LABORATORY COLONIZATION AND MAINTENANCE OF *TOXORHYNCHITES MOCTEZUMA*

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ABSTRACT. A colony of *Toxorhynchites moctezuma* was established at the Caribbean Epidemiology Centre in Trinidad in 1984. *Toxorhynchites moctezuma* was maintained in cages with high humidities. Eggs were deposited most frequently in a cut bicycle tire containing water. A minimum of 42 h was required for hatching, but 94% hatched between 43 and 51 h. *Aedes aegypti* larvae were supplied as prey. Larval development times varied with the quantity of prey offered, but when fed *ad lib*, peak developmental time was 18 days. Mean pupal developmental time was 5.5 days. Although only 12% of larvae survived to pupation in 3 years of production, our experience indicates this species would be a likely candidate for mass production and release.

INTRODUCTION

The use of *Toxorhynchites* mosquitoes as control agents was first proposed by Colledge (1911), and subsequent studies on the use of this genus of mosquito in pilot control projects were reviewed by Gerberg (1985). Because of increasing resistance of *Aedes aegypti* (Linn.) to insecticides in the Caribbean (Georghiou et al. 1987, Rawlins and Ragoonansingh 1990), resistance of householders to placing insecticides in their drinking water supply (Nathan and Giglioli 1982) and the increasing cost of insecticides, we started a project to colonize *Toxorhynchites moctezuma* (Dyar and Knab) with the ultimate goal of using this mosquito as a biological control agent for *Ae. aegypti*. *Toxorhynchites moctezuma* appeared to be a suitable candidate, as the immature stages occur in peridomestic habitats in Trinidad and Tobago (Heinemann et al. 1980, Chadee 1985).

Initial attempts to colonize *Tx. moctezuma* in Trinidad started in April 1984, and by the end of that year a viable colony was achieved. This paper reports on the colonization and maintenance of *Tx. moctezuma*.

MATERIALS AND METHODS

Specimens for the colony were collected at Chaguaramas Forest, northwest Trinidad, and consisted of eggs, larvae and pupae, which were reared separately. Adults were placed in an aluminum screened cage (61 × 61 cm) and maintained in an outdoor insectary. High humidity was maintained by placing a thin plastic sheeting (Saran Wrap) over the top, back and sides of the cage. Both a hygrometer and a maximum-minimum thermometer were placed inside the cage. The humidity in the cage varied between 84 and 96%, while the temperature ranged from 23 to 30°C. No attempt was made to regulate photoperiod in the insectary. Adult *Tx. moctezuma* were offered honey, soaked in a cotton pad attached to the top of the cage. Three types of containers with water were placed in the cage for oviposition; a cut bamboo 20 cm high × 5 cm internal diameter, a cut bicycle tire 30 cm long × 3 cm deep, and a 2-liter plastic ice cream container painted black.

Following oviposition, eggs were removed with a black plastic spoon that was modified for scooping eggs off the surface and quantifying before they were placed in rearing dishes 30 × 27 × 13 cm deep, half-filled with water. Approximately 300 predator eggs were placed in each dish to which about 10,000 (0.018 ml) *Ae. aegypti* eggs were introduced. Thus, when the predator larvae hatched a ready supply of prey larvae was available. As the predator larvae matured, approximately 12,000 second and third stage prey larvae were introduced on days 3, 6, 9, 12 and 13.

Pupae were removed with a pipette from the larval rearing trays and placed in a 2-liter white plastic container half-filled with water. This container was placed in the adult cage each day except on Saturdays, Sundays and public holidays. After the emergence of all pupae, the exuviae were sexed and recorded. Dead adults were also removed from the cage and recorded each working day.

For prepupal killing behavior studies, 65 *Tx. moctezuma* larvae were used for these observations. Single eggs were placed in plastic containers with 200 ml of tap water. Ten *Ae. aegypti* first instar larvae were offered to each predator larva until they molted to the second instar. Thereafter, 10 second or third stage prey larvae were offered daily until the predator entered the pupal stage. When the adults emerged, the sexes were noted and recorded.

RESULTS AND DISCUSSION

Females oviposited in all 3 types of containers, but the cut bicycle tire was preferred. For example, of 10,853 eggs deposited in November,

Table 1. Development and prey consumption observations on 60 *Toxorhynchites moctezuma*, reared from egg to adult in the laboratory and offered 10 prey (*Aedes aegypti*) larvae per day.

	Instar 1	Instar 2	Instar 3	Instar 4	Total
Mean no. days ± SD	2.9 ± 1.0	4.4 ± 1.6	18.4 ± 7.0	37.2 ± 10.2	63.9 ± 11.3
Mean no. of prey consumed ± SD	14.5 ± 6.6	23.8 ± 9.4	56.4 ± 18.3	170.1 ± 39.7	266.4 ± 44.6
Mean no. prey consumed per day	5.0	5.4	3.1	4.5	
Mean no. prey killed ± SD	0	0	0	5.3 ± 3.6	—

Table 2. Larval and pupal development times (in days) of *Toxorhynchites moctezuma* when varying numbers of prey were offered per day.

