 3,134 eggs recovered during the 28 days of study, 1,548 (49.4%) were from the transects through the wooded area and 1,586 (50.6%) were recovered in the 3 tire pile sites (Table 1). *Toxorhynchites amboinensis* overwhelmingly preferred to oviposit in shaded tires. Of the 1,586 eggs recovered from the tire piles, 55% were from site I, 42% from site II and 3% were recovered from site III.

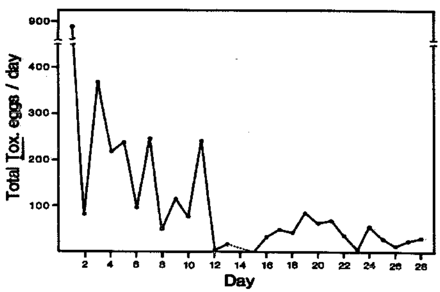

Fig. 2. Total *Toxorhynchites amboinensis* eggs recovered daily from 86 sampled tires. Dotted line indicates a single day of missing values.

The *Toxorhynchites* repeatedly oviposited in tires located in shaded habitats. With few exceptions over half of all tires from shaded sites received at least 1 egg every 4 days throughout the 28 day period (Table 1). Around 90% of the 42 eggs from the exposed tire site were found in the first 12 days after the release. The 3 tires from this site that received eggs were the 3 sampled tires closest to site II, a distance of only 4 m.

When the 10 previously unsampled tires from each of sites I and II were examined for *Toxorhynchites* larvae on day 24, 47 larvae were found in 6 tires from site I (range 1–18) and 4 larvae were found in 2 tires from site II (range 1–3). All larvae were late third or early fourth instars. When *Toxorhynchites* larvae were found in the tires, relatively few prey larvae were found, but in tires void of *Toxorhynchites*, higher levels of *Aedes* and *Culex* larvae were found.

DISCUSSION

This study shows that *Tx. amboinensis* prefers to oviposit in shaded tires. The oviposition rate of 100% in shaded tires was significantly greater than the 80% positive ovitraps seen by Focks et al. (1979) in releases of *Tx. r. rutilus* into a Florida housing project or the 75% positive treeholes seen in earlier releases of *Tx. r. rutilus* in an Indiana woodlot (S. L. Durso, unpublished data). This preference for shaded tires may be even greater than the data suggest when it is considered that the only eggs from the sun-exposed site were found in 3 tires located adjacent to the partially shaded site.

This preference for shaded areas may be due either to differences in water quality and organic content between shaded vs. exposed tires, the different prey populations, or simply due to a reluctance of adult *Tx. amboinensis* to move into exposed areas. Our data supports the latter explanation. If the *Toxorhynchites* females can sense prey populations, tires in the exposed site containing large *Culex* populations should have attracted a higher rate of oviposition than observed. Furthermore, organic matter, as well as mosquito larvae, was flushed out of many tires in the shaded sites during the flooding, but the resultant change in the water quality and organic content did not affect oviposition.

The female *Tx. amboinensis* released into General Tire adapted well to this environment. Though the unusually heavy rainfall during the middle of the study prevented the females from ovipositing during those few days, they were able to survive and continue oviposition soon afterwards. The relatively high percentage of tires receiving eggs on any one day demonstrates that female *Toxorhynchites* distribute eggs throughout available sites thereby maximizing their use of prey resources.

Table 1. *Toxorhynchites amboinensis* eggs recovered from the 4 tire habitats over 4 day intervals and the percentage of tires positive for eggs during that period.

	Eggs recovered (% positive tires)				
Days	Woods	I	II	III	Total
1–4	921 (69)	552 (60)	49 (20)	0 (0)	1522 (51)
5–8	249 (92)	112 (55)	245 (70)	12 (15)	618 (60)
9–12	176 (53)	33 (30)	199 (70)	26 (5)	434 (40)
13–16*	30 (34)	4 (10)	11 (40)	0 (0)	45 (22)
17–20	84 (76)	69 (65)	91 (75)	4 (5)	248 (57)
21–24	54 (53)	67 (80)	47 (70)	0 (0)	168 (51)
25–28	34 (46)	33 (70)	32 (60)	0 (0)	99 (44)
Totals	1548 (100)	870 (100)	674 (100)	42 (15)	3134 (80)
% total eggs	49.4	27.8	21.5	1.3	

* Denotes time period of heavy rainfall.

