SUPPRESSION OF LARVAL *AEDES AEGYPTI* POPULATIONS IN HOUSEHOLD WATER STORAGE CONTAINERS IN JAKARTA, INDONESIA, THROUGH RELEASES OF FIRST-INSTAR *TOXORHYNCHITES SPLENDENS* LARVAE[1]

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ABSTRACT. Weekly releases of first-instar *Toxorhynchites splendens* larvae were made in household water storage containers in a neighborhood in Jakarta, Indonesia, between April 1987 and April 1988. A single larva was placed in each container surveyed. Forty-one percent of all containers in the treatment area were treated each week and the average container was treated once every 2.4 weeks. *Aedes aegypti* populations were suppressed but not controlled by treatment. It is hypothesized that first-instar *Toxorhynchites* larvae were poor control agents due to their inability to withstand periods of starvation and to their accidental removal from containers during the act of water consumption.

INTRODUCTION

In Jakarta, Indonesia, dengue fever is highly endemic (Suharyono and Lubis 1982). In many areas of the city, piped water is not available. Water for household use must be purchased from roving vendors and stored in a variety of containers in and around the house. These containers provide larval habitat for dengue vectors such as *Aedes aegypti* (Linn.).

The ability to reduce dengue transmission by larviciding in water storage containers has been demonstrated (Suroso 1982). Currently, the Department of Health recommends the use of 1% temephos in household water for *Ae. aegypti* control (Anonymous 1981). However, in some areas there has been resistance to the use of chemicals due to perceived negative effects on the taste and smell of treated water.

The use of *Toxorhynchites* mosquitoes as biological control agents of container breeding mosquitoes has received much attention, although the degree of success achieved in field trials has varied (Steffan and Evenhuis 1981). Failure in early attempts may have resulted from inadequate knowledge of the species involved (Focks et al. 1979). More recently, pilot projects involving innundative releases of both eggs (Gerberg and Visser 1978) and adults (Focks et al. 1982) have indicated promise for the potential use of *Toxorhynchites* in biological control programs.

We decided to test the efficacy of *Tx. splendens* (Wiedemann) as an alternative to temephos for *Ae. aegypti* control in household water storage containers. Since local residents already tolerate the presence of pest larvae in their water supply, we felt that the addition of predator larvae would not be objectionable to them.

Laboratory studies have shown that weekly introduction of a single first-instar larva can provide a high degree of long term control in containers with populations of *Ae. aegypti* similar to those found in the field (Bailey et al. 1981, Castner and Bailey 1984). In light of these findings, we decided to make weekly introductions of first-instar *Tx. splendens.* In addition to the good control seen in laboratory experiments, the first instar would have the advantage as a control agent in that it need not be fed prior to release, thereby reducing the number of prey mosquitoes which must be reared in the laboratory as food.

MATERIALS AND METHODS

Two neighborhoods without piped water were chosen for study. Both were in the district of Bahari, Tanjung Priok, North Jakarta. This is a densely populated urban area typified by small, closely spaced houses and narrow streets. The control area was RW 09, a neighborhood of 108 houses occupied by 196 families and measuring 40 × 50 m. RW 04 was selected as the treatment area, measuring 70 × 150 m, with 126 houses

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occupied by 560 families. The 2 neighborhoods were separated by a distance of approximately 500 m. The major types of containers used for water storage in these areas were 55-gallon drums and "bak mandi," concrete cisterns approximately 1 m^3.

For colony maintenance, *Toxorhynchites* larvae were reared individually in 6-dram vials and fed *Ae. aegypti* larvae daily. Upon pupation, pupae were removed from vials, rinsed, and placed in "Mosquito Breeders" (BioQuip Products) in 250 ml of fresh water at a maximum density of 50 pupae per container until adult emergence.

Adults were kept in a 61-cm^3 cage and fed 10% sugar solution from dental wicks inserted in the necks of Erlenmeyer flasks. A 10-cm diameter black plastic bowl was placed in the cage for oviposition. The insectary was maintained at a temperature of 24–29°C and 50–85% RH.

Eggs to be hatched for larval releases were placed individually in wells of 24-well tissue culture plates. Larvae were held in these containers until release. Releases were made on Wednesday and Thursday of each week. Egg collection for each week's releases began the preceding Friday. Allowing 24–48 hours for hatching, larvae were between 24 and 96 hours old when released. Larvae were not fed prior to release.

To insure randomness in prey surveillance and predator releases, treatment and control areas were divided into quarters, each containing approximately equal numbers of houses. Each week, houses were selected by number from a table of random numbers and all containers in a house surveyed until a total of 100 containers had been surveyed in each quarter. Containers were recorded as either being positive or negative for larvae. In the treatment area, one first-instar *Tx. splendens* larva was placed in each container surveyed.

Pretreatment observations were carried out in both areas for 3 weeks in March 1987. Predator releases were begun in April 1987. The project was terminated in mid-April, 1988, when the treatment neighborhood was demolished to make room for the expansion of a railway line.