No. of observations	No. prey offered	Larval development		Pupal development
		Range	Mean ± SD	Mean ± SD
65	10	39–98	63.1 ± 11.3	5.5 ± 0.7
13	20	32–55	32.9 ± 1.0	6.4 ± 0.6
12	50	25–33	27.1 ± 2.7	6.0 ± 1.1
12	100	20–29	23.8 ± 3.1	5.6 ± 1.3
*	*Ad lib*	14–26	—	—

* Normal daily maintenance of the colony (ca. 300–400 *Tx. moctezuma* eggs/tray).

1985, 8,844 (81.5%) were deposited in the cut bicycle tire, while only 11.5 and 7% were deposited in the ice cream carton and bamboo section, respectively.

Eggs and hatching: Eggs were laid singly and usually from a height during a characteristically elliptical looping motion. The eggs were white when deposited, which later turned pink and finally grey.

Observations on 869 eggs showed that a minimum of 42 h is required for hatching. However, 93.9% of the eggs hatched between 43 and 51 hours. Of 60 eggs observed individually, 47 (78%) hatched, and of these, 23.4% died during the first instar and 21% in the second instar. All of the other larvae pupated and emerged as adults.

Larval and pupal development: When 65 *Tx. moctezuma* larvae were offered 10 prey larvae per day, the mean larval development times were 2.9 (± 1.0), 4.4 (± 1.6), 18.4 (± 7.0) and 37.2 (± 10.2) days for the first, second, third and fourth instars, respectively (Table 1). The mean development time for all larval stages was 63.1 (± 11.3) days and 5.5 (± 0.7) days (Table 2) for the pupae. No difference in the development times of males and females were noted. During the course of development male *Tx. moctezuma* consumed a mean of 269.5, and female 258.1 prey larvae.

Development time of larvae was reduced when more prey was offered (Table 2). Mean development time was 63.1 (± 11.3) days when 10 prey larvae per day were offered but only 23.8 (± 3.1) days when 100 prey larvae were offered. In the routine maintenance of our colony, predator larvae were fed *ad lib* with prey. In a series of observations in which 15,788 larvae pupated, development time varied between 14 and 26 days, peaking at day 18. However, 87.4% developed between 16 and 21 days. Mean pupal development time was not affected by prey density or length of the larval stage (Table 2).

Prepupal killing behavior: Prepupal killing behavior defined as killing but not eating, or at least not eating completely, has been reported for several *Toxorhynchites* mosquitoes (Steffan and Evenhuis 1981). We experimented to determine to what extent killing behavior occurs in *Tx. moctezuma.*

Table 3 presents the results of these observations. Of the 30 male *Tx. moctezuma* larvae under observation, 28 (93%) and 22 (62.9%) of the 35 females showed evidence of prepupal killing behavior. Killing behavior was noted as early as 5 days before pupation, but this activity

Table 3. Prepupal killing behavior exhibited by *Toxorhynchites moctezuma* larvae in the laboratory when offered 10 *Aedes aegypti* larvae per day.

Days before pupation	No. killed (%)	
	Male	Female
1	155 (55.0)	73 (73)
2	70 (24.8)	23 (23)
3	36 (12.8)	2 (2)
4	18 (6.4)	1 (1)
5	3 (1.1)	1 (1)
Total	282	100

became intensified by day one prior to pupation. At this time, 55.0% and 33.2% of the prey larvae were killed by the predator larvae, which eventually emerged to males and females, respectively.

Over the 5-day period in which killing behavior occurred, 20% of 1,400 prey larvae were killed by 28 predator larvae that pupated and emerged to males. Predator larvae emerging as female adults killed 7.1% of the prey larvae over the 5-day period. Killing behavior also occurred in a closely related species *Tx. theobaldi* (Dyar and Knab), but killing behavior was noted 3 days before pupation (Rubio and Ayesta 1984).

The total number of eggs deposited rose each year from 249,050 in 1985 to 598,023 in 1987. Despite the increased number of eggs produced, the percentage of these surviving in the larval instars to produce pupae remained a constant 12%. Only in one month (May 1985) were we able to rear to the pupal stage, 32.1% of the eggs harvested. The low rate of larval survival might have been due to cannibalism. Preliminary observations also suggested that when *Tx. moctezuma* eggs were handled shortly after oviposition, hatching rates decreased.

The colonies at CAREC are now maintained in 30 × 30 × 30 cm cages with wooden frames and bottom made out of plyboard. The sides top and back are covered with nylon mesh, while a stockinette sleeve is attached to the front. To maintain high humidity, a wet sponge 20 × 10 × 1.5 cm is placed on top of the cage, and the entire cage except the front is covered with a thin plastic sheeting (Saran Wrap). Each cage contains no more than 200 pairs of adults. Although the colony continues to be viable, it might be useful to introduce additional material from nature to prevent inbreeding and to avoid laboratory adaptation to the point of being nonfunctional when released.

Although only 12% of the larvae survived to pupation, this species was easy to colonize and maintain. Its potential as a biocontrol agent for *Ae. aegypti* was suggested by Tikasingh and Eustace (1991) in a field trial on Union Island, Saint Vincent and the Grenadines.

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