DIURNAL PATTERN AND BEHAVIOR OF OVIPOSITION OF *TOXORHYNCHITES THEOBALDI* IN THE FIELD

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ABSTRACT. The diurnal pattern and oviposition behavior of *Toxorhynchites theobaldi* natural populations were studied in 25 artificial containers in the field. The mosquito exhibited a bimodal oviposition pattern with the lower peak at 1100 hr and a mean of 15.7 eggs per container. The higher peak was observed at 1900 hr with a mean of 80.9 eggs per container. Each female flew from 21 to 58 elliptic vertical circles before ejecting one egg upon the surface. In 270 oviposition events, the average was 31.4 ellipses, and the frequency distribution of flights number with different ellipse numbers was fitted to a Poisson distribution. There was a significant linear correlation (r = 0.70) between the oviposition rate and the container surface area.

INTRODUCTION

Toxorhynchites is a genus of Culicidae which is not hematophagous as an adult, and its larval instars are predators of small invertebrates (Steffan and Evenhuis 1981), including larvae of other culicids. Consequently, all species of *Toxorhynchites* are potential biocontrol agents of some species of *Aedes*, because many species of this genus share the same breeding sites as *Toxorhynchites. Toxorhynchites theobaldi* (Dyar and Knab) is a mosquito species that is widespread in all American Neotropics and offers the potential to control *Aedes aegypti* (Linn.) in urban situations in any Latin American country, e.g., Mexico (Reyes et al. 1987).

There are numerous reports in the literature concerning the diel oviposition pattern of hematophagous mosquitoes (Haddow and Gillett 1957, Meek and Williams 1986). There are also similar studies for entomophagous mosquitoes, especially for *Toxorhynchites* species (Trimble 1979, Chadee et al. 1987). Likewise, many papers have been published on *Toxorhynchites* species with respect to oviposition behavior (Furumizo and Rudnick 1978, Trimble 1979, Horio and Tsukamoto 1985). Since information on some biological aspects is lacking for *Tx. theobaldi*, this paper describes the diurnal oviposition pattern, including the oviposition rate as related to the surface area of a container, and a quantitative description of the oviposition behavior. This information is necessary for establishing a mass rearing facility for species which will allow promulgating a program of experimental releases in urban areas with the hope of controlling the dengue vector, *Aedes aegypti.*

MATERIALS AND METHODS

The study was carried out in the graveyard of Valles City, San Luis Potosí, in northeastern Mexico. This is in the "Huasteca region" with a subtropical climate. Thirty samples were taken in the summer of 1987 (July–September). One day before the sampling date, all eggs oviposited that day were removed with a plastic scoop from each of the 25 containers after 2100 hr; thus we started with zero eggs on the sampling day. As there were not 25 containers of identical size and exposed surface in the memorial stones of the graveyard, containers of similar characteristics were selected randomly, but all under shady conditions. The same water level was maintained in all containers, using water from other containers. No experimental containers held flowers, although neighboring ones did. In each sampling which covered the entire day, we counted the eggs of *Tx. theobaldi* which were laid in each of the 25 containers at the end of 0600, 0700, 0900, 1100, 1300, 1500, 1700, 1900 and 2100 hr. At each count, eggs observed in each of the 25 containers were placed in a small vial and distributed in neighbor breeding sites not included in this study. This was done in order to avoid confusion in the counting and also not to reduce the native population of this mosquito predator.

In order to study the possible relationship between oviposition rate and exposed surface area of containers, the surface area of each one was measured in square centimeters. Finally, to describe qualitatively and quantitatively the oviposition behavior, 270 oviposition events were observed. For each one, the number of vertical elliptic flights was counted until the female ejected an egg over the surface of the container. In all the events, the observer was placed 1–2 m from the container.

All of the eggs collected in samples number 10, 20 and 30 were taken to the laboratory and were reared to the adult stage with a daily diet of 10–15 larvae of *Ae. aegypti* or *Culex quinquefasciatus* Say of the same instar as the predator larvae. Ninety percent (563) of eggs collected reached the adult stage, and all of them were determined as *Tx. theobaldi* with the key by Vargas (1953).

