EFFECTS OF SINGLE INTRODUCTION OF *TOXORHYNCHITES MOCTEZUMA* UPON *AEDES AEGYPTI* ON A CARIBBEAN ISLAND

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ABSTRACT. In March 1989, first instar *Toxorhynchites moctezuma* larvae were introduced into all potential *Aedes aegypti* oviposition sites ($n = 214$) that contained water in the village of Clifton on Union Island in St. Vincent and The Grenadines. One month after this introduction, the mean number of adult *Ae. aegypti* collected with a backpack aspirator in houses in Clifton dropped significantly. Sixty-four percent fewer males and 80% fewer females were collected after introduction of the predator. In the untreated village of Ashton, the overall drop was 24%, with males showing an increase of 27% and females a drop of 55%. The average wing length of females collected in Clifton increased slightly, whereas it remained the same in Ashton.

INTRODUCTION

The potential role of biological control methods for container-inhabiting mosquito species such as *Aedes aegypti* (Linn.) is becoming more important with the increased frequency of epidemic dengue and dengue hemorrhagic fever in the Americas and with the detection of insecticide resistance in this species. This study was initiated to determine if *Toxorhynchites moctezuma* (Dyar and Knab) was a viable alternative to chemical control procedures in permanent water storage sites on Union Island.

The potential efficacy of *Tx. splendens* (Wied.) (Annis et al. 1989), *Tx. rutilus* (Coq.) (Focks et al. 1982) and *Toxorhynchites* spp. (Focks 1985) as larval predators of *Ae. aegypti* has been reviewed. Recently, a study of the impact of *Tx. moctezuma* predation on this vector was initiated on Union Island, St. Vincent and The Grenadines (Tikasingh and Eustace 1989, unpublished data). They found reduced house and container indices, as well as reduced *Ae. aegypti* oviposition rates after introduction of this species. However, the most important outcome from any mosquito control strategy is a reduction in adult mosquito abundance. Monitoring changes in adult abundance may provide a better indication of the reduction in the risk of disease transmission achieved by control efforts (Service 1983).

This study was conducted on Union Island located at about 13°N latitude and 61°W longitude. The island has an area of approximately 16 km^2 and an average annual rainfall of 130 cm. The 1,400 occupants of the island reside primarily in 2 villages, Ashton in the south-central part of the island and Clifton on the southeastern corner of the island. The villages have approximately equal populations and share similar environmental, climatic and vegetation patterns. Houses in both villages consist of uncomplicated wooden and concrete structures, typically with one or 2 bedrooms, with ventilation openings that permit the easy entry and exit of mosquitoes. The absence of pipe-borne water also necessitates that residents store water in a variety of containers, ranging from 200 liter drums up to cisterns of 15,000 liter capacity.

MATERIALS AND METHODS

Before introducing *Tx. moctezuma* on Union Island, egg and larval surveys were conducted. Beginning on March 6, eighty ovitraps were placed in each village. Ovitraps consisted of 475-ml plastic cups fitted with wooden strips to which about 350 ml of tap water was added. The wooden strips (paddles) were changed and water was replenished at weekly intervals for 5 wk. At the conclusion of the trial, all paddles were examined and eggs were counted. From *Ae. aegypti* larval surveillance data, we calculated the percentage of tanks and cisterns, drums and other containers positive for larvae at the beginning and end of the study. Mirrors were used to reflect sunlight into the cisterns, especially along the water's surface near the cistern wall, to aid in the inspection.

During the week of March 13, we evaluated the pretreatment adult *Ae. aegypti* density with a backpack aspirator constructed after the model of Meyer et al. (1983). Working systematically through all rooms, the aspirator operator and his assistant with a flashlight and insect net made an effort to collect all mosquitoes at rest and in flight throughout the house. In each village, we surveyed 20 widely separated houses where entry was allowed by the occupant. Adult mosquitoes were identified to species, separated by sex, counted and discarded.

On March 12, we transported *Tx. moctezuma* eggs from the Caribbean Epidemiology Centre, Port of Spain, Trinidad, where this species had been maintained for 5 years, to Union Island.