In July 1987, surveillance was expanded to include monitoring of the adult *Ae. aegypti* population. This was accomplished by using both ovitraps and man landing-biting counts. Five ovitraps were placed in randomly selected houses in each quarter of the treatment and control areas. Ovitraps were plastic containers, 15 cm high and 10 cm in diameter, painted black and containing approximately 500 ml of water. Wooden tongue depressors, 15.5 × 2.0 cm, were placed inside each ovitrap. These were collected weekly and returned to the laboratory where the eggs were counted and reared to adults for species identification.

Man landing-biting collections were carried out weekly by 2 collectors each collecting for 10 min in each of 6 randomly selected houses in each quarter. Collections were made indoors between 1000 h and noon. Landing mosquitoes were aspirated from exposed lower legs.

To test the ability of various larval instars of *Tx. splendens* to withstand starvation, 192 0 to 24 h-old larvae were placed individually in the wells of 24-well tissue culture plates. The plates were divided into 4 groups, which received the following treatments: group 1, food withheld; group 2, food withheld after molting to the second instar; group 3, food withheld after molting the third instar, and group 4, food withheld after molting the fourth instar. Larvae receiving food were given 10 *Ae. aegypti* larvae of the same instar daily. Each day, counts were made of the number of surviving *Tx. splendens* larvae.

RESULTS AND DISCUSSION

The mean number of water storage containers in use per household each month varied from 6.2 to 7.7 in the control area and from 6.3 to 9.1 in the treatment area (Fig. 1). As a result, the number of households receiving treatment fluctuated during the study period. The largest number treated occurred in June of 1987 when just over 50% of houses received treatment. The lowest was in February 1988 when 35% of the houses were treated. The mean treatment rate for the entire study period was 41%. Therefore, on the average, each container in the treatment area received a first-instar predator larva once every 2.4 weeks.

Aedes aegypti populations were directly related to rainfall. The Breteau index (number of infected containers per 100 houses) in the control area was most highly correlated with mm of

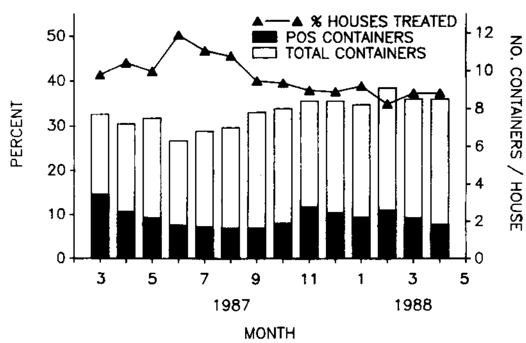

Fig. 1. Percentage of houses treated with *Toxorhynchites splendens* larvae each month in relation to the mean number of containers in use per house in a neighborhood in north Jakarta, Indonesia.

rainfall in the preceding month (n = 13, r = 0.822, $P < 0.001$). There were also significant correlations between the Breteau index and days of rainfall (n = 13, $r = 0.753$, $P < 0.003$) and mm of rainfall (n = 13, $r = 0.770$, $P < 0.002$) in the current month. Although *Ae. aegypti* was not dependent on rainfall for larval habitat, which was supplied by man and remained relatively constant, some other factor associated with rainfall, such as relative humidity, undoubtedly affected adult survival. Similar associations between *Ae. aegypti* populations and rainfall have been noted (Riviere 1985).

Throughout the study, *Ae. aegypti* population indices were higher in the treatment than control area (Fig. 2). An estimate of the impact of predator releases was obtained by comparing the percent change in the Breteau index from the initial pretreatment level in the treatment and control areas. As shown in Fig. 3, the percentage decline in the treatment area was greater than that in the control area in the latter part of the rainy season of 1987. Populations in both areas were lowest in September 1987 and began increasing when the rainy season resumed in October. While the Breteau index in the control area increased in 1988 to levels above those seen in 1987, it remained below its prior year levels in the treatment area.

While some suppression of the *Ae. aegypti* population in the treatment area was apparent, releases of first-instar *Tx. splendens* larvae did not bring about adequate control. After one year of releases, the Breteau index in the treatment area was similar to that in the area receiving no treatment. That this level of suppression was inadequate was evidenced by active dengue transmission in the treatment area during the epidemic of January and February, 1988.

There may be several reasons for the discrepancy between the poor performance of first-instar *Tx. splendens* larvae as control agents in the field and the good control seen in laboratory experiments using related species (Bailey et al.

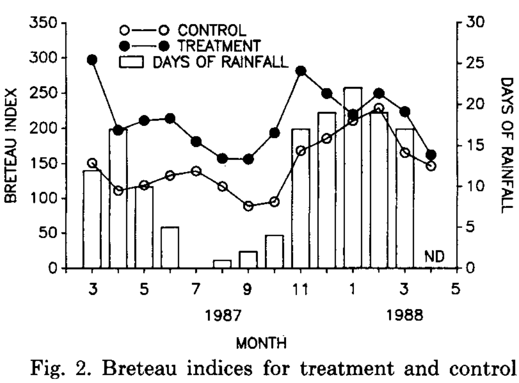

Fig. 2. Breteau indices for treatment and control areas in relation to rainfall in Jakarta, Indonesia.