RESULTS AND DISCUSSION

Toxorhynchites theobaldi exhibited an oviposition cycle only during the day, because eggs never appeared in the containers at 0600 hr, indicating that the females did not oviposition at night. This agrees with the reports published for other species of *Toxorhynchites* (Paine 1934, Bonnet and Hu 1951, Chadee et al. 1987) but not for *Tx. splendens* (Wied.), which sometimes oviposits during the night (Furumizo and Rudnick 1978). The mean number of eggs oviposited each two hr is shown in Fig. 1. The results showed that ovipository activity had a bimodal periodicity, with two peaks. The lower peak occurred at 1100 hr with a mean of 15.7 eggs per container, while the higher peak was observed 1 hr before twilight (1900 hr) with a mean of 80.9 eggs per container, five times higher than the morning peak. This pattern was similar to the one shown by *Tx. brevipalpis* Theobald (Bonnet and Hu 1951) but not for *Tx. splendens*, which only oviposits in the afternoon (Paine 1934). According to reports published by Chadee et al. (1987) for *Tx. moctezuma* (Dyar and Knab) [considered a junior synonym of *Tx. theobaldi* (E. S. Tikasingh, personal communication)], the oviposition cycle is in the afternoon, where it occurred between 1200 and 1800 hr, with a marked peak between 1400 and 1600 hr. This is different from the results obtained here for *Tx. theobaldi* (Fig. 1), because this species also oviposits in the morning. It is possible that these are different species, although the environmental factors may influence the diel pattern of oviposition of different populations of the same species. Detailed studies on the systematics of these species are necessary to identify and validate such studies.

The proportion of containers in which the predator oviposited were high (Fig. 2). The lowest percent was 72% for sample 26, while the highest was 100% for samples 4, 7, 10 and 29.

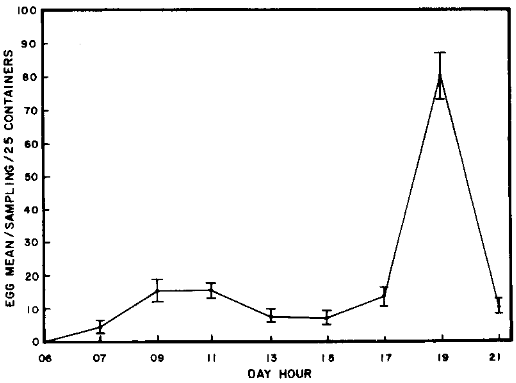

Fig. 1. Diurnal oviposition pattern of *Toxorhynchites theobaldi* in the field.

In conclusion, there was an mean percent oviposition of 89.9 per sample, which supports the concept that *Tx. theobaldi* may be a good biocontrol agent for urban *Ae. aegypti.*

In relation to the oviposition behavior, females of *Tx. theobaldi* made a series of vertical elliptic cycles before ejection of an egg on the water. The basic pattern of oviposition for this species is the same as the detailed description of Linley (1987b) for *Tx. amboinensis* (Doleschall), and very similar to that reported for *Tx. splendens* (Furumizo and Rudnick 1978) and *Tx. rutilus septentrionalis* (Dyar and Knab) (Williams et al. 1961, Trimble 1979). The elliptic cycles per oviposition flight fluctuated between 21 and 58. The average in 270 events was 31.4 ellipses per successful oviposition intent.

Some females oviposited three times and flew up to 100 ellipses without any rest at the same breeding site. The frequency distribution of flights with different number of ellipses is shown in Fig. 3. The distribution was fitted to a Poisson distribution, because the χ^2 value calculated was 36.50, which was lower than the table $\chi^2_{0.05}$ value with 24 *df*. For *Tx. amboinensis*, Linley (1987a, 1987b) stated that there was an average of 22.9 elliptic cycles in the morning; this number increased gradually in the afternoon up to 29.4, with a minimum and maximum of 6 and 43, respectively. These data do not coincide with the reports of Horio and Tsukamoto (1985); they stated that females of *Tx. manicatus yaeyamae* Bohart, *Tx.* sp. and *Tx. towadensis* (Matsumura) make one ellipse in each oviposition event, ejecting one egg at the end of an ellipse.

This general model of oviposition for all the *Toxorhynchites* species decreases the possibility of predation mainly by spiders, because the spiders make their webs over the surface of containers (Trimble 1979). In addition, some species have the ability to place eggs in very reduced

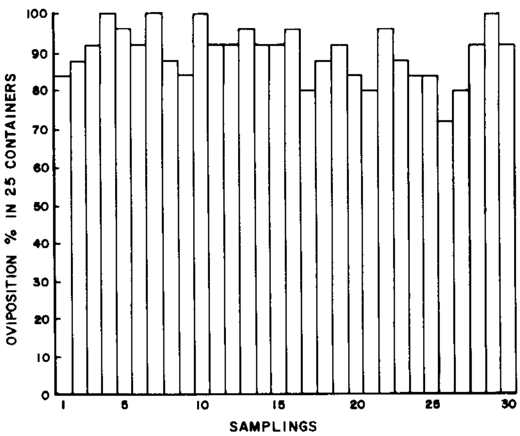

Fig. 2. Proportion of containers in which *Toxorhynchites theobaldi* oviposited.