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Approximately 60,000 *Tx. moctezuma* eggs were collected 30–36 h after oviposition. About 300 eggs were placed in polypropylene tubes (12 × 75 mm) containing 2.5 ml of water for transport. Eggs were hatched in plastic Petri dishes (30 × 27 × 13 cm) half-filled with water. About 1,000 *Tx. moctezuma* eggs were placed in each dish to which we added 5,000 first instar *Ae. aegypti* larvae as food for the emerging predators. For distribution in the field, approximately 300 first instar *Tx. moctezuma* larvae were placed into 120-ml polypropylene tubes containing 60 ml of water and taken to the field. Predator larvae were introduced into water storage containers between 0700 and 1100 hours.

On March 13 and 14, 1989, the first instar predator larvae were distributed to virtually all water holding containers in the village of Clifton. In addition to small containers such as vases, larvae were added to 92 cisterns and 103 tanks with volumes up to 15,000 liters. The number of larvae placed in each container varied from about 50 per drum to 200 per average-sized cistern, with an overall rate of approximately 50 larvae per 400 liters of water. In containers where the *Ae. aegypti* density appeared to be larger, the number of predators introduced was greater. The containers in Ashton served as an untreated control.

During the week of April 10, a post-treatment survey was conducted in the same houses that we had surveyed in March. Differences in the number of *Ae. aegypti* before and after the predator introduction were used as an indication of treatment efficacy. The paired *t*-test was applied to the data to demonstrate any significant differences before and after predator introduction.

Wing length, an indicator of body size, was measured on all adults collected during both periods, noting the distance from the axillary incision to the apical margin (Nasci 1986).

RESULTS

Of the 80 ovitraps examined weekly in each village for *Ae. aegypti* eggs, 12–40% and 11–43% were positive in the treated and untreated villages, respectively (Table 1). There was a noteworthy drop in mean number of eggs per positive ovitrap in Clifton, from 89 down to 33, whereas in Ashton, this number increased from 13 to 33. Also, the proportion of *Ae. aegypti* positive containers found before the predator release and at the end of the study were significantly different ($P = 0.011$) in Clifton. The differences were noted in tanks and cisterns, but not in other containers. Similar differences were not observed over the same period in Ashton (Table 2).

In neither village was *Tx. moctezuma* found in any container at the beginning of this study. One month after the predator introduction in Clifton, larvae and pupae were observed in 7% of the containers, but in none of the containers surveyed in Ashton.

There was a significant decline in the number of *Ae. aegypti* adults collected in Clifton after the introduction of the first instar *Tx. moctezuma*. Of the 20 houses sampled in Clifton, 54 ($\bar{x} = 2.7 \pm 1.1$/house) males and 116 ($\bar{x} = 5.8 \pm 2.0$/house) females were collected before the introduction of the predator larvae. One month later, the average number collected was reduced to 1.0 (± 0.3) male and 1.2 (± 0.4) females per house. The reductions in females ($P = 0.034$) and total *Ae. aegypti* ($P = 0.001$) were statistically significant.

In the untreated village, the mean adult *Ae. aegypti* density per house was similar to Clifton before treatment (2.6 ± 0.6 for males and 4.2 ± 0.8 for females). One month later, an average of 3.3 (± 0.7) males and 1.9 (± 0.5) females was collected in the same houses. The mean number

Table 1. Weekly collection of *Aedes aegypti* eggs on Union Island (March 13–April 10, 1989).

	Pre-treatment	Post-treatment			
Village	Mar 13	Mar 20	Mar 27	Apr 3	Apr 10
Clifton (treated)					
No. ovitraps	63	71	65	80	80
No. positive	14	29	15	10	21
% positive	22	41	23	12	26
Total no. eggs	1,244	713	781	267	690
Eggs/positive trap	89	25	52	27	33
Ashton (untreated)					
No. ovitraps	72	71	73	59	60
No. positive	31	8	16	24	16
% positive	43	11	22	41	27
Total no. eggs	392	513	323	506	532
Eggs/positive trap	13	64	20	21	33

Table 2. Proportion of *Aedes aegypti* positive containers before and 1 month after introduction of *Toxorhynchites moctezuma* larvae on Union Island. The Mantel Haenszel chi-square test was used to determine significance levels.