The continued oviposition for 28 days is comparable to the 30 days of oviposition observed in the earlier studies in Indiana woodlots. However, this period seems long in comparison to the greatly diminished oviposition rate which Focks et al. (1979) observed in their Florida release. The difference may be either climatic or due to their observed high emigration rate (6 females/day) into the surrounding wooded areas. Emigration of *Toxorhynchites* from the study site may have been minimized by surrounding physical barriers, but it was not measured.

The flooding of the tires was probably responsible for the discrepancies seen in the presence of *Toxorhynchites* larvae in the 10 sampled tires from each of sites I and II. Site II was severely flooded on day 14 while site I was less affected. The contents of many of the tires in site II were flushed out and presumably many *Toxorhynchites* larvae were lost as well. For this reason the presence of larval *Toxorhynchites* in 60% of the tires in site I is probably a more representative value than their presence in 20% of the tires in site II. The important observations from the larval samples are that *Toxorhynchites amboinensis* can develop within the aquatic tire environment and that they can exert control over prey populations.

The ovipositional preference of *Tx. amboinensis* indicates that use of this mosquito for control will be limited to those species occurring specifically in shaded tires. In tire yards in Northern Indiana, *Ae. triseriatus* occurs primarily in shaded tires, *Aedes atropalpus* (Coquillett) are common in sun-exposed tires and several species of *Culex* occur in both types of tires (J. C. Beier, unpublished data). Releases of *Toxorhynchites* in tire yards in the midwest may be appropriate primarily for the control of *Ae. triseriatus*, and secondarily for the control of *Culex*.

Toxorhynchites oviposition in tires may be influenced by the nature of the habitat surrounding the tire yard. The demonstrated emigration rates for *Toxorhynchites* (Focks et al. 1979) and their strong preference for shaded areas indicates that successful control should be expected only for releases in tire yards which are relatively isolated from surrounding wooded areas.

A recent EPA study estimated that 200,000,000 automobile tires are discarded into the environment each year (Deese et al. 1981). The growing theory that the majority of LaCrosse encephalitis cases are associated with tire-breeding *Ae. triseriatus* populations (DeFoliart and Lisitza 1980), indicates a potentially explosive situation. The utilization of *Toxorhynchites* mosquitoes in the control of tire-breeding populations of *Ae. triseriatus* offers one means of control. Future studies involving quantified larval surveys should be initiated to determine the degree of control that can be exerted upon vector or nuisance populations of container-breeding mosquitoes by *Toxorhynchites*.

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EVALUATION OF EMERGENCE TRAPS IN ASSESSING THE IMPACT OF A MACROPHYTE ON THE PRODUCTION OF CHIRONOMID MIDGES[1]

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ABSTRACT. Chironomid midge emergence was compared from portions of a residential-recreational lake kept free of the Eurasian water milfoil, *Myriophyllum spicatum* var. *exalbescens* and a portion infested with this aquatic weed, using floating and non-floating emergence traps. Quantitative and qualitative differences were observed using floating emergence traps in the hydrophyte free portion, while only quantitative variations were seen in the portion infested with *M. spicatum*. The differences found in the hydrophyte free portion were mainly due to increased catches of *Procladius* spp. and may have been due to pupal drift, which *Procladius* spp. reportedly exhibits prior to emergence. The results of this study suggest that the use of floating emergence traps in the portion of this lake containing hydrophytes provide a more accurate estimate of midge populations than non-floating traps.

A variety of aquatic habitats in and around residential and industrial areas in southern California produce tremendous numbers of nuisance chironomid midges necessitating periodic abatement measures (Ali and Mulla 1975, 1976; Mulla 1974, Mulla et al. 1975). Timing of midge control strategies and evaluating their efficacy rely in part on adult emergence collections obtained from submerged emergence traps (Ali et al. 1978, Johnson and Mulla 1981, Mulla et al. 1975). In some of these habitats, aquatic macrophytes completely cover the bottom and at times interfere with obtaining these collections. During a study comparing midge production from weed free portions of a residential-recreational lake to parts containing hydrophytes (Johnson and Mulla 1982), we saw the need for stationary adult emergence traps which floated above vegetation but remained below the water surface. This investigation provided an opportunity to compare the adult midge fauna, collected from submerged floating emergence traps an-

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