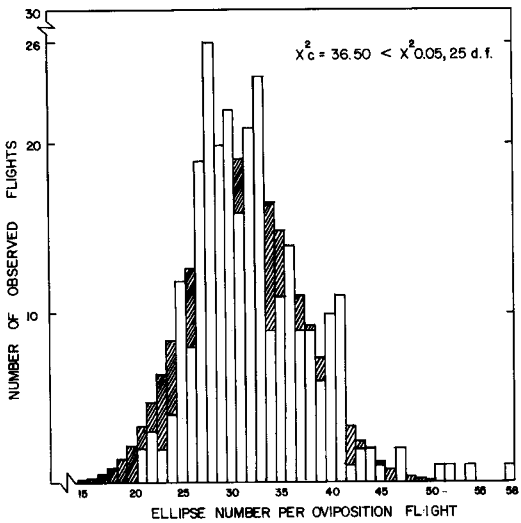

Fig. 3. Frequency distribution of oviposition flights with different number of ellipses by *Toxorhynchites theobaldi*. Expected frequencies: hatched bars. Observed frequencies: unhatched bars.

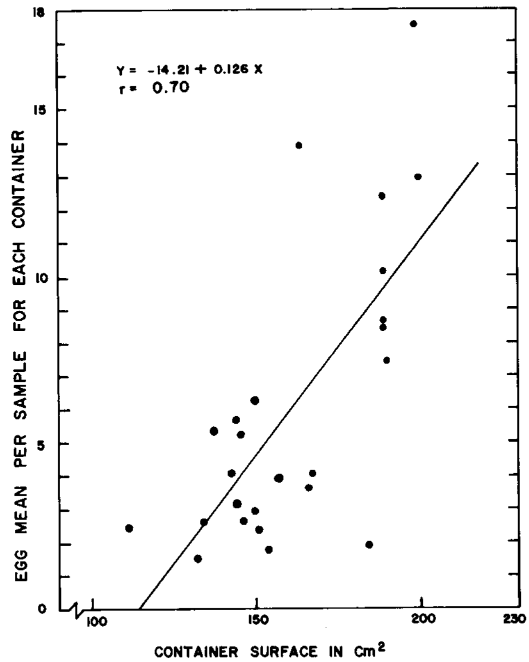

Fig. 4. Relationship between *Toxorhynchites theobaldi* oviposition rate and container's exposed surface area.

sites such as bamboo holes (Steffan and Evenhuis 1981). Linley (1987b) mentioned such advantages are of special relevance, because the females do not touch the water surface, and by this action they avoid spider predation. Furumizo and Rudnick (1978) stated that females have hygroreceptors to detect the water vapor of the breeding sites; however, *Tx. theobaldi* was observed at times touching the water with the legs in those containers with abundant organic wastes. This behavior was also observed in *Tx. brevipalpis* (Steffan and Evenhuis 1981). In order to detect such materials, it is possible that the females need to touch the water; this study suggests a hygroreceptive mechanism as well as a chemoreceptive apparatus in *Tx. theobaldi.*

It was noted that females of *Tx. theobaldi* which had an oviposition flight during the afternoon were approached by *Ae. aegypti* males making body contact with legs or wings. This behavior was always inhibitory to the oviposition flight and also for egg ejection. One reason for this may be the overlap that exists in the oviposition peak for both species. *Aedes aegypti* shows the oviposition peak well defined between 1600 and 1800 hr (Chadee and Corbet 1987), overlapping with the peak of *Tx. theobaldi.* It may be explained as a competitive interaction for space; however, Ikeshoji (1985) reported that the flight frequency for mate behavior in aedine mosquitoes is 460 Hz. Ten-day-old females of *Tx. theobaldi* showed a flight frequency which fluctuated from 450 to 500 Hz (Reyes, personal communication). These data suggest that females at the moment of oviposition exhibited a frequency very close to 450 Hz, and so they are attractive to *Ae. aegypti* males. The behavior of males can be explained as a coitus instinct rather than a competitive interaction for the oviposition site.

In respect to the relationship between oviposition rate and surface area, the oviposition rate shows a positive correlation with the surface area of the container (Fig. 4). This relationship is indicated by the linear equation, $Y = -14.21 + 0.126X$, where Y is the rate of oviposition and X is the surface area with a Pearson correlation coefficient of $r = 0.70$, significant at 0.05 level. These data quantitatively confirm the reports of several authors of the relation of species to oviposit more eggs when the container has a greater surface area (Focks et al. 1983, Reyes et al. 1987). In this case, the slope ($b = 0.126$) of the equation means that for each 1 cm^2 of the exposed surface, the oviposition rate increases by 0.13 eggs, and an increment of 7.9 cm^2 of the exposed surface is equivalent to one more egg in the container.

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