Village container type	Before (March)	After (April)
Clifton[1]		
Tanks and cisterns[3]	35/86 = 40.7%	16/85 = 18.8%
Drums[4]	49/96 = 51.0%	45/110 = 40.9%
Others[4]	12/32 = 37.5%	3/10 = 30%
Ashton[2]		
Tanks and cisterns	89/155 = 57.4%	38/74 = 51.4%
Drums	68/84 = 81.0%	38/44 = 86.4%
Others	10/10 = 100%	5/6 = 83.3%

[1] No significant difference between March and April.
[2] Overall significant difference $P = 0.011$.
[3] Tank and cistern index, March/April, significantly different, $P = 0.003$.
[4] Drums and other containers, no significant differences.

of females collected per house dropped significantly ($P < 0.05$), but the combined collection figure did not. Overall, the mean number of *Ae. aegypti* adults per house dropped from 6.8 (± 1.2) to 5.2 (± 1.1) in Ashton whereas in Clifton the reduction was from 8.5 (± 3.1) to 2.1 (± 0.6).

The mean wing length of males collected in the 2 villages before and one month after the predator release was constant (2.51 ± 0.02–2.53 ± 0.02 mm; $n = 140$). Female wing length over the same period varied from 2.96 (± 0.04) to 2.97 (± 0.03) mm in March and 3.10 (± 0.06) to 3.17 (± 0.07) mm in April ($n = 266$). The greatest wing length was obtained from the reduced *Ae. aegypti* population in Clifton. The 7% increase in size in mosquitoes collected in Clifton in April was statistically significant ($P < 0.05$), whereas the 2% increase in Ashton was not statistically significant.

DISCUSSION

The ovitrap data suggested similar oviposition patterns in the treated and untreated villages after treatment. This may reflect the inadequacy of the ovitrap sampling procedure, with only a maximum of 80 traps per area. However, the container indices showed differences with a significantly reduced number of positive containers, especially among the larger types, one month after the treatment in Clifton, and not in the untreated village. This reduced production of larvae in the treatment area coincided with significantly diminished presence (75%) of adult *Ae. aegypti* as opposed to a nonsignificant reduction of 24% in the untreated area.

There was no statistically significant change in the number of male *Ae. aegypti* collected in the 2 locations before and after the predator release, as there was in females. This reduction in the number of adult *Ae. aegypti* females inside houses may have been the result of the intervention since environmental conditions were similar in both villages. It is possible that the increased female body size observed in the mosquitoes collected in Clifton may have been the result of decreased intraspecific competition caused by the predator in this environment. Such changes in body size have been reported and discussed by Wada (1965) and Klowden et al. (1988). Linked to this is the enhanced blood feeding success (Nasci 1986) and greater probability of disease transmission (Service 1983). The relatively small sample size of males obtained and measured may have given an unreliable picture and the resultant absence of increased size of the Clifton male *Ae. aegypti*. If the increased body size of females was due to reduced larval competition, it is difficult to explain the similarity in male body sizes in the treated and untreated areas.

We do not have any evidence that *Tx. moctezuma* was able to sustain itself on this relatively arid island. After the initial 6-month *Tx. moctezuma* release program (Tikasingh and Eustace 1990, unpublished data), this species was not found in containers examined during extensive field surveys. Before implementation of a large-scale control effort with this species, additional research must define the environmental parameters under which it can survive and become established. The apparent inability to cope and survive in this environment severely limits this strategy since repetitive, and very expensive, introductions of *Toxorhynchites* would be required for *Ae. aegypti* control.

Conversely, there is evidence from a study conducted in Trinidad that in a moist environment *Tx. moctezuma* is able to survive in nature after release and establish itself in adjacent areas (Tikasingh 1989, unpublished data). Thus, repeated inundative releases of *Toxorhynchites* in a more suitable habitat may contribute to an effective mosquito control program; however, the costs may be prohibitive.

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