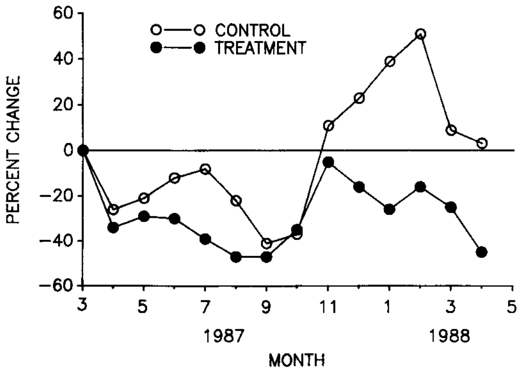

Fig. 3. Impact of releases of *Toxorhynchites splendens* larvae as measured by the percent change in Breteau indices in treatment and control areas.

Table 1. Number of days the various larval instars of *Toxorhynchites splendens* survived without food (food withheld following molt to the indicated instar).

Instar	No.	Mean ± *SD*	Minimum	Maximum
1	48	5.60 ± 0.95	2	7
2	48	7.33 ± 1.82	3	11
3	45	14.51 ± 4.26	3	21
4	31	58.23 ± 23.62	9	102

1981, Castner and Bailey 1984). One of the most important is most likely the limited ability of the first-instar larva to withstand starvation. In the laboratory, we found that first-instar *Tx. splendens* larvae survived an average of 5.6 days without food (Table 1). Some of the larvae we released were up to 4 days old. Assuming a mean age of 2.5 days upon release, the average *Tx. splendens* larva had only about 3 days to obtain food or die of starvation. It can be seen in Fig. 1 that a substantial majority of the containers into which *Toxorhynchites* larvae were released did not contain *Ae. aegypti* larvae. The average container index in the treatment area for the duration of the study was 28.2%. Based on the number of positive ovitraps, we estimate the average rate of colonization of uninfested containers by *Ae. aegypti* in the treatment area at only 17.7% per week (minimum 11.3%, maximum 25.0%). Thus, it would appear that as many as 50% of the larvae we released may have perished from lack of food. Assuming a maximum life span for *Tx. splendens* of 5 days without food following release and an average treatment rate of once every 2.4 weeks for each container, *Ae. aegypti* would have a minimum of 12 days to colonize a container unhindered if the predator larva did not find food within the first 5 days.

Therefore, under the conditions of our study, there was always an ample refuge of unoccupied containers for ovipositing female *Ae. aegypti*. To prevent this, a higher treatment rate would be necessary. Thus, when the *Aedes* container index is relatively low, first-instar *Toxorhynchites* larvae would appear to be unsuitable as control agents unless all containers in one area could be treated at frequent intervals.

An alternative would be to release later-instar larvae with greater resistance to starvation. As shown in Table 1, the ability to withstand periods of food deprivation increases with age. However, with the use of later-instar larvae greater inputs of time, manpower and resources would be required to rear them prior to release. There is also a trade-off between increased ability to withstand starvation and the amount of time an individual larva could be expected to provide control. The older the larvae, and therefore the more starvation resistant it is, the closer it is to pupation.

The small increase in starvation resistance exhibited by second-instar larvae would not seem to justify the extra effort required to rear them to that stage. However, the amount of time third-instar larvae survived without food was nearly triple that of first-instar larvae and double that of second-instar larvae. Considering the balance between inputs, survivability and duration of predatory activity, third-instar larvae may be the most desirable agents for such releases.

Another factor which may have contributed to the poor results seen in this study is accidental removal of the predator larvae. Since *Toxorhynchites* larvae are relatively slow moving and spend most of the time floating at the surface of the water, they may have been removed as people consumed water from the containers.

It could be argued that the effect of the predator larvae was to reduce the density of *Ae. aegypti* in containers without eliminating them completely. Such an effect was observed by Focks et al. (1982) in field experiments. In such a case, our method of larval surveillance (i.e., evaluating containers as positive or negative with no measure of prey density) would not have reflected the actual impact of predators upon the prey population. However, between the months of July 1987 and April 1988 when man landing-biting counts were made, there was a strong correlation ($n = 20$, $r = 0.934$, $P < 0.001$) between the monthly mean Breteau index and the mean number of bites per man-hour (Fig. 4). Therefore, we feel that changes in the Breteau index accurately reflected trends in the *Ae. aegypti* population. We conclude that predator losses due to starvation and accidental removal resulted in the lack of control achieved in this study.

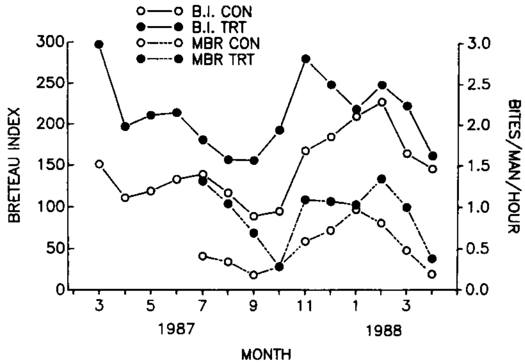

Fig. 4. Breteau indices and man-biting rates in treatment and control areas.

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